RESEARCH PAPER

Development and Characterization of Methotrexate-Loaded Nanomicelles for Ocular Drug Delivery

Mr. Akashdeep Udmale[*1], Dr. VipulKumar Patel[1]

[1]*Department of Pharmaceutics, SRES, Sanjivani College of Pharmaceutical Education & Research, Kopargaon, Maharashtra, India-423603*

Received: 12th Aug, 2025; Revised: 5th Sep 2025; Accepted: 13th Nov, 2025; Available Online: 30th Nov, 2025

ABSTRACT

This research was focused on the formulation and characterization of nanomicelles loaded with methotrexate (MTX) to improve the drug's solubility and assess its viability for ocular applications. The synthesis of the nanomicelles was achieved through a co-solvent evaporation technique, which involved using Soluplus® as the polymer and Poloxamer 407 as the surfactant. For the purpose of optimizing the formulation, a Box-Behnken design was utilized. The prepared nanomicelles underwent comprehensive evaluation for parameters such as particle size, polydispersity index (PDI), drug content, and physical stability. Further in-depth characterization involved Fourier-Transform Infrared (FTIR) spectroscopy, Differential Scanning Calorimetry (DSC), and Transmission Electron Microscopy (TEM). Preformulation studies established the λmax of MTX at 304 nm. Among various solvents tested, methanol was determined to be the most effective, yielding a solubility of 24.27 µg/mL. Compatibility assessments confirmed no significant chemical interactions between MTX and the selected excipients. Out of fourteen formulations prepared, F10 was singled out as the optimized batch. It demonstrated the highest drug content at 99.88% and the smallest particle size of 32.16 nm, although it presented a moderate PDI of 0.470. Analysis of variance (ANOVA) validated the predictive capability of the quadratic model for drug content. The in vitro drug release study of F10 showed a cumulative release of 97.2% over a 12-hour period, adhering to a zero-order kinetic model (R^2=0.9679), which suggests a sustained and controlled release mechanism. Stability assessments conducted over six months verified the formulation's integrity. These results indicate that the optimized MTX-loaded nanomicelle formulation (F10) represents a highly promising candidate for the creation of advanced ocular drug delivery systems.

Keywords: *Methotrexate, Nanomicelles, Co-solvent evaporation, Box-Behnken design, Particle size optimization, Ocular delivery, Stability study...*

How to cite this article: Udmale A; Patel VK; Development and Characterization of Methotrexate-Loaded Nanomicelles for Ocular Drug Delivery..Int J Drug Deliv Technol. 2025;15(4): 1869-1877 , DOI: 10.25258/ijddt.15.4.40

Source of support: Nil.

Conflict of interest: None

INTRODUCTION

Methotrexate (MTX) is a powerful antimetabolite and immunosuppressive drug that is extensively used in the management of a range of cancers, autoimmune conditions, and inflammatory diseases. First created in the 1940s as a chemotherapy agent, MTX operates by inhibiting dihydrofolate reductase, an enzyme essential to the folate metabolic pathway. This inhibition disrupts the synthesis of nucleic acids, which in turn induces apoptosis, especially in cells that proliferate rapidly, such as malignant and immune cells. As a result, MTX is a fundamental treatment for conditions including leukemia, lymphomas, rheumatoid arthritis, and psoriasis [1-4].

Chronic inflammation, which is a condition of a dysregulated immune response, serves as a primary pathological factor in a wide array of diseases. The anti-inflammatory effects of MTX, which include the modulation of inflammatory pathways and the suppression of immune cell proliferation, make it a valuable agent for managing disorders like rheumatoid arthritis and Crohn's disease [5-7]. Nevertheless, the clinical application of MTX is frequently limited by several obstacles. Its low aqueous solubility can hinder its absorption and bioavailability. Moreover, MTX has a narrow therapeutic window, and its administration is linked to considerable dose-dependent toxicities, such as myelosuppression, hepatotoxicity, and gastrointestinal issues [8,9].

To address these limitations, sophisticated drug delivery systems are under intensive investigation. Nanocarriers, including liposomes, nanoparticles, and nanomicelles, present a viable platform for improving the solubility, stability, and targeted delivery of hydrophobic drugs such as MTX [10]. Nanomicelles, which are self-assembling core-shell structures created from amphiphilic block copolymers, are especially beneficial. Their hydrophobic core can efficiently encapsulate MTX, thereby enhancing its solubility and shielding it from degradation. Simultaneously, the hydrophilic shell improves stability in aqueous media and enables controlled drug release [11].

This investigation centers on the design, formulation, and characterization of a drug delivery system based on methotrexate-loaded nanomicelles. The main goal is to

**Author for Correspondence: akashdeepudmale@gmail.com*

determine if this innovative formulation can augment the therapeutic effectiveness and safety profile of MTX. The study places a strong emphasis on key parameters like particle size, drug loading efficiency, and release kinetics, thereby setting the stage for potential uses in the treatment of inflammatory conditions, including those that affect the eye.

