



Formulation and Evaluation of Clarithromycin Loaded Nanostructured Lipid Carriers for the Treatment of Acne

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Authors' contributions

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ABSTRACT

Background: Clarithromycin is a macrolide antibiotic used in acne treatment, but it has poor solubility, which decreases its permeability through lipid barriers such as skin. Nanostructured lipid carriers can enhance the permeability of clarithromycin through the skin, thus improving its potential for controlling acne.

Aim: To formulate and evaluate Nanostructured lipid carriers of clarithromycin for topical delivery in acne treatment

Methods: Nanostructured lipid carriers were prepared by emulsification and ultrasonication methods using lipids such as glycerol monostearate and oleic with poloxamer 188 as stabilizer. These nano-carriers were optimized with the help of the Quality by Design (QbD) approach employing Design-Expert[®] software. The nanoparticles were characterized for particle size analysis, zeta potential, drug-excipient compatibility, entrapment efficiency, and surface morphology by Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM). The nano-carriers were also investigated for *in vitro* drug release and *ex vivo* permeation

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through excised goat skin. The optimized formulation was incorporated into topical carbopol gel base, formulated and examined for pH, viscosity, spreadability, *in vitro* drug release, *ex vivo* permeation, and stability under accelerated conditions.

Results: The average particle size of the optimized nanoparticles was 164.8 nm, and zeta potential was -39.2 mV. FTIR studies showed that drug and lipids are compatible with each other. The morphology study by SEM and TEM showed spherical shaped particles. The entrapment efficiency of the optimized formulation was found to be 88.16%. *In vitro* drug release studies indicated sustained release from the formulation due to diffusion through the lipid matrix of the particles. The *ex vivo* permeation study using goat skin produced greater permeation from the NLC gel (89.5%) than marketed gel (65%) due to the lipid solubility of the nanoparticles in the skin. The formulation was stable under accelerated conditions.

Conclusion: The optimized formulation can be considered as promising nano-carriers suitable for the sustained release of clarithromycin into the skin for effective control of acne.

Keywords: Nanostructured lipid carriers, clarithromycin, acne vulgaris, ultrasonic method.

1. INTRODUCTION

Nanostructured lipid carriers (NLC) are colloidal particles composed of physiological, biocompatible and biodegradable lipids, surfactants and co-surfactants mixed at specific ratios and dispersed in aqueous solutions. They have a solid matrix at room temperature and are considered superior to many other traditional lipid-based nanocarriers such as nanoemulsions, liposomes and solid lipid nanoparticles (SLNs) due to their enhanced physical stability, improved drug loading capacity, and biocompatibility [1]. In contrast to SLNs, the inclusion of liquid lipids in NLCs further improved the drug loading capabilities of the particles [2]. NLCs can protect the entrapped drugs from chemical degradation and, at the same time, improve the stability of photo-sensitive drugs from oxidation, hydrolysis and enabling the modulation of the drug release. In dermatological applications, NLC particles produce a close contact with the stratum corneum due to their small size and decrease the packing of corneocytes, promoting the penetration of drugs into deeper layers of the skin [3]. Due to the occlusive properties of the lipid nanoparticles, an increased skin hydration effect is observed.

Acne vulgaris a chronic inflammatory disease of the pilosebaceous unit, with a prevalence of 70–85% in adolescents. [4,5]. *Cutibacterium acnes* and *Staphylococcus aureus* are the two microbes responsible for developing acne vulgaris [6], characterised by inflammatory papules, pustules, nodules, and cysts that may contribute to scarring and pigmentary changes, and the lesions occur on the face, neck, chest, upper back, and shoulders [7,8]. Acne causes pimples, scars, redness and dullness of the skin. Acne

marks will remain for a month or a lifetime and affect a person's physical, psychological, and emotional behaviour [9]. Current therapy includes the use of several topical agents such as benzoyl peroxide, tretinoin, antibacterial agents and antibiotics such as clindamycin amongst many others in the form of ointments, creams or gels. However, the therapeutic success in acne depends on a regular application of the topical agents over a prolonged period of time [10,11]. Besides, the side effects associated with the constant use of these topicals such as skin peeling and reddening, considerably affect the patient compliance and obstruct the acne treatment. The use of nanoparticles as carriers for topical agents have helped in optimizing their efficacy by either modulating their physicochemical and biopharmaceutical properties or minimizing/eliminating the side effects associated with them, thus offering better patient compliance [12]. Thus NLCs are capable of improving the topical delivery of antiacne agents by enhancing their dermal localization with a concomitant reduction in their side effects.

