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Research Article

MICROEMULSION BASED HYDROGEL FORMULATION OF METHOXSALEN FOR THE EFFECTIVE TREATMENT OF PSORIASIS

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ABSTRACT

The aim of this research work was to enhance the retention of methoxsalen (MX) on the skin from novel microemulsion hydrogel formulation for the effective treatment of psoriasis. The microemulsion formulations of methoxsalen were prepared using ethyl oleate as oil, Tween 80 as surfactant and propylene glycol as a cosurfactant. Carbopol 934 gel matrix was used to construct the microemulsion hydrogel for improving the viscosity for topical application. Various concentration of surfactant: co-surfactant (1:1, 2:1, 3:1) was used for constructing pseudo ternary phase diagram. The microemulsion were extensively characterized for droplet size, polydispersity index, pH, conductivity and morphology. The *in vitro* skin permeation and skin deposition studies were carried out by using Franz diffusion cells fitted with human cadaver skin and compared with marketed formulation (Melanocyl lotion, Franco India Pharm Ltd.). Skin irritation studies were also performed to evaluate the possible noxious effect of formulations. Hydrogel formulation containing 1.5% Carbopol 934 had a most appropriate fluidity for topical administration. The photomicrograph shows the spherical shape and nano size range of droplet. The transdermal flux (J) of the drug from simple microemulsion MMX 4 was higher than its hydrogel formulation HMMX 4, while microemulsion based hydrogel formulation had lower permeation of drug as compared with its marketed formulation. Skin irritation studies confirmed no any sign of skin toxicity. Skin deposition studies confirmed the 10 fold higher deposition of methoxsalen as compared to marketed formulation. These results indicated that the studied microemulsion- based hydrogel may be a promising vehicle for topical delivery of methoxsalen.

Keywords: Methoxsalen, Microemulsion, Psoriasis, Topical delivery, Hydrogel

INTRODUCTION

Psoriasis is prevalent worldwide; however, the estimates of its prevalence vary by geographic location, being generally more common in the colder north than in the tropics.[1,2] Microemulsion based formulation have several interesting characteristics namely; enhanced drug solubilization, good thermodynamic stability and ease of manufacturing.[3] As topical vehicle, microemulsion can increase the local or systemic delivery of a drug by several mechanisms.[4] First, their composition and structure enable them to incorporate a greater amount of drug than other conventional topical formulations such as ointments, creams, gels and lotions. Microemulsion can enhance the solubility of poor water soluble drug via the finely dispersed oil droplet phase. Second, the diffusional barrier of the skin may be modified depending on composition of microemulsion. Third, an increase thermodynamic activity of the drug may favor its partitioning into the skin. However, most of the microemulsions possess a very low viscosity and therefore their application, especially in pharmaceutical industry may be restricted due to inconvenient application.[5] In order to overcome this disadvantage, some gelling agents such as Carbomer 940, xanthan gum and carrageenan have been used to increase the viscosity of microemulsion and form microemulsion based hydrogel which are more suitable for topical application when compared with microemulsion as a vehicle for drug delivery.[6-11] Biocompatible hydrogels with weak interaction with surfactants have recently been found to change the rheology properties of microemulsion.[12] Carbopol is a non-toxic, non- carcinogenic and non-immunogenic hydrophilic polymer, widely used to increase the viscosity of microemulsion for topical application.[13,14] The addition of hydrogel matrix into the microemulsion resulted in the formation of the microemulsion-based hydrogel, which is more suitable for topical application. Zhu et al.[14] reported microemulsion-based hydrogel (MBH) as a topical delivery system for penciclovir. Topical delivery of penciclovir in the forms of microemulsion, MBH and the commercial cream were evaluated *in vitro* and *in vivo*. The results of permeation test *in vivo* in mice showed that compared with the commercial cream, MBH and microemulsion could significantly increase the permeation of penciclovir into both epidermis and dermis. Methoxsalen (MX) is a photo active furocumarin derivative used for PUVA [psoralen (P) plus long-wavelength UV light A (UVA) irradiation] therapy.[15] It is widely used for the treatment of hyper-proliferating skin disease like psoriasis. Bioavailability of methoxsalen is highly variable because of its low water solubility and marked first pass effect. It also causes nausea, insomnia, nervousness and mental depression after oral administration. Because of these short comings topical delivery of methoxsalen by means of some topical formulation was attempted. This topical formulation were incorporated into a suitable cream base and evaluated for their performance. Grundmann -Kollmann et al.[16] and Stege et al.[17] have shown that the application of in o/w emulsion cream of methoxsalen to palm and soles regions provides a good localization of the drug. Thus, there is a need to develop novel topical delivery systems formulated with non-irritant component that can be applied throughout the body providing significant epidermal localization. The present study was focused on the screening of methoxsalen loaded microemulsion and construction of microemulsion based hydrogel formulation for the effective treatment of psoriasis.

