https://doi.org/10.48047/AFJBS.6.Si4.2024.451-461

African Journal of Biological Sciences

DEVELOPMENT & EVALUATION OF METHOTREXATE NANOSTRUCTURED LIPID CARRIERS FOR TOPICAL TREATMENT OF ATOPIC DERMATITIS

Mahesh Kumar Sharma [1], Tarun Pokhariyal[2], Aarti Mehta [3], Saravanakumar Kasimedu[4], Virendra Kumar [5], Shashi Kiran [6], Shivani Kalia [7], Subbarao Jampani*[8]

1. Associate Professor, Arya College of Pharmacy SP-40 RIICO Kukas Delhi Road Jaipur Rajasthan 302028
2. Associate Professor, Arya College of Pharmacy RIICO Industrial Area Kukas Jaipur 302028
3. associate professor, Arya college of pharmacy, kukas Jaipur 302028
4. Director & Professor in Pharmaceutics, Seven Hills College of Pharmacy (Autonomous), Venkatramapuram, Tirupati, Andhra Pradesh 517561, India
5. Assistant professor, Institute of pharmacy research centre, surajpur ,chhattisgarh pin 497229
6. Assistant Professor, KC Institute of Pharmaceutical Sciences, Una HP 177207
7. Assistant Professor, KC Institute of Pharmaceutical Sciences Una HP 177207
8. Professor, ChebroluHanumaiah Institute of Pharmaceutical Sciences, Chowdavaram, Guntur, Andhra Pradesh- 522019

Corresponding Author: Subbarao Jampani
Designation and Affiliation: Professor, ChebroluHanumaiah Institute of Pharmaceutical Sciences, Chowdavaram, Guntur, Andhra Pradesh- 522019
Email Id:subbapharmaco@gmail.com

Article History

Volume 6, Issue Si4, 2024
Received: 15 May 2024
Accepted: 05 June 2024
doi:
10.48047/AFJBS.6.Si4.2024.451-461

ABSTRACT

Objective: Formulation and characterization of Methotrexate loaded nanostructured lipid carriers (NLC) hydrogel offering improved performance in terms of drug loading and long-term stability for topical drug delivery and treatment of atopic dermatitis.

Methods: Drug-loaded NLC formulation was designed by solvent diffusion technique, which was optimized using 3^2 full factorial designs. The mean diameter and surface morphology of MTX-NLCs was evaluated. MTX-NLCs were integrated in 1%, 1.5% and 2.0% w/w Carbopol 934 P gel base, and in vitro skin deposition studies in human cadaver skin (HCS) were carried out.

Results: For drug loaded formulation the particle size was found in nanometric range. The optimized MTXNLCs were oval shaped, with an average particle size of 246 ± 7.98 nm, a zeta potential of −24.6±0.98 mV, and EE of 55.00±2.17%. Significantly higher deposition of MTX was found in HCS from MTX-NLC gel (70.45 ±3.98%) as compared to MTX plain gel (40.12±1.12%).

Conclusion: The formulated NLC is a potential approach for sustained release of drug which may reduce systemic side effects, increase skin retention time and duration of action. Further in vivo studies will confirm the effect of NLC to increase skin retention time, decreases systemic absorption of the corticosteroid thereby avoiding side effects.

Keywords: Methotrexate, NLC, Solvent diffusion method, Atopic dermatitis

1. INTRODUCTION

Atopic dermatitis (AD) is a chronic inflammatory skin disease that is extremely itchy and usually first manifests in early childhood (1). It is sometimes confused with eczema. Atopic dermatitis, which can have a significant impact on a patient's life, profession, choices, and social contacts, is often the initial sign of atopy and is linked to a personal or familial history of respiratory allergies (2). Over the past few decades, the prevalence of AD has increased dramatically in industrialised nations, affecting 15% to 30% of adults and 2% to 10% of children, respectively (3). Less than 2% of new instances of AD occur beyond the age of 20, with up to 60% of males and 55% of females diagnosed before the age of one (4).

The second generation of solid-lipid nanoparticles (SLN) is known as nanostructured lipid carriers (NLC), and it is made up of liquid lipids combined with solid lipid matrix (5). The lipid particles' small size guarantees close contact with the stratum corneum (SC) and may enhance the quantity of medication that penetrates the skin or mucosa. These carriers provide for a regulated release because of their solid lipid matrix. When the drug must be supplied over an extended length of time, to minimise systemic absorption, or when the substance irritates the body at high concentrations, this technique becomes crucial (6). More active compounds can be loaded into NLCs, and there is less chance of active molecules being ejected during storage (7). The small size of NLCs ensures intimate contact with the SC and increases the amount of medication that penetrates the skin (8).