Clarithromycin is a macrolide antibiotic that belongs to BCS Class II drugs. It is used in the treatment of severe acne and also treats soft tissue and skin infections. Clarithromycin is commercially available in the strength of 1% w/w in a gel formulation for topical use. Moreover, it is not very useful unless combined with benzoyl peroxide for acne control, probably due to its poor solubility [7]. So, we hypothesized that NLCs incorporated with clarithromycin in a gel base would provide better skin permeation of the antibiotic and the sustained release could ensure constant levels of the drug in acne affected areas of the skin.

2. MATERIALS AND METHODS

2.1 Materials

Clarithromycin (CLR) was a gift from Rawia International Healthcare Pvt Ltd. (Mumbai, India). Poloxamer 188 was procured from Yarrow Chem Products (Mumbai, India). Glycerol monostearate and oleic acid were from Loba Chemie Ptv Ltd. (Mumbai, India).

2.2 Compatibility Studies using Fourier Transform Infrared (FTIR) Spectroscopy

FTIR spectroscopy was used to find out the compatibility between drug and polymer. The FTIR analysis was carried out using approximately 10 mg drug and optimised formulation. The samples were placed directly into the instrument (Alpha Bruker IR spectrometer), and ATR-FTIR spectra were recorded at the mid-frequency range (4000-400 cm^{-1}) at 4 cm^{-1} resolution [13].

2.3 Preparation of Clarithromycin Loaded NLC

CLR loaded NLCs were prepared by emulsification and ultrasonication method. The lipid phase was prepared by mixing the required amount of glycerol monostearate and oleic acid at 70°C in a water bath, and to that lipid mixture, clarithromycin was added. Then surfactant (poloxamer 188) was dissolved in distilled water with magnetic stirring at a temperature of 70°C. Both phases were maintained at the same temperature and combined by adding an aqueous phase to the lipid phase with continuous stirring. Then the mixture was kept for mixing using a mechanical stirrer for 1 hour at 1500 rpm. The prepared emulsion was sonicated for 10 min for further size reduction and finally stored at 2-5°C [14].

2.4 Design of Experiments

Design Expert[®] 12 software (Stat-Ease Inc, Minneapolis, MN, USA) was used to study the various effects on the preparation of NLCs and 2³ factorial design was selected for optimisation of NLC. Independent variables include the concentration of solid lipid, liquid lipid, and surfactants. The particle size and zeta potential were considered dependent variables. Each factor includes high, medium, low values (+1, 0, -1), respectively. Ten runs were generated by the

design expert[®] software for the formulation of NLC [15,16].

2.5 Characterisation of Prepared Clarithromycin Loaded NLC's

2.5.1 Particle size and zeta potential

The particle size was determined by employing Dynamic Light Scattering (DLS) principle of the Malvern Zetasizer (Nano ZS Malvern, UK) under ambient conditions. The zeta potential was used to determine the electrostatic mobility and for the stability of NLC formulation. The nanoparticles with the zeta potential range above ± 30 mV have a high degree of stability [17, 18].

2.5.2 Entrapment efficiency

The percentage entrapment efficiency of the drug corresponding to the amount of drug encapsulated within and adsorbed onto the NLCs was calculated indirectly. Calculating the concentration of free drug in the dispersion medium. To determine entrapment efficiency, the dialysis bag method was used. The formulation was taken in the dialysis bag and was immersed in media containing phosphate buffer pH 5.5. The free drug was dialysed for 2 hours, and the concentration was determined using a UV visible spectrophotometer. The entrapment efficiency and drug loading were calculated the following formula:

$$\% \text{ EE} = (W_T - W_F / W_T) \times 100$$

$$\% \text{ DL} = (W_T - W_F / W_L) \times 100$$

W_T is the weight of drug taken, W_F is the analysed weight of the free drug in dialysis media, W_L is the weight of the total lipid [18].

2.5.3 Scanning electron microscopy (SEM)

SEM (Philips XL 30) was used to analyse the surface morphology of the formulations. The optimised NLC sample was freeze-dried, and the particle was sputtered with gold before the examination to make the surface of particles electro-conductive and was observed, and photomicrographs were taken [19].

2.6 Transmission Electron Microscopy (TEM)

TEM (Philips electron optics BV, Netherland) was used to analyse surface characteristics of nano lipid carriers. The sample was mounted on the carbon-coated copper grid and stained with a drop of 1%(w/w) aqueous solution of phosphotungstic acid [20].

