



Optimization and Evaluation of Topical Gel Containing Solid Lipid Nanoparticles Loaded with Metronidazole

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ABSTRACT

The present research was undertaken to develop, optimize, and evaluate a topical gel containing metronidazole-loaded solid lipid nanoparticles (SLNs) with the objective of improving the topical delivery of metronidazole and providing a suitable sustained-release formulation. Metronidazole is a nitroimidazole antimicrobial agent widely used in the treatment of infections caused by susceptible anaerobic microorganisms and protozoa. Conventional topical formulations may present limitations related to drug retention at the site of application and drug-release characteristics. Solid lipid nanoparticles offer several potential advantages as topical drug-delivery carriers, including nanoscale particle size, enhanced contact with the skin, controlled drug release, and protection of the incorporated drug.

Metronidazole-loaded SLNs were prepared using a suitable lipid-based nanoparticle preparation technique. The formulation was optimized by evaluating critical formulation parameters such as particle size, polydispersity index, zeta potential, entrapment efficiency, drug loading, and percentage yield. The optimized SLN formulation was incorporated into a suitable topical gel base and evaluated for appearance, homogeneity, pH, viscosity, spreadability, drug content, extrudability, and in-vitro drug-release characteristics. The release data were further analyzed using appropriate kinetic models to understand the drug-release mechanism.

The optimized metronidazole-loaded SLN formulation was expected to provide nanosized particles with satisfactory physicochemical characteristics and improved drug-retention/release properties. Incorporation of the optimized SLNs into a topical gel may provide a convenient and potentially effective platform for localized delivery of metronidazole. The developed formulation may therefore represent a promising approach for topical metronidazole delivery, subject to confirmation through appropriate biological and stability studies.

Keywords: Metronidazole, solid lipid nanoparticles, SLN, topical gel, nanoparticulate drug delivery, optimization, controlled release.

INTRODUCTION

Topical drug delivery involves the application of a pharmaceutical formulation directly onto the skin or other accessible external surfaces to achieve localized or, in some cases, systemic therapeutic effects. The topical route offers several advantages, including avoidance of gastrointestinal degradation, reduction of first-pass hepatic metabolism, ease of administration, and direct localization of the drug at the desired site of action.

However, effective topical drug delivery can be influenced by several physiological and physicochemical barriers. The stratum corneum represents the major barrier to penetration through intact skin. Drug molecular size, lipophilicity, solubility, formulation characteristics, skin hydration, and residence time can significantly affect topical drug delivery.

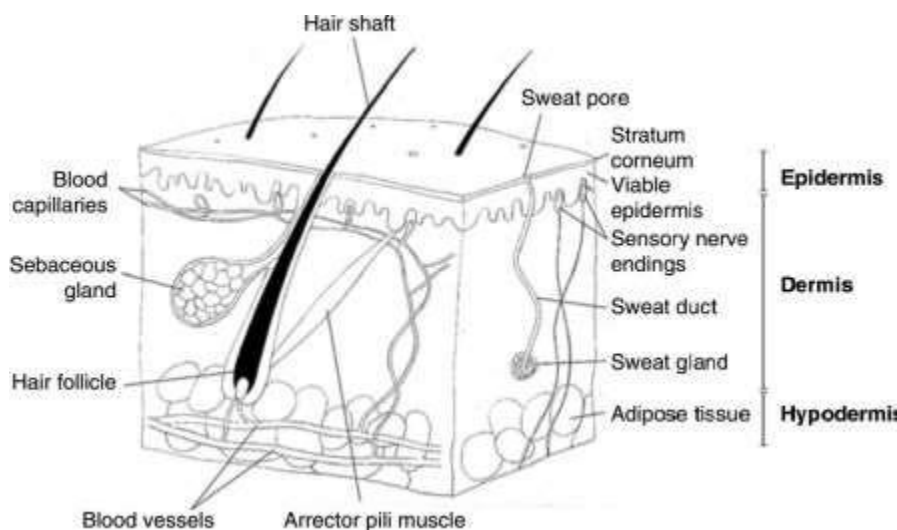
Nanotechnology-based drug-delivery systems have received considerable attention for improving the performance of topical formulations. Among these systems, solid lipid nanoparticles are colloidal carriers generally composed of physiologically acceptable solid lipids stabilized by surfactants. SLNs typically possess particle sizes in the nanometer range and can be used to incorporate drugs within or onto a lipid matrix.

SLNs may provide several advantages for topical drug delivery, including increased surface area, improved contact with the skin, controlled drug release, enhanced physical stability compared with some conventional lipid systems, and the possibility of improving drug localization within superficial skin layers.

Metronidazole is a synthetic nitroimidazole antimicrobial agent. It is active against a variety of anaerobic microorganisms and certain protozoal organisms. The drug is used in several dosage forms, including oral, intravenous, vaginal, and topical preparations. Topical administration can be advantageous when treatment is intended to act locally because it can potentially reduce unnecessary systemic exposure.

Despite the availability of conventional topical preparations, development of a nanoparticle-based gel may provide opportunities to modify drug release and improve the residence characteristics of metronidazole at the application site. Therefore, the present study was designed to formulate and optimize metronidazole-loaded solid lipid nanoparticles and subsequently incorporate the optimized nanoparticles into a topical gel.

The developed formulation was evaluated using relevant pharmaceutical and physicochemical parameters to determine its suitability as a topical delivery system.



