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Formulation and Development of Methotrexate Loaded Nano Emulsion for Treatment of Psoriasis

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ABSTRACT:
Psoriasis, a chronic inflammatory condition, is increasingly prevalent and associated with various life-threatening diseases. The formulation was assessed for physicochemical properties, entrapment efficiency, drug release kinetics, and stability. The final optimized Methotrexate (MTX) nano emulsion had a particle size of 30.69 nm, a PDI of 0.65, and an average entrapment efficiency of 76.57%. After 20 hours, the drug release kinetics indicated a 72.47% release at pH 5.5. FTIR analysis showed that the optimized MTX nano emulsion formulation effectively fluidized both the epidermis and dermis, potentially enhancing drug permeability and retention. In vivo studies on rabbit skin demonstrated that the increased penetration of methotrexate-loaded nano emulsion gel was not due to structural changes in the intercellular lipid layers of the stratum corneum. These results suggest that MTX nano emulsion based on neem oil could be a potential treatment for psoriasis and might reduce the recurrence of psoriasis-like symptoms.

INTRODUCTION

Psoriasis is a chronic, inflammatory skin condition characterized by the rapid proliferation of keratinocytes, leading to the formation of thick, scaly, and erythematous plaques. This autoimmune disorder affects millions of people worldwide, significantly impacting their quality of life due to its visible and often uncomfortable symptoms [1]. The exact cause of psoriasis remains elusive, but it is believed to result from a combination of genetic predisposition and environmental triggers. The condition can manifest in various forms, ranging from mild to severe, and is often associated with comorbidities such as arthritis, cardiovascular diseases, and depression. Despite extensive research, psoriasis remains incurable, necessitating ongoing management strategies to control symptoms and improve patients' overall well-being.[2]

The treatment of psoriasis, a chronic and debilitating skin condition, has witnessed significant advancements with the advent of nano emulsion technology. Nano emulsions are finely dispersed oil-in-water or water-in-oil systems, characterized by their small droplet size, which enhances drug solubility, stability, and bioavailability. By encapsulating therapeutic agents like methotrexate in nano emulsions, researchers aim to deliver these drugs directly to the affected skin areas, ensuring a targeted and controlled release [3]. This innovative approach not only maximizes the therapeutic efficacy of the treatment but also minimizes systemic side effects, offering a promising solution for patients suffering from psoriasis. The enhanced penetration and retention of the drug at the site of action provided by nano emulsions represent a significant leap forward in the quest for more effective and safer psoriasis therapies.[4]

Methotrexate, a cornerstone in the treatment of psoriasis, has long been utilized for its potent anti-inflammatory and immunosuppressive properties. However, its systemic administration is often accompanied by significant

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adverse effects, limiting its long-term use and patient compliance. To address these challenges, recent advancements have focused on the development of methotrexate nano emulsions. These nano-scale delivery systems enhance the solubility, stability, and bioavailability of methotrexate, allowing for targeted and controlled release directly to the affected skin areas. This innovative approach aims to maximize therapeutic efficacy while minimizing systemic exposure and side effects, offering a promising alternative for the effective management of psoriasis.[5]

METHODOLOGY

To evaluate the solubility characteristics of MTX medicines, a systematic methodology was employed to ascertain their interactions with various solvents at room temperature. Initially, 10 mg of medicine sample was carefully weighed and placed independently in 10 ml of each selected solvent, ensuring a consistent sample-to-solvent ratio across all experiments. These mixtures were then transferred into firmly capped tubes to prevent any evaporation or contamination. To promote uniform dispersion and solubilization, the tubes were subjected to continuous shaking using a mechanical shaker. This agitation facilitated the interaction between the medicine and the solvent, allowing for the establishment of a homogeneous biochemical dispersion. The solubility of each medicine was then assessed based on the extent of dissolution observed, and the results were systematically recorded in a table for comparative analysis. This method provided a comprehensive understanding of the solubility profiles of the two medicines in various solvents, which is crucial for optimizing their formulation and therapeutic efficacy [6].

