WORLD JOURNAL OF PHARMACY AND PHARMACEUTICAL SCIENCES

Volume 3, Issue 2, 1464-1480. Research Article ISSN 2278 – 4357

FORMULATION, DESIGN AND CHARACTERIZATION OF MICROEMULSION BASED SYSTEM FOR TOPICAL DELIVERY OF ANTIPSORIATIC DRUG

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Article Received on
25 October 2013,
Revised on 23 November 2013,
Accepted on 30 December 2013

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ABSTRACT

The purpose of this study was to develop a stable methotrexate (MTX) loaded microemulsion gel (MMG) for topical use in psoriasis to improve cutaneous deposition and local effect. The pseudo-ternary phase diagrams were developed for various microemulsion formulations composed of Capmul MCM - C8 as oil phase, Tween 20 as surfactant and polyethylene glycol 400 (PEG 400 as cosurfactant. Composition of microemulsion system was optimized using concentration of oil, surfactant/cosurfactant (1:1) and water as independent variables. The MTX- loaded microemulsion was characterized by droplet size and zeta potential. Microemulsion gel was prepared by adding 1% Carbopol 934 as a gelling agent. The transdermal ability of MTX from microemulsion gel was evaluated by in vitro permeation study. The results shows that optimized microemulsion formulation was composed Capmul MCM - C8 (7.5% w/w), Tween 20 (37.5% w/w), PEG 400 (12.5% w/w) and water (42.5% w/w). The optimized microemulsion was found to be relatively uniform in size of optimized (11.52 ± 0.6 nm). The MMG showed enhanced in vitro permeation ability with better drug deposition capacity compared MTX solution, gel and microemulsion. The results suggest that the MMG is promising formulation for topical delivery of MTX for psoriasis treatment.

Key words: Methotrexate; Microemulsion; Microemulsion gel; Topical delivery; Antipsoriatic.

1. INTRODUCTION

Psoriasis is a chronic immune-mediated inflammatory disorder that affects 1-3% of world population. [1,2] It is characterized by inflammation, overproduction of keratinocytes, and a decrease in epidermal differentiation Due to chronic nature of the disease, patients suffer from substantial psychological and financial burdens resulting in a significantly impaired quality of life. [3] The etiopathogenesis of the disease has not been clearly understood, but it is believed to be T cell mediated disorder occurring in genetically susceptible individuals, influence by environmental factors.[4]

Mehotraxate (MTX) is folic acid antagonist with antineoplastic activity used for the treatment of psoriasis. MTX inhibits DNA synthesis and, as a result, mitotic activity by competitively inhibiting the enzyme dihydrofolate reductase. It is administered by the oral or parenteral route over long periods of time. MTX when given orally has a short elimination half life and may provoke number of side effects including gastrointestinal disturbances, ulceration of mouth, stomatitis, diarrhoea, enteritis and hepatic toxicity.[5] MTX, 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.[6] A major problem is that the drug is hydro soluble and is mostly in the dissociated form at physiological pH, its capacity for passive diffusion is thus limited.[7] Several approaches such as microsphere, solid lipid nanoparticle, lipid nanospheres, nanostructure lipid carriers, polymeric nanocapsules and lecithin/chitosan nanoparticle 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, microemulsion 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.[8]

Microemulsion is a colloidal dispersion composed of aqueous phase, oil phase, surfactant and co-surfactant at appropriate ratios, which is single optically isotropic and thermodynamic stable liquid solution with droplet diameter usually within the range of 10-100 nm.[9,10] The ingredients of microemulsion could facilitate permeation rate of the drug by reducing the diffusion barrier of the stratum corneum.[11] However, due to low viscosity of microemulsion their less retentive capacity in the skin restrains its application in the pharmaceutical industry. [11,12] In order to overcome this disadvantage, gelling agent such as Carbopol 940, Xanthan gum and carrageenan have been added in microemulsion for forming MMG to increase its

viscosity which could be suitable for topical application.[13,14] Moreover, MMG prevents the absorption of drug in the blood stream and provide higher drug accumulation in the skin for efficient action. The present study was undertaken to develop a stable o/w MTX loaded microemulsion gel (MMG) for topical treatment of psoriasis with improvement in cutaneous deposition of MTX and to enhance the local effect.