Skin is divided by three layers like as ⁸⁵

- Epidermis
- Dermis
- Hypodermis

Epidermis

The epidermis is skin's outer layer, which is comparatively thin and hard. Epidermis at upper layer is keratinocytes. They developed out of cells in inner epidermis layer called basal layer. New keratinocytes hike smoothly up upper epidermis surface. Thickness is 100 μm . The main source of resistance to diffusion and permeation through skin is SC. Five layers of epidermis:

- Stratum basale
- Spinosum
- Granulosum
- Lucidum
- Corneum ⁶⁴

Dermis

Dermis is usually elastic, but is abundant in blood channels, lymphatic vessels, and nerve endings. A large network of dermal plexuses bind the essential circulation, with extensive horizontal branching in the dermis from arterioles and venules to form plexuses and flow from capillaries to hair and sweat glands. Dermal capillaries help to extract antigens outside of clear, fluid materials. ²³

Hypodermis

The hypodermis tissue supports upper layer and inner layer like as dermis and epidermis. It should be used as storage of fat. Hypodermis layer supports temperature balancing, provides nutrient support and physically protection. It moves blood vessels and ending nerves to skin, and may contain organs that feel pressure.

1.2 Solid Lipid Nanoparticles

Solid lipid nanoparticles (SLNs) are colloidal drug carrier system compressed of solid lipid diffuse in aqueous surface-active solution and size from 50 to 1000 nm particle size.

²¹ SLN offer better different to polymeric systems, colloidal systems and other NDDS owing to properties like good tolerability, physical stability, biodegradable, high bioavailability, protection of responsible drugs from decay, ease of preparation, and low toxicity. ^{38, 43, 67} In addition, production

process can be modified to release desired drug, protect decay of drug, and avoid organic solvents. This large-scale flexibility may be of prime importance in marketing of new products. Such quality makes SLN an interesting carrier system for better drug delivery.¹

1.2.1 Structure of solid lipid nanoparticles

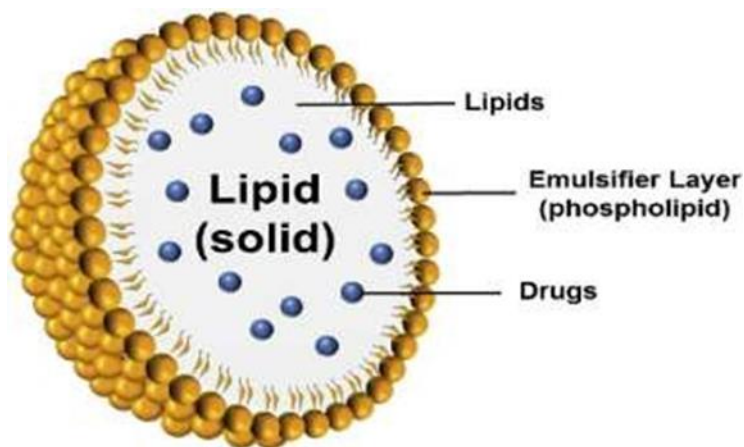


Figure 2: Structure of solid lipid nanoparticles.³⁶

Advantages of SLNs

1. Improve stability of pharmaceuticals.
2. Excellent biocompatibility.
3. Avoidance of organic solvent.⁷⁴
4. Long-term stability.
5. Improve bioavailability of trapped bioactive compounds.
6. Easy to scale up and sterilize.
7. Control and target drug release.²¹
8. Low toxicity.
9. Biodegradable and biocompatible

Disadvantages of SLNs

1. Poor drug loading capacity.⁶⁸
2. Less compatibility for hydrophilic drugs
3. Unsure gelation tendency.

2. Materials and Methods

2.1 Reagents and chemicals

Metronidazole received as gift sample from SMS Pharmaceuticals, INDIA, Carbopol 934 purchased from sigma Aldrich, Italy, Steric acid purchased from Fisher Scientific India Pvt. Ltd, Ethanol purchased from Merck, India, n-octanol purchased from SD Fine-chem. Ltd, Mumbai, Methanol purchased from Fisher Scientific India Pvt. Ltd, Poloxomer 188 purchased from Central Drug House (P) Ltd, Sodium Hydroxide, Potassium Dihydrogen orthophosphate, and Disodium hydrogen orthophosphate purchased from Thomas Baker, New Delhi.

2.2 Preformulation studies

2.2.1 Determination of the absorption maximum of metronidazole in ethanol

The absorption maximum of metronidazole determined as per standard protocol with some modification. In brief, stock solution of metronidazole developed at concentration of 1 mg/ml in methanol. Further, it followed by serial dilution to get concentration of metronidazole as 2, 4, 6, 8, 10 µg/ml,

and then it proceeded to UV spectrophotometric analysis at λ_{max} of 299 nm. Qualification is taken in triplicate and obtained data were analyzed statistically.⁴⁸

2.2.2 Determination of Aqueous Solubility

The determination of aqueous solubility of metronidazole estimated through Saturation shake - flask method. An optimum amount of metronidazole dissolved in distilled water and acetate buffer pH 5.5 then followed by vortex and centrifugation at 50 rpm at 37 °C for 48 hrs, resulting solution filtered and analyzed spectrophotometrically at 299 nm. Qualification is taken in triplicate.

2.2.3 Determination of lipophilicity

Lipophilicity of metronidazole determined through traditional shake flask method as described in protocol with some modification. In brief, an optimum uniform amount of metronidazole poured inside three different volumetric flasks and then measured quantity of lipids such as stearic acid, prectrol, dynasan 114 placed to each flask simultaneously. Resulting heterogeneous mixture proceeded to vortex and then centrifugation at 50 rpm at 37 °C for 48 hrs. Supernatant are separate and filtered using syringe filter of 0.22 μm . Filtrate then analyzed spectrophotometrically at 299 nm.¹⁴

The further partition coefficient of metronidazole determined using n-octanol and water partition system. Measured amount of metronidazole placed inside conical flask containing measured volumes of an n-octanol and aqueous buffer solution. flask shaken with uniform time interval for 48h to attain equilibrium and then resulting mixture placed to separating flask with final shaking and kept remains undisturbed to be separated inside two layers. Targeted measurement proceeded to be analyzed spectrophotometrically at 299 nm. Resulting values of both phases were determined in form of $\log_{10}P$ of ratio calculated. All qualification is taken in triplicate.¹⁶