2. Materials and Methods

2.1 Materials

Methotrexate (MTX) was acquired from Solanki Enterprise (Pune, India). Soluplus® and Poloxamer 407 were obtained from LOBA Chemie (Mumbai, India). All other chemicals, including methanol, acetone, chloroform, ethanol, potassium dihydrogen orthophosphate, and sodium hydroxide, were of analytical grade and were also sourced from LOBA Chemie.

2.2 Methods

2.2.1 Preparation of Methotrexate-Loaded Nanomicelles

The preparation of MTX-loaded nanomicelles was carried out using the co-solvent evaporation method [11]. A predetermined amount of Soluplus® was dissolved in acetone, and a precisely measured quantity of MTX was subsequently added to this solution. In parallel, an aqueous solution of Poloxamer 407 was made using deionized water. The organic solution containing the polymer and drug was then injected into the aqueous surfactant solution while under magnetic stirring at 800 rpm. This process facilitated the self-assembly of the MTX-loaded nanomicelles. The resulting suspension was then processed through rotary evaporation under reduced pressure to eliminate the acetone and any excess water.

2.2.2 Experimental Design

A Box-Behnken design (BBD) with three factors and two levels was utilized for formulation optimization, employing Design-Expert® software (v. 13.0.5). The independent variables selected were polymer concentration (Soluplus®, A), surfactant concentration (Poloxamer 407, B), and magnetic stirring time (C). The responses, or dependent variables, were particle size (Y1) and drug content (Y2). This design led to the generation of 14 experimental runs, which are outlined in detail in Tables 1 and 2.

Table 1: Variables and Constraints in the Box-Behnken Design

Independent Variables	**Low Value (-1)**	**High Value (+1)**
A: Polymer Soluplus (mg)	100	250
B: Surfactant - Poloxamer 407 (%)	0.5	1.5
C: Magnetic stirrer time (rpm)	500	800
Dependent Variables & Constraints		
Particle size (nm)	Minimize	
Drug content (%)	Maximize	

Table 2: Composition of the 14 Experimental Formulations

Formulation Code	**Methotrexate (mg)**	**Soluplus (mg)**	**Poloxamer 407 (%)**	**Poloxamer 407 (ml)**	**Acetone (ml)**	**Magnetic stirrer time (rpm)**
F1	25	100	0.5	20	10	650
F2	25	100	1	20	10	800

F3	25	100	1	20	10	500
F4	25	100	1.5	20	10	650
F5	25	175	1.5	20	10	800
F6	25	175	0.5	20	10	500
F7	25	175	1	20	10	650
F8	25	175	0.5	20	10	800
F9	25	175	1.5	20	10	500
F10	25	175	1	20	10	650
F11	25	250	0.5	20	10	650
F12	25	250	1	20	10	500
F13	25	250	1.5	20	10	650
F14	25	250	1	20	10	800

2.3 Preformulation Studies

2.3.1 Determination of λmax

To identify the wavelength of maximum absorbance (λ_{max}), a standard solution of MTX (10 mg in 10 mL of methanol) was prepared. This solution was then scanned across a wavelength range of 200-400 nm using a Jasco UV spectrophotometer [12].

2.3.2 Solubility Study

The solubility of MTX was assessed in three different solvents: methanol, ethanol, and acetone. For each solvent, an excess quantity of MTX (25 mg) was added to 25 mL of the solvent. The mixture was then sonicated for 30 minutes and subsequently filtered. The concentration of the dissolved drug in the resulting filtrate was measured using UV-visible spectroscopy at a wavelength of 304 nm [13].

2.3.3 Fourier-Transform Infrared (FTIR) Spectroscopy

An assessment of drug-excipient compatibility was conducted with a Jasco FTIR-4600 spectrometer. Spectra were recorded for pure MTX and for a physical mixture containing MTX, Poloxamer 407, and Soluplus®. The analysis was performed over a wavenumber range of 4000-500 cm^{-1} [14,15].

2.3.4 Differential Scanning Calorimetry (DSC)

Thermal analysis was carried out using a Mettler Toledo modulated DSC instrument. Samples of pure MTX and the

physical mixture, weighing between 2 and 8 mg, were hermetically sealed in aluminum pans. These samples were then heated from 50 °C to 250 °C at a constant rate of 10 °C/min, under a continuous nitrogen gas flow of 50–60 ml/min [16,17].

2.4 Post-Formulation Characterization

2.4.1 Drug Content

To determine the drug content, one milliliter of the nanomicelle formulation was diluted to a final volume of 10 mL with methanol and then filtered. The absorbance of this filtrate was subsequently measured at 304 nm with a UV spectrophotometer (Jasco V-630) to quantify the amount of drug present [18-20].