UV analysis of drug

To determine the authenticity of the samples through UV spectroscopic analysis, a detailed methodology was followed. Initially, each sample was subjected to a dilution procedure wherein it was dissolved in ethanol using a 10 ml volumetric flask. The solution was then further diluted up to 10 times to achieve the appropriate concentration range for UV spectroscopic testing. The diluted samples were then analyzed using a UV spectrophotometer to measure their absorbance at specific wavelengths. This process allowed for the determination of the maximum concentration at which the sample exhibits peak absorbance. Concurrently, a standard curve was established in ethanol by preparing a series of known concentrations of the sample and measuring their respective absorbance values. [7] This standard curve served as a reference to confirm the authenticity and concentration of the samples. By comparing the absorbance values of the test samples with the standard curve, the authenticity of the samples was accurately determined, ensuring the reliability of the analysis. (Figure 1)

UV analysis for Methotrexate:

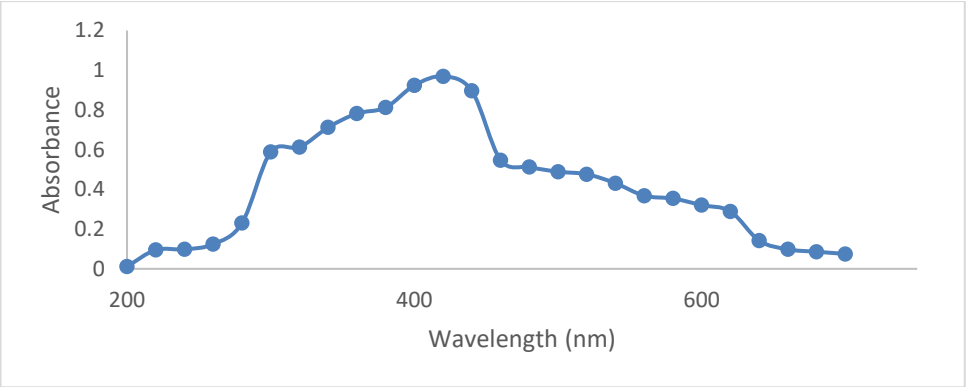

Figure 1: UV spectrum analysis of Methotrexate.

Screening of MTX Solubility in Neem Oil, Co-surfactants, and Surfactants:

To screen the solubility of MTX in neem oil, co-surfactants, and surfactants, an excess amount of the medication (approximately 250 mg) was added to 5 ml of selected neem oil, co-surfactants (polyethylene glycol 400, Tween 20, glycerin, Tween 80, and ethanol), and surfactants. (Figure 2) The mixture was homogenized using a vortex mixer. The blend was then subjected to continuous mixing for 24 hours in a shaker to ensure thorough solubilization. Following this, a sample from the

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saturated oil mixture was collected and centrifuged for 10 minutes at 3000 rpm. The supernatant from the centrifuged sample, which contains the dissolved oxiconazole in oils, surfactants, and co-surfactants, was carefully collected. From this, 1 ml of the supernatant was diluted to 10 ml using methanol. The concentration of MTX in the diluted sample was then determined using a UV spectrophotometer at a wavelength of 243 nm.[8]

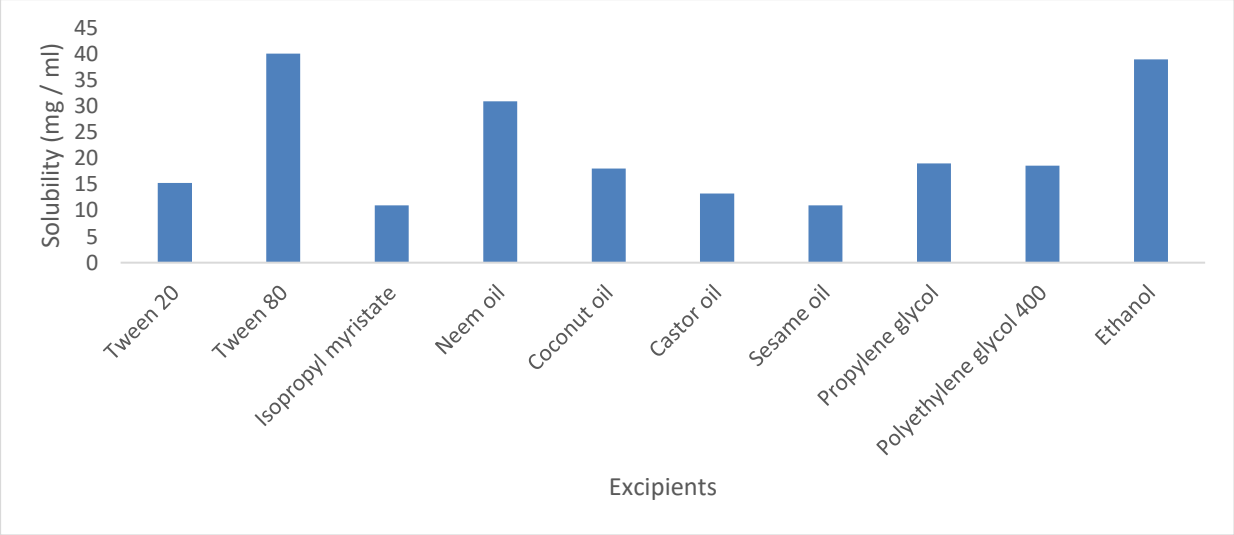

Figure 2: Graphical analysis of the solubility tests of Methotrexate.

