WORLD JOURNAL OF PHARMACY AND PHARMACEUTICAL SCIENCES
SJIF Impact Factor 6.647
Volume 6, Issue 7, 732-748 Research Article ISSN 2278 – 4357

FORMULATION AND EVALUATION OF NANO EMULSION BASED SYSTEM FOR TRANSDERMAL DELIVERY OF ANTIPSORIATIC DRUG

Umesh Acharya*, S. Rajarajan, Rukmagat Acharya, Ravindra Acharya, Gajendra Chaudhary and Saroj Ghimire

India.

Article Received on
27 April 2017,

Revised on 17 May l 2017,
Accepted on 07 June 2017,

DOI: 10.20959/wjpps20177-9430

***Corresponding Author**
Umesh Acharya
India.

ABSTARCT

The purpose of this study was to develop a stable methotrexate (MTX) loaded nanoemulsion gel (MNG) for topical use in Psoriasis to improve cutaneous deposition and local effect. The pseudo-ternary phase diagrams were developed for various nano-emulsion formulations composed of Isopropyl myristate as oil phase, Tween 20 as surfactant, PEG-400 as co surfactant and carbopol as gelling agent. Concentration of nano emulsion system was optimized using concentration of oil, surfactant/co-surfactant (1:3) and water as independent variables. The MTX-loaded nano-emulsion was characterized by droplet size and zeta potential. Nano-emulsion gel was prepared by adding 1% carbopol 934 as a gelling agent. The transdermal ability of MTX from nano-emulsion gel was evaluated by in vitro permeation study. The result shows that optimized nanoemulsion formulation was composed Isopropyl Myristate (8.5%w/w), Tween 20(37.5%w/w), PEG-400(10.5%w/w) and water (42.5%w/w). The optimized Nanoemulsion was found to be relatively uniform in size of (80±0.8 nm). The MNG showed improved in vitro permeation ability with better drug disposition capacity compared to MTX solution and gel. The result suggests that MNG is promising formulation for transdermal delivery of methotrexate for Psoriasis treatment.

KEYWORDS: Methotrexate; Nano-emulsion gel; Transdermal delivery, Anti-psoriatic.

INTRODUCTION

Nanoemulsion is a part of nanoparticle which is the branch of nanotechnology whose size ranges from at least one dimensions (1 to 100) nm. The term "*Nanoemulsion*" defined to a

thermodynamically stable isotropically clear dispersion of two immiscible liquids, for instance oil and water, stabilized by an interfacial film of surfactant molecules. The dispersed phase typically comprises tiny particles or droplets, with a size range of 5nm-200 nm, and has very low oil/water interfacial tension. The attraction of nanoemulsion for use in personal care and cosmetics as well as in health care is due to their small droplet size allowing them to deposit uniformly on substrates, possessing stability against sedimentation. Nanoemulsions are thermodynamically stable system that can improve the efficacy of a drug, increases bioavailability allowing the total dose to be decreased and thus minimize side effects. Psoriasis is a chronic immune-mediated disease that affects 1-3% of world population. Methotrexate, when delivered to the psoriatic site by means of transdermal drug delivery, has the potential to reduce side effects associated with this drug and to avoid first pass elimination.

Several approaches such as microspheres, solid lipid nanoparticle, polymeric nanoparticles, liposomes and nanoemulsion have been proposed to minimize side effects and to improve skin permeation and therapeutic concentration in the target tissues.

However, among all the colloidal drug delivery carriers, nanoemulsion offers several advantages over other dosage forms in terms of ease of preparation, high solubilization capacity for hydrophilic and lipophilic drugs, long term stability and improved dermal drug delivery.

The ingredients of nanoemulsion could facilitate permeation rate of the drug by reducing the diffusion barrier of the stratum corneum.

However, due to low viscosity of nanoemulsion their less retentive capacity in the skin decreases its application in the pharmaceutical industry. In order to overcome this disadvantage, gelling agent such as Carbopol 940 has been added in nanoemulsion for forming Methotrexate loaded nanoemulsion gel to increase its viscosity which could be suitable for topical application. Moreover, Methotrexate prevents the absorption of drug in the blood stream and provide higher drug accumulation in the skin for efficient action.

MATERIALS AND METHODS

1. MATERIALS USED

S.NO	MATERIALS	SUPPLIER
1	Methotrexate	Strides Labs, Bangalore.
2	Isopropyl Myristate	ABITEC Corporation, Janesville, WI.
3	Capmul MCM	ABITEC Corporation, Janesville, WI.
4	Tween 20	ABITEC Corporation, Janesville, WI.
5	Polyethylene glycol	ABITEC Corporation, Janesville, WI.
6	Carbopol	Yarrow chemical products, Mumbai.
7	Captex	Yarrow chemical products, Mumbai.
8	Tween 80	Yarrow chemical products, Mumbai.
9	Propylene glycol	Yarrow chemical products, Mumbai.

