Formulation, Optimization And Evaluation Of Methotrexate Loaded Nanoemulgel For Enhancing Transdermal Delivery In Rheumatoid Arthritis

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Abstract- *The preparation and assessment of a topical delivery system for methotrexate-loaded nanoemulgel for the treatment of rheumatoid arthritis was the goal of this investigation.*

METHODS*: Using peanut oil, Tween 20 as the surfactant, and PEG 400 as a co-surfactant, the pseudo ternary phase diagram was produced based on the nanoemulsion composition. The methotrexate-loaded nanoemulsion was made using the spontaneous emulsification technique. Badam gum was used as a gel matrix in the resulting nanoemulsion to create nanoemulgel. The methotrexate-loaded nanoemulgel pH, particle size, physical appearance, viscosity, spreadability, TEM, drug content, diffusion studies, release kinetics, and stability investigations were all evaluated and described. nanoemulgel was clear and had a particle size of 19.*

RESULTS*: A nanoemulgel comprising 8.6% peanut oil, 34.4% Tween 20 and PEG 400 as Smix (surfactant and co-surfactant mixture), 43% water, and 12.5% w/w badam gum was shown to be the ideal formulation. The generated nanoemulgel had a 195.1 nm particle size and a zeta potential of -0.278 mV. Its nature was transparent. The drug content and release of the improved formulation were determined to be 98.11±0.34% and 95.11±0.02%, respectively. The optimal pH, viscosity, and spreadability levels were found. The results of the stability analysis showed that the generated nanoemulgel remained stable at temperatures between -25 and +45°C. In conclusion, methotrexate-loaded nanoemulgel has been successfully created for topical drug delivery in rheumatoid arthritis treatment.*

Keywords- Methotrexate, Nanoemulgel, Rheumatoid arthritis, Topical delivery, Peanut oil.

I. INTRODUCTION

Rheumatoid arthritis, one of the more complex autoimmune ails, is associated with habitual seditious pain and lump of the joints, elbows, shoulders, ankles, and other body organs[1].The multitudinous connected co-physiological and inheritable factors that beget it stymie its pathophysiology and effective remedy discovery. In rheumatoid arthritis, the vulnerable system affects not just joints but also other tissue[2]. In extreme cases, the complaint may indeed affect the internal organs of the body[3]. common stuffings are impacted by rheumatoid arthritis, which leads to swelling. habitual inflammation brought on by rheumatoid arthritis can beget bone deterioration and common scars. The primary ideal of rheumatoid arthritis treatment is pain reduction. Reducing pain and inflammation is the main thing of rheumatoid arthritis treatment, and damage to joints[4,5].

To treat rheumatoid arthritis, drugs can be administered via a variety of ways, includingparenteral routes like intramuscular, intravascular, subcutaneous, intra-articular, or topical channels, as well as enteral routes including oral, buccal, and sublingual[4] .By combining the medication with an absorbable formulation, the topical drug delivery method applies the medication to the body surface. In addition to enabling simple and continuous drug delivery and reducing elevated drug plasma concentration (Cmax), topical distribution offers a number of advantages over oral delivery for the treatment of illness[7,9].

However, the amount of medication that may be administered by dermal application is significantly limited because some medications cannot penetrate the skin at a therapeutic rate because of the exterior stratum corneum membrane barrier. A topical delivery system that employs nanoparticles may also lead to a selection of their use in the treatment of rheumatoid arthritis, and it has been predicted that a system that uses nanoparticles to deliver drugs may offer a different possible approach for influencing the permeability of the drug[8,9].

Nanoemulsions are miscellaneous colloidal systems on the submicronic scale, whereas colloidal particulate structures are allowed of as isotropous dissipations that are

thermodynamically and kinetically stable and comprise two inharmonious factors, videlicet water and oil painting, stabilized by an associate face subcaste that includes an respectableco-surfactant and face active agent to produce a single- stage[9]. oil painting- in- water(oil painting dispersed in water phase), W/O(waterless phase dispersed in oil painting phase), andbi-continuous(microdomains of waterless phase and oil painting phase are connected in the device) are the three classes of nanoemulsions that have lately been described. To stabilize these mixes, face-active substances that are both hydrophilic and lipophilic are applied coincidently[10].By incorporating nanoemulsion into a hydrogel matrix, a nanoemulsion- grounded hydrogel known as nanoemulgel is created[13,14]. Among the many beneficial qualities of nanoemulgel are its ease of spreading, ease of removal, reduced oiliness, lack of staining, extended shelf life, thixotropic nature, aqueous solubility, bio-friendliness, acceptable appearance, and transparency[15,17].

II. MATERIALS AND METHODS

MATERIALS:

Samarth Life Science Pvt. Ltd., Mumbai, India, handed the methotrexate; Loba Chemie Pvt. Ltd., Mumbai, handed the Tween 20; Leonid Chemicals Pvt. Ltd., Bangalore, handed the Polyethylene Glycol 400; Merck supplied the benzyl alcohol; and Badam gum was prepared in the laboratory and of logical grade.