Optimization of Co-surfactants, Oils, and Surfactants:

The optimization of the concentrations of surfactants, oils, and co-surfactants was conducted using various ratios. The ratios of the co-surfactant to surfactant mixture (Smix) varied as 1:4, 1:3, 1:2, and 1:1. Additionally, the ratios of Smix to neem oil were adjusted to 9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8, and 1:9 (Table 1). Aqua distilled water was incrementally added using a titration method, and the mixture was stirred with a magnetic stirrer until it became translucent and no phase separation was observed.[9]

Table 1: Observation of combined ethanol and tween 80 (Smix) comparisons with neem oil.

S no.	Smix: oil	Observations of ethanol and tween 80 mixtures (Smix)			
		1:1	**2:1**	**3:1**	**4:1**
1.	9:1	+++	++	+++	++
		-	+	-	+
2.	8:2	+++	++	+++	++
		-	+	-	+
3.	7:3	+++	++	+++	++
		-	+	-	+
4.	6:4	+++	++	+++	++
		-	+	-	+
5.	5:5	+++	++	+++	++
		-	+	-	+

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6.	4:6	+++	++	+++	++
		-	+	-	-
7.	3:7	+++	++	+++	++
		-	+	-	-
8.	2:8	+++	++	++	++
		-	+	-	-
9.	1:9	+++	++	++	++
		-	+	-	-

+++ : Cloudy, ++ : Slightly cloudy, + : Translucent,

Nano emulsion Formulation:

The nano emulsion formulation was carried out using the spontaneous emulsification method. MTX was first added to the oil phase, which included standardized butylated hydroxytoluene. Next, the Smix solution, a mixture of co-surfactant and surfactant, was added to the oil phase. This mixture was then stirred using a magnetic stirrer until a homogeneous mixture was obtained. Following this, Aqua dart (aqua distilled water) was gradually added through titration while maintaining continuous stirring. The process continued until the formation of the nano emulsion, which was indicated by the development of a translucent solution.[10]

Evaluation of the nano emulsion

The evaluation of the nano emulsion after its formation was conducted based on several criteria. These included organoleptic properties, pH value, viscosity, type of nano emulsion, and tests for physical stability. Additionally, the size of the particles and the distribution of globules were assessed to ensure the quality and efficacy of the nano emulsion.[11]

RESULTS AND DISCUSSION

Study solubility of Methotrexate:

The solubility of Methotrexate in surfactant, oil and co-surfactant is demonstrated in Figure 2. The best solubility of Methotrexate from the findings is in Tween 80 (surfactant), neem oil (oil) and ethanol (co-surfactant).

FTIR Analysis:

The FTIR spectral analysis reports reveal that Methotrexate nitrate has shownits characteristic peaks at 3678.49 (O-H stretch), 3150.70-3220.11(O-H), 2700.81(C- H), 1276.59 - 1240.48 (C-O), 954.18 (C-Cl). Individual components such as neem oil, methanol, tween and Methotrexate with tween have commonly shown peaks in (Figure 3-6). The combination of the antifungal drug with different polymers shows the characteristic absorption peaks at 3422.08 (O-H), 2918.12 (C-H), 2359.79 (C=C), 1654.11 (C=O),1407.69-1488.06 (C-Cl), 1078.55 (C-O). From the above results, it is concluded that the absorption peaks of Methotrexate nitrate remain unchanged in drug-polymer admixture which indicates that there is not any prominent chemical reaction between Methotrexate nitrate and the polymers used in the formulation of niosomal gels. Table 2 represents the interpretation of IR spectral data of Methotrexate and polymers.