2. METHODS

I) Analytical Methods

a) Determination of λmax

100mg of methotrexate shaken with 100mL of 6.8 pH phosphate buffer in 100 mL volumetric flas and filter. Dilute 1mL of the filtrate to 100mL with the same solvent and determine the λmax using U.V. spectrophotometer.

b) Estimation of Methotrexate

In present study, the spectrophotometric method was adopted for the estimation of Methotrexate using double beam U.V. spectrophotometer.

c) Preparation of 6.8 pH phosphate buffer

Place 50mL of 0.2m potassium dihydrogen phosphate in a 200mL volumetric flask and 22.4ML of 0.2m sodium hydroxide and then add water to volume.

d) Preparation of Standard Stock Solution

50mg of methotrexate was dissolved in 50mL of 6.8pH phosphate buffer in 50 mL volumetric flsak of concentration 1mg/mL solution. From this stock solution different concentration of solution such as 4, 6, 8, 10, 12 µg/mL was prepared after dilution with same solvent. The absorbance of the resulting solution was measured spectrophotometrically at 259nm**.**

II) Preformulation Studies

a) **Screening of Oils, Surfactants and Cosurfactant by Solubility Studies of Drug**

Solubility of methotrexate in various oils, surfactants and cosurfactants was determined by

adding excess amount of drug (approx 500 mg) in screw-capped vials containing 2 mL of vehicle. The mixture was heated at 50°C in a water-bath to facilitate the solubilization using vortex mixer. Mixtures were shaken with shaker at 37°C for 48 h. After reaching equilibrium each vial was centrifuged at 3000rpm for 15min, and excess insoluble methotrexate was discarded by filtration using a membrane filter (0.45µm, 13mm, Whatman, USA). The concentration of drug was quantified by measuring the absorbance at 259nm using UV-Visible spectrophotometer (UV -1700 PharmSpec, Shimadzu, Japan).

b) Pseudoternary Phase Diagrams

Pseudoternary phase diagrams were developed using the aqueous titration method to identify nanoemulsion regions and the size of nanoemulsion region, For each phase diagram at a specific Surfactant was blended with cosurfactant in the ratio of 1:7, 1:8, 1:9, 1:10, 1:11 (i.e. Km, w/w). Volumes of each surfactant and cosurfactant mixture (Smix) were blended with oil in a ratio of 9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8, 1:9 (w/w),a transparent and homogenous mixture of oil and S/CoS was formed by vortexing for 5 minutes. Then each mixture was titrated with distilled water in a drop wise manner and visually observed for phase clarity and flow ability. The concentration of water at which transparency-to-turbidity transitions occurred was considered as the endpoint of the titration. And the amount of surfactant/Co-surfactant. These values were then used to determine the boundaries of the nanoemulsion domain corresponding to the chosen value of oils, as well as the S/CoS mixing ratio. To determine the effect of drug addition on the nanoemulsion boundary, phase diagrams were also constructed in the presence of drug using drug enriched oil as the hydrophobic component. Phase diagrams were mapped with sigma plot software.

c) Drug-excipient compatibility studies by FT-IR

Excipients are integral components of almost all pharmaceutical dosage forms. To investigate any possible interaction between the drug and the utilized excipients (Isopropyl Myristate, Tween 20, polyethylene glycol), IR spectrum of pure drug (Methotrexate) and its physical mixture was carried by using FTIR. The successful formulation of a stable and effective dosage form depends on the careful selection of the excipients, which are added to facilitate administration, promote the consistent release and bioavailability of the drug and protect it from degradation.Infrared spectroscopy is one of the most powerful analytical techniques to identify functional groups of a drug.

III) Preparation of Methotrexate Transdermal Nanoemulsion

There are many methods for preparation of nanoemulsion, such as; Ultrasonication, High speed homogenization, Phase titration, Phase inversion etc.

In this study formulations were prepared by Phase titration method. Appropriate amount of surfactant and co-surfactant were mixed and then added oily part, mix the formulation until completely dispersion occurs at room temperature. Then drug was added and the final mixture was mixed by vortexing until a clear solution was obtained. The formulation was equilibrated at ambient temperature for at least 48 hrs, and examined for signs of turbidity or phase separation prior and particle size studies. In the formulation using Isopropyl Myristate as oil phase and Tween 20/PEG-400 as surfactant/co-surfactant were designed.

Table: Formulation Design of Mtx Naonoemulsion

FORMULATION CODE	DRUG (%)	SURFACTANT + CO-SURFACTANT (%v/v) Tween20+polyethylene glycol	OIL_(%v/v)	Water(ml)
SN1	0.1	8(1:7)	1	2
SN2	0.1	9(1:8)	1	2
SN3	0.1	10(1:9)	1	2
SN4	0.1	11(1:10)	1	2
SN5	0.1	12(1:11)	1	2

IV) Evaluation of methotrexate nanoemulsion

A) Thermodynamic Stability

a) Heating cooling cycle: Six cycles between refrigerator temperature 4˚C and 45˚C with storage at each temperature of not less than 48 hrs was studied. Those formulations, which were stable at these temperatures, were subjected to centrifugation test.

b) Centrifugation

Passed formulations were centrifuged at 3500 rpm for 30min. Those formulations that did not show any phase separation were taken for the freeze thaw stress test.

c) Freeze thaw cycle

Three freeze thaw cycles between - 21˚C and +25 ˚C with storage at each temperature for not less than 48h was done for the formulations. Those formulations, which passed these thermodynamic stress tests, were further taken for the dispersibility test for assessing the efficiency of self-emulsification. The formulations were observed visually for any phase separation or color change.

d) Robustness to Dilution

These systems when diluted with excess of water and 0.1N HCl (500-900 mL) and were stored for 12 hours give no precipitation or phase separation

V) Transmittance Measurement

The percentage of transmittance of the optimized nanoemulsion formulation, as well as its 100 times dilution with 0.1N HCl and distilled water. Stability of optimized lipid formulation with respect to dilution was checked by measuring transmittance through U.V. spectrophotometer (UV-1700 SHIMADZU) at 259nm.