A folic acid antagonist with anti-neoplastic properties is methotrexate (MTX). When used orally or intravenously over an extended length of time, it is beneficial in treating psoriasis. Nevertheless, hepatotoxicity is one of the negative effects linked to MTX systemic use. (9) Numerous clinical trials have demonstrated the potential for improved therapy with fewer adverse effects when MTX is used topically. (10) Nevertheless, the drug's low skin penetration resulting from limited weak solubility and high molecular weight (454.56 g/mol) are significant disadvantages when used topically. (11) Therefore, there is a need to develop new delivery systems to solve this problem and improve the local bioavailability of methotrexate. One of the possibilities includes the development of NLCs to increase the penetration of MTX across the skin. Hence, the present work aimed to explore the potential benefits of NLCs in improving the topical delivery of MTX for the treatment of atopic dermatitis.

MATERIALS AND METHODS

Materials: Methotrexate was obtained as a gift from Yarrow Chem Pvt., Ltd., Mumbai, India. Compritol 888 was obtained as a gift sample from SD Fine chemicals, India. Capmul MCM C8 was received as a gift sample from CDH Laboratory, India. A dialysis bag (molecular weight cutoff of 14 kDa) was purchased from Hi-media Pvt. Ltd, Mumbai, India. Trehalose dehydrate was purchased from Sigma Chemicals Co., USA. Carbopol 940P (Noveon, USP) was obtained from BF Goodrich (Cleveland OH, USA), propylene glycol, and glycerol were purchased from Qualigens, Mumbai, India.

Preparation of MTX Loaded NLC: In an aqueous setting, drug-loaded NLCs were created using the solvent diffusion approach. (12) In summary, 4 mL of acidified isopropyl alcohol (IPA) was added to the melted Compritol 888 along with Capmul MCM and MTX (5%) and the resulting organic phase was heated to 65–70°C in a water bath until the entire lipid was dissolved in solvent. After that, this phase was mechanically agitated by adding dropswise to 25 mL of decanted water using a high-speed homogenizer called the Eurostar (IKA Labortechnik, Staufen, Germany) set at 1000 rpm for 10 minutes. After obtaining the pre-emulsion, it was put in a round bottom flask and rotated at 70°C to evaporate any remaining solvent at lower pressure. Drug-loaded NLCs were obtained by cooling the dispersion at room temperature while agitating it after the organic solvent was completely removed.

Following the addition of 0.1 M hydrochloric acid to bring the pH of the resulting NLC suspensions down to 1.20, the nanoparticles were combined and centrifuged for 30 minutes at 25,000 rpm (3K30, Sigma, Germany). After re-dispersion of the NLC particle in distilled water, the resulting dispersion was stored for five hours at −75°C in a deep freezer (Sanyo Ultra Low Temperature Freezer MDF-192, Moriguchi, Japan). After that, the sample was freeze-dried (Freezone 2.5 L, Labconco, Kansas City, MO, USA) with samples that had a weight ratio of 1:1 for trehalose, a cryoprotectant. A similar procedure was followed for the preparation of blank (without using the drug) NLC dispersion.

Experimental Design: The effect of the two independent factors, the amount of liquid lipid Capmul MCM and the amount of solid lipid Compritol 888, on the response variables of mean particle size (MPS) and entrapment efficiency (EE) was investigated by 32 factorial design optimisation of MTX loaded NLCs. There were three testing levels for each factor: −1, 0 and +1. In order to facilitate the computation of the coefficient in the polynomial equation, the values of the factors were converted. The response was assessed using interactive multiple regression analysis and F statistics, and the regression equations for the two responses were computed using the following formulas:

$$\textbf{Response}: \ \textbf{Y1}\ (\textbf{MPS}) = b_0+b_1X_1+b_2X_2+b_1^2X_{11}+b_2^2X_{22}+b_{12}X_1X_2 \quad (1)$$

$$\textbf{Response}: \textbf{Y2}(\textbf{EE})=b_0+b_1X_1+b_2X_2+b_1^2X_{11}+b_2^2X_{22}+b_{12}X_1X_2 \quad (2)$$

where b0 is the intercept and b1, b2 are the regression coefficients for the second order polynomial equations. Y1 and Y2 are dependent variables, i.e., MPS and EE, respectively, showing the quantitative impact of the formulation components. The level of independent variables is represented by the quantity of liquid (X2) and solid (X1) lipid. Three symbols represent the quadratic impacts of the variables: X12 (X11), X22 (X22), and X1X2. The interactions between two elements are shown by X1X2. Microsoft Excel was used to use multiple regression in order to determine the factors that were significantly influencing the responses. The factors in the model with a p-value of less than 0.05 were deemed to have a significant impact on the formulations. (13)

Contour Plots and Surface Response Curves The values of the response are shown by contour plots and surface response curves, which also aid in illuminating the link between independent and dependent variables. Using Microsoft Excel and STATISTICA® students' version 1998, the entire models were utilised to plot the contour plots and surface response curves at specified values of particle size and EE, with X1 and X2 values between −1 and +1. Equations 1 and 2, respectively, were used to create the full model equations for MPS and EE.