2. MATERIALS AND METHODOLOGY

2.1 Materials

Methotrexate was gifted by Zydus cadila (Goa, India). Capmul MCM - C8, Labrafac PG, Captex 355 EP/NF, Transcutol and Plurol oleique cc 497 were gifted by Abitec Corporation (Janesville, USA). Tween 20 was purchased from National Chemicals (Vadodara, India). PEG 400, Propylene glycol and Carbopol 934 were purchased from Suvidhinath Laboratories (Vadodara, India). Hydroxy propyl methyl cellulose K4M (HPMC K4M) and HPMC K100 were gifted by colorcon Asia Pvt Ltd (Goa, India). All other chemicals used were of analytical grade.

2.2 Solubility determination

2.2.1 Screening of component of microemulsion

Solubility of the MTX was determined in different oils such as Capmul MCM(C8), Labrafac PG, Labrafac lipophile, oleic acid, ethyl oleate, isopropyl myristate, olive oil and Captex 355 EP/NF , surfactants such as Tween 80 and Tween 20, and cosurfactants such as PEG 400, Transcutol P,Lauroglycol-90 and Plurol oleique CC 497. An excess amount of MTX was added to 5 ml of different oils, surfactants and cosurfactants and stirred at 25^{o}C for 24 hrs on magnetic stirrer followed by centrifugation for 10 min at 7500 rpm. The supernatant was filtered through membrane filter (0.45 µm) and the drug concentration in the filtrate was determined by UV spectrophotometer after appropriate dilution with Methanol: DMSO (2:1).[15,16] The oil, surfactant and co-surfactant that showed high solubility of MTX were used in the preparation of microemulsion.

2.3 Construction of pseudo-ternary phase diagrams

The pseudo ternary phase diagrams were constructed using water titration method to determine the appropriate components and their concentration ranges that can result in large existence area of microemulsion with different possible composition of oil, surfactant/co-surfactant, and water.[17]

The weight ratio of surfactant to co-surfactant (Smix) was varied as 1:1, 2:1, 3:1 and 4:1. These mixtures (Smix) were mixed with the oil phase to give the weight ratios of 9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8 and 1:9 at room temperature with continuous stirring. Water was added drop wise to the oil and Smix mixture under gentle stirring until the mixture became clear at a certain point. The concentrations of components were recorded in order to complete the pseudo-ternary phase diagrams. Pseudo-ternary phase diagrams were constructed with Sigmaplot Software Version (11). The contents of oil, Smix and water at appropriate weight ratios were selected based on these results.

2.4 Formulation optimization

D-optimal mixture experimental study was designed based on a three component system: the oil phase X_1(Capmul MCM C8), Smix X_2 (a mixture of Tween 20/ PEG 400, 3:1 w/w) and aqueous phase X_3 (water). The total concentration of the three phases summed to 100%. Based on the results of pseudo-ternary diagrams, appropriate range of the component was selected as follows: The ranges of oil and Smix were 5-10% and 30-60%, respectively. It was reported that hydration of stratum corneum significantly affects penetration of drug into the skin; water range was selected to be 30-65%. The blank o/w microemulsion was prepared by water titration method. Globule size (Y_1) and transparency (Y_2) of blank microemulsion were used as dependent variables to optimize the ranges of components.

2.5 Preparation of MTX-loaded microemulsion

Based on the results of blank microemulsion, MTX loaded o/w microemulsion was prepared by water titration method. For drug loaded microemulsion, surfactant and co-surfactant were mixed in fixed ratio to oil followed by MTX. Sonication was performed in bath sonicator for 5 minutes followed by heating at 75±5˚C for 5 minutes to dissolve drug. Preparation was allowed to cool at room temperature followed by drop wise addition of required quantity of water with stirring to form clear and transparent liquid.[16] The resulting microemulsions were tightly sealed and stored at ambient temperature.

2.6 Characterization of microemulsion

The pH values of the microemulsion were measured at 25^{o}C using pH meter (Macro Scientific Works Pvt. Ltd, India). The globule size and zeta potential were measured using photon correlation spectroscopy that analyzes the fluctuations in light scattering due to the Brownian motion of particle using Malvern Zetasizer Nano-ZS (Malvern Instruments Ltd., Worcestershire, UK). For determination of drug content, one gram of microemulsions was

diluted with appropriate amount of methanol: DMSO (2:1) mixture. The concentration of MTX was determined by UV spectrophotometer at 299 nm. The plain microemulsion without drug with the same composition was taken as blank after appropriate dilution with methanol: DMSO (2:1) mixture.