Table 1. List of variables in 2³ factorial design

Variables	Name	Units	Levels		
			Low	Medium	High
Independent variables					
A	Solid lipid	gm	1.2	1.5	1.8
B	Liquid lipid	ml	0.22	0.555	0.89
C	Surfactant	gm	0.6	0.8	1.0
Dependent variables		Units			
R1	Particle size	nm			
R2	Zeta potential	mV			

2.6.1 *In vitro* drug release

The drug release study was carried out using Franz diffusion cell. The cellophane membrane was soaked overnight in the phosphate buffer and then mounted between the donor and receptor compartments. The receptor compartment contained a phosphate buffer of pH 5.5, and the optimised NLC formulation equivalent to 10 mg was placed in the donor cell. The study was carried out at 37±0.5°C, and the medium was continuously stirred at 50 rpm in the receptor compartment. One ml of sample was extracted from the receptor compartment at stipulated time intervals and replaced with buffer to achieve sink conditions. These samples were analysed by using a UV spectrophotometer at 226 nm [21]. This study was repeated with an aqueous solution of the drug in a phosphate buffer of pH 4.0.

2.6.2 Kinetic analysis

The *in vitro* drug release data were interpreted using different mathematical models such as zero order, first order, Higuchi matrix, and Korsmeyer-Peppas model to understand the drug release kinetics. The best fit model was chosen based on the R and K values obtained from these models.

2.6.3 Preparation of CLR loaded NLC gel

Carbopol 934 (1%) was dissolved in distilled water and stirred using a mechanical stirrer for 2 hours. Then the solution was neutralised by adding triethanolamine slowly with constant stirring until the pH was about 6.2. Sufficient NLC dispersion was admixed with the neutralised gel so that the final drug concentration in the gel was 1% w/w [19].

2.7 Evaluation of CLR Loaded NLC Gel

2.7.1 Measurement of pH

The pH of a topical formulation is an essential factor that influences skin permeability and skin

irritation. The pH was measured using a calibrated electronic pH meter. The pH meter electrode was kept in constant touch with the gel and allowed to equilibrate for 1 min [22].

2.7.2 Viscosity

Viscosity indicates fluid resistance to flow; the higher the viscosity, the more excellent the resistance, and the greater the gel's thickness on application. The viscosity was determined using Brookfield viscometer (Brookfield DV-II+ Pro) using spindle number T-95 at different rpm i.e. 5, 10, 20, 50, 100 rpm [23].

2.7.3 Spreadability

A simple apparatus was used to determine this parameter and consists of 2 slides. One of the slides on which the formulation is placed is fixed. The movable slide is positioned with one end attached to a string to which load was applied using a pulley and a pan. Time taken for the upper slide to travel a distance of 0.5 cm apart from the lower slide under the direction of the weight was noted [24].

2.7.4 Drug content

The drug content study was done to determine the amount of drug present in the gel. About 1gm of the gel was taken and dissolved in 100 ml of phosphate buffer, pH 5.5, then shaken using a vortex mixer for 2 hours. Then it was then filtered, diluted using phosphate buffer pH 5.5, and absorbance at 226 nm was measured using a UV spectrophotometer [25].

2.7.5 *In vitro* drug release from NLC incorporated gel

In vitro drug release by dialysis was studied to determine the extent and duration of the release of the drug incorporated in the gel. This study was conducted using the same approach used for NLC particles as described earlier. Drug

release profile from the NLC gel was compared with that of pure drug-containing gel and that from a marketed formulation with similar clarithromycin concentrations (1% w/w). Release data from the NLC gel was subjected to kinetic analysis by fitting to various mathematical models such as zero order, first order, Higuchi's and Korsmeyer papas model to define the mechanism of drug release [21].

2.7.6 Ex vivo skin permeation study

Ex-vivo diffusion experiments were performed to test the permeation of clarithromycin administered through NLC gel and CLR marketed gel using excised goat skin. The sample of goat skin was collected from the nearby slaughterhouse. Skin samples were treated separately with the formulations (CLR loaded NLC gel and clarithromycin marketed gel). Then the skin was kept between the donor compartment and the receptor compartment of the diffusion cell. Quantities of NLC gel and marketed gel equivalent to 10 mg of CLR were placed in each donor compartment, while the receptor compartments were filled with phosphate buffer pH 5.5. The receptor medium was maintained at $37\pm 0.5^\circ\text{C}$ and stirred at 50 rpm. Samples of 1 ml were collected from the receptor compartment at stipulated time intervals and then replaced with fresh buffer to maintain the sink condition. These samples were analysed at 226 nm using a UV spectrophotometer [21].

2.8 Stability Studies

The CLR loaded NLC gel was placed in an airtight container. The formulation is kept at $40\pm 2^\circ\text{C}/ 75\pm 5\%$ RH for short-term stability studies as per the ICH guidelines. Samples are withdrawn and analysed every month for pH and drug content [25].