MATERIALS AND METHODS

Methoxsalen was supplied as a gift sample by M/s Inga Laboratories Pvt. Ltd. Mumbai, India. Isopropyl myristate, Isopropyl palmitate, Oleic acid, Tween 80 and Ethyl oleate were purchased from CDH Laboratory Pvt. Ltd. Mumbai, India. Propylene glycol was purchased from SD Fine Chemical, Mumbai, India. Carbopol 934 was procured from HiMedia Laboratories Pvt. Ltd. Mumbai, India. All other chemicals used were of analytical reagent grade and were used as received.

Screening of oils for microemulsion

The solubility of MX in various oils like Isopropyl myristate (IPM), Isopropyl palmitate (IPP), Oleic acid (OA), and Ethyl oleate (EO) was measured to determine the suitable oil which can be used as the oil phase in microemulsion and provide excellent skin permeation rate of MX.[11] An excess amount of MX was added to each oil, and then mixed by magnetic stirring (Coslab, India). After stirring for 72 h at 25°C, the equilibrated sample was centrifuged (CFC-FREE, C-24, Cooling centrifuge, REMI, India) for 10 min at 8000 rpm to remove the excess amount of undissolved MX. Then, the supernatant was filtered using Whatman filter paper (# 41) and then diluted with ethanol and the concentration of MX was determined using UV-

Spectrophotometer (UV-Double beam spectrophotometer 2101, Systronics, India) at 219 nm. The data are shown in table 1.

Table 1: Solubility of Methoxsalen In Various Oils And Oily Mixtures At 25°c

S. No.	Component	Solubility (g/ml)
1	IPM	0.150 ± 0.014
2	IPP	0.136 ± 0.011
3	OA	0.142 ± 0.008
4	EO	0.206 ± 0.011

Values represented as mean ± S.D. (n = 3)

IPM- Isopropyl myristate, IPP - Isopropyl palmitate, OA- Oleic acid, EO - Ethyl oleate

Construction of pseudo - ternary phase diagrams

Pseudo- ternary phase diagram was constructed using H_2O titration method at ambient temperature (25°C) to determine the concentration range of components for the existing range of microemulsion.[11,18] Three phase diagrams were prepared with the 1:1, 2:1 and 3:1 weight ratio of Tween 80 to propylene glycol (PG), respectively. For each phase diagram at a specific surfactant/co-surfactant weight ratio, the ratios of oil to the mixture of surfactant and co-surfactant were taken. The mixture of oil, surfactant and co-surfactant at certain weight ratios were diluted with water drop-wise, under moderate magnetic stirring. After being equilibrated, the mixtures were assessed visually and determined as being microemulsion, crude emulsions or gels. The pseudo-ternary phase diagram of various ratios of surfactant and co-surfactant are graphically presented in fig. 1.