Evaluation and Characterization of Prepared NLC:

Determination of Entrapment Efficiency (EE): The ultrafiltration method was used to calculate the EE of optimised MTX-NLC. (14) The free drug concentration in the NLC was used to calculate the amount of drug entrapped. The material was, in short, put in an Amicon Ultra-4 filtering unit (EMD Millipore) with a molecular weight limit of 30,000 Da, and centrifuged at 3,000 µg for 10 minutes (Centrifuge 5702; Eppendorf AG). With the UV detector set to 260 nm, the filtrate was examined using HPLC (HPLC 1200 series; Agilent Technologies, Santa Clara, CA, USA) and a Phenomenex C18 analytical column (5 µm, 4.6µ150 mm). The EE was calculated based on the following equation:

$$\text{Entrapment efficiency (\%)} = \frac{\text{Weight of MTX added} - \text{Free MTX}}{\text{Weight of MTX added}} \times 100 \quad \ldots\ldots\ldots\ldots\ldots\ldots 3$$

Particle size and zeta potential analysis: Using the Zetasizer Nano ZS (Malvern Instruments, Malvern, UK), the optimised NLC formulations' particle size, zeta potential, and polydispersity index (PDI) were ascertained. To guarantee measuring accuracy, the device was calibrated using a latex standard prior to the measurement.

Transmission electron microscopy (TEM) studies: Using a TEM device (Hitachi 7100S; Hitachi, Tokyo, Japan), the produced NLC formulations' form and surface appearance were assessed. Using a TEM, a drop of the diluted formulations was positioned on a copper grid coated with carbon, dyed with a 2% uranyl acetate aqueous solution, and monitored. (15, 16)

In vitro Release Study: By dispersing 20 mg of the lyophilized MTXNLCs in 25 mL of phosphate buffer saline (PBS, pH 7.4), adding the mixture to a dialysis bag (cutoff 14 kDa), and shaking with a magnetic stirrer at 100 rpm at 37±1°C, the in vitro drug release profile of the MTXNLCs was ascertained. At a set time interval, one millilitre of the dispersion was removed from the medium and replaced with the same volume of medium. A 0.4 µm filter (EMD Millipore, Billerica, MA, USA) was used for filtering, and UV-visible spectroscopy at 303 nm was used to analyse the drug release. In a similar manner, 2.5 mg of MTX was suspended in 25 mL of PBS (pH 7.4) to examine the in vitro drug release profile of the MTX solution. The experiments were run in triplicate and the average results are reported. (17)

PREPARATION OF MTX LOADED NLCs GEL

Various concentrations of Carbopol940 (0.5%, 1.0%, 1.5%, and 2.0% w/v) were used to create the hydrogels. Aqueous dispersion of Carbopol 940 was made with 5% v/v glycerol as the hydrating agent and the required quantity of Carbopol 940 was dissolved in filtered water. The hydrogels were allowed to equilibrate for a full day at room temperature after the mixture was agitated for ten minutes at 1,000 rpm and the aqueous dispersion was neutralised with triethanolamine to achieve the consistency needed for topical application. Using a Remi stirrer (Remi Lab World, Mumbai, Maharashtra, India) at 1,000 rpm for two minutes, the freshly manufactured MTX-NLC gels were mixed with carbopol hydrogels to create gels with a final concentration of 0.5% MTX-NLC. (18, 19)

Measurement of viscosity of MTX-NLC gel: Using a CP40 spindle, the prepared MTX-NLC gel's viscosity was measured using a cone (0.8°) and plate geometry viscometer (Brookfield-AMETEK, Middleboro, MA, USA). (20) At 37°C±1°C, the viscosity of the in situ gelling solutions was evaluated at various angular velocities. During a typical run, the angular velocity—or shear rate expressed in rotations per minute—was changed at a regulated ramp speed between 0.5 and 100 rpm. The velocity was increased to 100 rpm after 6 seconds at 0.5 rpm, with a comparable pause at each speed. Reversing the angular velocity hierarchy (100–0.5 rpm) resulted in a comparable 6-second wait. To investigate the behaviour of gels, rheograms were plotted using viscosity vs shear rate.