VI) Droplet Size Analysis

Nanoemulsion formulation (1 gm) containing 100 mg of Methotrexate was diluted to 20mL with distilled water a flask and was mixed gently by inverting the flask. The particle size so formed was determined by dynamic light scattering (DLS) technique using Zetasizer (Malvern Instruments, UK).

VII) Zeta Potential Determination

Nanoemulsion formulation (1 gm) containing 100 mg of Methotrexate was diluted to 20mL with distilled water a flask and was mixed gently by inverting the flask. The particle size so formed was determined by dynamic light scattering (DLS) technique using Zetasizer (Nano ZS, Malvern Instruments, UK).

VIII) Drug Content

The drug content of methotrexate nano-emulsion formulation was measured using UV spectroscopic method. The 10µg/mL of aliquot was prepared using nano-emulsion formulation using methanol as a solvent. The samples were measured as 259 nm using UV spectroscopic method.

IX) In- Vitro Studies

Franz diffusion cells (area 3.4618 cm2) with a cellulose membrane were used to determine the release rate of methotrexate from different nanoemulsion formulations. The cellulose membrane was first hydrated in distilled water at 25 ºC for 24 hours. The membrane was then clamped between the donor and receptor compartments of the cells. Each Diffusion cell was filled with 130 mL of phosphate buffer (pH =6.8). The receptor fluid was constantly stirred by externally driven magnetic bars at 300 rpm throughout the experiment. Methotrexate

nanoemulsion (5g) was accurately weighted and placed in donor compartment. At 0.5, 1, 2,3,4,5,6,7,8 and 12h time intervals, 5mL sample was removed from receptor for spectrophotometric determination and replaced immediately with an equal volume of fresh receptor medium. Samples were analyzed by UV visible spectrophotometer (BioWaveII, WPA) at 259nm. The results were plotted as cumulative drug release % versus time.

Data Analysis (Curve fitting analysis)

To analyze the mechanism of the drug release rate kinetics of the dosage form, the data obtained were plotted as:

a. Cumulative percentage drug released Vs time (In-Vitro drug release plots)
b. Cumulative percentage drug released Vs Square root of time (Higuchi's plots)
c. Log cumulative percentage drug remaining Vs Time (First order plots)
d. Log percentage drug released Vs log time (Peppas plots)

X) Ex- vivo Diffusion Studies

Ex-vivo permeation studies were performed using healthy male Wistar rat skin. Rat skin was selected for the ex-vivo studies owing to its structural similarities to human skin. The rats were housed in cages with adequate facility of food and water prior to use. Hairs from the abdominal region of the rats were shaved with electronic hair remover. The rats were sacrificed and abdominal skin was excised. The excised skin was kept into phosphate buffer pH 6.8. The skin was dipped in hot water and subcutaneous fat was removed with a scalpel. The specific portion of the skin was cut and used for the permeation study after washing with distilled water. Franz diffusion cell with effective surface area of 3.14 cm^2 was used for the experiment. The rat skin was paced between the donor and receptor compartments of Franz diffusion cell with the stratum cormeum facing upwards. Methotrexate solution, plain Methotrexate gel, Methotrexate nanoemulsion and Methotrexate nanoemulsion gel were placed on stratum cormeum. The receptor chamber was filled with 130mL diffusion medium (Phsophate buffer pH 6.8). The receptor medium was maintained at 37 ± 1^0C and was magnetically stirred at 50rpm for 24h. Samples were withdrawn (5mL) at predetermined time intervals, filtered through 0.22µ filter and were analyzed by UV spectrophotometer at 259nm. Fresh buffer solution was immediately replaced in the receptor compartment after each sampling. After 24 hrs release study, the surface of the skin was thoroughly washed with diffusion medium, sonicated for 20 min. The supernant was analyzed at 259 nm by UV spectrophotometer, for determination of percent drug remained on the skin. The percent of

drug penetrated into (and retained and localized in) the skin was estimated by substraction of the sum of the percent of drug retained on the skin surface and drug permeated through the skin from the initial amount of drug used in the donor cell, taken as one hundred percent.]

XI) Accelerated stability studies

Stability studies of the methotrexate nanoemulsion samples were carried out by subjecting them to temperature stability and centrifugation. The temperature stability study was carried out by keeping the sample at two different temperatures ($40^{O}C \pm 2^{O}C$, Room temperature) for 45days and visual inspection was carried out by drawing samples at intervals for the subsequent days.

In order to estimate metastable systems, the optimized formulation was diluted with purified distilled water. Then formulation was centrifuged at 1000 rpm for15 minute at 0°C and observed for any change in homogeneity of nanoemulsion formulation.

