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Original Article

Design and Evaluation of Hydrogel-Thickened Microemulsion for Topical Delivery of Minoxidil

Sampathi Sunitha[a*], Wankar Jitendra[b], Dodoala Sujatha[c], Mankala Santhosh Kumar[d]

[a]Department of Pharmaceutics, National Institute of Pharmaceutical Education and Research (NIPER-HYD), Balanagar, [b]Junior research fellow, Center for Cellular and Molecular Biology (CCMB), Tarnaka, Hyderabad, A.P., India, [c]Department of Pharmacology and Toxicology, National Institute of Pharmaceutical Education and Research (NIPER-HYD), Balanagar, [d]Department of Pharmaceutics, Srikrupa Institute of Pharmaceutical Sciences, Vill: Velkatta, Mdl: Kondapak, RD: Siddipet, Dist: Medak, Andhra Pradesh - 502277, India.

Abstract

The available minoxidil formulations for topical application suffer with major drawback having less contact time with the scalp which requires repeated application. Hence, the present study was aimed to investigate the effect of microemulsions and microemulsion based hydrogel systems (MEHs) for increased percutaneous penetration of minoxidil. Minoxidil microemulsions were developed by following conventional titration method using oleic acid as oil phase, Tween 80 as surfactant and polyethyleneglycol 200 as co-surfactant. Smix is a surfactant and co-surfactant mixture, whose ratios in the mixture were optimized using the pseudo ternary phase diagram. The physicochemical interaction between the drug and polymer were investigated by FTIR. Prepared microemulsions and MEHs were evaluated for drug content, viscosity, pH, *in vitro*, *ex vivo* permeation, skin irritation and stability studies. The drug content and viscosity in prepared microemulsions was found ranged from 56.77±2.88 to 92.85±1.59 %, and 89.12±1.801 to 144.24±0.95 cps respectively. The *Ex vitro* skin permeation from these microemulsions was sustained over 24 h with drug release around 32±3.26 to 99±3.78 % with more retardation in formulation F4 (oleic acid:Smix:water 58%:40%:2%). F4 was incorporated into hydroxypropyl cellulose gel to get MEH formulation and both were compared with the marketed topical solution. Marketed preparation was diffused at faster rate in comparison to the microemulsion and MEH. The drug release order was found to be Higuchi's with non-Fickian "anomalous" mechanism at controlled rate. The flux of the microemulsion F4 and MEH was found around 70.11±10.81 and 90.26±11.46 (μg/cm^2/hr) with permeation coefficient around 27.18±6.69 and 30.21±5.16 (cm/hr). The microemulsion did not show any dermatological reactions when tested. The microemulsion was found stable on storage and results suggested that microemulsions and MEHs could be more promising for topical delivery of minoxidil in hair loss treatment in comparison to solution based formulations.

Key Words: Hydrogel, minoxidil, microemulsion, oleic acid, polyethyleneglycol 200, Tween 80.

Corresponding Author: Dr. Sampathi Sunitha, Department of Pharmaceutics, National Institute of Pharmaceutical Education and Research (NIPER-HYD), Balanagar.
Tel: + (91) 9866078442
E-Mail: sunithasampathiphd@gmail.com
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1. Introduction

Alopecia is a serious problem in most of the individuals irrespective of gender and age. It is also reported as one of the important side effects with many drugs. Minoxidil is an antihypertensive agent widely used in topical formulations for the treatment of hair loss in women and men who suffer from androgenic alopecia (AGA) [1]. AGA is a hereditary and progressive androgen dependent thinning of the scalp hair which will follow a definite pattern of hair loss. The US food and drug administration approved treatment for androgenic alopecia with oral finsateride at a dose of 1 mg per day and as topical solutions of 2 and 5 % of minoxidil [2]. Hair growth can be stimulated by different mechanisms like increasing the linear growth rate of hair, increase the diameter of the hair fibre, alter the hair cycle, shortening telogen or prolonging anagen, or act through a combination of these effects. Minoxidil is reported to act on hair cycle and increase the hair diameter which is attributed to its vasodilatory effect mediated by enhancing the flux of potassium ions by acting on plasma membrane adenosine triphosphate (ATP) sensitive potassium channels [3].

Though many drugs are used for the treatment of alopecia, minoxidil is one of the drugs available in the market as 2% or 5% topical aqueous or organic solutions suffering from the major drawback of less contact time with the scalp. As the mechanism of hair growth is by local vasodilatation the less contact time of the drug solution with the scalp indicates repeated applications for the therapeutic benefit. Hence there is a need to increase the contact time by which the local drug concentration level increases to cause better vasodilatation. In the present study, an attempt has been made to formulate and evaluate the microemulsion and microemulsion based hydrogels of minoxidil for increasing contact time of the drug with the scalp and to attain control release of a drug for a longer time period, which may help in reducing the frequency of application and thereby increasing the patient compliance.