Determination of drug content and pH of MTX-NLC gel: The drug content was ascertained by dissolving 10 mg of MTX-Equivalent gel in 20 millilitres of methanol within a 50 millilitre volumetric flask. After the solution was passed through a 0.45 µm membrane, HPLC was used to determine how much MTX was left in the filtrate. A digital pH metre (pH Tutor Bench Metre; Thermo Fisher Scientific) was used to measure the pH values of the optimised MTX-NLC hydrogel and placebo gel. In summary, 0.5 g of MTX gel formulation was evenly distributed in distilled water, and the pH metre was used to determine the dispersion's pH. Every experiment was carried out three times.

Determination of spreadability of MTX-NLC gel: The parallel plate method, as previously reported, was used to determine the spreadability test. In short, a glass plate was covered with a second glass plate after 0.1 g of gel was deposited inside a premarked circle with a diameter

of 1 cm. For five minutes, a 200 g weight was left to lie on the upper glass plate. It was observed that the diameter increased as a result of gel spreading. (21, 22, 23)

In vitro Skin Deposition Study: To eliminate subcutaneous fat, hot distilled water was used to thoroughly cleanse HCS. The process of preparing a full thickness HCS membrane involved shaving the skin, punching out a disc with an area of about 2.5 cm^2, and then using a Davis Dermatome-7 (Anthony Products, Indianapolis, IN, USA) to slice the disc to a thickness of 500 mm. Before being used, these slices were hydrated with PBS for a full day at room temperature. (24) After that, 0.1 g of gel was weighed, applied to the skin, and left to stand at room temperature for 36 hours. By three PBS washes of the skin surface and measuring the drug's absorbance in PBS at 303 nm, the amount of drug still present on the skin surface was ascertained. After carefully wiping off any remaining washing solvent with a cotton swab, the skin was digested overnight at 40°C in PBS containing 3% w/v sodium lauryl sulphate (SLS) to allow the drug to escape. The amount of drug that escaped was measured at 303 nm using a UV-visible spectrophotometer to determine how much drug had been deposited in the skin.

3. RESULTS AND DISCUSSION

Factorial Design: The solvent diffusion approach was used to prepare nine batches of MTX-NLCs using 3^2 statistical factorial design experiments. Table 1 lists the MPS and EE of several batches of MTXNLCs together with their real and transformed values. Table 2 displays the findings of the whole model of MTXNLCs' regression output and response. Multiple regression analysis was used to determine the mean particle size (dependent variable) of MTX-NLCs obtained at different values of the two independent variables (X1 and X2). The approach produced a full model second-order polynomial equation. The MPS values showed a wide variation ranging from 179±12.87 to 246±7.98 nm, while EE values varied from 35±1.31% to 55.66±2.17%. Highest EE was achieved (55.66±2.17%) at a high level (+1) of X_1 (100 mg) and medium level (0) of X_2 (30 mg) in batch MN8. The following equations (4 and 5) are the quantitative effect of the formulation components on independent variables X_1 and X_2. These equations were derived by the best-fit method to describe the relationship between the particle sizes (Y_1), the concentration of solid lipid (X_1), and the amount of liquid lipid (X_2). Similarly, it also describes the relationship between the entrapment efficiency (Y_2) and the variables X_1 and X_2.

$$Y_1(\text{MPS}) = 233 + 18X_1 - 28.3333X_2 + 2X_{11} - 7X_{22} - 4.75X_1X_2 \quad (4)$$

$$Y_2(\text{EE}) = 52 + 4.5X_1 - 1.5X_2 - 2.5X_{11} - 7.5X_{22} + 1.75X_1X_2 \quad (5)$$

Using the p-values given in Table 2, the importance of each coefficient in equations 4 and 5 was ascertained. The amount of solid and liquid lipid is significant when the p-value is smaller, which indicates that the associated coefficient is more significant. Table 2 makes it evident that the independent variables' major effects (X1 and X2) were important predictors of MPS and EE because their p-values were less than 0.05 ($p<0.05$), However, because the interaction term X1X2's p-values were determined to be over 0.05 ($p>0.05$), it had minimal predictive value. The models are highly significant (p-value<0.05) based on the model F values for MPS and EE, which were 52.78 and 16.78, respectively, and their associated Ftab values, 3.68 and 3.14. The value for X2 (b2 = -27.45) was found to be greatest when the coefficients of the two independent variables in equation 4 were compared, and as a result, it was thought to be a significant contributing factor impacting the particle size of the MTX loaded NLCs. In this case, a positive sign denotes a rise in one variable's level that results in an increase in the corresponding response parameter, and a negative sign denotes a rise in one

variable's level that results in a reduction in the response parameter. It was discovered that the particle size was significantly impacted by changes in the ratio of Capmul MCM to Compritol 888. In a similar vein, equation 5's result for X1 (b1 = 4.5) was similarly the highest of the two independent variables, pointing to a significant contributing factor as an EE variable. However, a quadratic component of X2 (X22) was found to be greatest (−7.5), indicating that the interaction between X2 had a negative effect on the EE. The goodness of fit of the model was checked by the determination coefficient (R2).

