RESEARCH ARTICLE

Topical Methotrexate Loaded Nanogel for Rheumatoid Arthritis: Optimization using Box-Behnken Design and Evaluation of Anti-Arthritic Activity

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ABSTRACT

The present study emphases on formulation, optimization, and assessment of a methotrexate-loaded nanogel (MXT-NGs) for current topical treatment of rheumatoid arthritis. MTX, a potent antimetabolite, faces encounters as systemic toxicity, poor solubility, and non-specific distribution when administered conventionally. To overcome these limitations, nanogel formulations were developed using Carbopol 934, HPMC K15, and Tween 80, and optimized through a Box-Behnken Design (BBD) approach. Seventeen formulations (MXT-NGs-F1–MXT-NGs-F17) were evaluated for physicochemical properties including pH, viscosity, spreadability, entrapment efficiency, zeta potential, drug content, and particle size. DSC, FTIR, SEM, and XRD analyses inveterate compatibility, amorphization of drug, and nanoscale morphology. The optimized formulation (MXT-NGs-F16) exhibited favorable characteristics: entrapment efficiency (76.65%), high drug content (96.45%), small particle size (123.74 nm), and sustained drug release up to 12 hours. *Ex vivo* permeation also *in vivo* studies in a CFA-induced arthritis rat model demonstrated significantly reduced paw edema, arthritis index score, and biochemical markers of inflammation (ALT, AST, ALP, TNF-α, IL-1β) with enhanced antioxidant levels (GSH, SOD, CAT). These conclusions highlight probable of optimized methotrexate nanogel as a promising transdermal delivery system for enhanced management of rheumatoid arthritis with reduced systemic side effects.

Keywords: Methotrexate, Nanogel, Box-Behnken Design, Rheumatoid Arthritis, Topical Delivery, Sustained Release, Antioxidant Activity, Drug Permeation, Anti-inflammatory, CFA-induced Arthritis Model.

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INTRODUCTION

Methotrexate (MTX) is a folate antagonist widely recognized for its therapeutic efficacy in treatment of various autoimmune and neoplastic conditions that leads to progressive joint destruction, disability, and decreased quality of life[1]. Although MTX remains the first-line disease-modifying antirheumatic drug (DMARD) for RA, its clinical use is often challenged by poor aqueous solubility**,** non-specific tissue distribution, short biological half-life, and dose-limiting systemic toxicity**,** which can result in hepatotoxicity, myelosuppression, and gastrointestinal irritation[2]. These drawbacks significantly limit its therapeutic index and patient adherence to long-term treatment regimens.

To over awed these limitations and improve localized beneficial probable of methotrexate in inflammatory joint disorders, innovative drug delivery schemes such as nanogels have gained significant attention. Nanogels are nano-sized hydrogel particles designed through crosslinking of hydrophilic polymer networks. They offer exclusive advantages like high drug loading, responsive drug release, and exceptional tissue compatibility. In addition, nanogels are perfect for targeted anti-arthritis treatment since of their EPR outcome, which consents them to collective especially in inflammatory tissues[3]. In context of RA, topical or transdermal nanogels can deliver MTX directly to affected joints, thus enlightening site-specific drug concentration and lessening systemic side effects[4].

Anti-arthritis action of methotrexate is predictable to its ability to suppress immune cell activation, decrease pro-inflammatory cytokines corresponding TNF-α and IL-6, and inhibit proliferation of synovial fibroblasts, thus reducing joint degradation. Delivering MTX through a nanogel-based topical route not only sustains beneficial concentration at site of inflammation but similarlyimprovesbeneficialeffects in preclinical arthritis models by reducing paw edema, joint stiffness, and inflammatory cell infiltration in synovial tissue.

To design an optimal MTX-loaded nanogel by desirable properties as small particle size, suitable viscosity, and controlled release, it is critical to relate statistical tools for systematic formulation development. Response Surface Methodology (RSM)**,** particularly the BBD**,** is a powerful method that permits evaluation of multiple formulation variables and their interactions by a minimal number of experiments. BBD is particularly suitable for optimizing

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critical quality attributes (CQAs) in complex formulations and facilitates the development of second-order polynomial models for robust and reproducible product design[5].
Consequently, existing study was undertaken to formulate and illustrate methotrexate-loaded topical nanogels using the BBB statistical design. The study designed to optimize formulation by respect to particle size, viscosity, and overall stability, althoughconfirmingactual anti-arthritis actionoverimproved topical delivery. By falling systemic revelation and improving local bioavailability, the developed nanogel system holds capacity as a safer and more effective alternative to conventional MTX therapy in management of rheumatoid arthritis.

MATERIAL AND METHODS

Material

Materials used were of analytical and pharmaceutical grade. Methotrexate was procured from Sigma-Aldrich, Mumbai (India). HPMC K15, Carbopol 934, Glycerol, Triethanolamine, and Tween 80, received from Loba ChemMumbai (India), were used as polymer, gelling agents, humectant, pH adjuster, and stabilizer, respectively. Double-distilled water was used through study.

Methods

Formulation and Categorization of Methotrexate Loaded Nanogel

Factorial Design

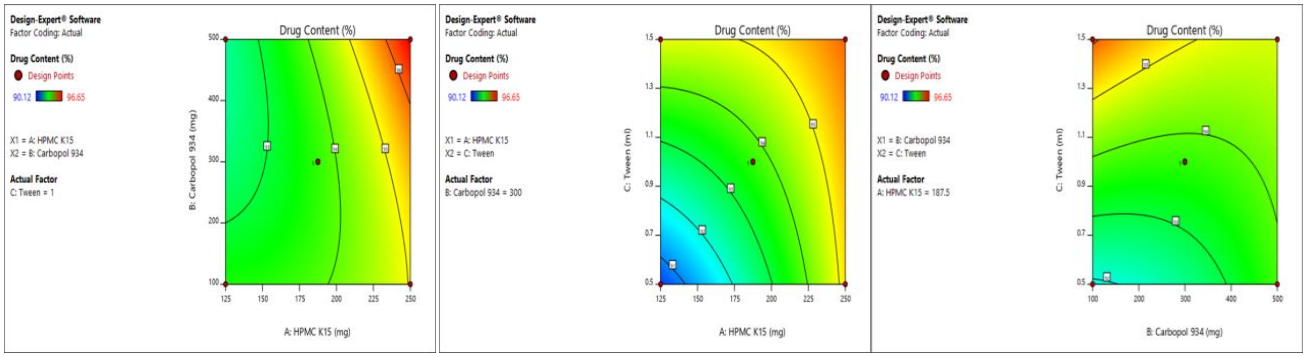
The optimization of Nanogel containing methotrexate was carried out using a two-factor Three-level BBD. We used A-HPMC K15, B-Carbopol 934, and C-Tween as our independent variables. The three levels of selection for these independent variables (factors) were low (-1), medium (0), and high (+1). (Table 1) displays the factor levels and the results. Particle size (Y2, nm) and drug content (Y1 %) were dependent variables (response) examined in this study. One hundred and seventeen iterations of research were assessed for results (Y1) and (Y2)[6].