RESULTS

Preformulation studies: Standardization method of estimation of METHOTREXATE.

I. Analytical method:

Standard calibration curve for methotrexate

Table : Concentration and absorbance obtained for calibration curve of Methotrexate in Phosphate buffer 6.8

Concentration (µg/mL)	Absorbance
2	0.125± 0.001
4	0.248± 0.002
6	0.396± 0.001
8	0.483± 0.002
10	0.621± 0.002

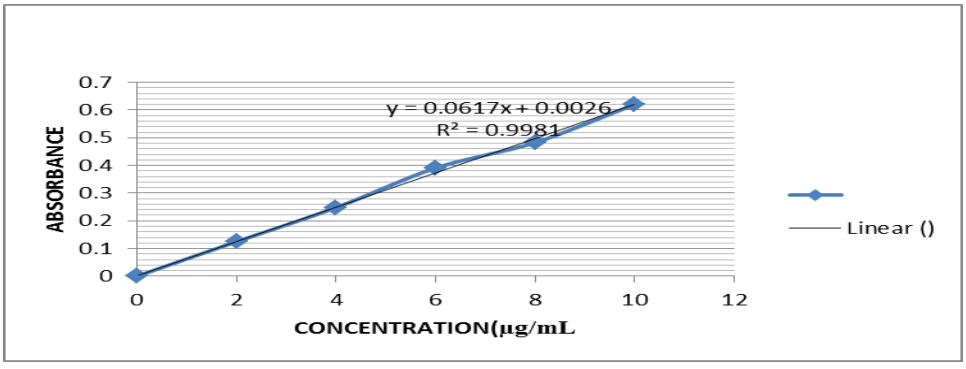


Figure : Calibration curve of Methotrexate

Solubility study

Solubility studies of methotrexate in different oils

Table: Solubility of Methotrexate in different oils

Oils	Solubility (mg/mL)
Captex	4.23
Isopropyl Myristate	5.8
Capmul MCM	5.2

In surfactant and cosurfactant

SURFACTANTS	SOLUBILITY mg/mL
Tween 20	12.18
Tween 80	6.880
PEG	94.418
1,2 Propylene glycol	23.478

Table no 6: Solubility of Methotrexate in different surfactant& co-surfactants

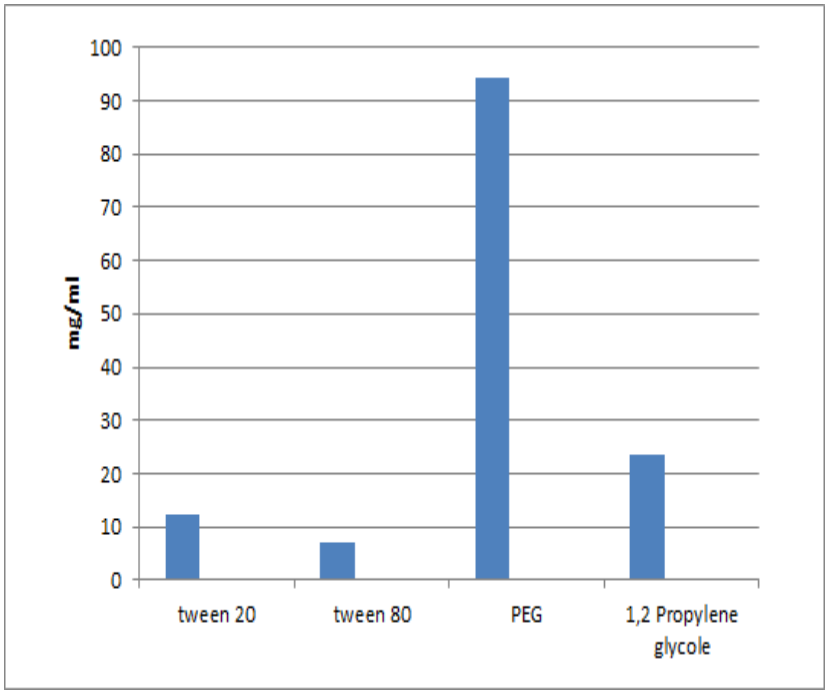


Figure: Schematic diagram of drug solubility in different surfactants &co-surfactants