The percentage transmittance of microemulsions was measured using UV spectrophotometer (UV 1601, Shimadzu Corp., Japan) at 630 nm. The viscosity of various microemulsions was determined at 25^{o}C using Brookfield DV III Rheometer (Brookfield Engineering Labs, U.S.A) with LVDV – III U spindle. Electric conductivity of the microemulsions was determined using conductivity meter (CM-180 ELICO, India). The centrifuge tests at 10000 rpm for 30 min were carried out to assess the physical stability of the microemulsions.

2.7 Formulation and characterization of MMG

Different concentration of Carbopol 934 (0.5%, 1%, 1.5%, 2% and 2.5%), HPMC K4 M (1%, 2% and 3%) and HPMC K100 (1%, 2% and 3%) were used as a gelling agent for the optimized microemulsion formulation to formulate microemulsion based gel. Based on physical appearance of plain gel, Carbopol 934 (1%) gels was optimized for preparation of microemulsion gel of MTX. Carbopol 934 (1%) and Propylene glycol (5%) were allowed to hydrate in sufficient quantity of water for 4-5 h at room temperature. Microemulsion containing MTX was added in gel phase and left over night for gelling. Triehanolamine was added to obtain MMG with adequate consistency suitable for topical application.

The pH of the MMG was determined by taking one gram of gel, dissolved in 100 ml distilled water and stored for two hours. The pH values of MMG were measured at 25^{o}C using pH meter (Macro Scientific Works Pvt. Ltd, India). The drug content and viscosity of microemulsion gel was determined by same procedure describe for microemulsion.

2.8 Stability study

The optimized microemulsions and microemulsion gel were stored at 25 ± 2^{o}C/60 ± 5% RH and 40 ± 2^{o}C/75 ± 5%RH for three months. The clarity, phase separation and drug content were performed for the stored drug loaded microemulsion using the same procedures adopted for the fresh samples. The centrifuge tests were carried out to assess the physical stability of the microemulsions. The pH value, globule size and zeta potential of microemulsions were also determined using previously describe procedure.

2.9 Ex-vivo diffusion study of MTX containing formulations

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.[18] The study performed was approved by Institutional Animal Ethics Committee of Department of Pharmacy, Sumandeep Vidyapeeth, Vadodara, India (Protocol No. 947/ac/06/CPCSEA). 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 7.4. 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.[7,19] MTX solution, plain MTX gel, MTX microemulsion and MTX microemulsion gel were placed on stratum cormeum. The receptor chamber was filled with 20 ml diffusion medium (Phsophate buffer pH 7.4). The receptor medium was maintained at 37 ± 1^oC and was magnetically stirred at 50 rpm for 24 h. Samples were withdrawn (5 ml) at predetermined time intervals, filtered through 0.22µ filter and were analyzed by UV spectrophotometer at 303 nm. 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 (5X) with diffusion medium, sonicated for 20 min. The supernant was analyzed at 303 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 subtraction 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.

3 RESULTS

Screening of component of microemulsion

The solubility of MTX in various oils, surfactants and co-surfactants was estimated as shown in Table 1. Among the various oily phases that were screened, Capmul MCM C8 provided the highest solubility of MTX so was selected for further study. Tween 20 and PEG 400 were selected as surfactant and co-surfactant, respectively.

Construction of pseudo-ternary phase diagram

The pseudo-ternary phase diagram was constructed to obtain the appropriate concentration ranges of the component of microemulsion for different S_{mix} ratio viz. 1:1, 2:1, 3:1 and 4:1. From the four phase diagrams (Fig. 1), the largest microemulsion region was observed in S_{mix} 3:1.