Table 1: Variables as well as their levels in BBB design

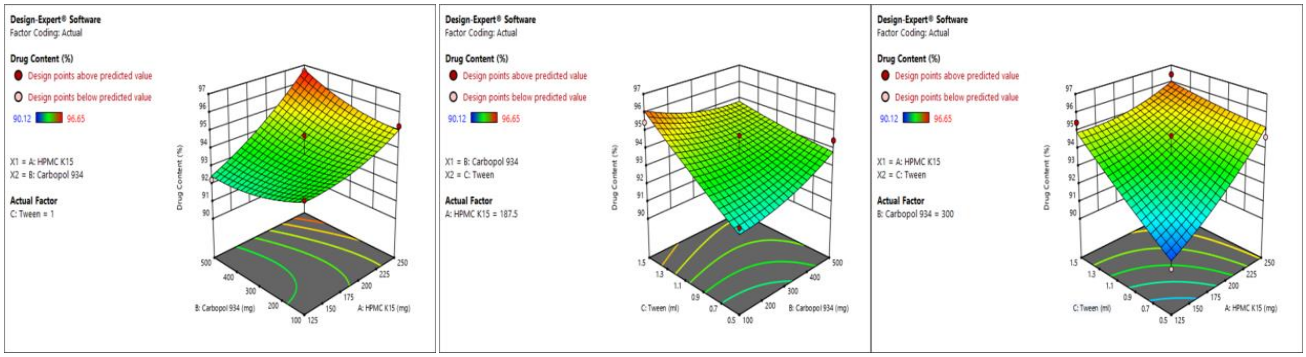
S. No.	Formulation Variables			
	Independent variables	Level		
		Low (-)	Medium (0)	High (+)
1	A: HPMC K15 (mg)	125	175	250
2	B: Carbopol 934 (mg)	1 %	1.5 %	2 %
3	C: Tween (ml)	0.5	1.0	1.5
	Response variables			
1	Y1: Drug Content (%)			
2	Y2: Particle size (nm)			

Preparation of Methotrexate Loaded Nanodispersion

Methotrexate nanodispersion was prepared using a modified emulsification-diffusion method. The following was done: dissolve 100 mg of methotrexate in 10 ml of ethyl acetate that included HPMCK15. Under high-speed homogenization (5,000-10,000 rpm) using a T-10 Ultra Turrax, this organic phase was added drop by drop (0.5 ml/min) to 30 ml of an aqueous Tween 80 solution. Organic phase was added at 0.5 ml/min using a syringe that was inserted with a needle straight into the water-based stabilizer solution. Sonication for 5-10 minutes surveyed through further homogenization at 10,000-25,000 rpm for 6 minutes yielded the final mixture. To encourage solvent

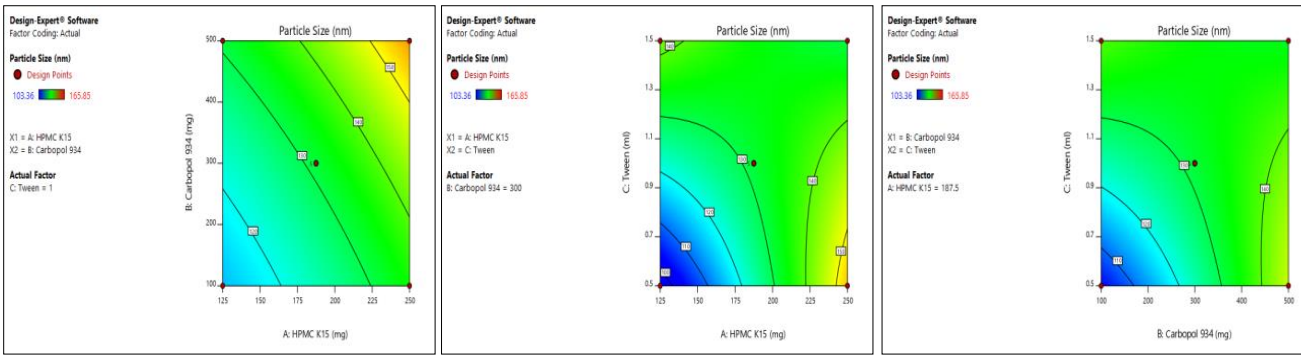


(HPMC K15 vs. Carbopol 934) (HPMC K15 vs. Tween 80) (Carbopol 934 vs. Tween 80)

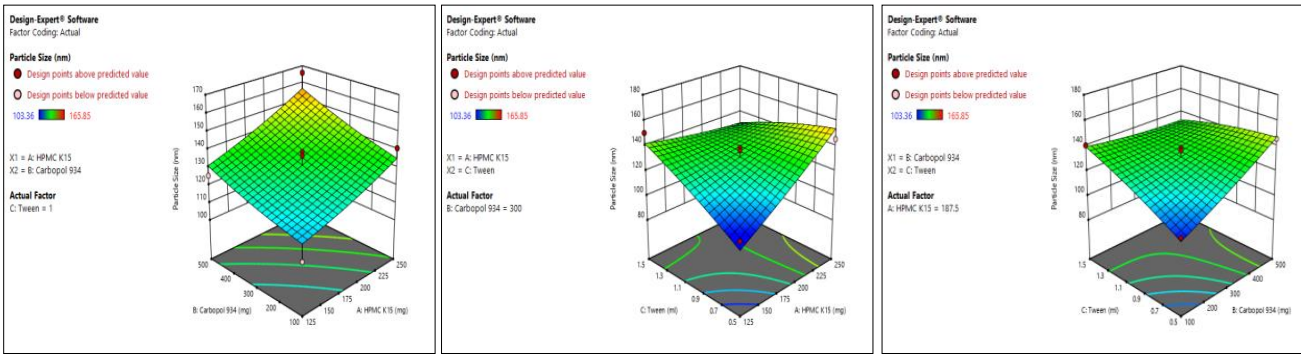


(HPMC K15 vs. Carbopol 934) (HPMC K15 vs. Tween 80) (Carbopol 934 vs. Tween 80)

Figure 1: Response surface plots for drug content



(HPMC K15 vs. Carbopol 934) (HPMC K15 vs. Tween 80) (carbopol 934 vs. Tween 80)



(HPMC K15 vs. Carbopol 934) (HPMC K15 vs. Tween 80) (carbopol 934 vs. Tween 80)

Figure 2: Response surface plots for particle size

diffusion and create the nanodispersion, double distilled water was added progressively while stirring continuously for 1 hour[7].

Preparation of Methotrexate Loaded Nanogel

To make the nanodispersion gels, a high-speed stirrer was used to disperse carbopol 934, a gel forming agent, into the nanodispersion. Triethanolamine was then used to adjust the pH to 7.0. The finished nanogel containing Methotrexate was left at room temperature for storage[8].

Coded Factors Final Equation

Drug Content (%) = +93.70+1.44 A+0.1550 B+1.29 C+0.7000 AB-0.8825 AC-0.8000 BC+0.3807 A^2+0.3732 B^2+0.0908 C^2

Final Equation in Terms of Coded Factors

Particle Size (nm) = +131.39+11.39 A+9.30 B+5.56 C+1.30 AB-17.23 AC-12.60 BC+1.59 A^2+1.46 B^2-2.21 C^2

Characterization of Nanogel Preparation

FTIR Spectrum

The FTIR was used to capture the KBr technique FTIR spectra of Methotrexate. Using a dried potassium bromide pellet, a baseline correction was performed. In a pressure compression machine, 3-5 mg of medicines and 100-150 mg of potassium bromide were ground to produce a about 1 mm diameter pellet. We scanned the sample pellet from 4000 cm-1 to 400 cm-1 while it was installed in the infrared chamber.

Differential Scanning Colorimetry

To capture the temperature profiles, a differential scanning calorimeter was used. Amounts ranging from 5 to 10 milligrams were carefully measured and sealed in aluminum pans with flat bottoms. Alumina assisted as reference standard although samples were heated in a nitrogen mesosphere (200 ml/min) at a constant rate of 100 °C/min from 50 to 400 °C.

Determination of Physicochemical Properties

Visual scrutiny was done for organoleptic potentials, opacity, washability, clearness, and physical exterior of the gel. To regulate the Methotrexate nanogel's pH, a pH meter was used. Quantities were noted three times, and mean was calculated[9].