2.2.4 Fourier Transform Infrared (FTIR) Spectroscopy

The spectral analysis is for metronidazole and stearic acid performed by Win-IR, Bio-Rad FTS spectrophotometer. Individual sample assorted with potassium bromide and later proceeds for spectroscopical observation under range of 4000 to 400 cm^{-1} .²⁹

2.3 Preparations of metronidazole loaded solid lipid nanoparticles (SLN)

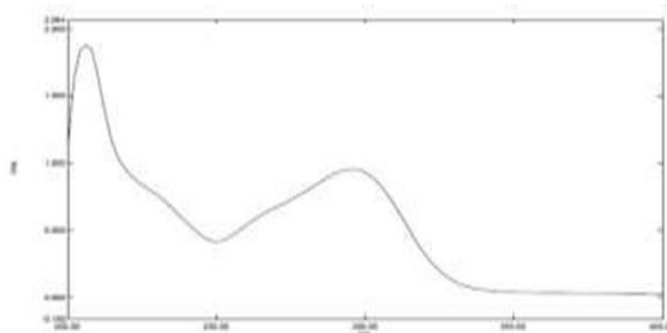
The SLN prepared using referenced protocol of solvent diffusion method with some modification. Briefly, known amount of metronidazole and stearic acid placed inside 5 ml of ethanol and heated at $60 \pm 3.0^\circ\text{C}$ on water bath. Obtained solution placed inside 5 ml of aqueous poloxomer 188 solutions at $4 - 8^\circ\text{C}$ under magnetic stirring at 2000 rpm with help of syringe. SLN formed instantly and recovered by centrifugation at 2000 rpm for 30 min at 4°C . Obtained heterogeneous mixture further proceeded to high-pressure homogenization via APV 2000 homogenizer at 1200 bars. Obtained mixture placed to be stable at room temperature, which turns to clear nanocrystals by recrystallization of dispersed lipid.

Results and discussion

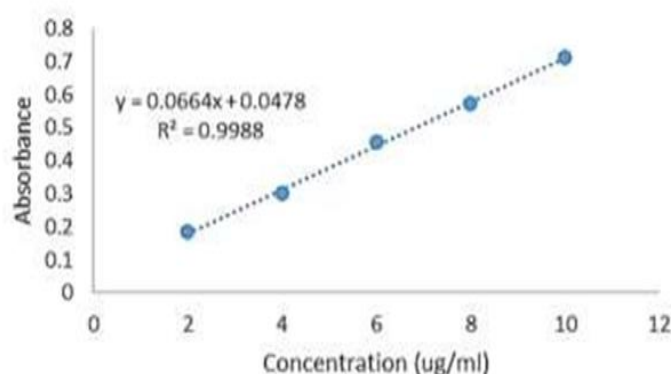
2.4 Preformulation study of drug

2.4.1 Determination of the absorption maximum of metronidazole in ethanol

The potential drug absorption calculated as per standard protocol through absorption maximum of metronidazole at 299 nm λ_{max} against concentration 2-10 $\mu\text{g/ml}$. regression equation and coefficient were found to be $0.0664x - 0.0478$ and 0.998 respectively. Associated aim to determine metronidazole absorption maxima and method validation is for qualitative and quantitative analysis.⁴⁸



Absorption maxima of luliconazole in methanol



Regression coefficient of luliconazole against concentration (2-10 µg/ml)

Figure 7: Absorption maxima of metronidazole and regression coefficient against the different concentration of metronidazole (µg/ml)

Physicochemical studies of metronidazole were conducted to evaluate physicochemical properties of drug. Studies conducted to evaluate metronidazole hydrophilic and lipophilic compatibility. Result shows that metronidazole having poor solubility potential with water which found to be 0.00585 ± 0.293 mg/ml, and solubility of metronidazole in stearic acid, prectrol, dynasan 114 obtained 23.754 ± 0.47 , 18.314 ± 0.85 , 22.875 ± 0.32 mg/ml. Besides, non-aqueous solubility obtained 17.984 ± 0.52 mg/ml for metronidazole in n-octanol. $\log_{10}P$ value of metronidazole in stearic acid, prectrol, dynasan 114, and n-octanol obtained as 3.98, 3.30, 3.87, and 3.65 simultaneously.

The FTIR analysis performed of metronidazole and stearic acid for better compatibility analysis of leading moiety before and after formulation. FTIR spectra of metronidazole is shown in Figure 7; Table 6. principal IR absorption peaks of metronidazole at 2979.43 cm^{-1} (C-H stretch), 2198.82 cm^{-1} (C≡N stretch), 1554.35 cm^{-1} (C-H aromatics stretch), 1471.35 cm^{-1} (C=C-C aromatic ring stretch), 820.41 cm^{-1} (para C-H distribution) and 759.46 cm^{-1} (C-Cl stretch) were all detected in spectra of luliconazole. These detected principal peaks confirmed purity and authenticity of metronidazole as similar to referenced report.⁵²

Table 6: FTIR interpretation of luliconazole

Characteristics Peaks	Reported (cm^{-1})	Observed (cm^{-1})
C-H stretch	2850 - 3000	2979.43
C≡N Stretch	2100 - 2400	2198.82
C=C aromatic stretch	1450 - 1650	1554.35
C=C-C Aromatic ring stretch	1510 - 1450	1471.35
para C-H distribution	860 - 800	820.41
C-Cl stretch	600 - 800	759.46