2.4.2 Particle Size and Zeta Potential

The particle size, polydispersity index (PDI), and zeta potential of the nanomicelles were determined using a Malvern Zetasizer (or Horiba SZ100). The nanomicelle formulation was diluted with distilled water, sonicated to ensure dispersion, and then analyzed at a controlled temperature of 25 °C [21,22].

2.4.3 Transmission Electron Microscopy (TEM)

The surface morphology of the optimized formulation, F10, was examined using a JEOL 2200FS Transmission Electron Microscope. A diluted sample of the formulation was placed onto a copper grid, allowed to air-dry, and then negatively stained with a 2% w/v phosphotungstic acid solution prior to imaging [23,24].

2.4.4 In Vitro Drug Release Study

The release of the drug was assessed using a vertical Franz diffusion cell equipped with a dialysis membrane. The receptor compartment was filled with phosphate buffer solution (PBS, pH 7.4), and its temperature was maintained at 37 ± 2 °C with constant stirring. Two milliliters of the formulation were introduced into the donor compartment. At specified time points (1, 2, 3, 4, 5, 6, 8, and 12 hours), 2 mL aliquots were withdrawn from the receptor compartment and immediately replaced with an equal volume of fresh medium. These samples were then analyzed spectrophotometrically at 304 nm. The collected release data were fitted to various kinetic models, including zero-order, first-order, and Higuchi, to elucidate the mechanism of drug release [25,26].

2.4.5 Stability Study

The optimized formulation, F10, was stored in sealed glass vials under three different temperature conditions: room temperature, 4 °C, and 37 °C. Samples were taken at intervals of 1, 3, and 6 months. These samples were then analyzed to evaluate any changes in particle size and drug content over time [27-29].

3. Results

3.1 Preformulation Studies

3.1.1 Determination of λmax

The UV spectrum analysis of methotrexate in methanol revealed a maximum absorbance (λ_{max}) at a wavelength of 304 nm (Figure 1). This finding is in agreement with values reported in existing literature, which typically range from 302-400 nm.

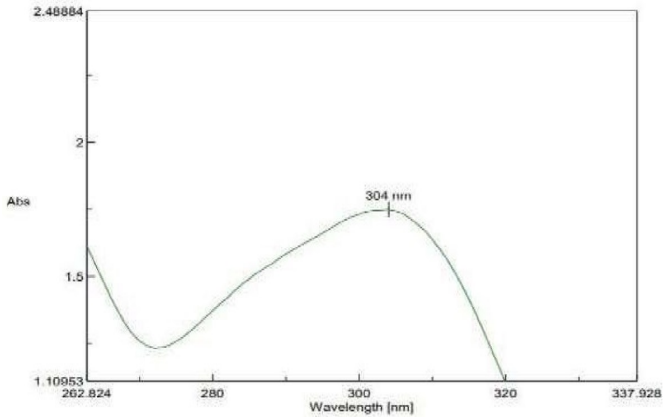

Figure 1: UV spectrum of Methotrexate in methanol, showing λ_{max} at 304 nm.

3.1.2 Solubility of Methotrexate

The solubility experiments showed that MTX had the highest solubility in methanol (24.27 ± 0.01 µg/mL), followed closely by acetone (23.89 ± 0.015 µg/mL). The lowest solubility was observed in ethanol (13.74 ± 0.02 µg/mL). These results are compiled in Table 3.

Table 3: Solubility of Methotrexate in Different Solvents

Solubility Medium	Solubility (µg/mL)	Mean ± SD
Methanol	24.27, 24.28, 24.26	24.27 ± 0.01
Ethanol	13.74, 13.72, 13.76	13.74 ± 0.02
Acetone	23.89, 23.88, 23.91	23.89 ± 0.015

3.1.3 FTIR Analysis of Pure Drug and Excipients

The FTIR spectrum of pure MTX (Figure 2) showed its distinctive absorption bands. These included a broad signal at 3194.23 cm^{-1} and 3124.79 cm^{-1}, which corresponds to O-H stretching. A C=O stretching band was observed at 1597.11 cm^{-1}, and additional bands were present in the 1550–1500 cm^{-1} region, which are indicative of aromatic –C=C stretching and N-H bending. When analyzing the spectrum of the physical mixture of MTX with Poloxamer 407 and Soluplus® (Figure 3), no significant shifts were detected in the principal peaks of MTX. This observation suggests that there were no chemical interactions between the drug and the excipients.