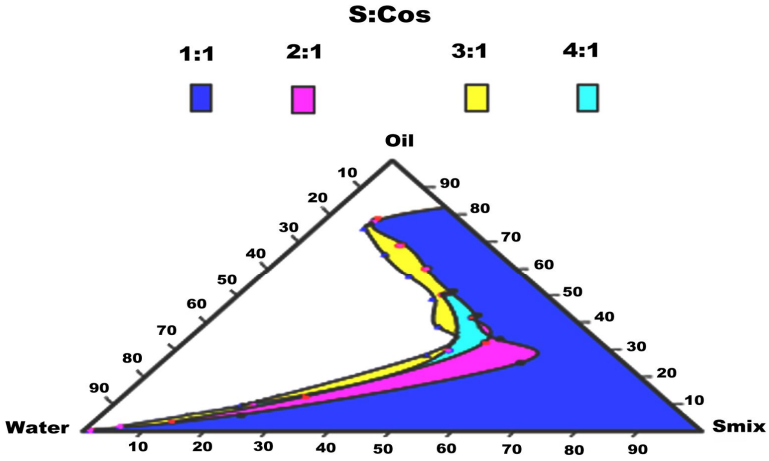


Figure 1 Pseudo-ternary phase diagram of microemulsion composed of oil (Capmul MCM C8), Smix (Tween 20: PEG 400) and water

Table 1 Methotrexate solubility in various oils, surfactants and co-surfactants

Excipients	**Solubility (µg/ml) ± SD**
Oils	
Ethyl oleate	415.65 ± 1.05
Oleic acid	143.76 ± 3.65
Capmul MCM C8	**997.71 ± 0.50**
Isopropyl myristate	72.96 ± 1.14
Olive oil	53.41 ± 4.84
Captex 355 EP/NF	365.67 ± 0.08
Labrafac PG	35.90 ± 1.02
Labrafac lipophile	134.21 ± 5.09
surfactants	
Labrasol	1188.15 ± 1.56
Tween 80	813.43 ± 3.35
Tween 20	**841.52 ± 4.38**
co surfactants	
PEG 400	**701.07 ± 2.26**
Transcutol P	623.55 ± 3.07
Lauroglycol-90	196.02 ± 0.45
Plurol oleique CC 497	136.46 ± 0.62

Values are expressed as mean ± SD; n=3

Formulation optimization of microemulsion

Blank microemulsion were prepared to show the effect of oil phase X_1 (Capmul MCM C8), Smix X_2 (a mixture of Tween 20/ PEG 400, 3:1 w/w) and aqueous phase X_3 (water) on globule size (Y_1) and transparency (Y_2). The responses of these formulations are summarized in Table 2. The microemulsion with smallest globule size and higher transparency were chosen to prepare drug loaded microemulsion.

Table 2 - Optimization of blank microemulsion formulations

Batch No.	Oil (%)	S_{mix} (%)	Water (%)	Size ± SD (nm)	% T± SD (λmax-630nm)
M1	5	30	65	13.28 ± 1.05	99.6 ± 0.20
M2	5	40	55	12.55 ± 0.39	99.6 ± 0.31
M3	5	45	50	12.05 ± 1.54	99.4 ± 0.16
M4	5	50	45	11.28 ± 0.88	100.1 ± 0.10
M5	**5**	**55**	**40**	**9.92 ± 1.06**	**99.1 ± 0.23**
M6	**5**	**60**	**35**	**8.23 ± 0.34**	**99.4 ± 0.15**
M7	7.5	30	62.5	46.82 ± 3.32	96.4 ± 0.08
M8	7.5	40	52.5	21.04 ± 2.28	99.2 ± 0.24
M9	**7.5**	**45**	**47.5**	**12.06 ± 0.26**	**99.9 ± 0.21**
M10	**7.5**	**50**	**42.5**	**10.07 ± 1.08**	**99.5 ± 0.18**
M11	7.5	55	37.5	13.04 ± 1.59	100.1 ± 0.11
M12	7.5	60	32.5	15.38 ± 0.69	99.7 ± 0.25
M13	10	30	60	---	---
M14	10	40	50	155.10 ± 4.26	94.2 ± 0.38
M15	10	45	45	89.0 ± 4.26	96.3 ± 0.40
M16	10	50	40	65.58 ± 3.48	98.6 ± 0.24
M17	**10**	**55**	**35**	**12.16 ± 1.11**	**99.6 ± 0.23**
M18	**10**	**60**	**30**	**11.56 ± 0.56**	**99.3 ± 0.12**

Values are expressed as mean ± SD; n=3

Table 3 shows the globule size, zeta potential and transparency of MTX loaded microemulsion. The factors X_1, X_2 and X_3 at 7.5%, 50% and 12.5% provided the optimum response of Y_1: 11.05 nm and Y_2: 99.9%. The zeta potential of optimized microemulsion was found to be -4.58 ± 0.88 mV (Fig. 2). Carbopol 934 (1%) gel was used to incorporate MTX microemulsion into gel.