1. Tween 20, 2.tween 80, 3. PEG 4. 1,2 Propylene glycol.

FT-IR analysis

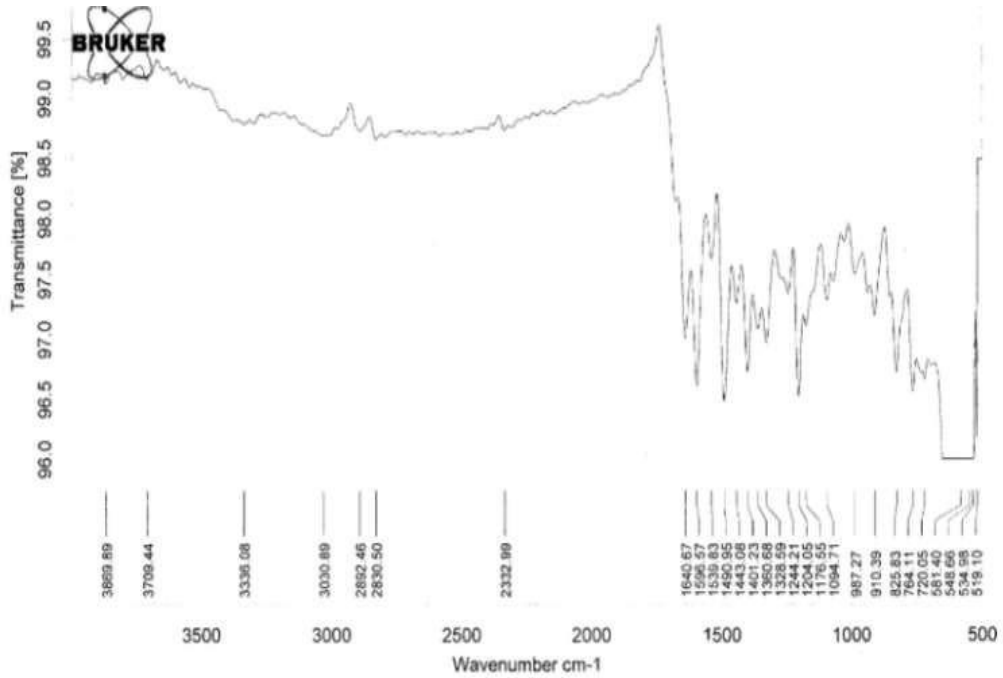


Figure: FTIR Spectrum of Methotrexate

FTIR-Methotrexate and Isopropyl myristate

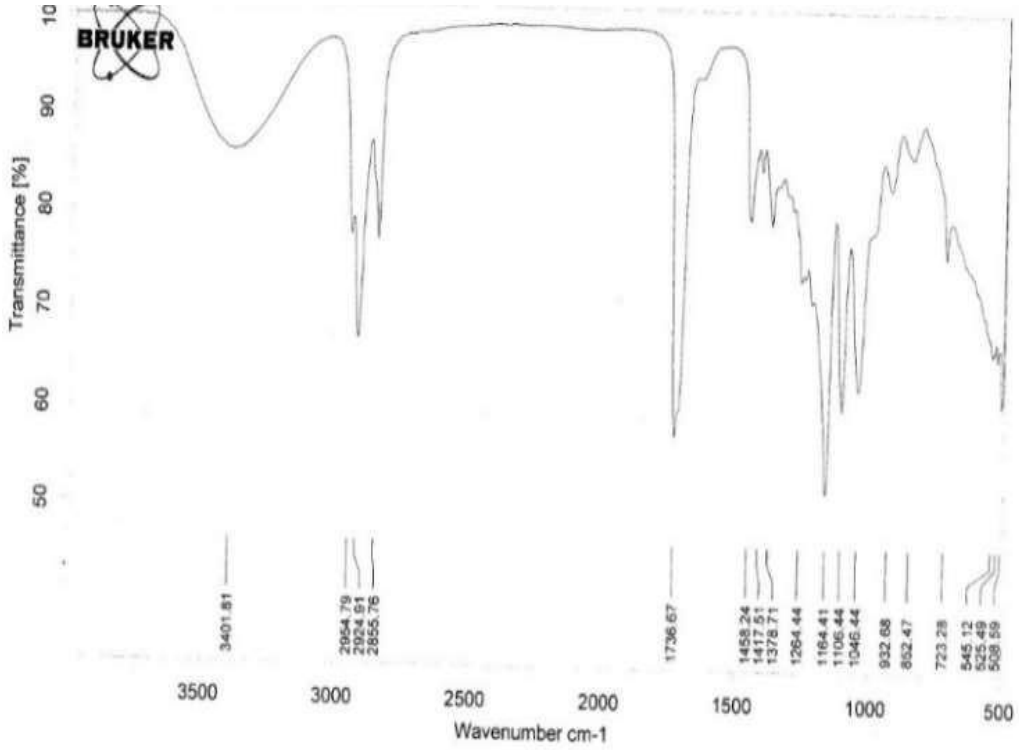
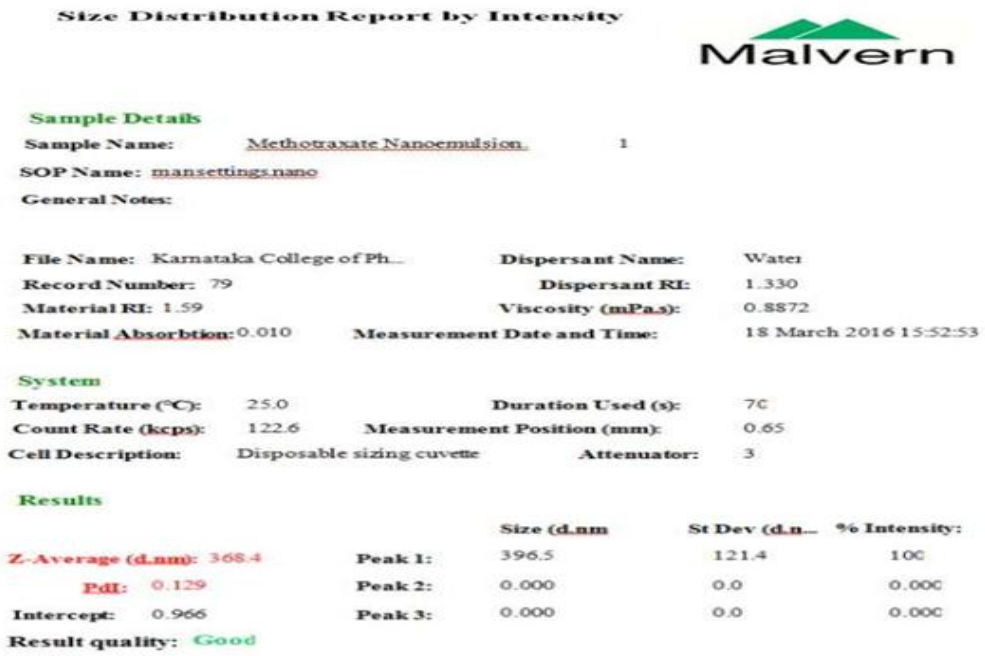