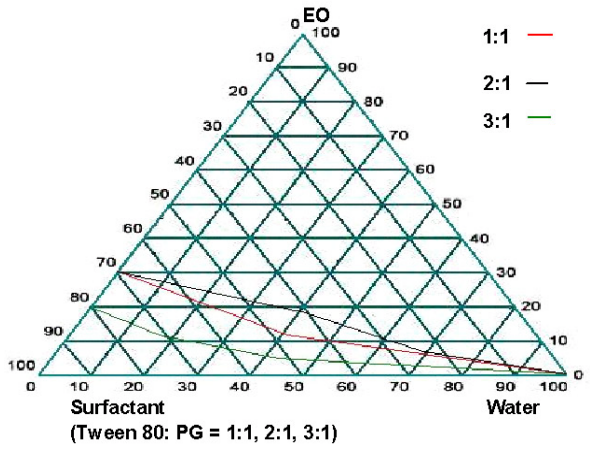

Fig. 1: Pseudo-ternary phase diagrams of the oil - surfactant - water system at 25°c. PG-propylene glycol, EO- ethyl oleate

Preparation of Methoxsalen loaded microemulsions

Methoxsalen was dissolved to the mixture of oil, surfactant, and co-surfactant with varying component ratio as shown in the table 2 and then an appropriate amount of water was added to the mixture drop by drop while continuous stirring with magnetic stirrer at ambient temperature, until a clear oily phase was obtained.

Preparation of microemulsion based hydrogel

Carbopol 934 was swelled in distilled water for 24 h and a highly viscous solution was obtained, and then the MX loaded microemulsion was slowly added to this viscous solution of Carbopol 934 under magnetic stirring. And the clear microemulsion-based hydrogel was obtained.

Characterization of microemulsion

The average droplet size and polydispersity index of the microemulsion were characterized at 25°C by photon correlation spectroscopy (Malvern Zetasizer Nano ZS90, Malvern instrument Ltd., UK). The viscosity of microemulsion based hydrogel formulations was measured at 25°C using a Brookfield digital viscometer (Model DV-II, Brookfield, USA). The pH values of different microemulsion formulation were determined by digital pH meter (Systronics digital pH meter 335, INDIA). The conductivity of different microemulsion formulations was determined using conductometer (Elico, CM 180 conductometer, India).

Microemulsion was visualized by TEM using a Hitachi electron microscope (Hitachi H-7500, Japan). Samples were negatively stained on a carbon -coated copper grid with 1% aqueous solution of phospho-tungustic acid (PTA) and viewed under the microscope with an accelerated voltage of 100 kV.

Determination of MX solubility in microemulsions

Excess MX was added to 10 ml of the microemulsion, and left at 37°C in the dark with magnetic stirring for 96 h. At 24, 48, 72, and 96 h, aliquots of the microemulsion were taken and centrifuged it for 10 min at 11000 rpm. The supernatant was filtered through 0.45 µm polyvinyl difluoride filters (to remove drug in suspension), and was then diluted with an appropriate volume of ethanol. MX was quantified in the filtrate by spectrophotometrically at 219 nm. Six replicate assays were performed.

***In vitro* skin permeation and retention study**

Excised human cadaver skin from the abdomen was obtained from Department of Anatomy and Physiology, Chhattisgarh Institute of Medical Sciences (CIMS), Guru Ghasidas University, Bilaspur, India. The full-thickness human cadaver skin was used for the *in vitro* permeation experiment using locally fabricated Franz diffusion cell. The skin was stored in formalin solution at 4°C. It was first immersed in purified water at 60°C for 2 min and the epidermis was then peeled off. The skin was clamped between the donor and the receptor chamber of diffusion cell with an effective diffusion area of 2.8 cm^2 and a cell volume of 7 ml. The receptor chamber was filled with freshly prepared phosphate buffer pH 5.5. The diffusion cell was maintained at 37°C and the solution of the receptor chamber was stirred continuously at 100 rpm by using magnetic stirrer with hot plate (Remi, India). The formulation (1.0g) was gently placed in the donor chamber. At 1, 2, 3, 4, 5, 6, 12, 18 and 24 h, 5.0 ml of the solution in the receptor compartment was removed and replaced immediately with an equal volume of fresh buffer. *In vitro* skin permeation of selected hydrogel formulation of microemulsion (HMMX 2) and marketed product melanocyl lotion (Franco Indian Pharm. Pvt. Ltd. India) in cadaver skin was also performed. Each experiment was performed in triplicate.