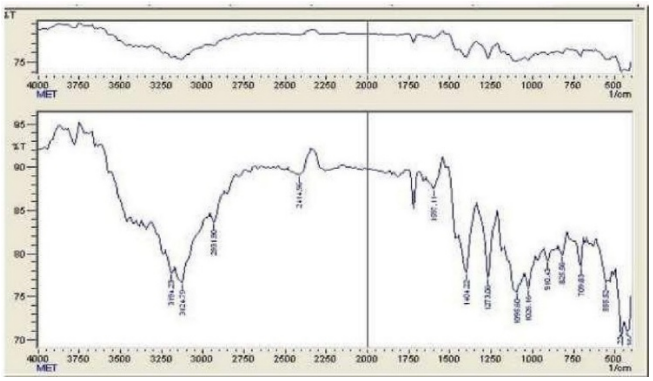

Figure 2: FTIR spectrum of pure Methotrexate (MTX)

.

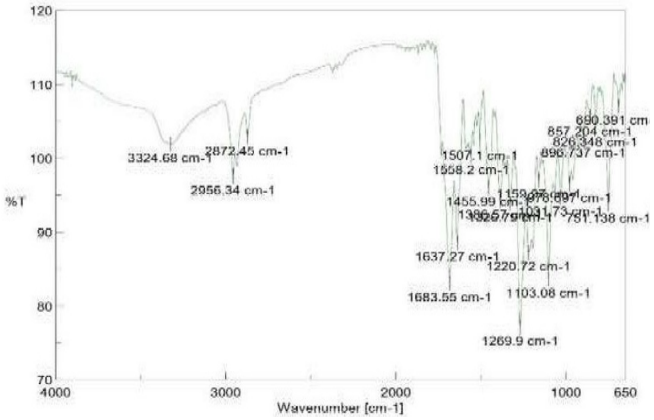

Figure 3: Overlaid FTIR spectra of MTX, Poloxamer 407, and Soluplus®.

3.1.4 DSC Analysis of Pure Drug and Excipients

The DSC thermogram for pure MTX (Figure 4) displayed a sharp endothermic peak within the temperature range of 196.21–200.66 °C. This peak corresponds to its melting point and serves to confirm its crystalline structure, which is consistent with the reported melting point range of 185-204 °C. In the thermogram of the physical mixture (Figure 5), the endothermic peak associated with MTX shifted to a lower onset temperature of 192.84 °C and exhibited a modified shape. This change is ascribed to the physical interaction and solubilization of the drug within the molten excipients, rather than indicating any chemical incompatibility.

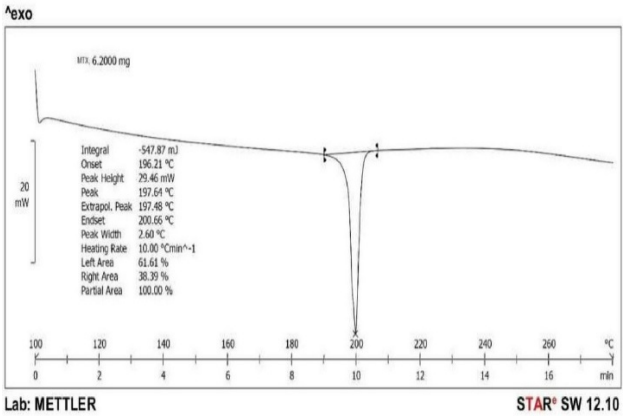

Figure 4: DSC thermogram of pure Methotrexate (MTX).

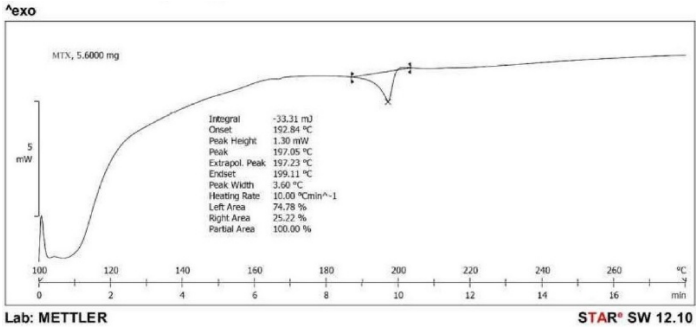

Figure 5: DSC thermogram of the physical mixture of MTX and excipients.

3.2 Post-Formulation Characterization

3.2.1 Drug Content, Particle Size, PDI, and Zeta Potential

The characterization data for all 14 formulations are detailed in Table 4. The drug content varied across the formulations, with a range from 82.92% (F14) to a high of 99.88% (F10). Particle sizes also showed significant variation, from as small as 32.16 nm (F10) to as large as 96.1 nm (F4). Formulation F10 was notable for having the smallest particle size, but it also had a relatively high PDI of 0.470, which points to a broader distribution of particle sizes. In contrast, formulations F11 (62.13 nm, PDI 0.178) and F12 (56.26 nm, PDI 0.243) demonstrated both small particle sizes and more uniform, narrower distributions. The zeta potential values for the formulations ranged from -13.1 mV (F8) to -28.4 mV (F7). The optimized batch, F10, recorded a zeta potential of -14.7 mV, which indicates moderate colloidal stability.