Figure: FTIR of Methotrexate +Tween 20

Particle Charaterization.

Particle size analysis.


Size Distribution Report by Intensity

Malvern

Sample Details

Sample Name: Methotraxate Nanoemulsion 1

SOP Name: mansettings nano

General Notes:

File Name: Karnataka College of Ph... Dispersant Name: Water

Record Number: 79 Dispersant RI: 1.330

Material RI: 1.59 Viscosity (mPa.s): 0.8872

Material Absorbtion: 0.010 Measurement Date and Time: 18 March 2016 15:52:53

System

Temperature (°C): 25.0 Duration Used (s): 70

Count Rate (kcps): 122.6 Measurement Position (mm): 0.65

Cell Description: Disposable sizing cuvette Attenuator: 3

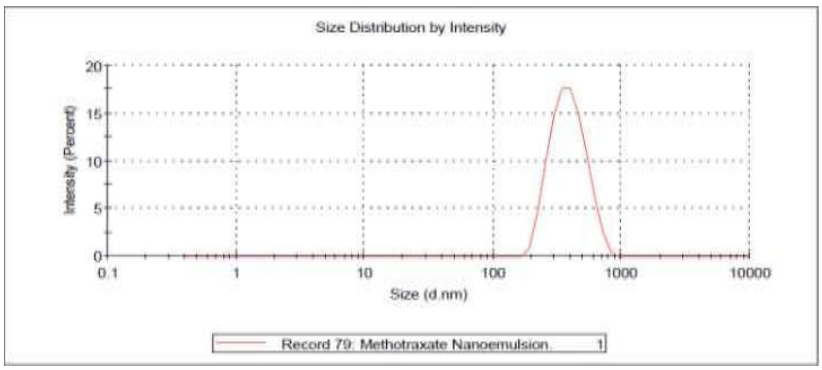
Results

		Size (d.nm	St Dev (d.n...	% Intensity:
Z-Average (d.nm): 368.4	Peak 1:	396.5	121.4	100
PdI: 0.129	Peak 2:	0.000	0.0	0.000
Intercept: 0.966	Peak 3:	0.000	0.0	0.000

Result quality: Good

Figure: Droplet size of Nanoemulsion

Table: Particle size determination curve.



Invitro release study

Table :*Invitro* drug release studies of formulations SN1-SN5

Time in hours	SN1	SN2	SN3	SN4	SN5
1	18.62	17.58	20.01	19.24	18.33
2	20.23	21.97	22.37	21.66	22.16
3	22.89	26.66	30.09	29.36	31.4
4	30.5	31.34	36.75	33.81	35.36
5	42.61	39.37	46.35	41.42	43.33
6	48.94	49.67	56.92	57.31	63.52
7	56.66	68.05	67.50	70.95	68.18
8	68.6	70.12	74.22	71.64	74.83

Stability study

Table no 16: Stability Test characterization of the formulation SN3

Time (Month)	Tempoc	Robustness test	Thermodynamic test	Phase separation	Crystallization and colour change
1	40oc±2oc/75% RH	√	√	√	√
	Room temp	√	√	√	√
2	40oc±2oc/75% RH	√	√	√	√
	Room temp	√	√	√	√

Where, √-Passedand

×-Failed

DISCUSSION

The present study was carried out to formulation and evaluation of topical nanoemulsion of methotrexate by phase titration method. Hence it was necessary to find suitable excipients with good compatibility.

1) Standard Calibration Curve of Methotraxate

It was found that the estimation of Methotraxate by UV Spectrophotometric method at λ_{max} 259 nm in 6.8 pH phosphate buffer had good reproducibility and this method was used in the study. The correlation coefficient for the standard curve was found to be closer to 1 that is 0.9983, at the concentration range, 4 - 12µg/ml. The regression equation generated was y = 0.024x + 0.056.

2) Pre-formulation studies

Pre-formulation studies were carried out by mixing the drug with various excipients in different proportions and kept for a month at different temperature and humidity conditions reveals that no significant change appear in the sample at all mentioned conditions, so no incompatibilities were observed between drug and excipients.

2) Solubility studies

Solubility studies are carried out in various oils, surfactant and cosurfactant and the oil, surfactant and cosurfactant which shows more solubility are selected for the preparation of nanoemulsion i.e Isopropyl Myristate, Tween 20 and Polyethylene glycol respectively.