Homogeneity and Grittiness

To find out how persistent the nanogel was, we enfolded a little bit of it among our index fingers and thumbs. The constancy of the gel was resolute by carefully examining it for presence of any gritty particles on fingertips.

A slight amount of gel was rubbed into skin on rear of hand to determine gel's homogeneity[10].

Determination of Particle Size

The chosen nanogels had their mean size then polydispersity index of size distribution measured by a Malvern Mastersizer 2000 MS. A record was made of the average particle size[11].

Zeta Potential

Malvern Mastersizer 2000 MS, was used to test zeta potential of chosen formulation[12].

Entrapment Efficiency

As a significance of centrifugation, extent of unentrapped drug recovered in supernatant was used to estimation amount of drug entrapped in Methotrexate loaded nanogel. A cooling centrifuge was used to spin the nanodispersion at 15,000 RPM for 10 minutes at 10°C. The unentrapped medicine was formerly measured in supernatant by UV Vis spectroscopy. To get the % Entrapment Efficiency, total

Table 2: Characterization of various gel formulations F1 to F8

Specifications	F1	F2	F3	F4	F5	F6	F7	F8
After feel effects	Smooth							
Colour	Transparent							
Homogeneity	Good							
Consistency	pourable							
pH*	6.85 ± 0.03	6.35 ± 0.05	6.89 ± 0.04	6.74 ± 0.03	6.78 ± 0.06	6.39 ± 0.04	6.78 ± 0.03	6.82 ± 0.05
Entrapment Efficiency* (%)	63.32 ± 0.45	65.85 ± 1.32	68.85 ± 1.65	63.32 ± 1.74	67.78 ± 1.33	65.58 ± 1.47	64.47 ± 0.58	68.85 ± 0.33
Spreadability*	9.85 ± 0.15	10.32 ± 1.02	9.85 ± 1.74	12.25 ± 0.85	13.36 ± 0.65	14.47 ± 0.36	13.22 ± 0.45	12.15 ± 0.74
Viscosity*	2875 ± 15	3265 ± 22	3345 ± 18	3265 ± 35	3174 ± 29	3098 ± 24	3265 ± 18	3145 ± 13
Zeta potential	-32.25	-34.65	-33.32	-30.15	-29.98	-30.55	-32.74	-33.74
Drug Content* (%)	92.25 ± 0.12	93.65 ± 0.25	94.74 ± 0.15	94.65 ± 0.33	95.25 ± 0.32	93.65 ± 0.15	93.12 ± 0.22	95.45 ± 0.85
Particle Size (nm)	125.45	130.37	138.85	145.58	140.85	105.65	125.65	150.45
% CDR after 12 hrs	69.85 ± 0.32	66.58 ± 0.25	64.47 ± 0.15	74.98 ± 0.33	79.25 ± 0.24	68.74 ± 0.32	63.32 ± 0.36	75.98 ± 0.74

*Average of three determinations

Table 3: Characterization of various gel formulations F9 to F17

Specifications	F9	F10	F11	F12	F13	F14	F15	F16	F17
After feel effects	Smooth								
Colour	Transparent								
Homogeneity	Good								
Consistency	pourable								
pH	6.85 ± 0.05	6.78 ± 0.02	6.88 ± 0.08	6.75 ± 0.03	6.88 ± 0.08	6.74 ± 0.05	6.62 ± 0.08	6.82 ± 0.02	6.74 ± 0.05
Entrapment Efficiency (%)	68.85 ± 0.85	74.58 ± 0.35	72.25 ± 0.68	69.98 ± 0.85	68.78 ± 0.65	66.45 ± 0.85	68.85 ± 0.33	76.65 ± 0.74	70.25 ± 0.65
Spreadability	11.25 ± 0.12	13.36 ± 0.22	12.25 ± 0.15	11.65 ± 0.22	13.32 ± 0.18	12.44 ± 0.21	13.25 ± 0.22	13.65 ± 0.24	12.25 ± 0.23
Viscosity	3145 ± 15	3210 ± 20	3385 ± 18	3147 ± 16	3245 ± 13	3365 ± 17	3214 ± 22	3645 ± 11	3145 ± 25
Zeta potential	-35.58	-39.85	-37.74	-36.65	-35.85	-34.65	-35.65	-41.85	-32.25
Drug Content (%)	96.65 ± 0.50	94.47 ± 0.45	93.32 ± 0.35	94.47 ± 0.63	93.65 ± 0.14	95.45 ± 0.22	92.25 ± 0.41	96.45 ± 0.33	90.12 ± 0.44
Particle Size (nm)	165.85	130.25	136.65	145.85	125.45	140.65	105.85	123.74	103.36
% CDR After 12 hrs	86.85 ± 0.22	73.36 ± 0.74	75.45 ± 0.15	85.33 ± 0.98	88.85 ± 0.32	63.32 ± 0.25	71.21 ± 0.15	91.15 ± 0.47	88.85 ± 0.33

*Average of three determinations

drug quantity andquantity of unentrapped drug in supernatant were entered into following equation[13].

Spreadability

Graph Pad in Stat 9 is used for statistical examination, and entirely outcomes are existing as means ± standard error of mean (SEM). Group alterations were considered important when p-value was less than 0.05[14].

Extrudability Study

The extrudability of generated nanogel was assessed by filling collapsible tubes by it and assessing weight in grams desirable to create a 0.5 cm ribbon of gel in 10 seconds[15].

Viscosity

At 37 °C, by spindle No.7, the nanogel's viscosity was dignified using DV-E, Brookfield Engineering Laboratories, MA, USA. An adequate amount of nanogel was useful to center of viscometer plate, just under spindle, and viscosities were restrained[16].

Total Drug Content

The resulting nanogel, which weighed half a gram, was diluted by ten milliliters of methanol and passed through a 0.45 µm filter. Calibration curve method was used to evaluation the total drug content by UV spectrophotometry at 314 nm.

$$\%\ Drug\ entrapment = \frac{\text{Conc. of total drug} - \text{Conc. of unentrapped drug}}{\text{Conc. of unentrapped drug}} \times 100$$

Scanning Electron Microscopy

The morphology of nanogel is measured by scanning electron microscopy (Nova NanoSEM NPEP303). In a preparatory step earlier SEM analysis, 100µl of nanogel formulation comprising methotrexate was placed to a 10mm glass slide and left to dry overnight in a vacuum desiccator at room temperature. The nanogel research was covered by gold using gold sputter module in a higher

Table 4: Experimental statistics by predicted response

Run Order	Formulation Code	Parameters	Actual Value	Predicted Value
8	MXT-NGs-F16	Particle size (nm)	123.74	130.50
		Drug content (%)	96.45	96.02
		Zeta potential (mV)	-41.85	
		PDI	0.187	

Table 5: Spectral interpretation of EDS-Spectroscopy

EL	AN	C Atom (%)	Signal wt (%)
C	6	69.94	8.82
O	8	26.33	4.43
N	7	3.73	1.54

Table 6: Regression analysis data of optimized nanogel MXT-NGs-F16formulation

Batch	Zero Order	First Order	Higuchi	Korsmeyer Peppas	
	R^2	R^2	R^2	R^2	'N' value
F16	0.9679	0.9664	0.9879	0.9758	0.4921

Table 7: Analysis data of optimized nanogel formulation MXT-NGs-F16

Batch	Parameters	Values
	Flux	0.0033µg/cm^2/min
F16	Permeability Coefficient	0.00066

Table 8: Effect of optimized combined nanogel of methotrexate against Freund's adjuvant induced arthritis in rats

Group	Drug and Dose	Paw Volume		
		Day 7	Day 14	Day 21
Group I	Normal Control	2.5 ± 0.20	2.8 ± 0.25	3.0 ± 0.22
Group II	Complete Freund's adjuvant (CFA)	8.5 ± 0.50	9.8 ± 0.55	10.2 ± 0.60
Group III	CFA + methotrexate (1%)	7.2 ± 0.40	6.5 ± 0.35	5.8 ± 0.30
Group IV	CFA + Marketed formulation of methotrexate cream/ointment (1%)	7.0 ± 0.42	6.2 ± 0.33	5.5 ± 0.28
Group VIII	CFA + optimized formulation of methotrexate nanogel (1%)	6.2 ± 0.33	5.5 ± 0.30	4.5 ± 0.22

vacuum evaporator afterward it was placed on an appropriate support. The experiment was showed at a voltage of 15 kilovolts and a range of magnifications[17].