The cumulative amount of methoxsalen permeating across the cadaver skin was plotted against time. The skin retention studies of different formulations were performed in order to analyze the content of methoxsalen in the skin after 24 h of diffusion[19]. The skin samples were washed up with water and methanol on both sides and carefully dried. A defined amount of methanol was added to each piece of skin. The samples were vortexed (Vortex mixer, Fischer Scientific, USA) for 10 min and stirred overnight. The samples were analyzed by HPLC. The experiments were performed in triplicate.

Quantification of MX by HPLC

For quantification of MX in skin, the skin was first washed with ethanol (25 ml), left in 10 ml of ethanol in the dark for 48 h. The ethanol was then filtered through filter paper, and evaporated to dryness at 50°C under vacuum. The dried residue was then dissolved in 1.0 ml of HPLC mobile phase and centrifuged for 10 min at 11000 rpm. The supernatant was filtered through 0.45 µm polyvinyl difluoride filters, and MX was quantified in the filtrate by HPLC.[20]

The HPLC system consisted of a pump (LC 10-AT vp, Shimadzu, Japan), an SPD-M10A vp diode array UV/UV detector (RF-551, Shimadzu, Japan) and a 5 cm-C_{18} column (Phonomex, USA). A Spherisorb 5 ODS analytical column, 100 mm x 4.6 mm I.D., particle size 5 µm (Pye Unicam), was used. The mixture of acetonitrile and 0.01 M phosphoric acid (34:66, v/v, pH 2.82) was used as the mobile phase at flow-rate 1 ml/min and temperature 40°C. The mobile

phase was filtered before use and continuously degassed using helium. The UV detector was set at 248 nm (detection limit 15 ng/ml of samples). The calibration curve was linear in the studied concentration range of 50 - 1500 ng/ml. Retention time for MX was found to be 7.6 min.

Skin irritation studies

The selected microemulsion was used for skin irritation studies. All samples were applied to the shaved skin on the back of six Balb/c mice, and then the mice were secured. The animals were observed and evaluated for any sign of erythema, oedema or erosion for a period of 7 days. The study protocol including handling, care and dose administration were permitted by Institutional Animal Ethics Committee with Reg. No.1230/a/08/CPCSEA. Studies were carried out as per the guidelines of Council for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India.

Statistical analysis

Data shown as mean± S.D. (n = 5). Statistical data were analyzed by the Student's t-test at the level of $p < 0.05$.

RESULTS AND DISSCUSION

Screening of oils for microemulsion

For the development of microemulsion formulations for topical delivery of poorly water soluble drug methoxsalen, the optimum oil need to be chosen. Screening of oils was performed to investigate the most appropriate oil component for methoxsalen solubility, which can be determined by the solubility of methoxsalen in individual oil component. The solubility of methoxsalen in IPM, IPP, OA and EO was found to be 0.150 ± 0.014, 0.136 ± 0.011, 0.142 ± 0.008 and 0.206 ± 0.011 g/ml, respectively (Table 1). The solubility of methoxsalen was highest in EO (0.206 ± 0.011 g/ml), followed by IPM, IPP and OA. Thus, ethyl oleate was used as an oil phase in the preparation of microemulsion due to better solubilizing effect for methoxsalen.

Construction of pseudo-ternary phase diagram

The ratio of surfactant and co-surfactant is the key factor in the preparation of microemulsion. The construction of phase diagram makes it easy to find out the concentration range of components for the existence range of microemulsions. The pseudo-ternary phase diagrams with various weight ratio of Tween 80 to propylene glycol are described in fig. 1. The translucent microemulsion region was found under the area of plotted lines. The area of microemulsion region was found to be in order of 2:1 > 1:1 >3:1. According to the solubility of Methoxsalen and pseudo -ternary phase diagrams, Ethyl oleate and 2:1 phase ratios of Tween 80 to PG were selected for the preparation of microemulsion.

Preparation of microemulsion

Microemulsion formulations of methoxsalen were prepared by selecting 2:1 ratio of Tween 80 to Propylene glycol. Propylene glycol was incorporated into the microemulsion system not only reduce the interfacial tension between the oil phase and the aqueous phase but also to makes the lipophilic drug soluble in the system (Table 2).