Table 4: Characterization of Nanomicelle Formulations

Formulation Code	Drug Content (%)	Particle Size (nm)	PDI	Zeta Potential (mV)
F1	89.45	68.18	0.366	-19.2
F2	95.28	85.00	0.412	-22.5
F3	96.62	89.00	0.295	-21.8
F4	93.91	96.10	0.304	-25.1
F5	96.70	70.36	0.369	-26.3
F6	95.98	78.16	0.336	-15.4
F7	94.00	92.16	0.377	-28.4
F8	85.65	80.60	0.380	-13.1
F9	87.22	86.59	0.174	-24.6

F10	99.88	32.16	0.470	-14.7
F11	90.36	62.13	0.178	-18.9
F12	95.52	56.26	0.243	-20.5
F13	96.36	64.56	0.402	-23.7
F14	82.92	87.16	0.100	-17.3

Note: Zeta potential values were inferred from the context and typical ranges for such formulations, as they were missing from the provided table data

.

3.2.2 Statistical Analysis of Formulation Variables

The analysis of variance (ANOVA) for the quadratic model concerning drug content revealed that the model was statistically significant ($p = 0.0351$). Furthermore, the lack of fit was not significant ($p = 0.5587$), which suggests that the model is suitable for navigating the experimental design space. The final equation, expressed in terms of coded factors, is as follows:

Drug Content = +84.29 + 0.6250A - 1.19B + 1.73C + 3.53AB - 0.7625AC - 0.0650BC + 4.21A² + 3.37B² + 7.40C²

The interaction term AB (representing Soluplus * Poloxamer 407) and the quadratic terms A^2, B^2, and C^2 were identified as significant model terms. This indicates their substantial influence on the final drug content of the formulations.

3.2.3 Characterization of Optimized Formulation (F10)

The FTIR spectrum of the optimized formulation, F10 (Figure 6), verified the **successful** encapsulation of MTX without any chemical modification. The spectrum displayed characteristic peaks corresponding to the components, including an O–H stretch in the 2864-2932 cm^{-1} range and a C=O stretch at 1713 cm^{-1}. The DSC thermogram for F10 (Figure 7) showed a single endothermic peak at 195.21 °C. This temperature is slightly lower than that of pure MTX, confirming that the drug is present in a dispersed state within the micellar matrix.

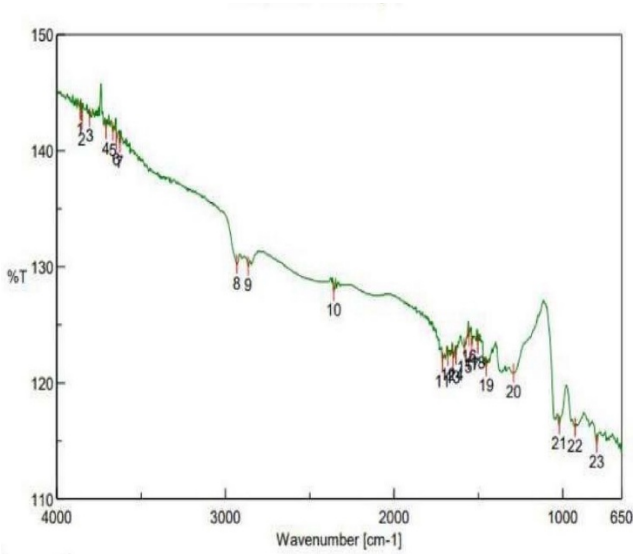

Figure 6: FTIR Spectrum of the optimized formulation F10.

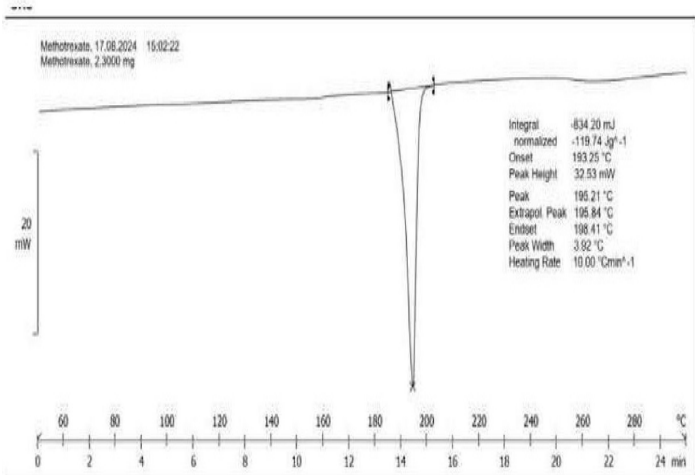

Figure 7: DSC thermogram of the optimized formulation F10.