3. RESULTS AND DISCUSSION

3.1 Compatibility Studies using FTIR

FTIR spectroscopy investigations were carried out on clarithromycin drug and optimised NLC formulation. The IR spectrum of the pure clarithromycin showed characteristic peaks at 2889.37 cm^{-1} , 1112.01 cm^{-1} , due to C-H stretching, C-O stretching, and optimised formulation showed the characteristic peaks at 2914.13 cm^{-1} and 1111.02 cm^{-1} similar to the peaks which are generated in the drug. There is no interaction between drug and lipids. So, this

observation indicates that the drug and lipids are compatible with each other [26].

3.2 Preparation of Clarithromycin Loaded NLC

NLC formulation was prepared by using emulsification and ultrasonication methods. The glycerol monostearate is used as solid lipid, oleic acid as liquid lipid, and Poloxamer188 is used as a surfactant in the preparation of CLR loaded NLC.

3.3 Evaluation of Clarithromycin Loaded NLC

3.3.1 Particle size and zeta potential

Based upon the preliminary evaluations, variables *viz.* Concentrations of solid lipid, liquid lipid and surfactants, have been found to affect the characteristics of NLCs significantly. Design Expert® (Stat-Ease; MN Trial Version 12.0.3.0) was therefore used for the preparation of different combinations of the above mentioned independent variables within a defined limit, *i.e.* solid lipid concentration (1.2 to 1.8 gm), liquid lipid concentration (0.22-0.89 ml) and surfactant concentration (0.6-1 gm). 23 Complete factorial architecture was used to study the effect of the independent variable on the characteristics of the NLC. Table 2 shows a set of 10 runs generated and their respective responses.

Effect of Particle size: The particle size for the NLC formulations ranged from 159.8 nm to 281.2 nm. The final equation in terms of coded factors is given in the following equation:

$$\text{Particle size} = + 229.86 + 41.92A - 9.45 B - 8.75 C + 5.38 AC \quad (1)$$

The concentration of the lipid had a positive effect on particle size, and a negative effect was seen for the surfactant concentration. The model generated for the particle size had a P-value of <0.05 and F value of 173.13, indicating the model to be significant. The value of 1.22 indicates a non-significant lack of fit, implying the model to be appropriate to calculate the particle size. The difference between the adjusted (R^2 0.9871) and the predicted (R^2 0.9751) model R-square value was less than 0.2, indicating reasonable agreement between the two. The amount of the solid lipid and surfactant had a significant effect on the particle size, as shown in the above equation [27].