UV visible spectrophotometer:

Before being filtered, 100 ml of pH 6.8 phosphate buffer was used to dissolve 100 mg of counted methotrexate in a volumetric beaker. To make 100 ml, pipette out 1 ml and also add the phosphate buffer. The λmax of methotrexate was also measured using a UV visible spectrophotometer.

Fourier transfrom infrared analysis (FT-IR):

The FT- IR spectrophotometer(8400S, Shimadzu Kyoto, Japan) was used to determine the medicine- excipient comity of the pure medicine and the physical admixture. To produce KBr bullets or thin flicks, the pure drug methotrexate and the physical admixture were combined independently with KBr in a mortar and pestle. They were also compressed in a KBr press(Technosearc Instrument, Mumbai, India) for five twinkles at a pressure of five tons, and FT- IR gamuts were recorded in the wavelength range of 4000- 400 cm- 1[14].

SELECTION OF EXCIPIENTS:

The least prickly and least sensitive excipients are chosen for topical operation in emulgel phrasings. For the drug to remain answerable in the nanoemulsion, it must be answerable in the oil painting phase. In order to keep the nanoemulsion stable, both advanced and lower HLB values of the surfactant andco-surfactant were taken into account[15].

SCREENING OF OIL, SURFACTANTS AND CO-SUFACTANT:

Numerous oils, surface-active agents, and co-surfactants have undergone primary study to ascertain their lodging potential toward methotrexate.By using a vortexing mixer to add more methotrexate to 2 milliliters of a selected oil, surface-active agent, and co-surfactant one after the other in a separate 10milliliters of volumetric flask, the drug miscibility in a range of oils, surface-active agents, and co-surfactants has been ascertained.To achieve equilibrium, these volumetric flasks were maintained at 25±1.0°C for 72 hours in an orbital shaker incubator (Remi, CIS24 BL, Mumbai, India).Following their removal from the shaker, the equilibrated samples were centrifuged for 15 minutes at 3000 RPM. After that, the resulting supernatant was collected and filtered through a filter of paper. UV spectroscopy at 259 nm has been used to determine the content of methotrexate in a particular oil[19,20].

CONSTRUCTION OF PSEUDO-TERNARY PHASE DIAGRAM:

The pseudo ternary phase diagrams were created using the aqueous titration technique to determine the position and size of the nanoemulsion zone. The surfactant and co-surfactant (Smix) were mixed at the following ratios to create each pseudo-ternary phase diagram: 1:1, 1:2, 1:3, 3:1, and 2:1 (i.e., Km, w/w). The amounts of each Smix have then been combined with the oil in the following ratios: 9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8, and 1:9 (w/w). Five minutes of vortexing with a vortexer produced a transparent, homogenous mixture of oil and the Smix component[18].Each admixture was also titrated using the drop system with distilled water and observed nearly for phase chastity and flowability. The volume of water at which the transition from clarity to turbidity passed was supposed to be the titration's endpoint. This titration system has been used to determine the rate of surfactant toco-surfactant(Smix). These reckoned values were used to determine the region of the nanoemulsion expression that included the named quantum of oil painting, surfactant, andco-surfactant(Smix). Next, the mock ternary phase illustration was used to dissect the medicine's effect on the nanoemulsion border. The mock ternary phase illustration was made using the ProSim Ternary Diagram software[21, 22].

PREPARATION OF METHOTREXATE LOADED NANOEMULGEL:

Formulation of Methotrexate nanoemulsion:

The pseudo ternary phase diagram contains Tween 20, PEG 400, and peanut oil ratios (table 1) were mixed with 1% methotrexate. As a result, once the water was added dropwise, thereforementioned solution was stirred at ambient temperature[21].

Table 1: Composition of nanoemulsion with selected oil, surfactant and co-surfactant

Nanoemulsion code	Smax*	Excipients(w/w%)		
		Peanut oil	Tween 20 : PEG 400 (Smix)	Water
S1	1:1	15	45	40
S2	2:1	10	45	45
S3	3:1	10	40	50

Smax represents the ratio of surfactant to cosurfactant

Formulation of Methotrexate nanoemulgel:

Expression of methotrexate nanoemulgel A determined volume of badam gum was completely mixed with distilled water using a stirrer. The gel was stirred unevenly and also placed in the refrigerator for a full day. The expression(F1- F3) was prepared using S3 nanoemulsion with a 31 Smix rate, as indicated in table 2 [5].