Characterization of microemulsion

The characterization parameters of microemulsion formulations are presented in table 3. The droplet sizes of all the microemulsions were less than 100 nm, which was within the diameter range of microemulsion. The microemulsions droplet diameter was found to be in nanometric range (124-355 nm). Formulation MMX 2 has 124 nm droplet diameters which was minimum droplet size among them. The increase in conductivity (Table 3) could be attributed to the large attractive interaction forces among the droplets leading to the permeation of the electrolyte.[21]

Table 2: Formulation Code and Composition of the Selected Microemulsion Formulation (% W/W)

Component	MMX 1	MMX 2	MMX 3	MMX 4	MMX 5
Methoxsalen	0.05	0.05	0.05	0.05	0.05
Ethyl oleate	3	6	6	6	6
Tween 80	40	20	30	40	50
Propylene Glycol	20	10	15	20	25
Water	36.95	63.95	48.95	33.95	18.95

MMX $_{1-5}$ - Microemulsion formulation of methoxsalen

Table 3: Physicochemical Parameters of the Various Microemulsion Formulations

Formulation code	Droplet size (nm)	Polydispersity Index	pH	Conductivity (µS/cm)
MMX 1	188	1.000	5.10	117
MMX 2	124	0.472	5.62	270
MMX 3	421	0.432	5.41	206
MMX 4	325	0.540	5.79	252
MMX 5	355	0.318	5.50	307

MMX $_{1-5}$ - Microemulsion formulation of methoxsalen

In this study, the electrical conductivities of several selected microemulsions were measured, and the results indicate that the electrical conductivities reached above 100 µS/cm. On the basis of the above results the investigated microemulsions are roughly identified as the o/w system.[22] The pH of prepared microemulsion formulation MMX 1, MMX 2, MMX 3, MMX 4 and MMX 5 were found to be 5.10, 5.62, 5.41, 5.79 and 5.50, respectively. Hence all the microemulsion formulation pH shows nearly the pH of skin. It reveals no interaction of microemulsions with skin and easily penetration into the skin.[4]

The shape and morphology of the microemulsion droplet was determined by TEM (Transmission electron microscopy). The photomicrograph shows the spherical shape and nano size range of droplet (Fig.2). It also reveals that decreasing the nano size range cause increasing the transparency of the microemulsion.

Preparation of microemulsion based hydrogel

Carbopol 934 as an aqueous gel matrix in continuous phase, displayed non-covalent intermolecular associations deriving from disparate forces such as coulombic, van der waals and hydrogen-bond interaction and showed a weak gel behavior. These physical interactions could lead to the formation of the three-dimensional gel network and the dispersed oil droplets were reasonably hosted within the meshes of the three-dimensional gel network.[12] The incorporation of Carbopol 934 into microemulsions (MMX 4)

resulted in the significant increase of viscosity of microemulsions. The viscosities of prepared hydrogel were determined by digital viscometer and it was found to be 21.4, 22.0, 23.0, 24.0 PaS containing 1%, 1.5%, 2%, and 2.5%, of carbopol, respectively. The result reveals that viscosity increases with increasing concentration of carbopol. However 2.5% carbopol results a too high viscosity (24.0 PaS) and 1% carbopol led to a low viscosity of (21.4 PaS). Hydrogel formulations containing 1.5% Carbopol 934 had a most appropriate fluidity for topical administration.

Solubility studies of MX in Microemulsions

The solubility of MX was higher in MMX 4 (high lipid content and low water content) than in other formulations. This may be attributable to the fact that MX is lipid soluble, and thus has higher affinity for the oil phase: since this is the external phase in the MMX 4 microemulsions, a greater amount of drug is solubilized. The order of solubility of MX in prepared microemulsions were found to be MMX4> MMX3> MMX1> MMX2> MMX5 (Table 4).