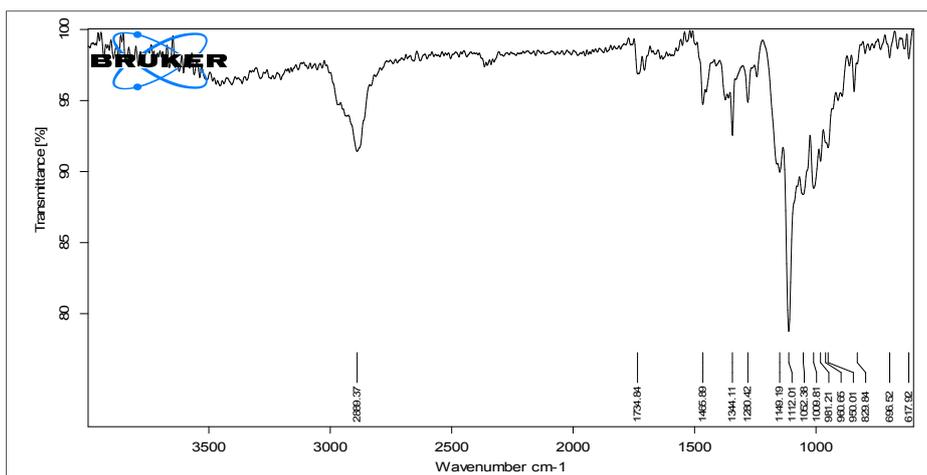


Fig. 1. FTIR spectra of clarithromycin

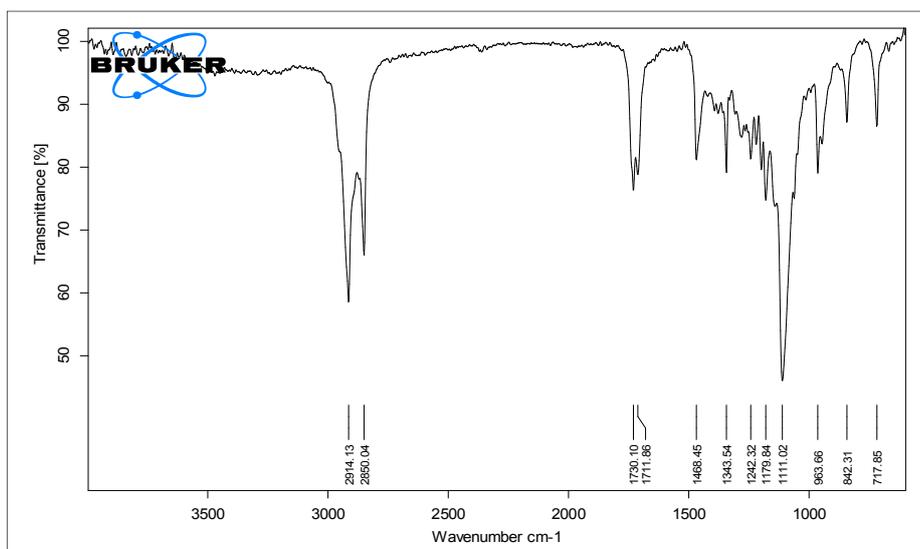


Fig. 2. FTIR spectra of optimised formulation

Table 2: 2³ factorial design

Run	Factor 1 A:Solid lipid gm	Factor 2 B:Liquid lipid ml	Factor 3 C:Surfactant Gm	Response 1 Particle size nm	Response 2 Zeta potential mV
1	1.5	0.555	0.8	231.4	-35.3
2	1.5	0.555	0.8	237.6	-35.7
3	1.2	0.22	0.6	209.8	-33.3
4	1.2	0.89	0.6	192	-37.1
5	1.8	0.89	1	258.7	-32.8
6	1.8	0.22	1	275.8	-36.5
7	1.2	0.89	1	159.5	-40.8
8	1.2	0.22	1	185.8	-34
9	1.8	0.22	0.6	281.2	-31
10	1.8	0.89	0.6	266.8	-38.7

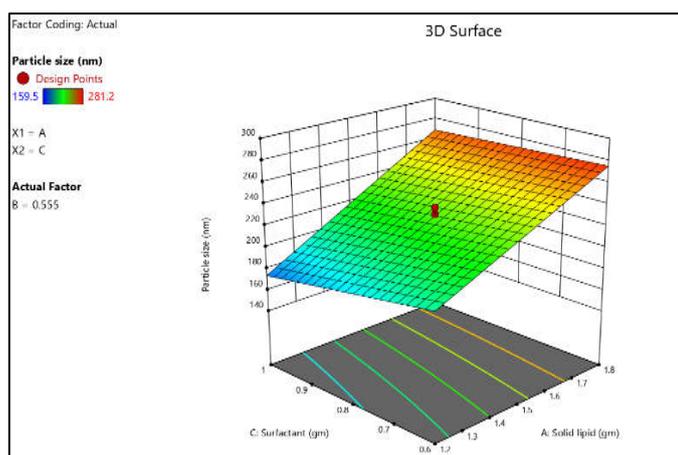


Fig. 3. 3D plot of particle size (nm)

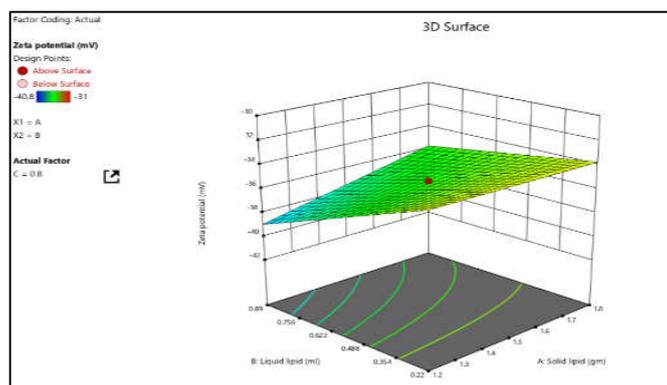


Fig. 4. 3D plot of zeta potential (mv)

Table 3. Predicted and actual responses of optimised NLC formulation

Factors			Responses	
Solid lipid	Liquid lipid	Surfactant	Particle size (nm)	Zeta potential (mV)
1.2	0.890	1.0	Predicted 164.36	-40.795
Percentage Error			Actual 164.8	-39.2
			0.26	3.9

Effect on Zeta potential: The zeta potential for NLC formulations ranged from -40.8 mV to -31 mV. The final equation in terms of coded factors is given in below equation:

$$\text{Zeta potential} = -35.52 + 0.7750A - 1.82B - 0.5000C + 0.8250AB + 0.6000AC + 1.05 + 1.80ABC \quad (2)$$

The concentration of solid lipid did not affect zeta potential, and a slightly negative effect was seen because of surfactant concentration.