Table 2: Composition of 0.5% w/w methotrexate loaded nanoemulgel

S.No.	Materials	F1*	F2*	F3*
1	Methotrexate	0.5	0.5	0.5
2	Peanut oil	12.5	8.6	8.6
3	Tween20 : PEG400(S_{mix})	38.4	38.7	34.4
4	Water	34.4	38.7	43
5	Badam Gum(1.5%)	12.5	12.5	12.5
6	Badam Alcohol	1.5	1.5	1.5

F1, F2, and F3 represent the formulation of Methotrexate loaded nanoemulgel.

Figure 1:Formulation of Methotrexate nanoemulgel

OPTIMIZATION OF MTX-NLC BY DoE:

Screening study:

The most important factors and their associations can be evaluated and ascertained by minimizing experimentation and experimental design. One-factor-at-a-time investigations, which are commonly employed in formulation studies, require a considerable number of trials to identify the key parameters and the possibility of any interaction between them. It is possible to divide statistical designs into two main categories. Finding the important parameters is the aim of the screening research. Consequently, a resolution V 2 factorial design was used, where k ¼ 5 factors (the first number in parenthesis) were assessed and p ¼ 1 factors (the second number in parenthesis) were generated from the interactions of a full factorial design. Numerous aspects, including as drug loading, lipid type and amount, aqueous phase volume, surfactant type, homogenization rate and duration, and previous research and literature, affect the quality of NLC formulation.Particle size, polydispersity index (PDI), and EE of MTX-NLC are significant quality characteristics of the NLC formulation that influence the results in the optimization studies.Thus, the homogenization speed (A), homogenization length (B), stabilizer concentration (C), drug loading (D), and amount of lipid (E) were assessed using the particle size, PDI, and EE of MTX-NLC.The design matrix was created using the statistical software application Design-Expert (Design Expert 8.0.7.1 software, Stat-Ease Inc., Minneapolis, MN).

Optimization study:

The MTX- NLC flyspeck size, PDI, and EE were set up to be significantly told by the factorial design, homogenization speed(A), stabilizer attention(C), and lipid content(E). The Box- Behnken design was used to optimize the crucial rudiments. The three criteria employed to estimate the pivotal characteristics were flyspeck size, PDI, and EE.

The Design Expert software generated 17 tests in which the midpoint represents each edge of a multidimensional cell with five center points.

Table 3: Factors and their level in 2(5-1) factorial design.

Factors	Levels	
	-1	+1
A=Homogenization speed(rpm)	17000	23000
B=Homogenization time (min)	15	30
C=Stabilizer conc.(%w/v)	0.5	2
D=Drug loading (%w/w)	1	3
E=Lipid amount(%w/v)	1	5

EVALUATION OF METHOTREXATE LOADED NANOEMULGEL:

a) Transimission electron microscope (TEM):

The size and shape of nanoemulgel were examined by transimission electron microscope (TEM) with the image software. The drug spectroscopy by using zeta sizer (ZS 90, Malven Instrument Ltd, UK). The prepared nanoemulgel was diluted with deionized water (1:1000) and measured for the particles size[24,25].

b) Physical appearance:

The physical characteristics of nanoemulgelColor, pH, homogeneity, stability, and consistency of the prepared nanoemulgel were visually assessed[26].

c) Determination ofpH:

A digital pH meter was used to determine the pH for prepared nanoemulgel. To measure pH, electrode of pH meter was first cleaned with distilled water before being dipped into the mixture[27].

d) Viscosity:

Measurement using spindle number 63 at 30–50 RPM, the Brookfield Viscometer (Brookfield DV II+, USA) was used to measure the viscosity of the nanoemulgel[23].

e) Spreadability test:

The diameter of the spreading of 1g of formulation between two glass plates and for one minute while a standard weight of 20g was applied to the top plate was used to measure spreadability[27].

$$S = \frac{ML}{T}$$

Where S isspreadability (cm. g/sec), M is upper slide weight (g), L is glass slide length (cm) and T is time taken for slide separation (sec).

III. CONCLUSION

Due to its affordability and ease of use, topical drug delivery is the recommended method for the local application of medicinal drugs. Because of improved patient compliance, it has also been more well-liked by the public in recent years. As a result, an in vitro research and successful formulation of a possible methotrexate-loaded nanoemulgel employing peanut oil as the oil phase and badam gum as a gelling agent were conducted. The optimized formulation (F3) outperformed the other formulation in terms of physicochemical parameters and demonstrated 95.11% in 12 hours. At every storage condition specified in the approach, the product has a satisfactory stability profile. Thus, methotrexate is a disease-modifying antirheumatic medication with a longer retention period, according to this research investigation.Hence, the study demonstrated that the prepared nanoemulgel was found to be an operative tool in the management of rheumatoid arthritis when applied topically with a longer stability profile. At every storage condition specified in the approach, the product has a satisfactory stability profile. Thus, methotrexate is a disease-modifying antirheumatic medication with a longer retention period, according to this research investigation.Hence, the study demonstrated that the prepared nanoemulgel was found to be an operative tool in the management of rheumatoid arthritis when applied topically with a longer stability profile.

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