Compatibility study by FTIR analysis

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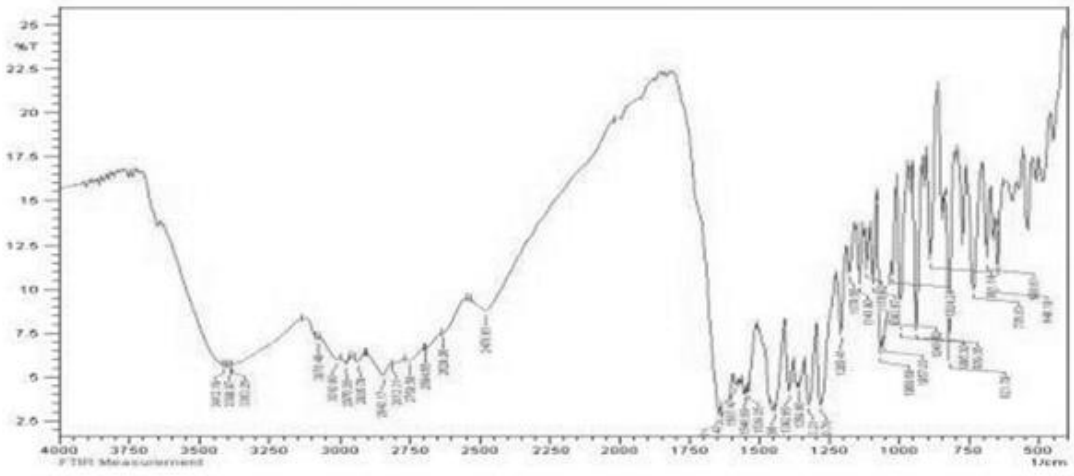

Figure 3: FTIR spectrum of pure drug Methotrexate.