The model generated for zeta potential had a P-value of <0.05 and F value of 269.89, indicating the model to be significant. The value of 0.01 indicates a non-significant lack of fit, implying the model to be appropriate to calculate the zeta potential. The difference between the adjusted (R^2 0.09952) and the predicted (R^2 0.9945) model R-square was less than 0.2, indicating reasonable agreement between the two. The amount of solid lipid and liquid lipid had a significant effect on the zeta potential of the NLC formulation shown in the above equation [28].

Where A, B, C represents the coded values for solid lipid, liquid lipid and surfactant respectively, and AB, AC, ABC are interaction effect between the concentration of solid lipid and liquid lipid, the concentration of solid lipid and surfactant, concentration solid lipid, liquid lipid, and surfactant.

3.3.2 Entrapment efficiency

The drug entrapment efficiency was carried out using the dialysis bag method. The percentage drug entrapment efficiency of the optimised nanostructured lipid carrier was found to be 88.16%. The drug loading capacity of the NLC was found to be 4.21% due to the unique arrangement of NLC matrices. In the NLC, the solid lipid matrix is surrounded by a small portion of oil in which the solubility of the drug is

significantly higher than enhances the overall drug loading capacity. The larger amount of liquid lipids significantly increases entrapment efficiency.

3.3.3 Scanning electron microscopy (SEM)

The SEM was used to examine the shape and morphology of the optimised NLC formulation. The CLR loaded NLC were spherical with a more or less smooth surface.

3.3.4 Transmission electron microscopy (TEM)

Studies show that the photomicrograph from the TEM studies showed the particles have a spherical shape, and the average size was found to be 200 nm.

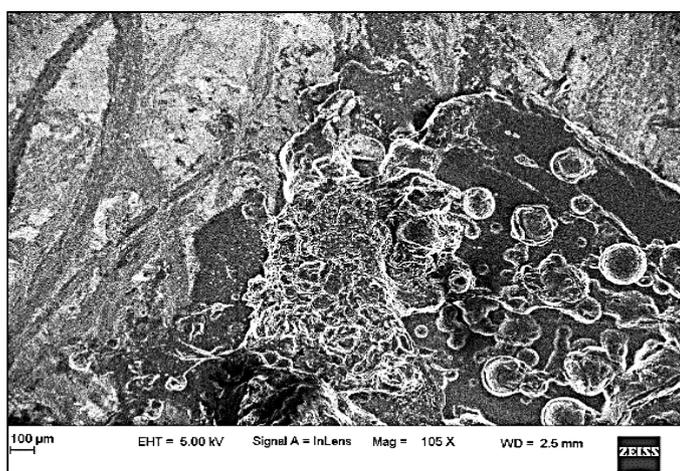


Fig. 5. SEM image of CLR loaded NLC

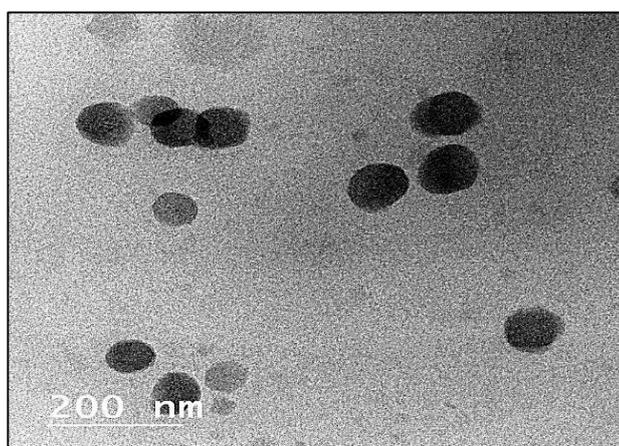


Fig. 6. TEM image of CLR loaded NLC

3.3.5 *In vitro* drug release

The drug release from the optimised NLC was investigated using the Franz Diffusion Cell for 8 hours, and a comparison was made with that obtained from an aqueous solution of the drug. The drug release profiles are given in Fig 7. The drug release from the aqueous solution was $95.6 \pm 0.29\%$ at 7 h, while the optimised NLC formulation showed $55.56 \pm 0.20\%$ at 8 h. As is expected, the drug release from the solution was more rapid than from the NLC since the lipid matrix of the latter sustained the drug diffusion.