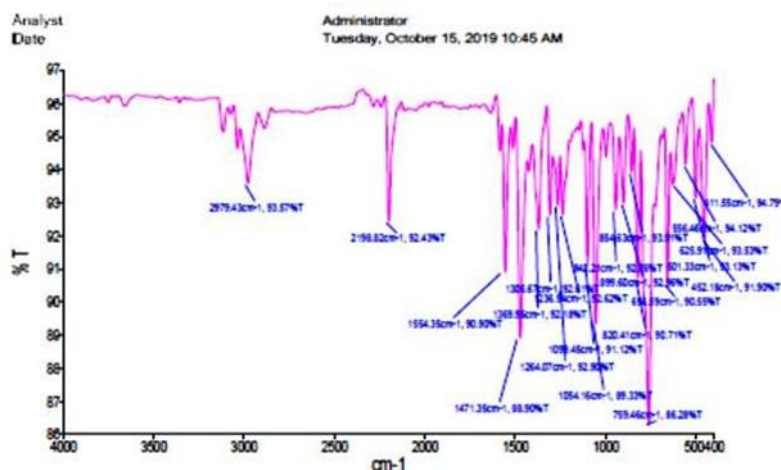


Figure 8: FTIR spectrum of luliconazole

The FTIR spectra are interpretation of stearic acid shown in Figure 9 and Table 7. principal IR absorption peaks of stearic acid at 2915.20 cm^{-1} (C–H stretch alkanes), 2848.02 cm^{-1} (C–H stretch aldehyde), 1700.89 cm^{-1} (C=O stretch saturated), 1471.59 cm^{-1}

(C–C stretch), 1295.47 cm^{-1} (C–O stretch, aromatic aster), 936.42 cm^{-1} (O–H bend), 719.89 cm^{-1} (C=C bend) and 547.03 cm^{-1} (C–I stretch) were all detected in spectra of stearic acid. These detected principal peaks confirmed purity and authenticity of stearic acid as similar to reported data.⁶⁶

Table 7: FTIR interpretation Stearic acid

Characteristics Peaks	Reported (cm^{-1})	Observed(cm^{-1})
C–H stretch alkanes	2850 - 3000	2915.20
C–H stretch aldehyde	2800 - 2860	2848.02
C=O stretch saturated	1700 – 1730	1700.89
C–C stretch	1400 - 1500	1471.59
C–O stretch, aromatic aster	1250 - 1310	1295.47
O–H bend	910 – 950	936.42
C=C bend	665 - 730	719.89
C–I stretch	500 - 600	547.03

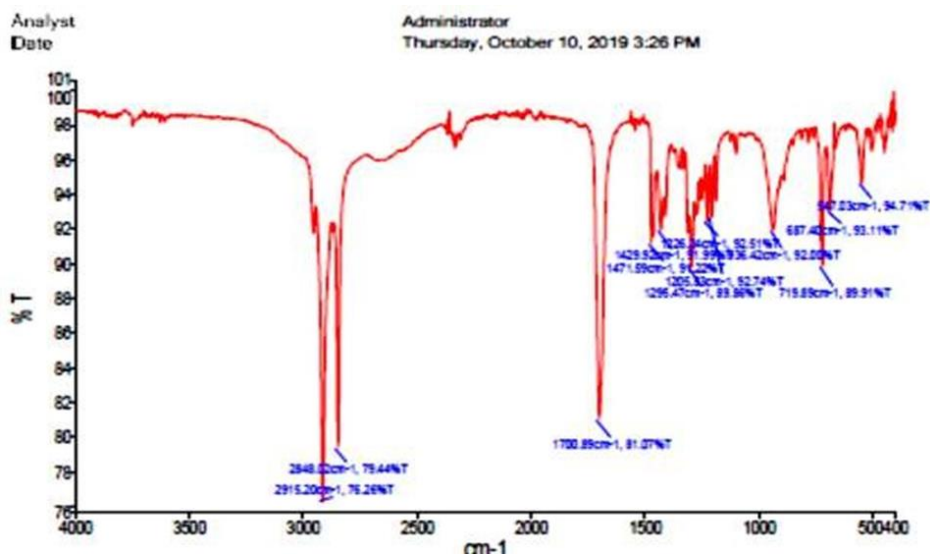


Figure 9 FTIR spectrum of stearic acid

2.5 Development of the method for metronidazole loaded solid lipid nanoparticles (SLN)

The method deals with various modified nano-precipitation methods for optimization of SLN in respect of EE of metronidazole at both treatment segments, i.e. nano-precipitation and cooling sonication probe. Temperature controlled by 4°C and 25°C in both segments. Instant adding of organic phase to aqueous phase conserved at 4°C which gives immediate precipitation due to hyphenation with anti-solvent. Temperature controlled at initial phase of nano-precipitation which helped to achieve homogeneity. High-pressure homogenization supports to get uniform homogeneity by decreasing larger crystals size and bead milling aggregation.⁸⁷ Further, in optimization of SLN, method archived step by step with alternate changes in concentration of stearic acid and poloxomer 188 (w/v) ranging from 0.5-2 %. All prepared groups of SLN were coded successfully and proceed to quantitate percent entrapment of active moiety spectrophotometrically at 299 nm. Obtained data were evaluated statistically. SLN which deal with high entrapment of metronidazole chosen as optimized SLN and proceed for further evaluation.

Table 8: Preparation of different metronidazole solid lipid nanoparticles

SLN code	Luliconazole% (w/v)	Different concentration of leading reagents for the formation of SLN	
		Stearic acid % (w/v)	Poloxomer 188 % (w/v)
F1	1	0.5	1
F2	1	0.7	1
F3	1	1	1
F4	1	2	1
F5	1	1	0.5
F6	1	1	0.7
F7	1	1	1.5
F8	1	1	2

2.6 Evaluation of SLN

2.6.1 Evaluation of entrapment efficiency of SLN

Initially, in pre-formulation studies, metronidazole characterized physio-chemically and spectroscopically. After successful formation of different batches of nanoparticles, percentage EE of metronidazole determined. Percentage of EE evaluated spectrophotometrically at 299 nm. Thereafter results reveal that SLN F6 and SLN F1 have highest and lowest % EE of metronidazole loaded SLN by 92.13%±0.975 and 53.78%±1.052 w/w respectively. Similarly, study cited by Ige et al. reported maximum % EE by 90–95% w/w.³⁹ Therefore, based on percent drug entrapment, SLN F6 selected as an optimized SLN and proceed for further evaluation includes physicochemical properties and gel formation. Percent drug entrapment of all SLN groups have been shown graphically in Figure 10