XRD Analysis

A sample is exposed to a barrage of X-rays during XRD check, and the diffraction pattern that emerges is then noted. This pattern may be castoff to decide distinct phases, degree crystallographic purity, and assessment crystallite size and

Table 9: Effect of optimized combined nanogel of methotrexate on arthritis index score in rats

Group	Drug and Dose	Arthritic Score
Group I	Normal Control	0 ± 0.0
Group II	Complete Freund's adjuvant (CFA)	3.5 ± 0.548
Group III	CFA + methotrexate (1%)	1.5 ± 0.84
Group IV	CFA + Marketed formulation of methotrexate cream/ointment (1%)	1.05 ± 0.84
Group V	CFA + optimized formulation of methotrexate nanogel (1%)	0.95 ± 0.53

Standards are stated as mean ± S.E.M. (n = 6). Standards are statistically significant at $p<0.05$ (One-way ANOVA followed by Dunnett's test).

Table 10: Effect of optimized combined nanogel of methotrexate on serum biomarkers i.e. ALT, AST, ALP and SODin rats

Group	ALT (IU/L)	AST(IU/L)	ALP(IU/L)	SOD (U/mg protein)
Group I	45.2 ± 2.10	50.5 ± 2.20	80.2 ± 2.50	5.80 ± 0.18
Group II	98.6 ± 3.50	105.3 ± 3.80	190.4 ± 4.20	2.10 ± 0.12
Group III	78.4 ± 3.20	85.7 ± 3.40	145.8 ± 3.80	3.90 ± 0.14
Group IV	75.8 ± 3.10	80.6 ± 3.30	138.2 ± 3.70	4.00 ± 0.14
Group V	65.4 ± 2.80	72.5 ± 2.90	125.7 ± 3.40	4.60 ± 0.17

Standards are stated as mean ± S.E.M. (n = 6). Standards are statistically significant at $p<0.05$ (One-way ANOVA followed by Dunnett's test).

orientation. It also offers information onorganization of atoms in material[18].

EDX Analysis

In order to define elemental structure of reaction mixture, EDX were used. The sample of methotrexate nanogel was examined by an EDX by means of a scanning electron microscope. The presence of phases is often shown by the EDX[19].

In-vitro Drug Release of Methotrexate Loaded Nanogel

Using Franz's diffusion cell, an *in vitro* drug release investigation was carried out. Receiver cell capacity was 10 ml, and the effective permeation area was 0.196 cm2. Over the receptor cell, which was fullby phosphate buffer saline (pH 7.4), donor cell containing the nanogel gel was positioned. By a clamp, a dialysis membrane that had been pre-treated and had a molecular weight cutoff of 12–14 kD was inserted between the donor and receptor compartments. The experiment lasted 12 hours at a temperature of 37 ± 1°C while being continuously stirred at 600 rpm using magnetic stirring.

We used a UV spectrophotometer set at 314 nm to measure the drug content in samples taken from the receptor cell at predetermined intervals (0.5, 1, 2, 4, 6, 6, 8, 10, and 12 hours) and then added new release medium to the receiver

Table 11: Effect of optimized combined nanogel of methotrexate on serum biomarkers i.e. Lipid peroxidation, GSH, CAT, TNF-α and IL-1β in rats

Group	Lipid peroxidation	GSH (U/mg protein)	CAT (U/mg protein)	TNF-α (U/mg protein)	IL-1β (U/mg protein)
Group I	1.05 ± 0.10	12.80 ± 0.52	14.80 ± 0.58	12.50 ± 0.45	8.90 ± 0.35
Group II	3.45 ± 0.15	5.20 ± 0.34	5.50 ± 0.29	35.20 ± 1.20	25.50 ± 1.00
Group III	2.30 ± 0.12	9.60 ± 0.42	9.80 ± 0.35	22.80 ± 1.05	16.80 ± 0.75
Group IV	2.10 ± 0.12	9.00 ± 0.39	9.20 ± 0.37	23.60 ± 1.00	15.20 ± 0.80
Group V	1.80 ± 0.10	11.20 ± 0.47	12.50 ± 0.46	15.20 ± 0.88	12.30 ± 0.60

Standards are stated as mean ± S.E.M. (n = 6). Standards are statistically significant at $p<0.05$ (One-way ANOVA followed by Dunnett's test).

compartment at the same time to maintain a constant sink condition. Various release kinetics models were used to the data in order to determine the nanogel's release kinetics[20].

Ex-vivo Skin Permeation Studies of Nanogel Loaded with Methotrexate

In order to determine measures of medicine that penetrated skin into the body, researchers conducted *ex-vivo* skin permeation investigations. Skin of goats was procured from a nearby slaughterhouse and preserved in a buffer pH 7.4 until it was prepared to be put on a Franz diffusion cell. After removing the hair from the goat's skin with a razor, it was cleaned off with isopropyl alcohol. A layer of skin was applied to separate the donor and receptor compartments. A 13-milliliter buffer solution is placed in receptor compartment, while 5 milligrams of methotrexate-loaded nanogel is applied to the skin in donor compartment.

Using a magnetic stirrer, temperature of diffusion cell was controlled at 37°C and the stirring rate at 100 RPM. To keep sink condition, 3 ml of samples were taken at 0, 30, 60, 120, 240, 360, 480, 600, and 720 minute intervals and mixed with the same amount of freshly prepared buffer solution. Each sample was studied three times, and the average was used for further calculations.

At 313 nm, samples were inspected by a UV. Permeation parameters, such as flow and permeation coefficient, were applied to the results. An x-axis graph showing % CDR and a y-axis graph showing time was used to compute the parameters.

Freund's Adjuvant-induced Arthritis

The sub-plantar region of the left hind paw of each rat was injected with 0.1 ml of complete Freund's adjuvant, which included 6 mg of mycobacterium per ml, in order to induce arthritis[21]. The 21-day dosing period for the isolated substance and standard began on the same day. On the day of injection and at regular intervals on the 7th, 14th, and 21st days, plethysmographic measurements of paw volumes and body weight were taken. Visual evaluation and grading of arthritis based on secondary lesions followed this system. There were thirty rats utilized in the study. The following is a breakdown of the rats into their five groups, with six animals per group:

Group I: Control rats were applied topically with a gel base for 21 days

Group II: Arthriticcontrol rats received 0.1 ml complete Freund's adjuvant or CFA (6 mg mycobacterium in each ml)

Group III: Rats treated with Plain gel methotrexate (1%, Physical mixture) for 21 days

Group IV: Rats treated with the marketed formulation of methotrexate cream/ointment (1%) for 21 days

Group V: Rats treated with the optimized MTX-NGs-F16 formulation of methotrexate nanogel (1%) for 21 days