More than 98% of the total changes were described by the model, according to the R2 values for MPS (0.9887) and EE (0.965) for the entire model. Since an R2 value close to 1 indicates a strong correlation between the independent variables, the adjusted determination coefficients (adj R2) for the entire model were similarly quite high (0.97 for MPS and 0.91 for EE). All of the aforementioned factors suggest that the model was extremely significant and that it can account for 99% of the fluctuations around the mean value.

Contour Plots and Surface Response Curves: Predicted MPS values at various Compritol 888 and Capmul MCM levels are displayed on the contour plot (Figure 1). It is evident that whereas MPS decreased when the amount of Capmul MCM increased, MPS increased when the amount of Compritol 888 increased. As the quantity of solid lipid increases, the surface response curve likewise demonstrates larger particle sizes. Lipid concentration has a significant impact on the size of lipid nanoparticles, which is explained by lipid's propensity to agglomerate at high concentrations. Stoke's law states that the density differential between the internal and exterior phases explains this behaviour. 3^2 Conversely, a decrease in particle size was seen as the quantity of liquid lipid increased. The surface response curve's small curvature suggests that quadratic effects are not very significant. (X22). Nevertheless, as we haven't looked into Capmul MCM at doses lower than 18 mg, it would be challenging to determine with certainty how significant the quadratic effects will be. When Compritol 888 was raised up to 105 mg with a fixed amount of Capmul MCM (30 mg), Figure 1 shows a rise in EE, while the particle size was found to increase beyond 100 mg. Consequently, it can be said that at dosages of 60–100 mg for Compritol 888 and 20–40 mg for Capmul MCM, respectively, the best results (minimal PS and maximum EE) can be obtained.

Table 1: Formulation of Methotrexate-Loaded NLCs by 3^2Factorial Design: Factors, Their Levels and Transformed Values, Response: MPS and EE

Code	Compritol 888 (mg)	Capmul MCM (mg)	X1	X2	X11	X22	X1X2	MPS ± SD* (nm)	EE ± SD* (%)
MN1	60	20	-1	-1	1	1	1	239±12.5	41±1.23
MN2	60	30	-1	0	1	0	0	232±12.5	42±1.65
MN3	60	40	-1	1	1	1	-1	187±18.6	37±0.85
MN4	80	20	0	-1	0	1	0	246±7.96	51±2.65
MN5	80	30	0	0	0	0	0	235±9.6	44±2.87
MN6	80	40	0	1	0	1	0	212±9.3	46±3.33
MN7	100	20	1	-1	1	1	-1	233±13.6	55±4.54
MN8	100	30	1	0	1	0	0	243±11.6	49±3.12
MN9	100	40	1	1	1	1	1	234±11.4	45±1.98

Note: *Data are represented as mean ± SD (n=3).

Table 2 Response of Full Model for Methotrexate-Loaded- NLCs

Response	Mean Particle Size (MPS)		%Entrapment Efficiency (%EE)	
	X Coeff.	p-value	X Coeff.	p-value
X1	18	0.165477	4.5	0.008793
X2	−27.45	0.003919	−1.5	0.134292
X12	2	0.001876	−2.5	0.144688
X22	−7	0.620082	−7.5	0.009798
X1X2	4.75	0.149443	1.75	0.147502
Intercept	233	*9.76 E-06 (significant)	52	3.8 E-05 (significant)

Note: *Statistically significant ($p<0.05$)