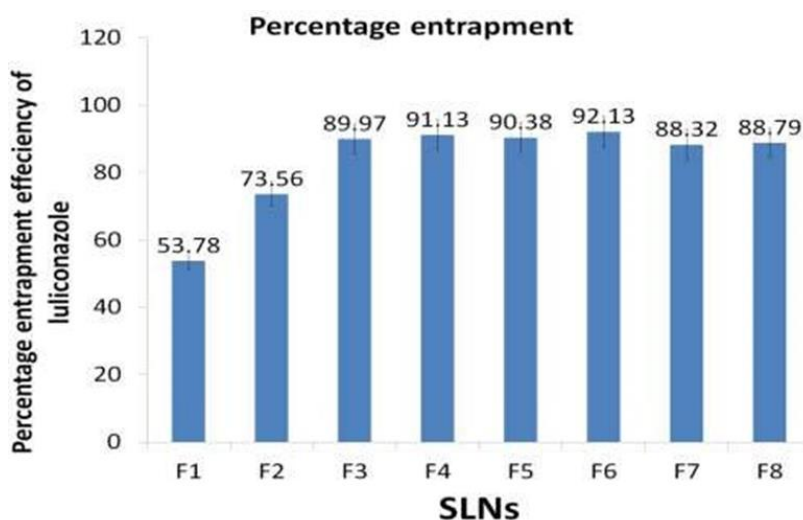


Figure 10: Percentage entrapment efficiency of metronidazole in SLN

2.6.2 Physicochemical property

The SLN F6 evaluated based on their physicochemical characteristics such as color, odor, pH stability, and aqueous solubility. physicochemical results reveal that SLN has white transparent color with homogeneous and uniform texture, aromatic odor, better stability at

2.7 pH, and water solubility found 0.01819 ± 0.035 mg/ml, i.e. much enough than metronidazole solubility.

2.7.1 Zeta potential and particle size and size distribution identification

The particle size analysis and zeta potential measurement of metronidazole SLN were identified successfully using nano ZS90 zetasizer system. Zeta potential is one of important parameters used to forecast physical stability of nanoparticles. Stability of nanoparticle system depends on high zeta potential value which points toward better stability of nanosystem since it could deliver deterring force between nanoparticles.⁸¹ As shown in Fig. 11, SLN shows quite high value of zeta potential by ~ 18.8 mV and states to high stability of nanosystem. In particle size analysis, SLN unveiled with mean particle diameter by ~ 344.3 nm, unimodal size distribution, polydispersity index (PDI) by 0.168, intercept value 0.98 and 92% peak intensity. PDI is parameter that represents dissemination factor with low aggregation of nanoparticles when PDI value would be < 0.5 .⁸⁸

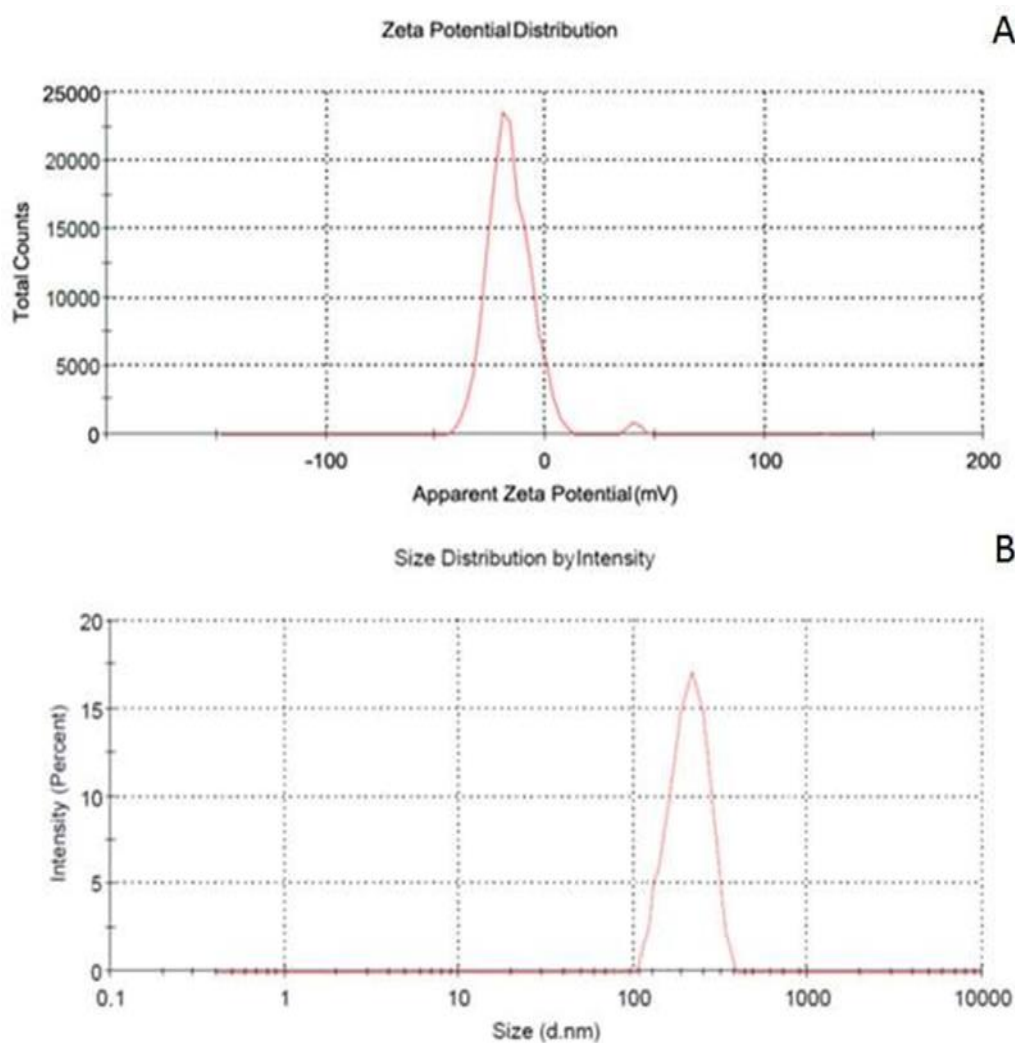


Figure 11 zeta potential, particle size and size distribution of metronidazoleSLN F6