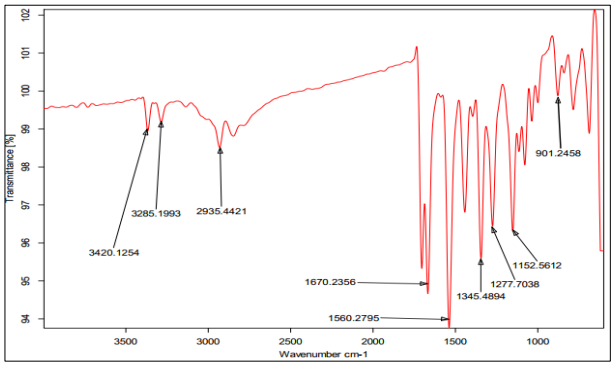


Figure 3: FT-IR of pure Methotrexate

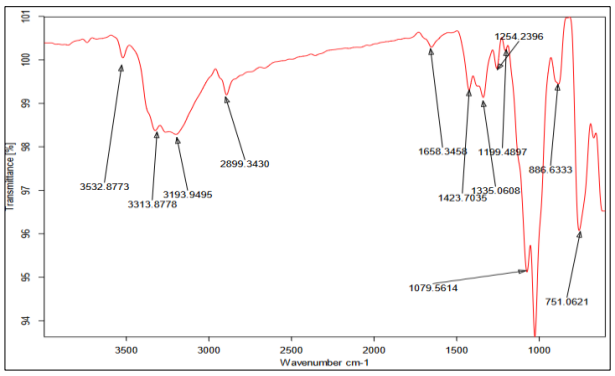


Figure 4: FT-IR of Physical Mixture of Methotrexate + Carbopol 934 + HPMC K 15

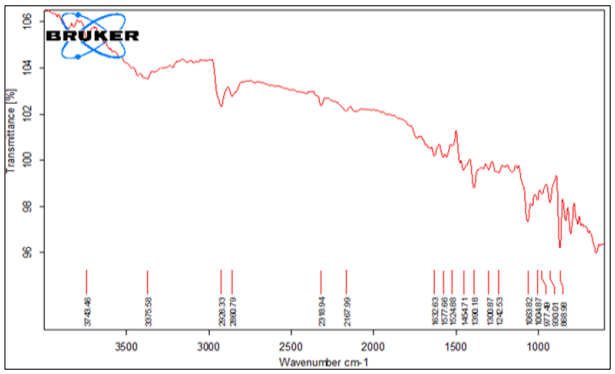


Figure 5: FT-IR of the optimized formulation of Methotrexate nanogel lyophilized powder MXT-NGs-F16