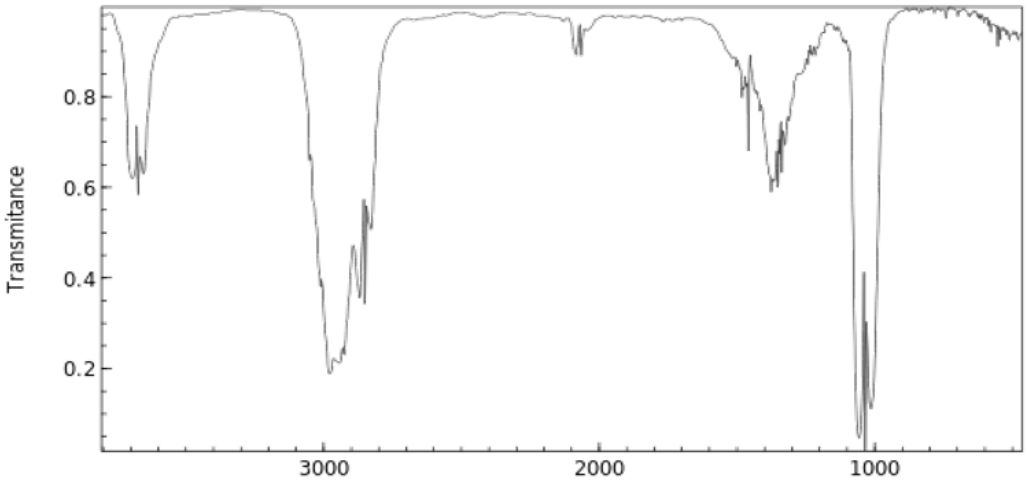

Figure 4: FTIR analysis of Methanol

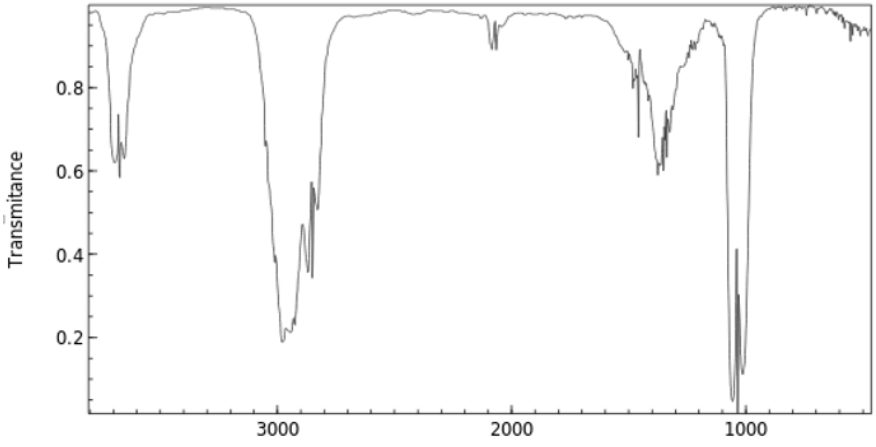

Figure 5: FTIR analysis of Tween 80

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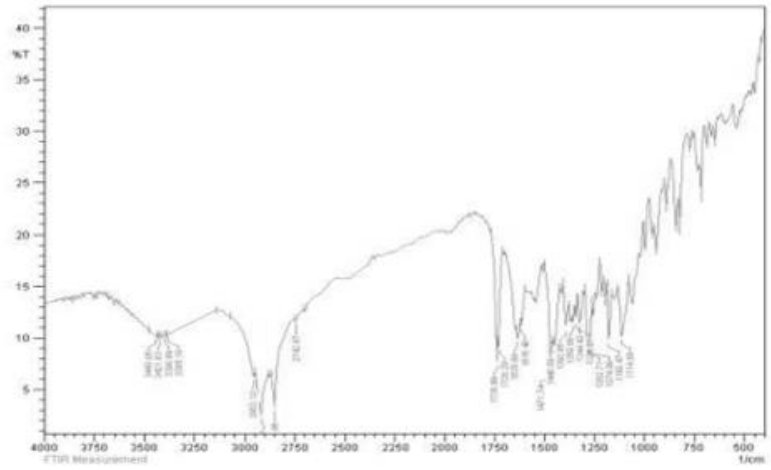

Figure 6: FTIR spectrum of Methotrexate and Tween

Table 2: Interpretation of IR spectral data of Methotrexate and polymers

Wave number observed (cm ^{1})	**Functional group**
2857	C-H (methylene, CH2)
1468, 1466	C=N
1345 – 1326	N-O (of cis isomer)
3174 – 3082	C-H (aromatic)
542.98, 607.00	C-Br stretch
689.43, 851.67, 903.85	CH out of a plane
777.55, 983.86	C-Cl stretch, P-H bend
1117.07, 1147.21, 1202.66, 1252.62, 1286.47, 1322.94	C-F stretch, C-O stretch, C-N stretch
1427.19	S=O sulfate ester
1448.70, 1490.66	C-C ring stretch
1534.20, 1600.81, 1639.51	N=O nitroso, C=C stretch
2929.21, 3103.79	CH stretch
3239.43, 337689	Dimer OH
3400.97, 3505.72	NH stretch, OH stretch
606.24	C-Br stretch
776.68, 946.16	C-Cl stretch, C-H out of a plane
1062.43, 1117.96, 1146.85	P-H bend, C-N stretch, C-O stretch
1326.22, 1382.48, 1454.17	CH stretch, S=O sulfate esters
1533.77, 1599.31	N=O nitroso
1639.21, 2932.17	C=C stretch, C-H stretch
3400.24, 3504.48	NH stretch, OH stretch

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EVALUATION OF NANOEMULSION:

Organoleptic:

Formulas F9, F8, F10, F11 and F12 are soft yellow, slightly viscous, transparent and homogeneous in appearance and have no phase distinction. This means that the neem oil (oil), tween 80(surfactant) and ethanol 96 percent (co-surfactant) concentrations used are sufficient.

The formulation quickly dissolves and leaves a subtly oily nuance when added to the skin. This is because the concentration of Tween 80 is very high.

pH:

In the pH spectrum of the skin (4.5 – 6.5) which is the desired pH of the formulation. pH should not be too acidic so that it can irritate the skin because it can cause scaly skin, similarly it should not be too alkaline [12]. All formulations produced have a pH that is still within the skin's pH spectrum. (Table 4)

Viscosity:

The viscosity was measured using a Brookfield viscometer. This emulsion comes in five different viscosity formulas. (Table 4) The globule's size will shrink as the quantity of 80 rises. The globule's smaller size raises the number of particles, which is proportional to the system's effort to maintain a constant density. The viscosity of globule contact could be improved by raising the number of tiny globules. [13,14,15, 16].

Table 3: Optimization of drug loaded nanoemulsion

S no.	Formulation	Composition (%)			
		Methotrexate	Neem oil	Ethanol	Tween 80
1	F1	3	0.6	1.0	3.7
2	F2	3	1.2	2.0	7.4
3	F3	3	1.8	3.0	11.1
4	F4	3	2.4	4.0	14.8
5	F5	3	3.0	5.0	18.5
6	F6	3	3.6	6.0	22.2
7	F7	3	4.2	7.0	25.9
8	F8	3	4.8	8.0	29.6
9	F9	3	5.4	9.0	33.3
10	F10	3	6.0	10.0	37
11	F11	3	6.6	11.0	40.7
12	F12	3	7.2	12.0	44.4