Table 3 – Optimization of methotrexate loaded microemulsions

Batch No.	Capmul MCM C8 (%)	Tween 20: PEG 400 (%)	Water (%)	Size (nm)	Zeta Potential (mV)	% T (λmax-630nm)
MTX	5	55	40	9.97 ± 1.20	-1.87 ± 0.50	99.0 ± 0.28
MTX	5	60	35	9.82 ± 0.95	-7.11 ± 1.60	98.9 ±0.18
MTX	7.5	45	47.5	14.81 ±	-2.53 ± 0.95	99.8 ± 0.08
MTX	**7.5**	**50**	**42.5**	**11.05 ±**	**-4.58 ± 0.88**	**99.9 ± 0.11**
MTX	10	55	35	12.95 ±	-2.98 ± 1.19	99.9 ± 0.05
MTX	10	60	30	12.84 ±	-5.67 ± 0.38	99.9 ± 0.90

Values are expressed as mean ± SD; n=3

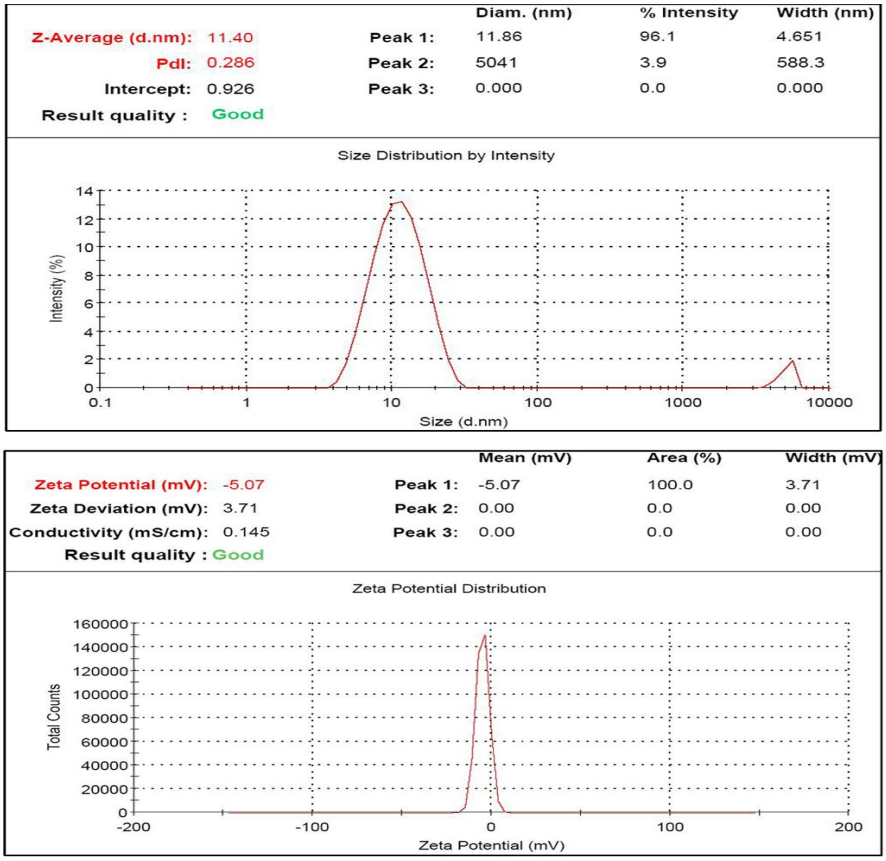


Figure 2 Particle size and Zeta potential of optimized microemulsion

Table 4 - Stability study of microemulsion and MMG at room condition and accelerated condition

