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DEVELOPMENT AND CHARACTERIZATION TECHNIQUES FOR METHOTREXATE LOADED NANO EMULSION

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ABSTRACTS

Psoriasis is an autoimmune disorder of the skin characterized by relapsing episodes of inflammatory lesions and hyperkeratosis plaques with worldwide occurrence of around 2–5%. Psoriasis is a disease known to be caused by multitude of both genetic and environmental factors such as trauma, drugs, infection, alcohol, smoking and stress but its accurate origin is still not known. Psoriasis, a chronic inflammatory condition, is increasingly prevalent and associated with various life-threatening diseases. Nanotechnology based drug delivery system has immense potential to enhance the bioavailability and effectiveness of drugs in their dosage forms, especially lipophilic drugs. Lipid based carrier system can overcome the lipid imbalance and normal moisturizing factors. The formulation was assessed for physicochemical properties, entrapment efficiency, drug release kinetics, and stability. FTIR analysis showed that the optimized Methotrexate 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 Methotrexate nano emulsion based on neem oil could be a potential treatment for psoriasis and might reduce the recurrence of psoriasis-like symptoms.

KEYWORDS: *Nano emulsion, Methotrexate, Psoriasis*.

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 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]

VARIOUS METHOD OF PREPARATION OF NANO-EMULSION

There are basically two methods of preparation of nanoemulsion as shown in figure 1. High energy emulsification methods generate highly disrupting forces that break down the oil and water phases, causing them to intersperse and form nanometer-sized droplets, whereas low energy emulsification methods include heat, stirring and phase inversion.[6]

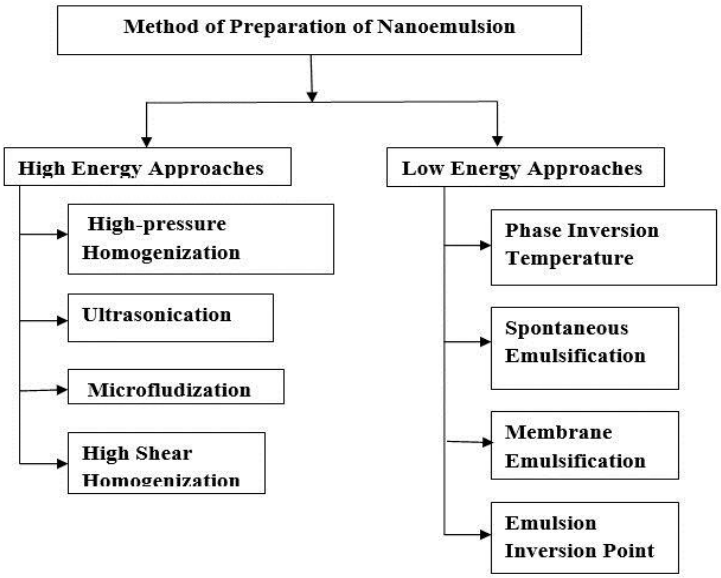

Figure 1: Method of preparation of Nano-emulsion.

HIGH PRESSURE HOMOGENIZATION

This is highly efficient method of preparation of nanoemulsion in which forcefully introduction of oil and water along with surfactants, cosurfactants are passed through a small orifice at high pressure. At first, emulsion is formed with large volume fraction of dispersed phase, which may be diluted later on. Excess amount of surfactants are added to avoid coalescence.[7]

MICROFLUIDISATION

In this method water and oil are introduced through small orifice by pressure pump from opposite direction into mixing area, where they mixed with other high shear and converted into small droplets which in turn used to prepared nanoemulsion.[8]

SONICATION

It is widely used method in which probe sonicator is placed in the mixture oil and water with surfactants, cosurfactants to give mechanical force by which dispersion is converted into small sized droplets.[9]

PHASE INVERSION TEMPERATURE TECHNIQUE

In this technique at room temperature oil, water and surfactants are mixed and then temperature is increased, then surfactant mixed in the oily phase. Due to change in temperature phase inversion prevents coalescence and produce stable nanoemulsions.[10]

SOLVENT DISPLACEMENT METHOD

In this method nanoemulsions can be prepared by pouring the organic phase containing oil dissolved in a solvent into aqueous phase having surfactants at room temperature. The preparation of nanoemulsion occurs by diffusion of organic solvent, evaporated by vacuum. Small sized droplets of nanoemulsion can be prepared by taking appropriate ratio of solvent to oil.[11]

SPONTANEOUS EMULSIFICATION

In the solution of oil and surfactant water is added at constant temperature and mixed lightly to produce o/w nanoemulsions. The preparation of nanoemulsion depends on surfactant structure, its concentration, interfacial tension, interfacial and bulk viscosity, phase transition region.[6,12,13]

Nano emulsion Formulation

The nano emulsion formulation was carried out using the spontaneous emulsification method. Methotrexate (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.[19]

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.[14]

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.[15] 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 2).