2.7.2 Optical microscopy

Optical microscopy of optimized preparation i.e. SLN F6 defined with help of digital light optical microscope at 100x magnification and observation shows that metronidazole SLN is effectively localized with homogenous and uniform texture within SLN dispersion. It states that only particles with mean diameter higher than $2.5 \mu\text{m}$ which were visualized clearly against microscopy resolution power. Moreover, SLN preparation has even no self-assembled structures observed. Micellar structures were not observed during observation of optical microscopy. Optical microscopy images of metronidazole loaded SLN F6 shown in figure12

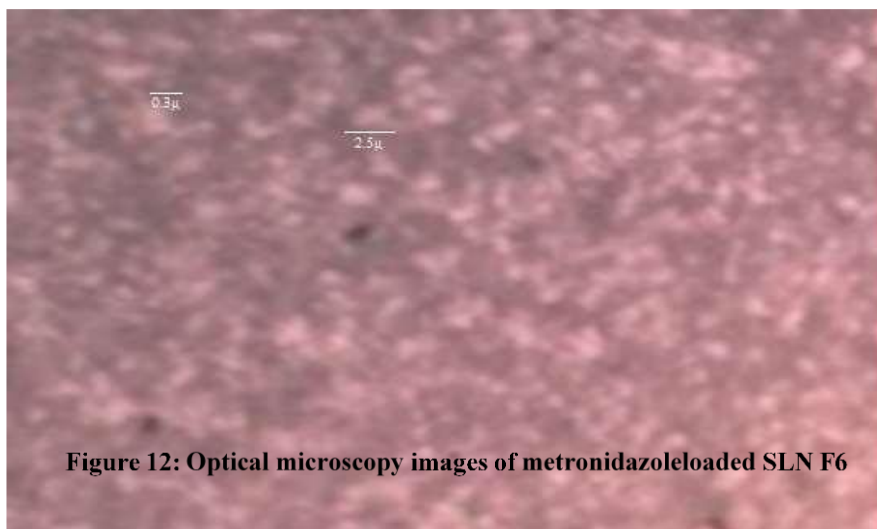


Figure 12: Optical microscopy images of metronidazoleloaded SLN F6

2.7.3 Drug-excipient comparability study by FTIR

FTIR analysis of SLN F6 performed to determine possible interaction between drug and drug additives. spectral data reveal principal absorption peaks of metronidazole at 2955.75 cm^{-1} for C-H stretching, 2523 & 2647 cm^{-1} for S-H stretching, 2201.52 cm^{-1} for $\text{C}\equiv\text{N}$ stretching, 1556.90 cm^{-1} for $\text{C}=\text{N}$ stretching, 1471.88 cm^{-1} for $\text{C}=\text{C}$ aromatic ring stretching and 720.33 and 1101.29 cm^{-1} for C-Cl stretching. Whereas, principal absorption peaks of stearic acid were found at 2914.97 cm^{-1} & 2848.05 cm^{-1} in high-frequency region attributed to $-\text{CH}_2-$ band asymmetric and symmetric stretching vibrations, whereas and 1698.03 cm^{-1} for $-\text{COOH}$ stretching is attributed in low-frequency region. Spectral analysis of optimized SLN confirmed that there are no more changes in metronidazole after successful formation of SLN. Spectral data strongly supports referenced values as reported.⁵²

Table 9: FTIR interpretation of SLN F6

Characteristics Peaks	Reported (cm^{-1})	Observed(cm^{-1})
C-H stretch	2850 - 3000	2955.75
		2914.97
		2848.05
$\text{C}\equiv\text{N}$ Stretch	2100 - 2400	2201.52
C=C alkene stretch	1650 - 2000	1698.03
C=C Aromatic stretch	1450 - 1650	1463.82
C-Cl stretch	550 - 850	609.29