On subjecting to kinetic analysis, the *in vitro* release of clarithromycin from optimised NLC formulation was found to follow zero-order kinetics with a regression coefficient (r^2) of 0.983. The drug release mechanism was determined by using the Korsmeyer-Peppas model for which the R^2 was found to be 0.9842. From the values of the release exponent 'n', which was 0.7084, it was deduced that this formulation followed the Non-Fickian or anomalous diffusion process.

3.4 Evaluation of Clarithromycin Loaded NLC Gel

3.4.1 Measurement of pH

The pH of a topical formulation should be as close to the skin pH as possible to minimise irritation at the application site. The normal pH of the skin is slightly acidic and may range from 4.7 to 5.75. A topical preparation with a pH outside this range may have irritation potential. The pH of NLC gel was 6.17, which is closest to the skin's pH and therefore can be considered free of irritation potential.

3.4.2 Viscosity

The viscosity was measured by using the Brookfield viscometer. The viscosity of optimised NLC formulation was found to be in the range of 577.3 to 54 cps. The graph shows that as the shear rate increases, the viscosity is decreased and vice versa, thereby indicating that this system has pseudoplastic properties.

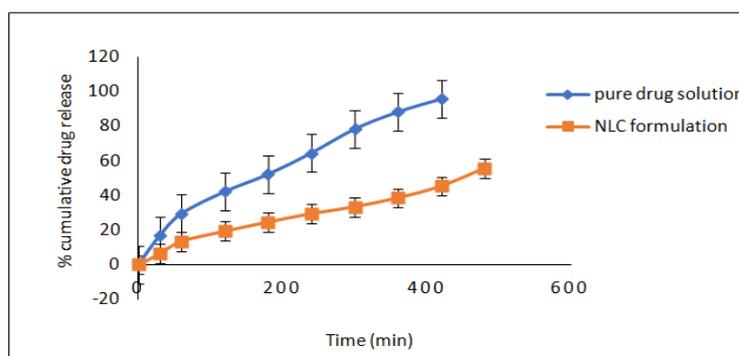


Fig. 7. Drug release of CLR from NLC and aqueous solution

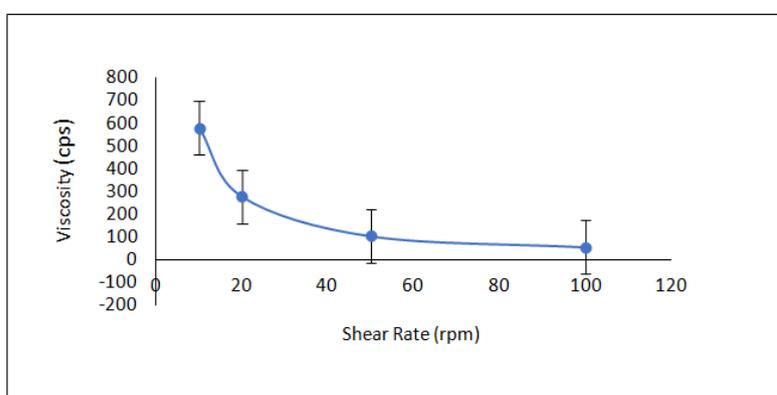


Fig. 8. Viscosity of optimised NLC gel

3.4.3 Spreadability

The spreadability was found to be 17.20 g/cm². If the spreadability is low, then eventually, it hampers the drug residence time on the skin, which may lead to poor bioavailability. Therefore, as per the literature suggested, the study was conducted, and the results were satisfactory, which showed good spreadability of the topical formulation [29].

3.4.4 Drug content

The drug content of optimised NLC formulation was analysed, and the result was found to be 84.84%. The drug content of the gel was carried out to ensure uniformity. The uniformity of the drug content is necessary for semi-solid preparation to confirm the homogeneity of the dispersed drug throughout the formulation.

3.4.5 *In vitro* drug release from NLC incorporated gel

The drug release profiles, as shown in Fig. 9, indicate that both the pure drug gel and the marketed gel showed a significantly greater release than from the NLC gel. This can be explained by the fact that drug was solubilised in the gel base in the former two, thereby allowing for greater release. In the SLN gel, the drug is entrapped in the lipid matrix of the particles, which allows for the slow diffusion of the drug through the matrix, resulting in a sustained release of about 50% at the end of 8 h. Release from the marketed gel was slightly superior (96% at 8 h) to that from the pure drug gel (93% at 8 h).