3) Fourier Transform Infrared spectroscopy

The IR spectrum of methotrexate shown in Figure 10, reveals characteristic shoulders in the Methotrexate. IR spectrum that occur at 3336.08cm-1 for the N-H stretching, 1596.5 for the C=C stretching, 1640.67 for CO-NH stretching. These bands were also observed for the physical mixture of polymers along with methotrexate at the same absorbance as shown in Figure 11, 12, 13 and 14. From these results, it can be confirmed that there is no interaction between methotrexate and polymers/excipient in the physical mixture.

4) Phase diagram Study for Nanoemulsion

According to phase diagram study, 1:7, 1:8, 1:9, 1:10 and 1:11 ratio of surfactant: co-surfactant were done for Tween 20: Polyethylene Glycol. 1:9 ratio gives more nanoemulsion area than other ratio of surfactant: co-surfactant, so1:9 ratio of surfactant: co-surfactant was selected for formulation of nanoemulsion.

5) Thermodynamic Stability Studies

Nanoemulsions are thermodynamically stable systems and are formed at a particular concentration of oil, surfactant and water, with no phase separation, creaming or cracking. It is the thermostability which differentiates nano-or microemulsion from emulsions that have kinetic stability and will eventually phase separate. Thus, the prepared formulations were subjected to different thermodynamic stability stress tests like heating cooling cycle, centrifugation and freeze thaw stress tests. On the basis of the results were shown in Table no 8. It was found that NE3 formulations were stable in centrifugation test and are submitted for further characterization and evaluation.

6) Percentage Transmittance

The clarity of nanoemulsions was checked by transparency, measured in terms of transmittance (%T). Formulation SN3 has % 99.36 transmittance values greater than 99%. These results indicate the high clarity of nanoemulsion. Due to higher particle size, oil globules may reduce the transparency of nanoemulsion and thereby values of % Transmittance.

7) Droplet Size Distribution (PSD) and Zeta-Potential analysis

Photon correlation spectroscopy (Malvern instrument, UK) using dynamic light scattering was employed to measure particles sizes of pre-concentrate generated nanoemulsion. The samples were loaded onto $1cm^2$ cuvette in a thermostated chamber. For nanoemulsion

particles size ≤100 nm. It has been reported that the smaller particle size of the emulsion droplets may lead to more rapid absorption and improve the bioavailability.

The formulations NE3 was subjected for particle size measurement. The optimal batch is NE3 with mean particle size of 368.4 nm in water.

The magnitude of the zeta potential gives an indication of the potential stability of the colloidal system. A dividing line between stable and unstable aqueous dispersions is generally taken at either +30 or -30mV. Particles with zeta potentials more positive and negative +30mV and -30 mV are normally considered stable. The optimal batch is NE3 with-8.83 mV zeta potential which shows these is considerable as stable product.

8) Drug Content Estimation

Nanoemulsion of methotraxate with Isopropyl Myristate, Tween20 and Polyethylene Glycol were prepared by spontaneous emulsification method. The percentage of drug content of all the formulations varied from 89% to 93% as shown in the table no 9. This result indicates that there was uniform distribution of the drug throughout the batch.

9) In vitro drug release study of Methotrexate nanoemulsion

In vitro drug release test results indicate complete dissolution of drug from all its nanoemulsion within1 to 8 hour which is depicted in table 12; The formulation NE1 i.e., the nanoemulsion of methotrexate with Tween20, Polyethylene Glycol (1:9 ratio) prepared by spontaneous emulsification method showed 80.57% release within 8 hours. Drug release from all nanoemulsion formulations was found to be significantly higher as compared with that of pure methotrexate drug (about 34.26% release after 8 hours). It could be suggested that the nanoemulsion formulation resulted in spontaneous formation of a nanoemulsion with a small droplet size, which permitted a faster rate of drug release into the aqueous phase, much faster than that of pure methotrexate drug. Thus, this greater availability of dissolved methotrexate from the Nanoemulsion formulation could lead to higher absorption and higher oral bioavailability. The maximum drug release up to 8 hours was found to be NE3 formulation (80.57%) and results were shown in figure no 19.

10) Ex-vivo Diffusion studies of Methotrexate containing Formulation:

The Methotrexate loaded nanoemulsion gel (1% Carbopol) showed enhanced Ex-vivo permeation ability with better drug deposition capacity compared methotrexate solution, gel

and nanoemulsion which is shown in Table no 15. The results suggest that the methotrexate loaded nanoemulsion gel is promising formulation for topical delivery of methotrexate for psoriasis treatment.

11) Stability studies:

Stability studies of the nanoemulsion samples were carried out by subjecting them to temperature stability and centrifugation. The temperature stability study was carried out by keeping the nanoemulsion sample at temperatures (40±2^{O}C, Room temperature and 75±5% RH) for 45 days and visual assessment carried out. As per the results evidence of phase separation or any flocculation or precipitation was observed in nanoemulsion formulation. NE3 formulation shows no sign of phase separation when subjected to centrifugation at 10000 rpm for 30 minutes. Thus, it was concluded that the nanoemulsion formulation was stable thermally as well as under stressful condition

CONCLUSION

The present investigation was focused on the development of transdermal nanoemulsion of Methotrexate by Phase Titration method, in which the surfactants and co-surfactant ratios were prepared using different ratios. The drug was dissolved in oil, to this mixture Smix (surfactant and co-surfactant mixture) was added by continuous stirring until clear dispersion is formed. Aqueous phase was added in required amount and was added drop wise until clear solution is formed. These are then kept for equilibrium for 48 h.