Microemulsion						
Test	**Room condition (25 ± 2ºC, 60 ± 5%RH)**			**Accelerated condition (40 ± 2ºC, 75 ± 5%RH)**		
	1 month	**2 month**	**3 month**	**1 month**	**2 month**	**3 month**
Transmittance (%)	99.8 ± 0.21	99.8 ± 0.34	99.6 ± 0.11	99.8 ± 0.09	99.6 ± 0.11	99.5 ± 0.18
Assay (%)	98.84 ± 0.15	98.91 ± 0.22	97.6 ± 0.45	99.1 ± 0.37	98.55 ± 0.46	97.12 ± 0.85
pH	5.52 ± 0.14	5.48 ± 0.17	5.36 ± 0.09	5.44 ± 0.25	5.47 ± 0.20	5.40 ± 0.39
Globule size (nm)	12.7 ± 6.9	14.91 ± 3.41	16.13 ± 4.78	11.7 ± 1.69	14.15 ± 2.24	17.97 ± 6.88
Zeta potential (mV)	-4.19 ± 2.31	-3.96 ± 3.69	-3.23 ± 1.56	-3.86 ± 2.89	-2.75 ± 1.50	-2.20 ± 5.47
MTX loaded Microemulsion Gel (MMG)						
Assay (%) ± SD	98.68 ± 0.28	98.25 ± 0.88	97.13 ± 2.45	98.9 ± 1.23	97.56 ± 2.66	97.35 ± 3.51
pH ± SD	5.92 ± 0.38	5.84 ± 0.14	5.80 ± 0.56	5.99 ± 0.14	5.80 ± 0.33	5.86 ± 1.26
Transparency(Visually)	+	+	+	+	+	+
consistency	++	++	++	++	++	++

Values are expressed as mean ± SD (n=3); + Transparent and clear; ++ Suitable for application on skin

Characterization of microemulsion and MMG

The pH value of microemulsion and MMG were 5.5 and 6.10, respectively. The drug content of microemulsion was 1.03 ± 0.01 g/ml. The drug content of MMG was 0.1% w/w. The transperancy of microemulsion and MMG were proven by >99% transmittance. The conductivity of the microemulsion was 0.150 ± 0.11 ms. The viscosity of MMG (4500± 26 Cp) increase significantly as compared to microemulsion (112.4 ± 1.6 Cp). The centrifuge tests showed that all the microemulsion had good physical stability.

Stability studies of the optimized formulations

The optimized formulations were stable when stored at 25 ± 2ºC/60 ± 5% RH and 40 ± 2ºC/75 ± 5%RH for three months where there was no obvious change in visual appearance. The pH, drug content and transparency of the fresh and stored formulations do not showed any significant change. The consistency of the MMG was found to be stable at room condition as well as accelerated condition for three months (Table 4).

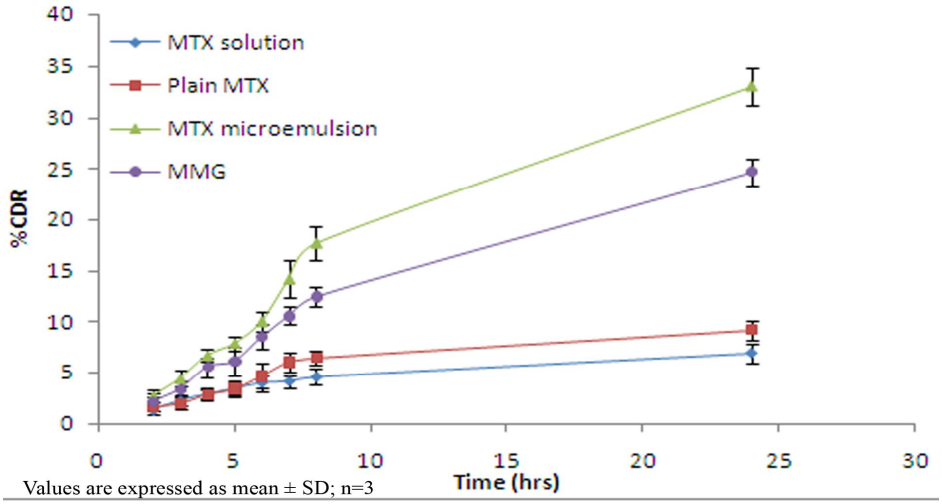
Ex-vivo diffusion study of MTX containing formulations

The permeation profiles of MTX solution, plain MTX gel, MTX microemulsion and MMG through rat skin are shown in Figure 3. The percent of drug remained on the rat skin and estimated to be retained and localized in the skin are shown in Table 5

Table 5 – Percentage drug present in different compartments after 24 hrs release study