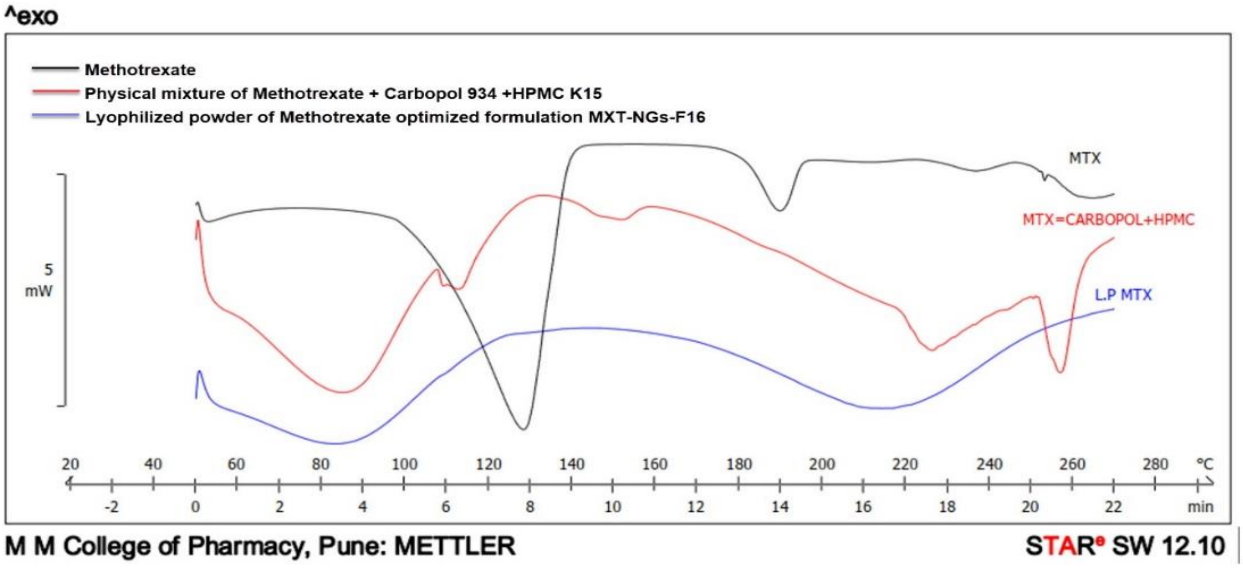


Figure 6: Overlay DSC thermogram

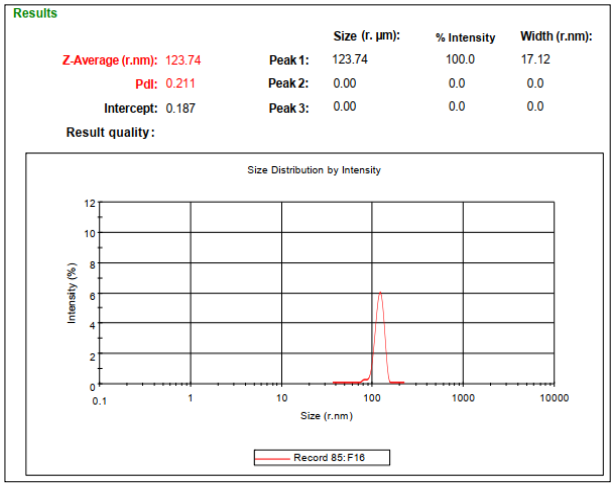


Figure 7: Particle size of Optimized formulation MXT-NGs- F16

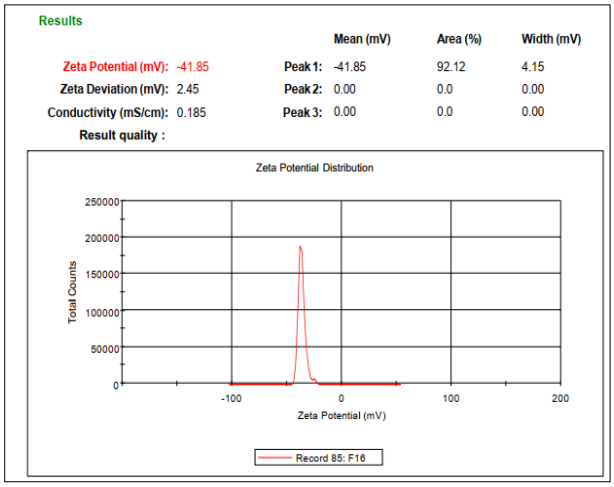


Figure 8: Zeta potential of Optimized formulation MXT-NGs- F16

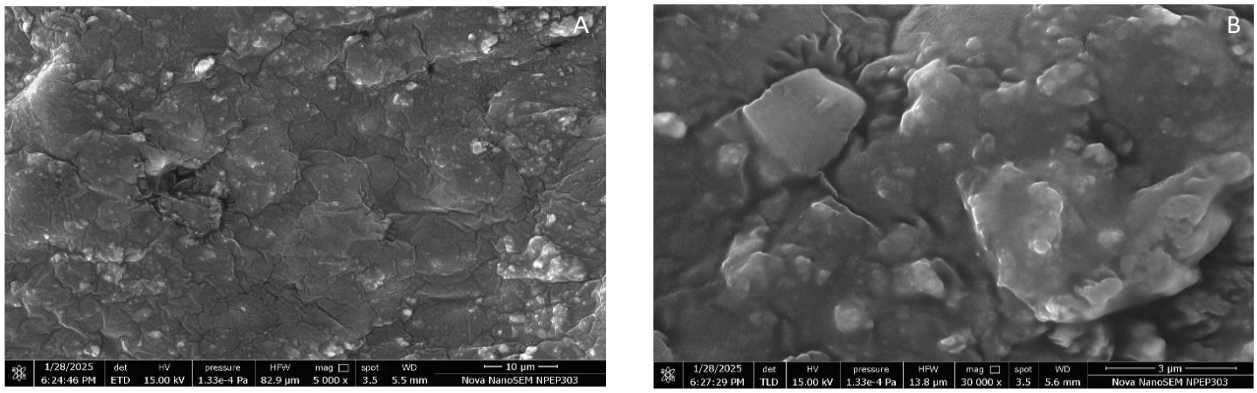


Figure 9: Images of SEM of optimized MXT-NGs- F16 formulation

On day 1st, Group I served as the normal control, receiving only a plain gel base, while Group II was the arthritic control, receiving CFA without treatment. Group III was treated with plain gel formulations containing methotrexate (1%), to assess the efficacy. Group IV received commercially available methotrexate gel as reference treatments.

Group V was treated with experimental nanogels of MTX-NGs-F16, designed for enhanced drug delivery. After measuring the paw volume and arthritis index score for the study period, all rats were terminated via cervical

decapitation on the 21st day. Centrifugation was used to extract serum from blood samples acquired via retro-orbital puncture. Various biochemical markers were measured, including ALT, AST, ALP, SOD, GSH, lipid peroxidation, CAT, tumor necrosis factors (TNF-α), and IL1-α.

Blood Sampling

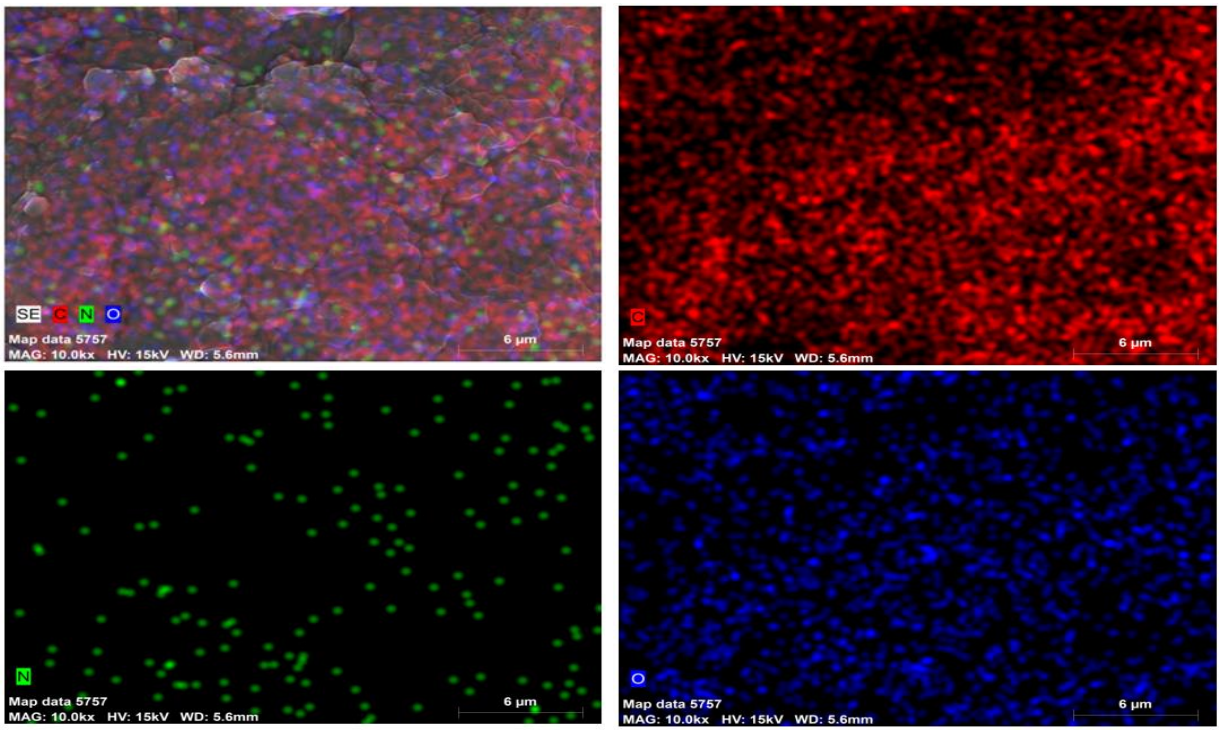


Figure 10: EDS images of Lyophilized Powder of Optimized formulation of MTX-NGs-F16