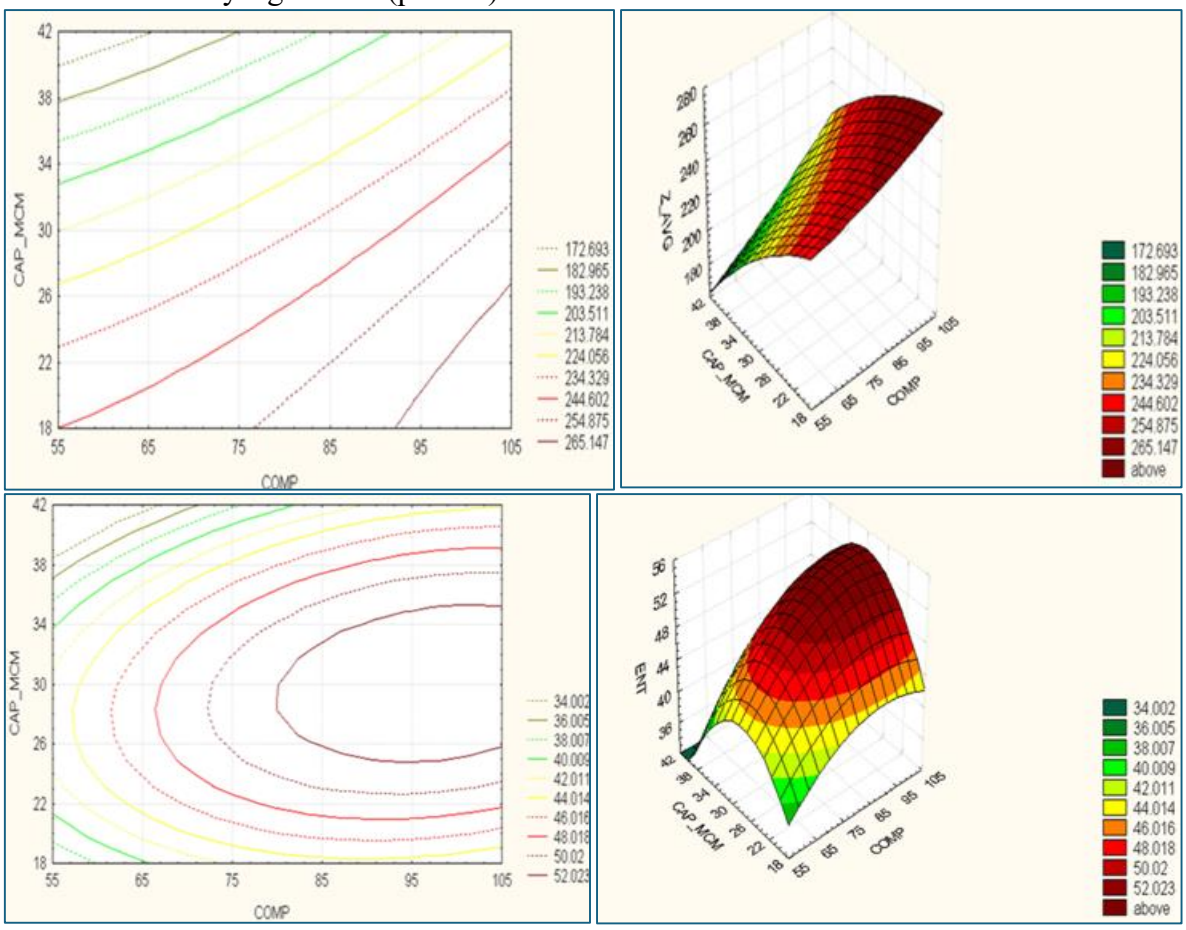

Figure 1: (A) Contour plot of Zavg versus the amount of Compritol 888 and Capmul MCM; (B) response surface plot of Zavg versus the amount of Compritol 888 and Capmul MCM; (C) the contour plot of % drug entrapment vs the amount of Compritol 888 and Capmul MCM; (D) response surface plot of % drug entrapped vs the amount of Compritol 888 and Capmul MCM.

Surface Morphology: The TEM investigation of the NLCs revealed oval-shaped, non-aggregated NLCs with a restricted size distribution (Figure 2). The Malvern particle size analyzer data and the sizes of the particles shown in the micrographs agree fairly well. (Table 1).