3.4.6 *Ex vivo* skin permeation study

The *ex vivo* skin permeation study was performed using Franz Diffusion Cell, and the permeation study was conducted on goat skin because it resembles the human epithelium and shows a similar absorption pattern. As seen in Fig.10, the permeation profiles for NLC gel, pure drug gel and the marketed gel are very different from the *in vitro* release data. Although the maximum release *in vitro* was the lowest for the NLC gel, the percentage drug permeated was the highest (89.46%). This could be attributed to the ability of the NLC particles to permeate through the skin due to their lipid solubility. On the other hand, the pure drug gel showed poor drug permeation (42% in 8 h) through the skin, although it readily released the drug *in vitro*. Comparatively, the marketed gel showed better drug permeation (maximum of 65% in 8 h) than the pure drug gel, probably due to the presence of solubilisers in the former gel base. The lipid nature of the NLCs could bring about the accumulation of these particles in the superficial layers of the skin itself. For acne treatment, the drug must accumulate in the skin for better effectiveness [29].

3.4.7 Stability studies

The optimised NLC formulation gel was subjected to stability studies for a period of 2 months. The formulation was evaluated every month for the pH and drug content. The results are given in Table 4. It can be concluded that the prepared NLC gel was stable at accelerated stability conditions and refrigerated conditions.

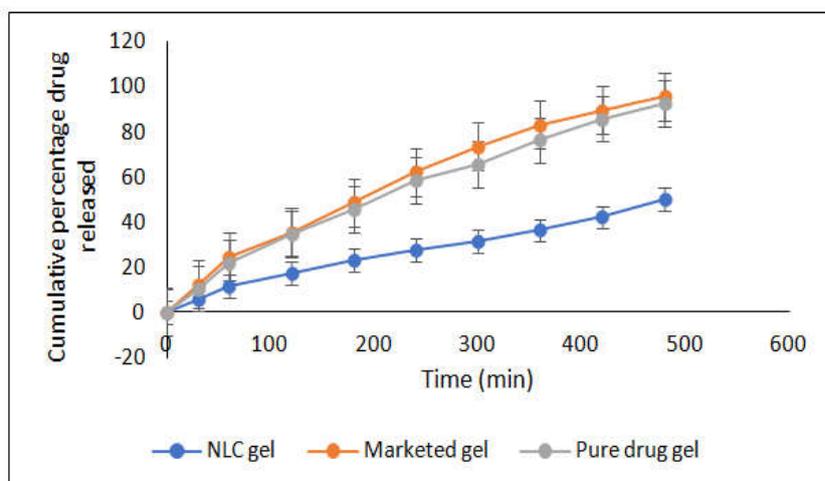
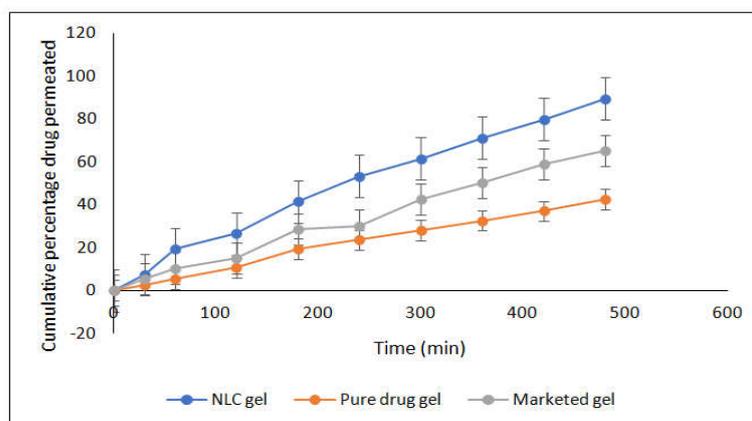


Fig. 9. Comparison of *in vitro* drug release profiles from NLC gel and marketed gel

Table 4. Data for stability studies

Storage condition	Days	pH	%Drug content
4°C	0	6.16	84.84
	30	6.14	84.79
	60	6.15	84.66
40°C	0	6.16	84.84
	30	6.18	84.74
	60	6.20	84.62

**Fig. 10. Comparison of ex vivo drug release study of NLC gel, pure drug gel, and marketed gel**

4. CONCLUSION

Clarithromycin loaded nanostructured lipid carriers in acne treatment were successfully developed by using the ultrasonication and emulsification method and characterised for various properties including drug release and skin permeation. The NLC gel showed good skin permeation and sustained release of the antibiotic, indicating that the formulation could provide sufficient drug concentrations in the skin which is important for better control over acne.

To support this surmise, further studies need to be carried out in a suitable disease model or dermal pharmacokinetic studies can be done to determine the extent of accumulation of clarithromycin in the skin.

DISCLAIMER

The products used for this research are commonly and predominantly products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of

knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

Not applicable.

ETHICAL APPROVAL

Not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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