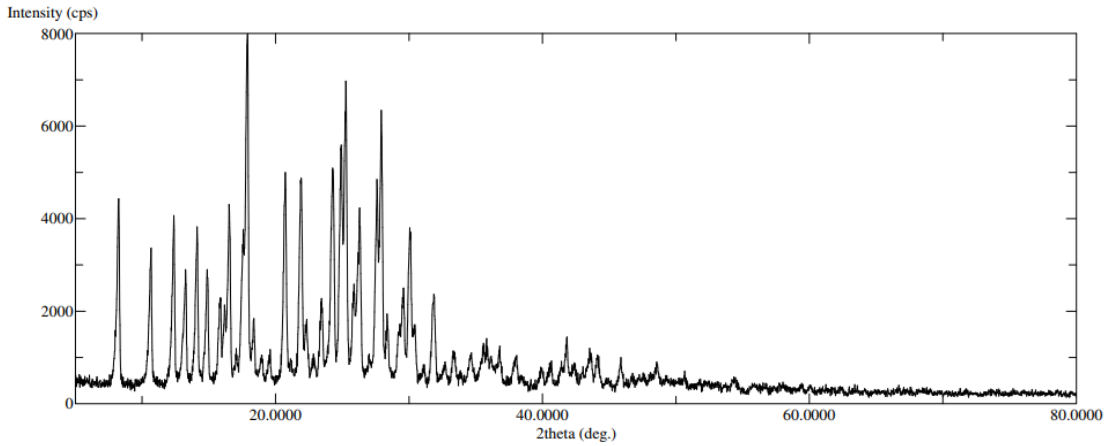


Figure 11: Graph of XRD of Pure drug methotrexate

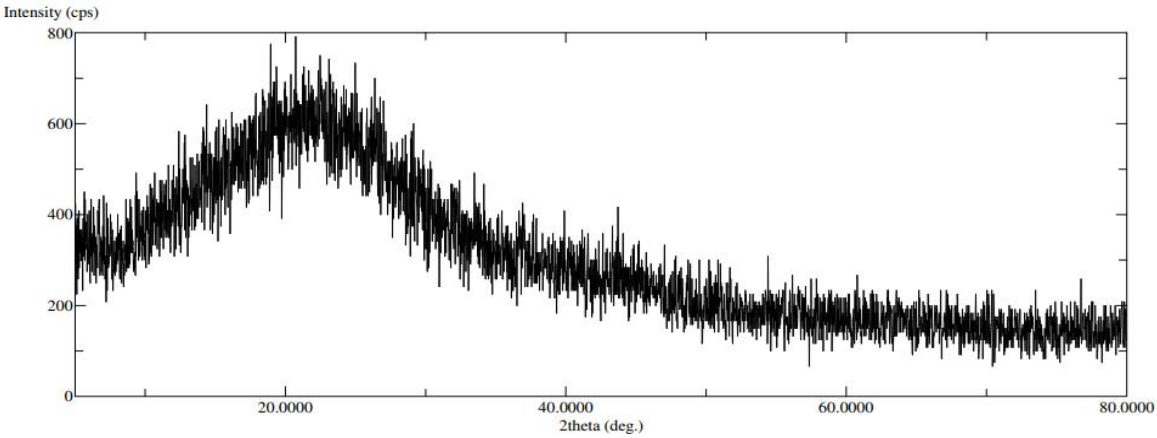
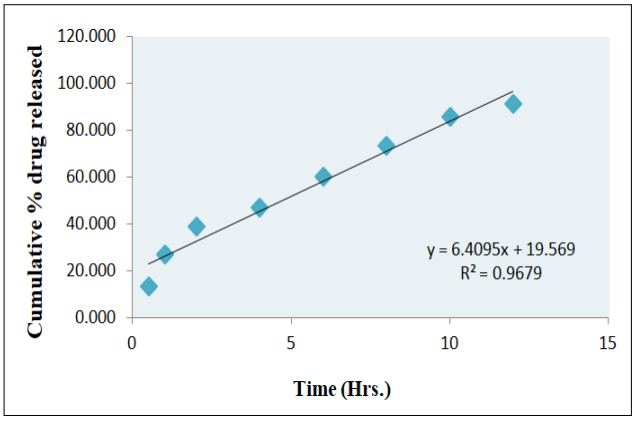
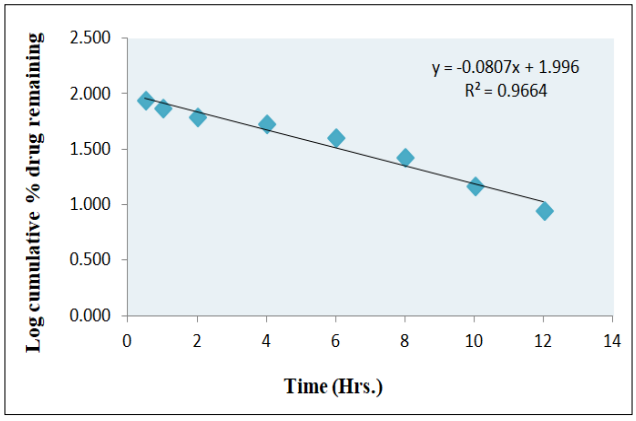


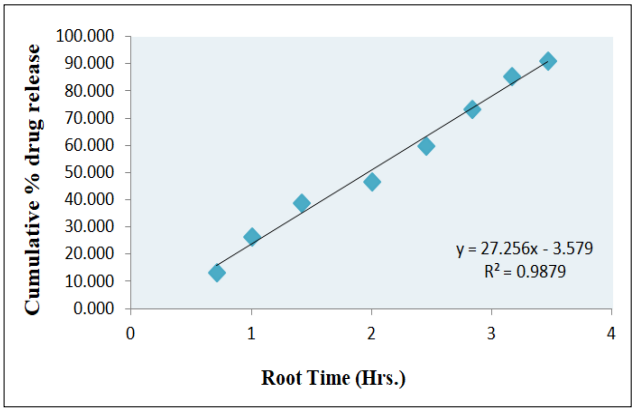
Figure 12: Results of XRD of methotrexate lyophilized MXT-NGs-F16



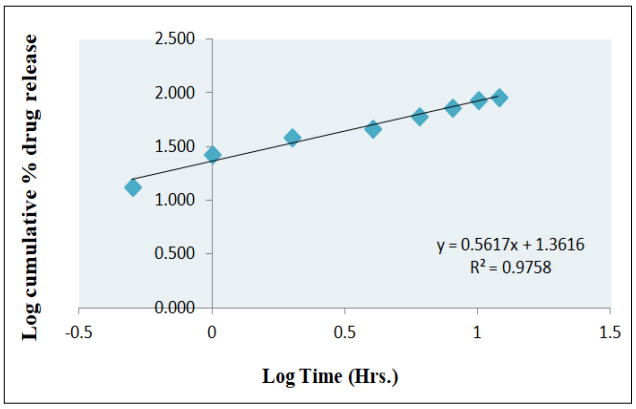
Cumulative % drug released Vs Time
(Zero Order Kinetics)



Log cumulative % drug remaining Vs. time
(First Order Kinetics)



Cumulative % drug release vs. root time
(Higuchi Release Kinetics)



Log Cumulative % drug release Vs Log time
(Korsmeyer Peppas Model)

Figure 13: Different release kinetics models of MTX-NGs-F16

Tail snipping, which included taking blood from the individual using the heparinized capillary tube, was used for glucose analysis. Gently pressing the incision for a few seconds after collection halted the bleeding. The time needed to collect 30 to 50 µL of blood was under 1 minute. To evaluate the lipid profiles and other biochemical parameters, a retroorbital bleeding method was employed to obtain blood from the retro-orbital plexus.

Statistical Analysis

Graph Pad in Stat 9 is used for statistical analysis, and all outcomes are existing as means ± standard error of mean (SEM). Group differences were deemed majorwhen p-value was less than 0.05.

RESULTS AND DISCUSSION

Methotrexate-loaded nanogel formulations (MXT-NGs-F1–MXT-NGs-F17) were successfully developed and evaluated for critical physicochemical parameters essential for topical drug delivery. All formulations were start to be transparent, smooth, and homogeneous in consistency, indicating satisfactory aesthetic and sensory qualities suitable for patient compliance. The pH of all formulations extended among 6.35 and 6.89, which is compatible by skin physiology and unlikely to cause irritation (Table 2). The entrapment efficiency varied across formulations, with MXT-NGs-F16 exhibiting the highest entrapment efficiency of 76.65% (Table 3), indicating efficient encapsulation of methotrexate within the nanogel matrix. Among all batches, formulation MXT-NGs- F16 showed superior performance in terms of drug content (96.45%), particle size (123.74 nm), and zeta potential (−41.85 mV), demonstrating enhanced drug loading, nanoscale particle uniformity, and colloidal stability (Figure 1, Figure 2). The BBB was effective in optimizing formulation variables, as shown by the close agreement among actual and predicted values for both particle size and drug content. The low polydispersity index (PDI = 0.187) further confirmed the homogeneity of nanogel particles (Table 4).

The structural integrity and compatibility of drug by excipients were confirmed by FTIR spectroscopy. Spectra of the optimized formulation (MXT-NGs-F16) showed no significant shifts or disappearance of characteristic peaks, indicating no chemical interaction between methotrexate and excipients (Figures 3, Figure 4, Figure 5 and Figure 6). Thermal analysis through DSC also revealed no major incompatibilities, while XRD analysis demonstrated the transformation of methotrexate from a crystalline to an amorphous state within the formulation, contributing to enhanced solubility (Figure 7). SEM images of MXT-NGs-F16 revealed well-defined spherical particles with smooth surfaces, and EDS analysis confirmed the elemental

composition consistent with the methotrexate-loaded system (Figure 8, Figure 9, Figure 10, Figure 11).

In vitro drug release studies specified a sustained release profile from MXT-NGs-F16 over 12 hours, achieving 91.15% cumulative release, whereas the marketed formulation (Roxate Gel) released most of the drug within the first 2 hours. Drug release from F16 best fitted the Higuchi kinetic model ($R^2 = 0.9879$), indicating a diffusion-controlled mechanism (Figure 12). The Korsmeyer–Peppas model yielded an 'n' value of 0.4921, suggesting Fickian diffusion as the primary release mechanism.

Ex vivo skin permeation studies using Franz diffusion cells revealed that MXT-NGs-F16 achieved a cumulative permeation of 2.3487 µg/cm² in 12 hours, with a steady-state flux of 0.0033 µg/cm²/min and a permeability coefficient of 0.00066. These findings indicate that the nanogel is capable of delivering methotrexate effectively through the skin barrier.