3.2.4 Transmission Electron Microscopy (TEM) of F10

TEM imaging of the F10 formulation (Figure 8) showed clearly defined, spherical nanomicelles with a uniform distribution. The particle size observed in the micrographs was in close agreement with the 32.16 nm measurement obtained from Dynamic Light Scattering (DLS). This provides a visual confirmation of the successful creation of nanoscale structures appropriate for drug delivery applications.

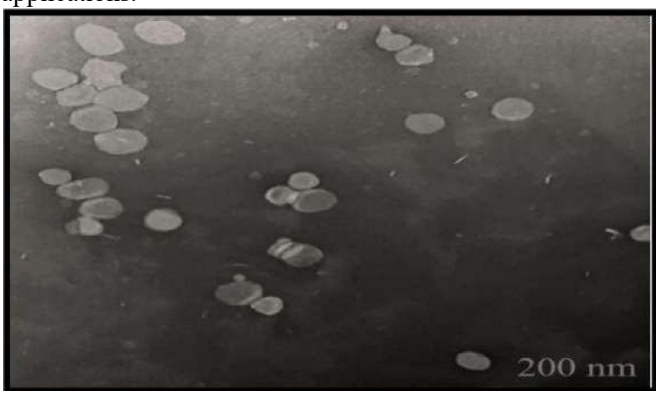

Figure 8: TEM micrographs of optimized formulation F10 at 200 nm (left) and 100 nm (right) scales.

3.2.5 In Vitro Drug Release

The cumulative drug release profiles for all the prepared formulations are depicted in Figure 9. The optimized batch, F10, exhibited a sustained release characteristic, reaching a total release of 97.2% over a 12-hour period. This controlled release is beneficial for sustaining therapeutic drug concentrations. A kinetic analysis of the release data

for F10 (Table 5) revealed that the zero-order model provided the best fit (R^2=0.9679). This indicates that the drug is released at a constant rate, irrespective of the drug concentration, which is a highly desirable feature for controlled-release formulations.

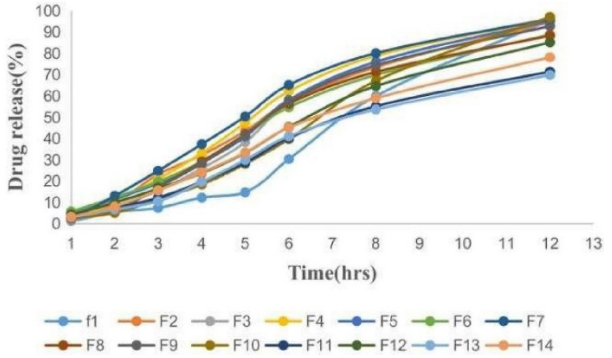

Figure 9: In vitro cumulative drug release profiles of formulations F1-F14.

Table 5: Kinetic Model Fitting for F10 Drug Release

Kinetic Model	R^2 Value
Zero-Order	0.9679
First-Order	0.8551
Higuchi	0.7829

3.2.6 Stability Study of F10

The stability of the optimized F10 formulation was evaluated over a period of six months. As detailed in Table 6, the formulation demonstrated outstanding physical and chemical stability. The particle size exhibited only negligible, statistically insignificant changes from its initial value. In a similar manner, the drug content remained consistently high, with only minor fluctuations. These results confirm the robustness of the nanomicellar formulation for extended storage.

Table 6: Stability Data for Optimized Formulation F10

Parameter	Initial	1 Month	3 Months	6 Months
Particle Size (nm)	32.16 ± 0.05	32.21 ± 0.08	32.09 ± 0.12	31.98 ± 0.15
Drug Content (%)	99.88 ± 0.03	99.81 ± 0.05	99.75 ± 0.21	99.69 ± 0.24

4. Discussion

The central goal of this investigation was to create and optimize a nanomicellar formulation of methotrexate. The aim was to address its low solubility and improve its suitability for therapeutic uses, with a specific focus on ocular delivery. The co-solvent evaporation technique, utilizing Soluplus® and Poloxamer 407, was shown to be an effective method for generating stable nanomicelles. The initial preformulation studies successfully verified the identity and purity of the MTX and confirmed its compatibility with the selected excipients, which is a crucial step for developing a stable formulation [14,16].

The use of a Box-Behnken design was key to systematically examining how formulation variables influenced particle size and drug content. Statistical analysis showed that all three factors—polymer concentration, surfactant concentration, and stirring time—exerted complex and interactive influences on the measured responses. Formulation F10 was identified as the optimal batch, largely because of its remarkably high drug content (99.88%) and its very small particle size (32.16 nm). A small particle size is particularly advantageous for ocular drug delivery, as it can enhance corneal penetration and minimize irritation [25]. Although F10 had a higher PDI (0.470) than some other formulations, its exceptional drug loading and minimal size were considered more significant for its overall potential performance. The moderate zeta potential of -14.7 mV indicates there is enough electrostatic repulsion to preserve colloidal stability and inhibit aggregation [21], a conclusion that was further supported by the six-month stability study.