Formulation	% drug released after 24 hrs ± SD	% drug retained into skin after 24 hrs ± SD	% drug remained on skin after 24 hrs ± SD	Mass balance ± SD
MTX Solution	6.88 ± 1.78	8.05 ± 1.15	82.43 ± 3.30	97.36 ± 6.23
Plain MTX Gel	9.15 ± 2.36	20.54 ± 2.52	67.05 ± 1.14	96.74 ± 6.02
MTX Microemulsion	32.98 ± 2.20	31.22 ± 1.69	33.87 ± 2.55	98.07 ± 5.44
MTX Microemulsion Gel	24.64 ± 1.75	48.71 ± 2.41	25.29 ± 1.72	98.64 ± 5.88

Values are expressed as mean ± SD; n=3



Values are expressed as mean ± SD; n=3

Figure 3 Ex vivo permeation profile of MTX from microemulsion, solution, plain gel and microemulsion gel

3. DISCUSSION

Screening of components of microemulsion

For the selection of components for microemulsion system solubility study was carried out in different oil, Surfactant & cosurfactant. It was very important to find out appropriate solvent to dissolve MTX and then formed microemulsion, because only the dissolved drug can permeate skin. The results shows that the solubility of the MTX was higher in Capmul MCM – C8 oil (997.71 ± 0.50 µg/ml) compared to other oil studied. Tween 20 (841.52 ± 4.38 µg/ml) and PEG 400 (701.07 ± 2.26 µg/ml) were selected as surfactant and cosurfactant respectively based on their higher solubility for MTX. Amongst various surfactants, Labrasol shows higher solubility of MTX but due to the unstability of microemulsion prepared with Labrasol, Tween 20 was selected as surfactant.

Construction of Pseudo ternary phase diagram

The phase diagram facilitates the determination of the components concentration range for the existence of microemulsion. Formation of microemulsion systems (colored area) was observed at room temperature. Phase behavior investigations of this system demonstrate the suitable approach to determining the water phase, oil phase, surfactant concentration and co-surfactant concentration with which the transparent, one phase low-viscous microemulsion system was formed. Study was carried out by plotting pseudo ternary phase diagram as shown in figure 1. The phase study with different S:Cos ratios (1:1, 2:1, 3:1, 4:1) revealed that the microemulsion region was found maximum when the S:Cos ratio was 3:1 compared to other ratios. This may be because of optimum concentration of S:cos mixture at 3:1 ratio beyond which the microemulsion may become turbid at higher concentration of tween 20.So

based on maximum microemulsion region in S:Cos ratio 3:1, same ratio of S:Cos was selected for further study.[20]

Formulation optimization of microemulsion and microemulsion gel

Based on the results of pseudo-ternary phase diagrams, appropriate range of components were chosen to prepare 18 experiments. As shown in table 2, lowest globule size was obtained in run 6 (X_1: 5%, X_2: 60%: X_3: 35%), where proportion of oil is lowest and Smix were highest. It indicates that microemulsion globules tent to constrict and get stabilized with higher amount of Smix when proportion of oil decrease significantly. Amongst the various batch, M5, M6, M9, M10, M17 and M18 were selected based on their transparency and globule size. The batches serve as a primary trial to prepare MTX loaded microemulsion (MTX 1 to MTX 6). MTX 4 batch was selected as optimized batch of microemulsion based on the results of globule size, zeta potential and transparency.

Many trials were made to transform this optimized microemulsion into gel form. Microemulsion gel was obtained by addition of 1% Carbopol 934 in order to make it suitable for topical application. The concentration of Carbopol 934 was selected from the preliminary experiments which were carried out to get the optimum viscosity of the gel for topical application so as to avoid the chances of formulation getting drained out. The viscosity of MMG was increase as compared to microemulsion. The optimized MMG contains about 94% MTX microemulsion, 5% Propylene glycol as plasticizer and 1% Carbopol 934 as gelling agent.

Characterization of microemulsion and microemulsion gel

As the system was showing conductance, it was proved that the system was o/w type microemulsion preparation. The developed microemulsion showed 99.9% transmittance which proved good transparency of the system. Dilution of microemulsion with water (100X) showed satisfactory 98.1% transparency and 23.1 ± 8.4 nm globule size, which proved its higher stability at elevated dilution condition. The pH of the microemulsion was found to be 5.50 ± 0.19 which is suitable for application on skin and would not cause any irritation to the skin. Viscosity measurement was examined as a function of share rate. The optimized batch (MTX 4) was found to be pseudo plastics non- Newtonian fluid and this type of fluid has a decreasing viscosity with an increasing share rate. Viscosity of MTX 4 microemulsion batch was found satisfactory. The particle size distribution is one of the most important