The following conclusions can be made from the result obtained,

1. Preformulation studies were carried out to standardize the spectrophotometric method of estimation of Methotrexate and to investigate any possible drug polymer interaction, FTIR studies were carried out. The results revealed that there was no drug polymer interaction in the formulation when stored at 40±2ºC, 75±5% RH for 2 months.
2. Total of 5 formulations were prepared. All the formulations of Methotrexate nanoemulsions were characterized by physicochemical evaluation.
3. The Methotrexate nanoemulsion shows viscosity in the range of 0.8617-0.9010, which was significant result for development of formulation.
4. Amount of Methotrexate released from all formulations at 8 h ranges from 80.57%-76.67%.
5. The best formula was SN3 amongst the 5 formulation (based on the above results) was

considered as selected formulation for further studies.

Methotrexate nanoemulsions show a potential drug delivery system with good stability and release profile. All the other formulations were also equally good in their physicochemical characteristic.

REFERENCES

1. Shah P, Bhalodia D, Shelat P. Nanoemulsion: a pharmaceutical review. Sys Rev Pharm. 2010; 1(1): 24-32.
2. Kriwet, K., Müller-Goymann, C.C. Diclofenac release from phospho- lipid drug systems and permeation through excised human stratum corneum. Int J Pharm. 1995; 125: 231-242.
3. Charles L, Anthony A, Attama. Current state of Nanoemulsion in Drug delivery. Journal of Biomedicine and Nanotechnology 2011; 2: 626-639.
4. Rang HP, Dale MM. Ritter JW, Flower RJ. Pharmacology, 6th ed. 2007; 588-609.
5. Naimish S, Mayur N, Patel V, Amir A, Desai T. Emerging trend of nanoemulsion in formulation and research. IBDR 2001; 1(1): 54-83.
6. Kumar B, Jain SK, Prajapati SK, Mahor A. Development and characterization of transdermal nanoemulsion gel for an antiviral drug. Int J Pharm Sci Res., 2010; 1(6): 57-74.
7. Monzer F. Nanoemulsion, properties and applications, Transdermal drug delivery. CRC press Tylor and Francis Group. 144: 274-6.
8. Sedigeh D, Behzad S, Rahim F. Preparation and Evaluation of Self Emulsifying drug delivery system containing Loratadine. Int J ADV Pharm Sci., 2010; 239-48.
9. Eun-AL, Prabagar B. Nanoemulsion based Hydrogel Formulation of Metaprolol for Topical delivery. J Pharm Inv., 2010; 40(5): 305-11.
10. Dontella P, Steven N, Massimo F. Lecithin Nanoemulsions for the Topical administration of Ketoprofen: Percutaneous adsorption through human skin and In vivo human skin tolerability. Int J Pharm 2002; 44: 21-31.
11. Jose AS, Danilo CG, Bedor. Physicochemical Characterization and Development of a Nanoemulsion system for Transdermal use. J Dis Sci Tech., 2010; 31: 1-8.
12. Eun-AL, Prabagar B. Nanoemulsion based Hydrogel Formulation of Itraconazole for Topical delivery. J Pharm Inv., 2010; 40(5): 305-11.
13. Rohit R Shah, Kiran A Wadkar. Fluconazole Topical Nanoemulsion, Preparation and

Evaluation. Res J Pharm Tech., 2009; 2(2): 353-57.

14. Jadhav KR, Shetye SR, Kadam VJ. Design and Evaluation of Nanoemulsion based drug delivery system. Int J Adv Pharm Sci., 2010; 1: 156-66.
15. Gundogdu E, Alvarez IG, Karasulu E. Improvement of effect of water-in-oil nanoemulsion as an oral delivery system for Fexofen: Invitro and in vivo studies. IJN.2011; 6: 1631-40.
16. Suman R, Manish K, Vikas C, Vandana G. Formulation Development and Characterization of Nanoemulsions for Topical delivery of Glipezide. Der Pharmacia Lettre 2010; 2: 33-42.
17. Derle DV, Sagar BSH, Pimpale S. Nanoemulsion as a vehicle for Transdermal permeation of Nimesulide. Ind J Pharm Sci 2006; 68: 622-5.
18. Malakar J, Sen SO, Nayak AK. Development and Evaluation of Nanoemulsions for Transdermal delivery of Insulin. ISRN Pharmaceutics 2011; 3(7): 118-26.
19. Elena P, Eugenia MC. Positively charged Nanoemulsion for Topical application. Int J Pharm 2007; 346: 119-23.
20. Chong KK, Zhong GG, Han-GC. Physicochemical Characterization and Evaluation of a Nanoemulsion system for Oral delivery of cyclosporine A. Int J Pharm 1998; 161: 75-8.