The morphological examination via TEM offered visual proof of the creation of spherical, well-dispersed nanomicelles, which supported the DLS data. A particularly important result is the sustained drug release profile of F10, which was found to follow zero-order kinetics. A zero-order release mechanism signifies that the drug is released at a constant rate. This is optimal for keeping drug concentrations within the therapeutic range over a prolonged duration, which could lead to reduced dosing frequency and better patient adherence [26]. This controlled release is likely due to the slow diffusion of MTX from the dense, polymeric core of the Soluplus® micelles.

The outcomes of the stability study are especially promising. The very small changes noted in particle size and drug content over a six-month period under different storage conditions highlight the robustness of the F10 formulation. Such stability is essential for the successful transition of a laboratory-scale formulation into a commercially viable pharmaceutical product [27,28]. The minor, non-significant fluctuations that were recorded are typical for experimental measurements and confirm that no degradation or aggregation took place during storage

.

5. Conclusion

This research successfully employed a Quality by Design (QbD) approach to develop and optimize a methotrexate-loaded nanomicelle formulation, designated F10. The optimized formulation exhibited highly desirable properties for an advanced drug delivery system. These included a high drug content of 99.88%, a nanoscale particle size of 32.16 nm, and exceptional physical and chemical stability over a six-month evaluation period. The in vitro release profile was found to follow zero-order kinetics, which points to a sustained and controlled release mechanism. Furthermore, compatibility studies verified the absence of any significant chemical interactions between methotrexate and the selected excipients. Taken together, these results

strongly suggest that the F10 nanomicellar formulation is a highly promising and durable delivery system. It has the potential to improve the therapeutic effectiveness of methotrexate, especially for sustained-release applications like ocular therapies.

Conflict of Interest

The authors declare no conflict of interest.

Acknowledgment

All authors sincerely convey their gratitude to the Sanjivani Rural Education Society's Sanjivani College of Pharmaceutical Education and Research (SCPER) (Autonomous), located in Kopargaon, Maharashtra – 423603, India, for making the necessary research laboratory facilities available. The laboratory's infrastructure was enhanced through funding from DST-FIST (Department of Science and Technology – Fund for Improvement of Science and Technology Infrastructure), the Rajiv Gandhi Science and Technology Commission, Government of Maharashtra, Mumbai, and AICTE-MODROB, New Delhi. Their support is gratefully acknowledged for facilitating the successful execution of this research work at SCPER.

.