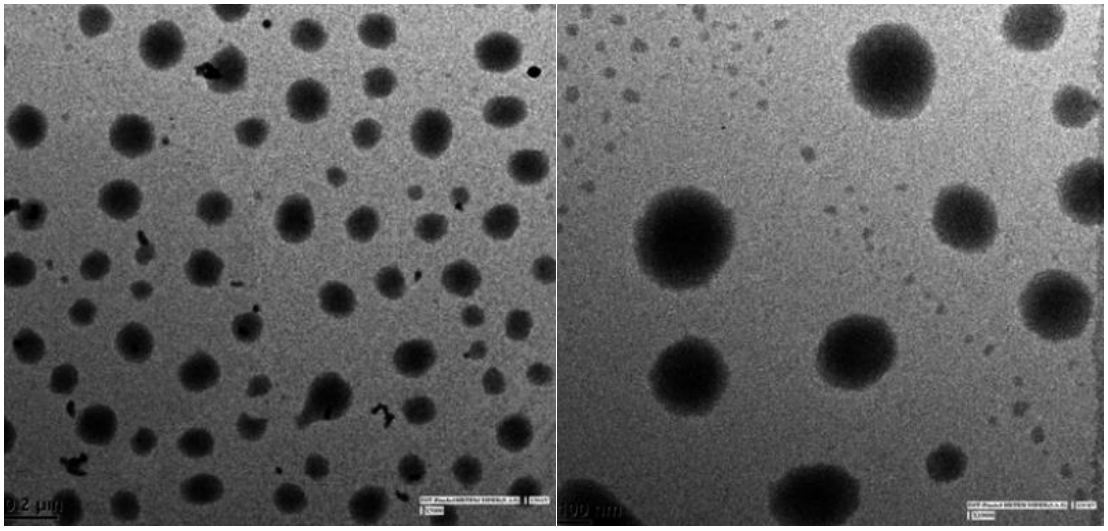

Figure 2: TEM image of MTX-NLC

In vitro Release Studies: Over the course of five and thirty hours, respectively, the cumulative percentage release of methotrexate from MTX suspension (MTX-S) and MTXNLCs was examined. Three duplicate analyses of each sample were performed, and Figure 3 displays the release curves. After 5 hours, MTX-S was found to have released 83.15±5.96% of the drug, but MTX-NLCs demonstrated a drug release of 31.46±2.18% after 10 hours. Moreover, MTX-NLCs demonstrated 62.03±2.34% of MTX release over the course of the trial (30 h) with a consistent and sustained release. The MTXNLC formulation showed comparatively sluggish and sustained drug release, which is explained by the drug's slow disintegration and diffusion from the NLCs after the water diffusion medium penetrates the hydrophobic lipid. The medication was liberated via zero-order diffusion from the lipid matrix. When applied topically, this type of medication release pattern is interesting because it allows the medicine to better penetrate the skin's deeper layers over an extended period of time. MTXNLCs offer a more effective MTX delivery mechanism than MTX suspension, which shows over 80% drug release after 5 hours. To determine the release mechanism, the drug release data from the MTXNLCs were fitted into the Korsemeyers Peppas model. For MTXNLCs, the release exponent "n" was 0.53, indicating a combination of diffusion and dissolution mechanism (anomalous diffusion - $0.5 < n < 1$) with R2 value of 0.992; this may indicate that the drug release is controlled by matrix diffusion-based kinetics.

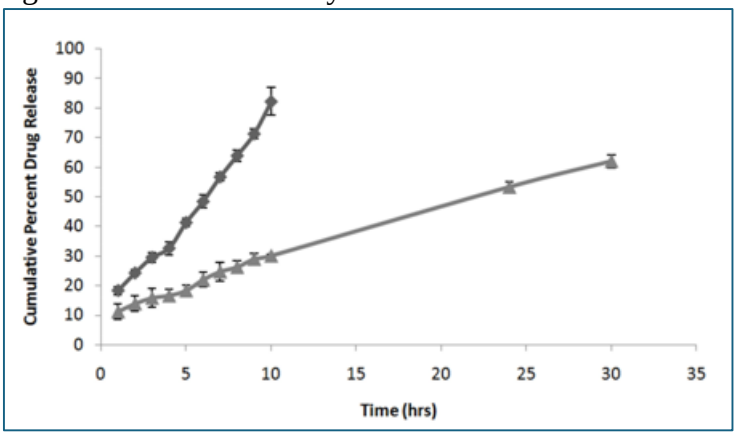

Figure 3: In-vitro drug release of MTX-NLC and MTX Suspension

Evaluation of Prepared MTX-NLC based hydrogel:

% drug content, pH and Spreadability of MTX-NLC based hydrogels: Table 3 presents the findings of an investigation into the physicochemical characteristics, including pH, drug

content, and spreadability, of three distinct concentrations of MTX-NLC-loaded Carbopol 940 (1%, 1.5%, and 2%). The results indicated that 1% Carbopol 940 hydrogel would work well for both the formulation of MTX-NLC and future research. Table 3 shows that the pH of the 1% Carbopol 940 gel based on MTX-NLC was 5.78±0.05, which is within allowable bounds for topical applications. It was discovered that the medication content of the MTX-NLC formulation was 99.98±3.44%. Spreadability is a key factor in patient compliance and aids in applying gel to the skin consistently. Table 3 displays the spreadability data for the different Carbopol 940 gel concentrations (1%, 1.5%, and 2%). To be more comfortable, the NLC hydrogel should spread more readily when applied to skin that is ill or irritated. The spreadability value of a topical preparation can potentially influence its medicinal efficacy. Table 3 illustrates that the 1% Carbopol 940 gel loaded with MTX-NLC had a spreadability value of 7.45±0.27 cm, which was considerably lower than the 1.5% and 2% formulations of Carbopol 940 gel. As such, it was deemed appropriate for topical administration.

Table 3: Physiochemical characteristics of optimized MTX-NLC gel (mean ± SD, n=3)

Code	Carbopol 940	Drug content %	pH	Spreadability (cm)
MNG1	1.0	99.98±3.44	5.78±0.05	7.45±0.27
MNG2	1.5	99.56±4.45	6.11±0.32	8.09±0.45
MNG3	2.0	78.66±4.39	5.85±0.21	9.66±0.31

NLC, nanostructured lipid carrier; SD, standard deviation.