UV analysis for Methotrexate

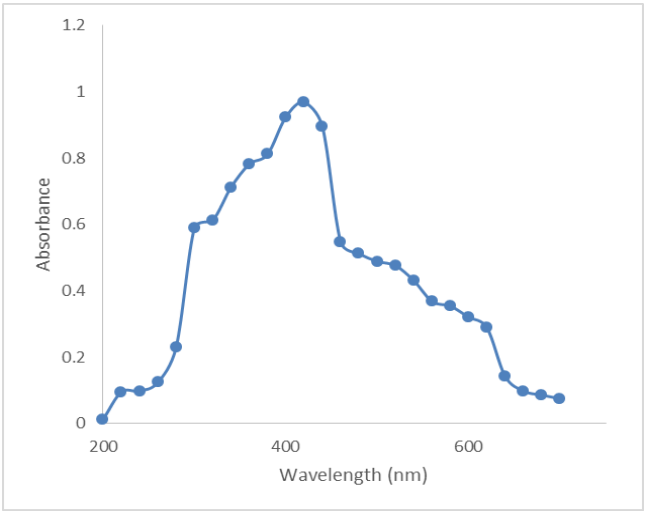

Figure 2: UV spectrum analysis of Methotrexate.

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 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.[16]

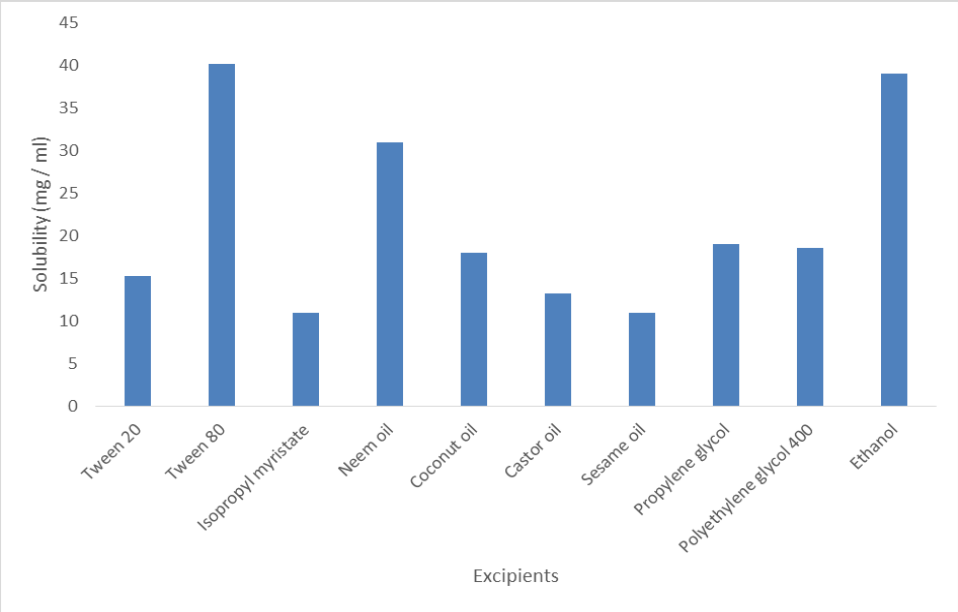

Figure 2: Graphical analysis of the solubility tests of Methotrexate.

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.[17]

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

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

CHARACTERIZATION OF NANOEMULSIONS

Determination of encapsulation efficiency

For determining the amount of drug entrapped in the formulation, weighed amount of formulation is dispersed in organic solvent by ultrasonication and the drug is extracted into suitable buffer. Drug content is estimated by analysing the extract spectrophotometrically at λmax of drug after making suitable dilutions against suitable blank. The entrapment efficiency (EE) and loading efficiency (LE) of the drug can be calculated by using the following Eqns.[18], drug EE = drug content in the product obtained (mg)/total amount of drug added (mg)×100 and drug LE = drug content in the product obtained (mg)/total product weight (mg)×100. Drug content could also be determined using reverse phase high-performance liquid chromatography (HPLC) techniques.

Determination of particle size and polydispersity index (PDI)

The particle size and PDI of nanoemulsions are analysed employing photon correlation spectroscopy (PCS) using Malvern Zetasizer, which monitors the variation in light scattering because of Brownian motion of particles as function of time. PCS is based on the principle

that the particles with small size travels with higher velocity as compared to particles with large size. The laser beam gets diffracted by sub-micron particles present in solution. Due to diffusion of particles, rapid fluctuations in laser scattering intensity occur around a mean value at a fixed angle and this is dependent upon particle size. The calculated photoelectron timecorrelation function generates a histogram of the line width distribution that can be related to the size of particle. For measuring particle size, weighed amount of formulation is dispersed in double-distilled water for obtaining homogenous dispersion and that has to be used instantly for measuring the particle size and PDI. The PDI can range from 0 to 1, where 0 (zero) stands for monodisperse system and 1 for a polydisperse particle dispersion.[19]

Determination of zeta potential

The zeta potential is a method for measuring surface charge of particles when it is placed in liquid. Zeta potential is used for predicting dispersion stability and its value depends on physicochemical property of drug, polymer, vehicle, presence of electrolytes and their adsorption. It is measured by Malvern Zetasizer instrument. For measuring zeta potential, nanoemulsion is diluted and its value is estimated from the electrophoretic mobility of oil droplets. Zeta potential of ±30 mV is believed to be sufficient for ensuring physical stability of nanoemulsion.