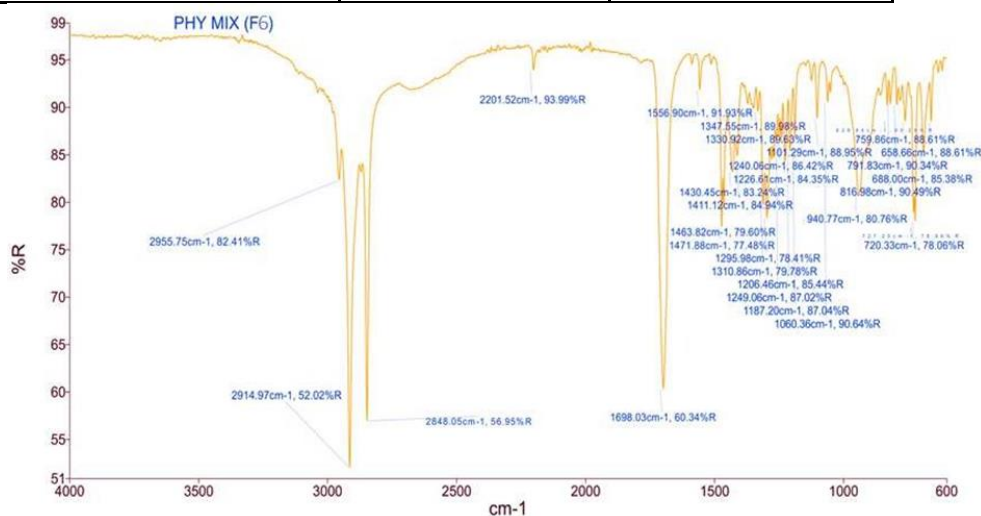


Figure 13: FTIR spectra of SLN F6

2.8 Optimization and evaluation of SLN gel

The topical gel containing SLN loaded with metronidazole prepared successfully by stirring method using carbopol 934 as gelling agent. Method of preparation of different SLN gel found unpretentious and robust. Initially, all four different preparations of SLN gel coded as G1, G2, G3, and G4, were evaluated to quantitate percent entrapment of metronidazole spectrophotometrically at 299 nm. Resulting data shows that SLN G3 with 1.5% carbopol w/w showing highest percentage of drug entrapment with $91.39\% \pm 0.187$. Thereafter optimized formulation is further evaluated to access physiochemical parameters includes visual appearance, pH, viscosity and spreadability. Resulting data reveals that viscosity of G3 gel as found to be 369cP, similar to gel viscosity as reported by Jana et al and pH found to be 6.12 ± 0.255 .⁴¹ further in spread ability evaluation, spreadability factor of prepared SLN gel found to be 4.5 and it states that prepared gel produces excellent spread ability as an ideal topical formulation. Spread ability is one of important physical properties of any topical formulation from patient's compliance point of view.¹⁸



Figure 14: visual appearance of SLN G3 gel

2.9 In vitro drug release and kinetics study

Statistical models are commonly used to forecast release mechanism and compare release profile. *in-vitro* release profile of drug performed in prepared buffer system using dialysis bag technique for 24 hrs. desolvation percentages of metronidazole from SLN are increased in proportion of time as illustrated in Figure 15 and Table 10. Pieces of evidence of release profiles show that developed SLN is proficient to release drug in regulated manner. Slow release of leading moiety from most SLN form is based on homogeneous drug entrapment throughout systems.⁶² Ekambaram et al. state same concept and claimed that controlled drug desolvation profile can be attained when drug is uniformly distributed in lipid matrix. Poloxamer 407 having immense efficacy against drug release rate from SLN then Cremophor RH40 because of its higher HLB value than cremophor RH 40.²⁰ Besides, Poloxamer 407 has high exterior spreadability so that it eases effects of interfacial tension between SLN and dissolution medium. It also reduces accumulation of drug particles and increases drug dissolution rate. Moreover, lipid mass in SLN can control size of nanoparticle and increase drug desolvation strength. Thickness of lipid surrounded nanoparticle increases length of drug disassociation resulting prolonged effect of drug release.¹⁷

Table 10: Percentage drug release profile of G3 and control gel.

Sr. no.	Time in hours	Percentage drug release of G3	Percentage drug release of control gel
1	0	0	0
2	0.25	7.375 ± 0.153	1.923 ± 0.011
3	0.5	14.002 ± 0.185	2.052 ± 0.155
4	1	22.064 ± 0.102	3.042 ± 0.158
5	2	32.289 ± 0.173	3.182 ± 0.162
6	3	40.622 ± 0.165	5.094 ± 0.122
7	4	47.048 ± 0.151	7.815 ± 0.205
8	6	55.582 ± 0.163	8.706 ± 0.215

Sr. no.	Time in hours	Percentage drug release of G3	Percentage drug release of control gel
9	8	62.309±0.134	9.387± 0.118
10	12	69.939±0.115	9.035± 0.205
11	24	79.578±0.213	9.773± 0.158

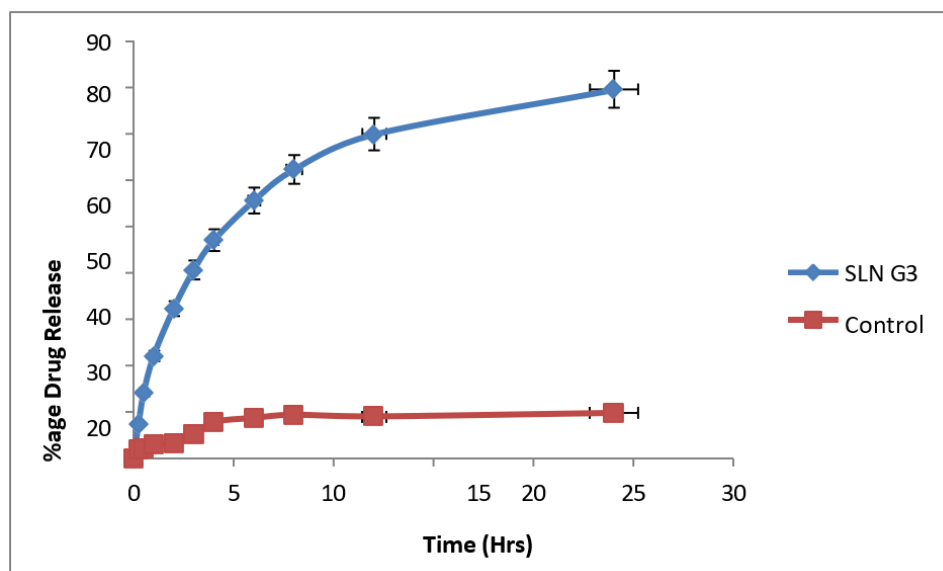


Figure 15: In-vitro drug release profile of SLN gel and control gel

Furthermore, an *in-vitro* drug release profile for optimized formulation applied to various kinetic models (zero-order, first-order, Higuchi, and KrosmyerPeppas model). To state kinetics profiling of drug release, obtained data were analyzed statistically in respect of rate constant and highest correlation. Best-fitted line found in all models except little suitability in zero-order equation. Resulting data describe dissemination of drug in controlled or regular manner from homogenous matrix systems and it states why drug disseminates at slower rate. Observations concluded that SLN G3 is far efficient as potential topical formulation for sustained drug delivery. This finding is almost similar to virtuous covenant as per previous shreds of evidence. Graphical representation of kinetics order of SLN G3 gel shown in Figure 16⁸⁴