Viscosity of MTX-NLC gel formulation: To investigate the impact of hydrogel type on the physicochemical characteristics of semisolid formulations, rheological flow patterns were established for hydrogels that solely contained MTX and hydrogels that contained MTX-NLC. Viscosity measurements were undertaken at various shear rates in order to examine the rheological behaviour of the NLC formulations. Shear thinning behaviour is characterised by the NLC formulation's viscosity gradually decreasing when the shear rate was increased from 0.5 rpm to 100 rpm. The behaviour of the MTX-NLC and MTX-NLC-loaded hydrogel formulations followed a pseudoplastic system, as seen by the rheograms that were produced (Figure 4).

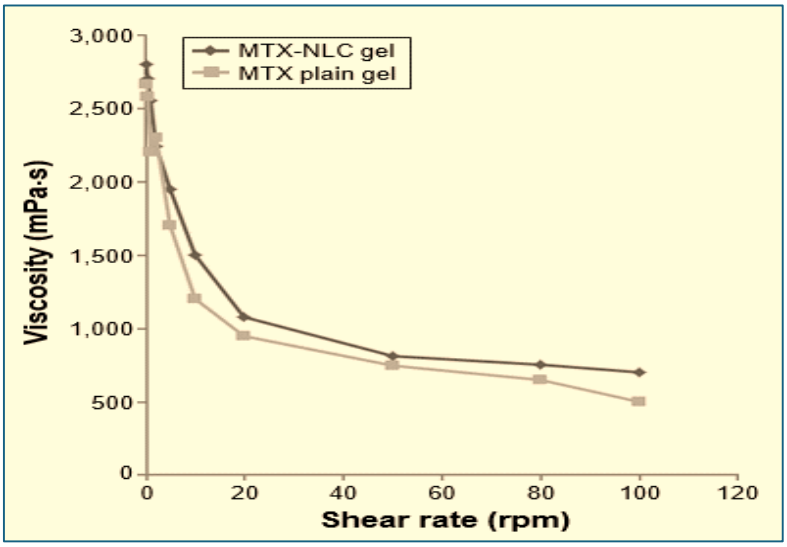

Figure 4: Viscosity of MTX-NLC gel and MTX plain gel under different shear rates

In vitro Skin Deposition Studies: Using HCS, in vitro skin deposition of MTXNLC gel and plain MTX gel was studied. The findings are summarised in Figure 5. The results of the investigation showed that MTX from MTXNLCs gel was deposited in the skin at a substantially higher rate (70.45±3.98%) than MTX gel (40.12 ±1.12%). The idea that adding MTX to NLC improves drug deposition into HCS was validated by the findings of these investigations. The outcomes also corroborate data that suggest SLN and NLCs enhance the

topical medicinal agents' skin localization. Improving MTX's dermal localization to lower systemic toxicity and better localised therapy of atopic dermatitis was one of the motivations behind using the NLC method for topical delivery.

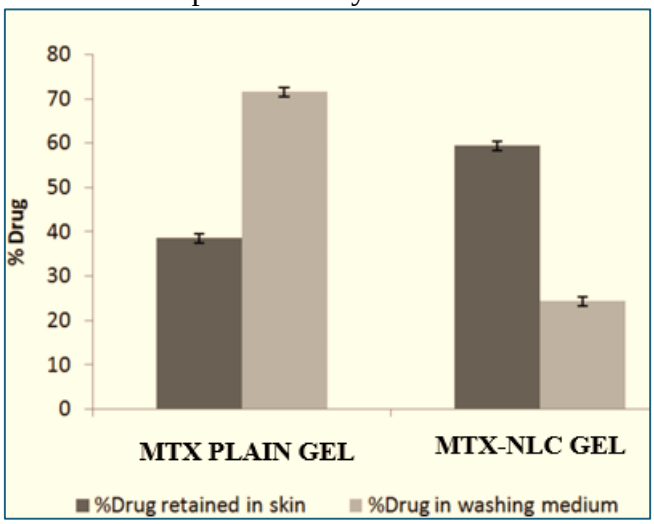

Figure 5: In vitro skin deposition study of MTX plain gel and NLCs.

4. CONCLUSION:

A 3^2 factorial experimental design was used to optimise the formulation of MTX-NLCs, choosing the liquid and solid lipid contents to achieve the best particle size and greatest entrapment efficiency. Under TEM, the resulting NLCs were smooth. The MTXNLC in vitro release study demonstrated a 30-hour sustained drug release. Studies on in vitro skin deposition revealed that MTX from MTXNLCs gel was deposited in the skin at a much higher ($p<0.05$) rate than that of regular MTX gel. The results of this study unequivocally show that MTXNLC gel has a promising future in the management of atopic dermatitis.

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