Table 4: Evaluation of nanoemulsion in the 1st week

S no.	Formula	Colour	Odor	Clarity	pH	Viscosity
1	F8	Soft yellow	Specific	Translucent	6.21	518.245
2	F9	Soft yellow	Specific	Translucent	6.09	526.025
3	F10	Soft yellow	Specific	Translucent	6.15	565.214
4	F11	Soft yellow	Specific	Translucent	5.63	569.210
5	F12	Soft yellow	Specific	Translucent	5.21	589.325

Both formulations involve oil-in-water (o/w) nano emulsions. This is because most components in the formula are hydrophilic or polar, causing the nano emulsion to remain in an oil-in-water (o/w) configuration despite the presence of a hydrophobic component. The findings align with the desired outcome, as the oil-in-water (o/w) nano emulsion is rapidly absorbed by the skin.

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As most of the formulation undergoes hydrolysis, the particle size increases from the first week to the eighth week, thereby reducing the efficacy of the nanoemulsion interface film layer of the globules. [17]. During storage, temperature modification can induce a decrease in the surfactants efficiency such that oil droplets begin to close together and gradually create larger droplets. The distribution of globule size is an essential aspect of the nano emulsion system as it can influence the release of the drug and the stabilization of the preparation [18]. The polydispersity index (PDI) is a measure of the particle size distribution of the emulsion, with a PDI near zero denoting the mono dispersion method and a PDI near 1.0, meaning that the emulsion has a very large size distribution. PDI values below 0.2 thus suggest homogeneous populations, while a value of 0.3 indicates heterogeneity. The high tolerance of two immiscible materials to remain combined as a single-phase emulsion is the attractive characteristics of formulation. Based on the sample form [19], the appropriate PDI value should be < 0.7. The findings of this analysis show that the PDI values are > 0.7 for the F8 and F9 formulas, indicating that the two formulas have a large range of particle size (polydisperse) and have an unstable formula. The PDI value of the F10 to F12 formula is below 0.7, so it belongs to the category of polydisperse and is still included in the category of stable formula. (Table 5) The particle size and globule size distribution calculation effects of the nano emulsion formula using PSA are shown in (Figure 7).

Table 5: The nano emulsion formula's particle size & PDI values

S no.	Formulation	Particle size (nm)	PDI	Drug release (%)	Entrapment efficacy (%)
1	F8	43.09	1.15	70.43	70.89
2	F9	39.00	1.25	69.56	70.67
3	F10	30.39	0.65	72.40	76.57
4	F11	45.98	0.71	70.59	72.78
5	F12	41.05	0.72	70.50	70.50

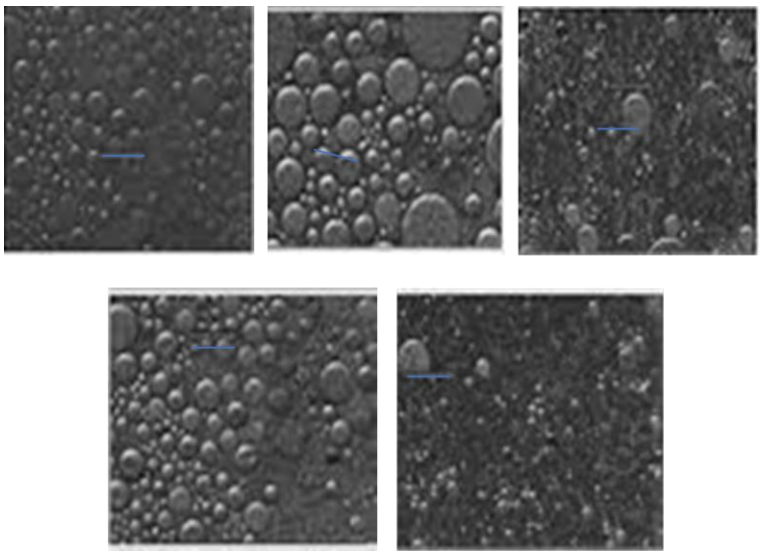

Figure 7: TEM images of nanoemulsion formulations

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CONCLUSIONS

Based on the study, it was concluded that after optimizing the co-surfactants, surfactants, and oils, a consistent Methotrexate nanoemulsion formulation was prepared. We developed twelve different formulations, labeled as F1 through F12. Considering parameters such as particle size, polydispersity index (PDI), drug entrapment efficiency (%), and drug release (%), the best results were achieved with formulation F10. This formulation comprised 5% neem oil, 36% Tween 80 as the surfactant, and 9% ethanol as the co-surfactant.

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