Fourier-transform infrared spectroscopy (FTIR) spectral analysis

FTIR analysis can be carried out for the assessment of drug excipient interaction, polymerization, crosslinking as well as drug loading in the formulation. It is also used for identifying the functional groups with their means of attachment and the fingerprint of the molecule. At low temperature a molecule exists in ground state and on absorbing the radiant energy, they get excited to higher energy states. IR spectroscopy is based on determining this energy difference (ΔE) between the excited and ground states of the molecule. For performing FTIR, sample can be prepared by employing suitable method such as potassium bromide pellet method, Nujol mulls and then sample is scanned in FTIR at moderate scanning speed between 4000- 400 cm-1.

Morphological study of nanoemulsion

The morphological study of nanoemulsion is carried by using transmission electron microscopy (TEM). In TEM, a beam of electron is incident on a thin foil specimen and passed through it. On interacting with the specimen, these incident electrons transform into unscattered electrons, elastically scattered electrons or inelastically scattered electrons. The

distance among the objective lens and the specimen and among the objective lens and its image plane regulates the magnification. The electromagnetic lenses concerted the unscattered or scattered electrons and cast them onto a screen that produce amplitude-contrast picture, a phase-contrast image, electron diffraction, or a phantom picture of distinct darkness, which is dependent upon the density of unscattered electrons. Bright field imaging at increasing magnification in combination with diffraction modes used for disclosing the size and form of nanoemulsion droplets. For performing TEM, few drops of nanoemulsion or a suspension of lyophilized nanoparticles is prepared in doubledistilled water and are placed onto holey film grid and immobilized. Excess solution has to be wicked off from the grid following immobilization and stained. The stained nanoparticles are then examined at particular voltage.[20]

Atomic force microscope (AFM)

AFM is comparatively a new technique being used these days for exploring the surface morphology of nanoemulsion formulations. AFM is carried out by diluting nanoemulsions with water followed by drop coating of the diluted nanoemulsion on a glass slide. Further the coated drops are dried in oven and scanned at of 100 mV/s.[21]

In vitro drug release study

In vitro drug release studies help to estimate the in vivo performance of drug formulation. The in vitro release rate of a drug is usually studied on a USP dissolution apparatus. Nanoemulsion or dried nanoparticles containing drug equivalent to 10 mg were dispersed in buffer and then it is introduced into dialysis membrane pouches and placed in a flask containing buffer. This study is carried out at 37±0.5° and a stirring speed of 50 rpm. Sample are withdrawn at periodic intervals and each time replaced by the same volume of fresh dissolution medium. Samples are then diluted suitably and the absorbance of sample is measured spectrophotometrically at a particular wavelength. Absorbance of the collected sample is used for calculating % drug release at different time intervals using calibration curve.[20]

In vitro skin permeation studies

Keshary Chine-diffusion cell is used for investigating in vitro and ex vivo permeation studies. For performing permeation studies, abdominal skin of adult male rats weighing 250±10 g is usually employed. The rat skin is positioned between the donor and the receiver chambers of diffusion cells. Temperature of receiver chambers containing fresh water with 20 % ethanol is

fixed at 37° and the contents of the chamber are continuously stirred at 300 rpm. The formulations are kept in the donor chamber. At specific time intervals such as 2, 4, 6, 8 h, a certain amount (0.5 ml) of the solution from the receiver chamber was removed for performing gas chromatographic analysis and each time replaced with an equivalent volume of fresh solution immediately. Each sample is performed three times. Cumulative corrections are done for obtaining total amount of drug permeated through rat skins at each time interval and are plotted against function of time. Slope of plot is used for calculating the permeation rates of drug at a steady-state.[22]

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.[24]

CONCLUSIONS

Based on the study, it was concluded that after optimizing the co-surfactants, surfactants, and oils, a consistent Methotrexate nanoemulsion formulation was prepared by spontaneous emulsification method. We will approach for twelve different formulations, labeled as F1 through F12 and various evaluation parameters such as particle size, polydispersity index (PDI), drug entrapment efficiency (%), and drug release (%) will be studied and the optimum concentration of the cosolvent and surfactant will be decided.

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