Table 4: Solubility of Mx At 37°C In The Different

S. No.	Microemulsion	Solubility (g/ml)[a]
1	MMX 1	0.406 ± 0.021
2	MMX 2	0.306 ± 0.021
3	MMX 3	0.521 ± 0.061
4	MMX 4	0.611 ± 0.055
5	MMX 5	0.217 ± 0.034

[a] Solubility values are mean±SD for six assays

Microemulsions

***In vitro* skin retention and permeation study**

The permeation parameters of microemulsion are presented in table 5 and permeation profiles of methoxsalen through cadaver skin are shown in fig. 3. A steady increase of methoxsalen in the receptor compartment with time was observed. The permeation profiles of microemulsions followed first order release kinetics (r^2= 0.99). Statistical comparison of the flux throughout 24 h showed that most of the microemulsions provided fluxes (P<0.05) higher than the marketed formulation. Result shows lowest permeation of MMX 2 when compared with other formulations. The order of transdermal flux (J) at steady state and permeation coefficient (P) of formulations were followed the pattern: Melanocyl lotion< MMX 2 < MMX 5 < MMX 3 < MMX4 < MMX 1. MMX 2, containing a lower amount of surfactant and the co-surfactant mixture, offered the lowest transdermal flux and permeation coefficient compared with other microemulsion formulations. The thermodynamic activity of drug in the formulation is a significant driving force for the release and penetration of drug into skin.[23] In the present work, the microemulsions had high concentrations of MX and permeation coefficient, so the high permeation rate of MX could be obtained. This phenomenon accorded with the results reported previously[7]. The content of surfactant mixture in microemulsions affected the skin permeation flux of triptolide significantly. This may be due to an increased thermodynamic activity of the drug in microemulsions at the lower concentration of surfactant and cosurfactants.[7,8,11] Since drug can be released from the internal phase to external phase and then from the external phase to the skin, the relative activities may monitor the skin permeation flux. On the other hand, percent retention of drug in human cadaver skin after 24 h from HMMX4, MMX4 and marketed formulation was found to be 7.29±0.69, 6.38±1.49 and 0.72±0.18 μg/cm^2, respectively. It was found that microemulsion based hydrogel formulation of methoxsalen had shown maximum drug retention into the skin as compared to microemulsion formulation and conventional marketed formulation (p<0.05). The comparative *in vitro* skin permeation study of microemulsion based hydrogel formulation of methoxsalen (HMMX 4) and its marketed formulation (Melanocyl lotion) was also carried out on human cadaver skin. The result showed that addition of Carbopol 934 into microemulsion decreased markedly the permeability of methoxsalen (Table 5). Peltola et al.[7] studied the influence of carbomer 940 on the permeability of estradiol. The addition of carbomer 940 into microemulsion decreased the permeability of estradiol and it might attribute to the increased viscosity and transform from microemulsion to lamellar structure or a highly ordered microstructure.[14,24] So a conclusion could be drawn that addition of Carbopol 934 in microemulsion would delay drug release.

Table 5: Permeation Parameters of Mx across Human Cadaver Skin

Formulation code	Study state Flux J_{ss} (μg cm^{-2} h^{-1})	Permeation coefficient K_p (×10^3 cm h^{-1})	Cumulative deposition of MX on skin (μg cm^{-2})
MMX 1	1.82 ± 0.71	7.28 ± 0.45	2.03±0.36
MMX 2	1.12 ± 0.24	4.49 ± 0.95	3.25±1.23
MMX 3	1.59 ± 0.36	6.36 ± 0.56	3.05±0.54
MMX 4	1.67 ± 0.11	6.66 ± 0.38	6.38±1.49
MMX 5	1.20 ± 0.13	4.81 ± 0.93	2.86±1.35
HMMX 4	1.32 ± 0.59	1.82 ± 0.71	7.29±0.69
Melanocyl lotion	0.68 ± 0.08	3.20 ± 0.45	0.72±0.18

Values represent as mean ± S D (n = 3)

MMX $_{1-5}$: Microemulsion formulation of methoxsalen, HMMX 4: Hydrogel formulation of microemulsion, Melanocyl lotion: Marketed product of methoxsalen

It was observed that the transdermal flux (J) of the drug from simple microemulsion MMX 4 was higher than its hydrogel formulation HMMX 4, while microemulsion based hydrogel formulation has significantly higher release of drug from the marketed formulation (p<0.05). This might be due to the partitioning of the drug into the oil phase of microemulsion hydrogel which decrease the steady state of drug release.