The anti-arthritic efficacy of the optimized nanogel formulation was assessed in a Complete Freund's Adjuvant (CFA)-induced arthritis rat perfect. MXT-NGs-F16 significantly reduced paw edema, with paw volume decreasing from 6.2 mm on day 7 to 4.5 mm on day 21, outperforming both the pure drug and the marketed formulation. Correspondingly, the arthritis index score was lowest (0.95) in the MXT-NGs-F16-treated group, indicating substantial amelioration of arthritic symptoms.

Biochemical assessments further validated the anti-inflammatory potential of MXT-NGs-F16. The treatment markedly reduced elevated liver enzymes—ALT, AST, and ALP—caused by systemic inflammation and restored SOD levels, indicating protection against oxidative damage. In addition, MXT-NGs-F16 significantly reduced lipid peroxidation and pro-inflammatory cytokines whereasimproving antioxidant limits like GSH and CAT. These results suggest that the topical nanogel formulation not only reduces inflammation and joint swelling but also addresses oxidative stress, contributing to a holistic anti-arthritic effect (Figure 13; Table 5 to Table 11).

CONCLUSION

In conclusion, optimized methotrexate nanogel formulation (F16), developed using Box-Behnken Design, demonstrated excellent physicochemical stability, sustained drug release, enhanced skin permeation, and superior therapeutic efficacy *in vivo*. These findings collectively support the potential of this nanogel as a promising alternative to conventional methotrexate therapy for the management of rheumatoid arthritis.

REFERENCES

1. Abdellatif AA, Mohammed HA, Khan RA, Singh V, Bouazzaoui A, Yusuf M, Akhtar N, Khan M, Al-Subaiyel A, Mohammed SA, Al-Omar MS. Nano-scale delivery: A comprehensive review of nano-structured devices, preparative techniques, site-specificity designs, biomedical applications, commercial products, and references to safety, cellular uptake, and organ toxicity. Nanotechnology Reviews. 2021 Oct 21;10(1):1493-559.
2. Abdel-Rashid RS, Helal DA, Omar MM, El Sisi AM. Nanogel loaded with surfactant based nanovesicles for enhanced ocular delivery of acetazolamide. International journal of nanomedicine. 2019 Apr 29:2973-83.
3. Avasatthi V, Pawar H, Dora CP, Bansod P, Gill MS, Suresh S. A novel nanogel formulation of methotrexate for topical treatment of psoriasis: optimization, *in vitro* and *in vivo* evaluation. Pharmaceutical development and technology. 2016 Jul 3;21(5):554-62.
4. Ahmad S, Patil K, Koli G, Rahman BA, Barde L, Deshpande M, Tare H. Design, Development and Characterization of Econazole loaded Nanoparticles for Topical Application. International Journal of Pharmaceutical Quality Assurance. 2023;14(2):358-362.
5. Barkat MA, Hasan N, Hassan MZ, Asiri YI, Nadaf A, Ahmad FJ, Kesharwani P. Formulation development of methotrexate lipid-based nanogel for treatment of skin cancer. Colloids and Surfaces A: Physicochemical and Engineering Aspects. 2024 May 5;688:133571.
6. Delgado-Pujol EJ, Martínez G, Casado-Jurado D, Vázquez J, León-Barberena J, Rodríguez-Lucena D, Torres Y, Alcudia A, Begines B. Hydrogels and Nanogels: Pioneering the Future of Advanced Drug Delivery Systems. Pharmaceutics. 2025 Feb;17(2):215.
7. Shivatare R, Jangra S, Gaikwad A, Kewatkar S, Bhutale N, Suryavanshi DS. Development and validation of ICPMS methods for simultaneous determination of elemental impurities in topical cream containing ximenynic acid. Future Journal of Pharmaceutical Sciences. 2023 Jun 2;9(1):47.
8. Duong VA, Nguyen TT, Maeng HJ. Preparation of solid lipid nanoparticles and nanostructured lipid carriers for drug delivery and the effects of preparation parameters of solvent injection method. Molecules. 2020 Oct 18;25(20):4781.
9. Farag RK, Mohamed RR. Synthesis and characterization of carboxymethyl chitosan nanogels for swelling studies and antimicrobial activity. Molecules. 2012 Dec 24;18(1):190-203.
10. Farooq U, Rasul A, Sher M, Qadir MI, Nazir I, Mehmood Y, Riaz H, Shah PA, Jamil QA, Khan BA. Development, characterization and evaluation of anti-fungal activity of miconazole based nanogel prepared from biodegradable polymer. Pakistan Journal of Pharmaceutical Sciences. 2020 Jan 2;33.
11. Ferrer MC, Dastgheyb S, Hickok NJ, Eckmann DM, Composto RJ. Designing nanogel carriers for antibacterial applications. Acta biomaterialia. 2014 May 1;10(5):2105-11.
12. Hamed KM, Dighriri IM, Baomar AF, Alharthy BT, Alenazi FE, Alali GH, Alenazy RH, Alhumaidi NT, Alhulayfi DH, Alotaibi YB, Alhumaidan SS. Overview of methotrexate toxicity: a comprehensive literature review. Cureus. 2022 Sep 23;14(9).
13. Kesharwani P, Jain A, Srivastava AK, Keshari MK. Systematic development and characterization of curcumin-loaded nanogel for topical application. Drug

development and industrial pharmacy. 2020 Sep 1;46(9):1443-57.
14. Mane V, Killedar S, More H, Salunkhe S. Development and Validation of a Novel Bioanalytical Method for Estimating Epigallocatechin 3 Gallate in Wistar Rat Plasma by RP-HPLC Employing Gradient Elution Techniques. Journal of Research in Pharmacy. 2023 May 1;27(3):1039-55.
15. Nnamani PO, Ugwu AA, Nnadi OH, Kenechukwu FC, Ofokansi KC, Attama AA, Lehr CM. Formulation and evaluation of transdermal nanogel for delivery of artemether. Drug Delivery and Translational Research. 2021 Aug;11:1655-74.
16. Nnamani PO, Ugwu AA, Nnadi OH, Kenechukwu FC, Ofokansi KC, Attama AA, Lehr CM. Formulation and evaluation of transdermal nanogel for delivery of artemether. Drug Delivery and Translational Research. 2021 Aug;11:1655-74.
17. Pathan IB, Dwivedi R, Ambekar W. Formulation and evaluation of ketoprofen loaded chitosan nanogel for pain management: *Ex-vivo* and *In-vivo* study. Ars Pharmaceutica (Internet). 2019 Jun 20;60(2):101-8.
18. Sinha P, Srivastava S, Mishra N, Singh DK, Luqman S, Chanda D, Yadav NP. Development, optimization, and characterization of a novel tea tree oil nanogel using response surface methodology. Drug development and industrial pharmacy. 2016 Sep 1;42(9):1434-45.
19. Suhail M, Rosenholm JM, Minhas MU, Badshah SF, Naeem A, Khan KU, Fahad M. Nanogels as drug-delivery systems: A comprehensive overview. Therapeutic delivery. 2019 Nov 1;10(11):697-717.
20. Mane V, Killedar S, More H, Nadaf S, Salunkhe S. Novel Phytosomal Formulation of Emblica officinalis Extracts with Its *In vivo* Nootropic Potential in Rats: Optimization and Development by Box-Behnken Design. Journal of Chemistry. 2024;2024(1):6644815.
21. Wadile KA, Ige PP, Sonawane RO. Preparation of itraconazole nanoparticles and its topical nanogel: Physicochemical properties and stability studies. International Journal of Pharmaceutical Sciences and Developmental Research. 2019 Jan 3;5(1):001-8.