In recent years, numerous drug penetration enhancement techniques were studied through the transdermal route [4-5]. Among them, one of the most promising found is the microemulsion formulations [6-11]. As colloidal carriers, microemulsions are one of the promising systems that now a day's have attracted the main interest in penetration enhancement. It is composed of oil phase, surfactant, co-surfactant and aqueous phase at appropriate ratios. Microemulsions have several specific physicochemical properties such as

transparency, optical isotropy, low viscosity and thermodynamic stability [12-13] which offer several advantages for pharmaceutical use like enhanced drug solubilization, ease of preparation, high drug loading capacity and small droplet size that make them promising in drug delivery. As a vehicle for transdermal systems microemulsions can increase the local or systemic delivery of drug by different mechanisms [14-15]. Three main mechanisms were proposed to explain the advantages of microemulsion for transdermal delivery of drugs. First, the high solubility potential for both lipophilic and hydrophilic drugs by microemulsion systems might increase thermodynamic activity towards the skin. Second, ingredients of microemulsion, acting as permeation enhancer, might weaken the structure of stratum corneum and increase the flux of drug via skin. Third, the drug release from microemulsion might be increased because the affinity of a drug to the internal phase could be modified easily [16]. Recently many drugs such as ketoprofen, triptolide, apomorphine, lidocaine and estradiol using microemulsion for transdermal delivery were been reported [17-21]. The choice of an appropriate vehicle for the transdermal delivery plays a key role to maximize the flux through the skin into systemic circulation [22-23]. In this study, minoxidil microemulsions were developed after screening oils and surfactants. Pseudo ternary phase diagrams were constructed to optimize the components and their concentration ranges, and the microemulsion was formulated by using oleic acid as an oil base and Tween 80 and polyethylene glycol 200 as surfactant and co-surfactant respectively.

Table 1. Formulae of minoxidil micro-emulsion with the selected percentages of oil, Smix and water from the pseudo ternary phase.

Smix	**Formulation code**	**Percent w/w of component in formulation**		
		Oil (%)	**Smix (S+ CoS %)**	**Water (%)**
Smix ratio = 1:1	F1	9.67	86.8	3.53
	F2	10	88	2
	F3	38	60	2
	F4	58	40	2
Smix ratio= 2:1	F5	8.94	75.61	15.38
	F6	10	80	10
	F7	14.24	80.40	5.34
	F8	14	82	4
	F9	8	78	15
Smix ratio= 3:1	F10	8.84	78.19	12.96
	F11	14.04	74.37	11.58
	F12	16	74	10
	F13	10	84	6
	F14	16	80	4

2. Materials and Methods

Minoxidil was obtained from Dr. Reddy's Labs, Hyderabad, India as a gift sample. Oleic acid was obtained from Loba chemicals, India. Polyethyleneglycol (PEG) 200, Tween 20, Propyleneglycol and Tween 80, hydroxypropyl cellulose was procured from SD Fine chemicals Ltd, Mumbai, India. Analytical reagent grade ethanol was obtained from Jiangsu Huaxi Int. Trade Co. Ltd. All other chemicals used were of analytical reagent grade only.

2.1. Construction of Pseudo Ternary Phase Diagram

To find out the existence range of microemulsions, pseudo ternary phase diagrams were constructed using water titration method at ambient temperature (25 ºC) [22]. Based upon on the available solubility profile of the drug, oleic acid was selected as an oil phase; Tween 80 and polyethylene glycol 200 were used as surfactant and co-surfactant respectively. The Smix (surfactant+Co-surfactant) ratios were selected to be 1:1, 2:1 and 3:1 v/v and used. For each phase diagram at specific Smix concentration the oleic acid was added from the range of 1:9 to 9:1, and the mixture was diluted with distilled water by sequential addition of 10 µL of water using a micropipette. Water was added drop by drop while mixing on a magnetic stirrer at room temperature, and the samples were marked as being optically clear or turbid. The microemulsion regions were identified as transparent and isotropic mixtures. The percentage of three different phases, that is oil, water, and the mixture of surfactant and co-surfactant were calculated (Table 1). From the endpoint compositions of titrated samples the mass percent composition of the components like oil, Smix and water was calculated and then plotted on triangular coordinate to construct the pseudo ternary phase diagram. The experiment was performed in triplicate to check the reproducibility.

2.2. Formulation of Minoxidil Microemulsion

From the pseudo ternary phase diagram, fourteen regions were identified with Smix ratios of 1:1, 2:1, and 3:1 as given in Table 1 and at these concentrations of Smix, oil and water 1% w/v of drug was added and mixed thoroughly to formulate minoxidil microemulsions (F1 –F14).