The bioavailability of drugs penetrating the skin can be enhanced by using microemulsion systems because the small droplet size ensures close contact with the stratum corneum. Small droplets have better chances to adhere to the skin and transport the drugs in a more controlled fashion.[24] It is difficult for nanoparticles transport into the skin in an intact form because of the limited voids in the lipid bilayers of stratum corneum. It is assumed that the microemulsion form films of densely packed spheres on the surface of the skin, which exert an occlusive effect, thus increasing skin hydration.[20,26]

CONCLUSION

Microemulsion-based hydrogel formulation containing 0.05% methoxsalen with a suitable viscosity for topical administration were successfully formulated by 1.5% Carbopol 934 in microemulsion system. Microemulsion-based hydrogel formulation serves as efficient promoters of the methoxsalen localization into the skin. The enhanced skin accumulation of methoxsalen could help significantly to optimize the targeting of the drug without a concomitant increase of the systemic side effects. It is concluded that microemulsion based hydrogel formulation could be a better vehicle for topical delivery of drug than conventional marketed formulation for the effective treatment of psoriasis.

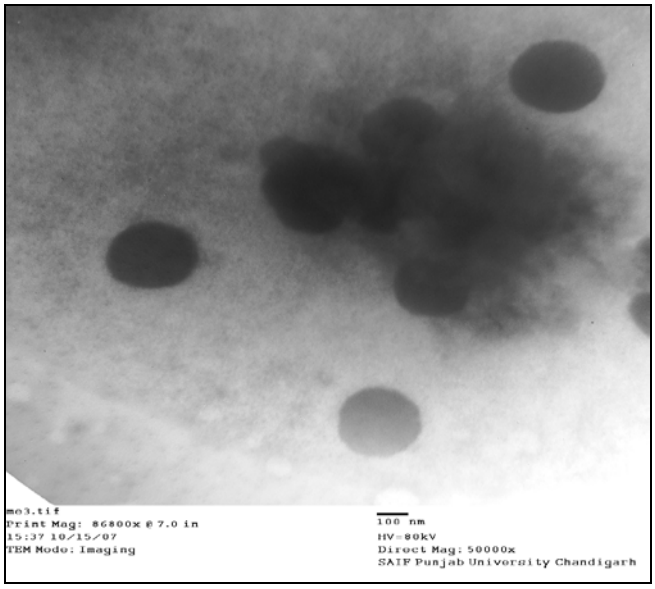

Fig. 2: Transmission electron photomicrograph [TEM] of microemulsion (mmx2) formulation (50,000x)

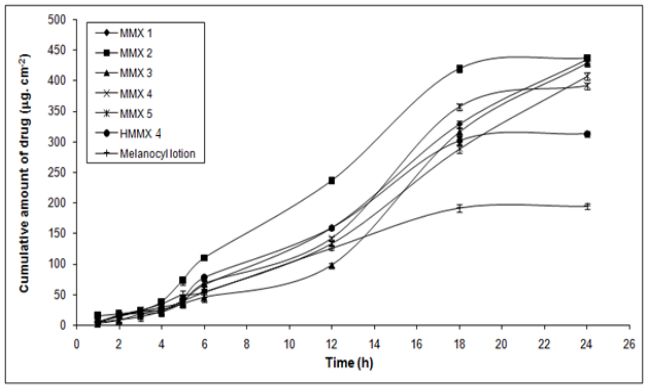

Fig. 3: Drug Permeation Profile of Different Formulations in Phosphate Buffer Ph 5.5 across Human Cadaver Skin

MMX $_{1-5:}$ Microemulsion formulation of methoxsalen; HMMX 4: Hydrogel formulation of microemulsion; 4, Melanocyl lotion: Marketed product.

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