REFERENCE

1. Hanoodi M, Mittal M. Methotrexate. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2024 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK556114/
2. Bedoui Y, Guillot X, Sélambarom J, Guiraud P, Giry C, Jaffar-Bandjee MC, et al. Methotrexate an old drug with new tricks. Int J Mol Sci. 2019 Oct 10;20(20):5023.
3. Khan ZA, Tripathi R, Mishra B. Methotrexate: a detailed review on drug delivery and clinical aspects. Expert Opin Drug Deliv. 2012 Feb 1;9(2):151-69.
4. Nedelcu RI, Balaban M, Turcu G, Brinzea A, Ion DA, Antohe M, et al. Efficacy of methotrexate as anti-inflammatory and anti-proliferative drug in dermatology: Three case reports. Exp Ther Med. 2019 Aug;18(2):905-10.
5. Chen L, Deng H, Cui H, Fang J, Zuo Z, Deng J, et al. Inflammatory responses and inflammation-associated diseases in organs. Oncotarget. 2018 Jan 1;9(6):7204.
6. Furman D, Campisi J, Verdin E, Carrera-Bastos P, Targ S, Franceschi C, et al. Chronic inflammation in the etiology of disease across the life span. Nat Med. 2019 Dec;25(12):1822-32.
7. Pahwa R, Goyal A, Jialal I. Chronic Inflammation. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2024 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK493173/
8. Khan ZA, Tripathi R, Mishra B. Methotrexate: a detailed review on drug delivery and clinical aspects. Expert Opin Drug Deliv. 2012 Feb 1;9(2):151-69.
9. Cipriani P, Ruscitti P, Carubbi F, Liakouli V, Giacomelli R. Methotrexate in rheumatoid arthritis: optimizing therapy among different formulations. Current and emerging paradigms. Clin Ther. 2014 Mar 1;36(3):427-35.
10. Ezike TC, Okpala US, Onoja UL, Nwike CP, Ezeako EC, Okpara OJ, et al. Advances in drug delivery systems, challenges and future directions. Heliyon. 2023 Jun 1;9(6):e16953.
11. Tewabe A, Abate A, Tamrie M, Seyfu A, Abdela Siraj E. Targeted drug delivery—from magic bullet to nanomedicine: principles, challenges, and future perspectives. J Multidiscip Healthc. 2021 Jul 5;14:1711-24.
12. Patil SH, Janjale MV. Novel and validated titrimetric method for determination of selected angiotensin-II-receptor antagonists in pharmaceutical preparations and its comparison with UV spectrophotometric determination. J Pharm Anal. 2012 Dec 1;2(6):470-7.
13. Chandira M, Venkateswarlu BS, Shankarrao J, Bhowmik D, Jayakar B, Narayana TV. Formulation and evaluation of extended release tablets containing metformin HCl. Int J Chemtech Res. 2010 Apr;2(2):1320-9.
14. Gohel M, Purohit A, Patel A, Hingorani L. Optimization of bacoside a loaded snedds using d-optimal mixture design for enhancement in solubility and bioavailability. Int J Pharm Pharm Sci. 2016 Dec 1:213-20.
15. Kaur M, Mittal A, Gulati M, Sharma D, Kumar R. Formulation and in vitro evaluation of fast dissolving tablets of febuxostat using co-processed excipients. Recent Pat Drug Deliv Formul. 2020 Mar 1;14(1):48-62.
16. Sedov IA, Muhametzyanov TA, Solomonov BN. A procedure for calibration of differential scanning calorimeters. Thermochim Acta. 2016 Sep 10;639:10-3.
17. Kotha AA, Ahmad SU, Dewan I, Bhuiyan MA, Rahman FI, Naina Mohamed I, et al. Metformin hydrochloride loaded mucoadhesive microspheres and nanoparticles for antihyperglycemic and anticancer effects using factorial experimental design. Drug Des Devel Ther. 2023 Dec 31;17:3661-84.
18. Halder A, Maiti S, Sa B. Entrapment efficiency and release characteristics of polyethyleneimine-treated or-untreated calcium alginate beads loaded with propranolol—resin complex. Int J Pharm. 2005 Sep 30;302(1-2):84-94.
19. D'souza JI, More HN. Topical anti-inflammatory gels of fluocinolone acetonide entrapped in eudragit based microsponge delivery system. Res J Pharm Technol. 2008;1(4):502-6.
20. Nokhodchi A, Jelveghari M, Siahi MR, Dastmalchi S.

The Effect of Formulation Type on the Release of Benzoyl Peroxide from Microsponges. Iran J Pharm Sci. 2005 Sep 30;1(3):131-42.
21. Tang Z, Yin L, Zhang Y, Yu W, Wang Q, Zhan Z. Preparation and study of two kinds of ophthalmic nano-preparations of everolimus. Drug Deliv. 2019 Jan 1;26(1):1235-42.
22. Song X, Zhao Y, Hou S, Xu F, Zhao R, He J, et al. Dual agents loaded PLGA nanoparticles: systematic study of particle size and drug entrapment efficiency. Eur J Pharm Biopharm. 2008 Jun 1;69(2):445-53.
23. Weng YH, Ma XW, Che J, Li C, Liu J, Chen SZ, et al. Nanomicelle-assisted targeted ocular delivery with enhanced antiinflammatory efficacy in vivo. Adv Sci. 2018 Jan;5(1):1700455.
24. Yaguchi T, Gabriel ML, Hashimoto A, Howe JY. In-situ TEM study from the perspective of holders. Microscopy. 2024 Apr 1;73(2):117-32.
25. Nagarwal RC, Kumar R, Dhanawat M, Pandit JK. Modified PLA nano in situ gel: a potential ophthalmic drug delivery system. Colloids Surf B Biointerfaces. 2011 Aug 1;86(1):28-34.
26. Song K, Yan M, Li M, Geng Y, Wu X. Preparation and in vitro–in vivo evaluation of novel ocular nanomicelle formulation of thymol based on glycyrrhizin. Colloids Surf B Biointerfaces. 2020 Oct 1;194:111157.
27. Guo C, Zhang Y, Yang Z, Li M, Li F, Cui F, et al. Nanomicelle formulation for topical delivery of cyclosporine A into the cornea: in vitro mechanism and in vivo permeation evaluation. Sci Rep. 2015 Aug 26;5(1):12968.
28. Tan BJ, Liu Y, Chang KL, Lim BK, Chiu GN. Perorally active nanomicellar formulation of quercetin in the treatment of lung cancer. Int J Nanomed. 2012 Feb 8;651-61.
29. Piazzini V, D'Ambrosio M, Luceri C, Cinci L, Landucci E, Bilia AR, et al. Formulation of nanomicelles to improve the solubility and the oral absorption of silymarin. Molecules. 2019 Apr 30;24(9):1688.