characteristics of emulsions for stability evaluation [21] and in vivo fate of emulsion.[22] At the same time the size of the nanoparticles plays a key role in their adhesion to and interaction with the biological cells. Globule size of optimized MTX 4 microemulsion was found to be 11.52 ± 0.6 nm. The nanometric size range of the globules was retained even after 100 times dilution with water which proved the system's compatibility with excess water. Zeta potential was found to be negative charge to the system (-4.97 ± 1.1). Hence the formulations will not cause any problem due to electrostatic interaction between the microemulsion and skin on topical administration. The o/w nature of emulsion was also confirmed by dilution and dye solubility tests.

The pH of the optimized ME gel was found to be 6.10 ± 0.25 which is suitable for application on skin and would not cause any irritation to the skin. Drug content was found to be 99.2 ± 0.68 which showing maximum utilization of drug. The prepared ME gel showed good acceptable transparency which is one of the ideal properties the topical gel should bear. The viscosity of optimized MTX microemulsion gel was found satisfactory for the application on the skin.

Stability studies of the optimized formulations

The optimized formulations were stable when stored at 25 ± 2ºC/60 ± 5% RH and 40 ± 2ºC/75 ± 5%RH for three months where there was no obvious change in globula size, drug content, transmittance, pH, globule size and zeta potential. Centrifugation test has shown that microemulsion was remaining stable even at centrifuging the microemulsion at 10,000 rpm for 30 minutes without phase separation. Based on parameters studied, microemulsion with MTX remained as clear liquid for a period of three months without the occurrence of phase separation or flocculation at the room temperature and refrigerator temperature.

Ex-vivo diffusion study of MTX containing formulations

Several studies have compared o/w and w/o microemulsions with other vehicles for the transdermal administration of medicines, such as lotions, suspensions, gels or emulsions.[23] It has been observed that microemulsions have a greater capacity to release drugs towards the skin. This may be due to the fact that drugs contained in microemulsions are in dissolved or suspended form, so that their absorption is faster and more effective. Here in this study it was observed that passive transdermal delivery of MTX from microemulsions was more effective than passive transdermal delivery from a simple solution.[24] In present study from drug

solution, % drug released in 24 hrs was only 6.88 ± 1.78 while in MTX microemulsion it was observed about 32.98 ± 2.20 %. From drug solution, % drug remained on skin was found to be 82.43 ± 3.30 which showed that drug in solution form had poor penetration through skin. While % drug remained on skin from plain MTX gel, MTX microemulsion and MTX microemulsion gel was found to be 67.05 ± 1.14, 33.87 ± 2.55 and 25.29 ± 1.72 % respectively. This showed MTX microemulsion gel has better drug penetration ability. Alvarez Figueroa and coworkers [15] had found 2.91 µg cm^{-2} amount of MTX remaining in skin after by passive diffusion of MTX across skin after 24 h. In present study amount of MTX remained in the skin was found higher in microemulsion gel i.e.15.51 µg cm^{-2} (48.71 ± 2.41 %) compared to microemulsion (31.22 ± 1.69%) and plain drug gel (20.54 ± 2.52%). So from above result, it has been concluded that microemulsion incorporated gel formulation had better skin penetration ability and also had better drug deposition capacity compared to other formulations.

4 CONCLUSION

In this study, microemulsion and MMG were prepared and evaluated. The results showed that microemulsion components had significant effect on the response. The miccroemulsion formulation containing 7.5% Capmul MCM C8, 50% Tween 20: PEG 400 (3:1) and 42.5% water was consider optimum. The MMG shows better retention in the skin and minimal irritation potential than microemulsion, solution and suspension form of MTX, which could be due to special characteristics of MMG. Thus, the drug loaded microemulsion gel could be a promising formulation to for topical delivery of anti-psoriatic drugs.

ACKNOWLEDGE

This is the time to say a sincere thanks to all those who have helped, supported and contributed to my work. I would like to give my warm thanks to Department of pharmacy, Sumandeep Vidyapeeth, Piparia, Vadodara, Gujarat, India for giving me opportunity to work in the field of topical novel drug delivery system in the university premises. I would also like to thanks zydus cadila, Goa and abitec corporation, USA for providing Methotrexate and Capmul MCM – C8 as gift samples respectively.

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