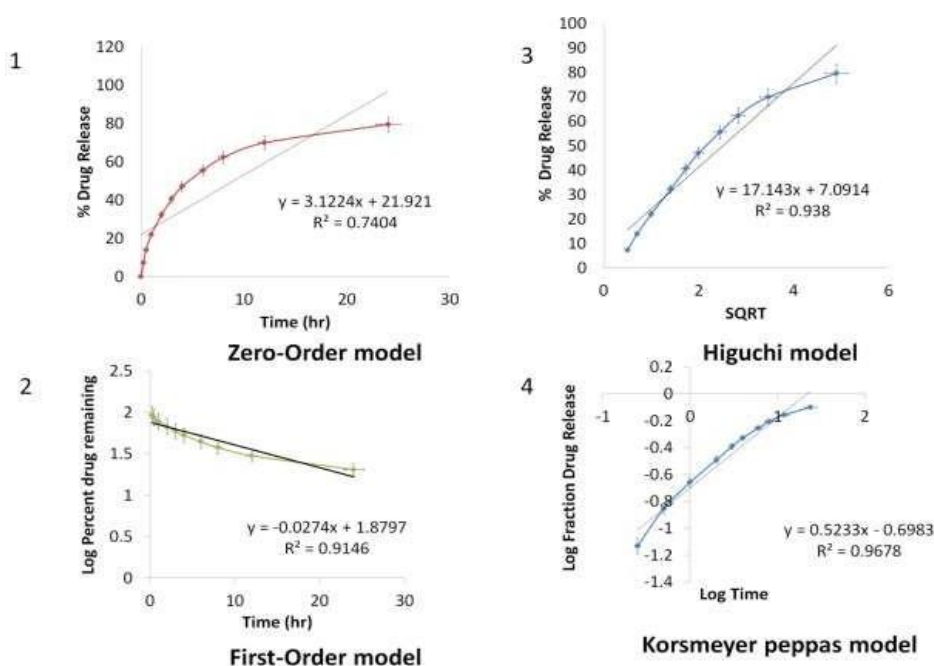


Figure16: kinetics order of SLN G3 gel

2.10 FTIR spectral analysis of SLN G3 gel

The FTIR spectral analysis of SLN gel G3 performed successfully to determine possible interaction between drug and drug additives and obtained spectral data matched with spectral data of metronidazole and stearic acid. Findings of spectral analysis show principal absorption peaks at 3331.36cm^{-1} for N-H stretching, 2961.88cm^{-1} for C-H stretching, 2193.49cm^{-1} for $\text{C}\equiv\text{N}$ stretching, 609.26 & 1044.56cm^{-1} for C-Cl stretching for luliconazole. Whereas, principal absorption peaks of stearic acid were ascribed to 2932.49cm^{-1} & 2863.16cm^{-1} in high-frequency region attributed to $-\text{CH}_2-$ band asymmetric and symmetric stretching vibrations, and 1639.31cm^{-1} for $-\text{COOH}$ stretching is attributed in low-frequency region. Spectral analysis of optimized formulation G3 reveals that no more possible interaction between drug and drug additives even after successive formation of topical gel. Hence it can be said that spectra show purity and authenticity of SLN G3 gel.

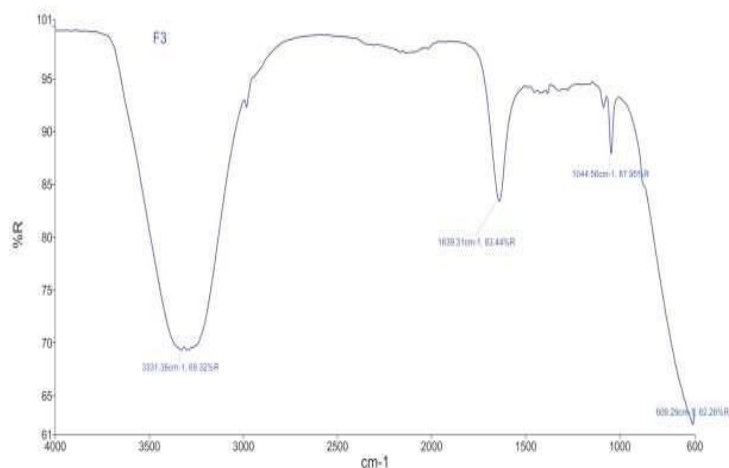


Figure17: FTIR spectra of SLN gel G3

Table 11: FTIR interpretation of SLN G3 gel.

Characteristics Peaks	Reported (cm^{-1})	Observed(cm^{-1})
N-H stretch	3300 – 2400	3331.36
C=C stretch	1638 – 1648	1639.31
CO - O – CO stretch	1040 – 1050	1044. 56
C – Cl stretch	550 – 850	609. 26

2.11 Scanning electron microscopy

The shape of enhanced formulation confirmed through SEM study and is shown in Figure

18. Most of vesicles is well specified, spherical, and discreet having large internal aqueous space. Low density of nanoparticles is shown in SEM analysis which may lead due to factor of dilution of nanosuspension before preparing SEM photographs. SEM studies reveal that metronidazole loaded SLN in gel had spherical shape with smooth surface.

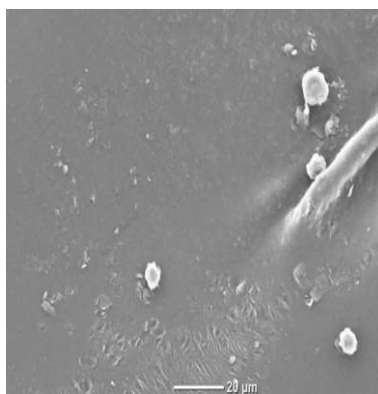


Figure 18: SEM analysis of SLN G3 gel

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