2.3. Formulation of Minoxidil Microemulsion Hydrogel

The best microemulsion formulation of minoxidil was incorporated into 2.5% of hydroxypropyl cellulose (Natrosol) to get a gel of microemulsion. Weighed quantity of the hydroxylpropyl cellulose was dissolved in 15 mL of distilled water and stirred thoroughly to get homogenous slurry. The best and stable microemulsion was incorporated and mixed thoroughly and the pH was adjusted to neutral with triethanolamine. Limonene 0.1% was added as the permeation enhancer.

Table 2. Results of Drug content and viscosity of the various microemulsion formulations.

Formulation Code	Drug content ±SD	Viscosity (cps)	pH
F1	88.36 ±1.47	89.96 ±8.928	7.20 ±0.086
F2	89.97 ±2.91	106.56 ±2.10	7.32 ±0.062
F3	85.97 ±3.50	132.38 ±1.88	7.34 ±0.045
F4	84.48 ±2.12	144.24 ±0.95	7.40 ±0.089
F5	86.45 ±1.26	99.84 ±0.970	7.42 ±0.094
F6	90.07 ±1.83	102.78 ±3.899	7.40 ±0.024
F7	90.67 ±2.13	113.44 ±0.993	7.38 ±0.021
F8	86.49 ±2.26	116.22 ±2.845	7.40 ±0.099
F9	87.97 ±1.57	89.12 ±1.801	7.20 ±1.220
F10	89.96 ±1.27	100.32 ±2.331	7.12 ±0.047
F11	92.85 ±1.59	112.22 ±0.902	7.36 ±0.078
F12	88.75 ±2.24	102.10 ±1.47	7.41 ±0.092
F13	89.55 ±1.27	117.21 ±1.747	7.42 ±0.012
F14	87.55 ±3.73	103.18 ±2.259	7.38 ±0.028

2.4. Characterization of Microemulsion Systems Formulations of Minoxidil

2.4.1. Drug content

An amount of microemulsion containing 10 mg of drug was taken and dissolved in 50 mL of phosphate buffer pH 7.2. The volumetric flasks were kept for 2 h over a shaker to mix it properly. The solution was filtered and drug content was measured spectrophotometrically at 288 nm.

2.4.2. Measurement of pH

The pH of minoxidil microemulsion formulations was determined by using digital pH meter. The measurement of pH of each formulation was done in triplicate and average values were calculated.

2.4.3. Viscosity

The measurement of viscosity of the prepared microemulsions was done with a Brookfield Viscometer DV II (LVDL-II+PX) at 25 ±0.3 °C.

2.4.4. Fourier Transform Infrared Spectroscopy (FT-IR)

FT-IR analysis was performed to know the interaction between the drug and the polymer used by taking spectra of drug, polymer and the physical mixture. About 1 mg of the samples were triturated with 300 mg of dry, finely powdered IR grade potassium bromide and compressed under vacuum at a pressure of about 800 Mpa. The obtained disc was scanned in FTIR from the range of 600-4400 cm^{-1}.

2.4.5. In Vitro Drug Diffusion Studies

In vitro diffusion study was performed by using Franz diffusion cell with an effective diffusion area of 2 cm^2. The dialysis membrane (cellulose acetate, Mol Wt 12000 D - Himedia) was clamped between the donor and the receptor chamber of Franz diffusion cell. Then, formulation was placed in the donor

compartment. The receptor chamber was filled with phosphate buffer pH 7.4 (PBS). The receptor medium was set at 37 ± 0.5 ºC and stirred at 600 rpm throughout the experiment. Samples were withdrawn at predetermined time intervals up to 24 h and fresh blank media was replaced in it. Withdrawn samples were analyzed by UV-spectrophotometer at 288 nm. The cumulative amount of drug permeated through dialysis membrane was plotted as a function of time.

2.4.6. Ex Vivo Studies

The best microemulsion formulation was studied for the *ex vivo* permeation by using rat abdominal skin as the membrane. *Ex vitro* skin permeation study was performed by using Franz diffusion cells with an effective diffusion area of 2 cm^2. Mice of weight 18-20 g (5-6 weeks old) were depilated and abdominal skin samples were excised, and clamped between the donor and the receptor chamber of Franz diffusion cells with the *stratum corneum* facing the donor chamber. 1mL of microemulsion containing 5mg minoxidil was placed onto the donor chamber. The receptor chamber was filled with phosphate buffer pH 7.4 (PBS). The receptor medium was maintained at 37 ± 0.5°C and stirred at 600 rpm throughout the experiment. For each experiment, 5 mL receptor medium was sampled at predetermined time intervals and then the same volume of pure medium was immediately replaced into the receptor chamber. All samples were analyzed by UV-Spectrophotometer at 288 nm. The release studies were conducted in triplicates.

2.4.7. Calculation of Permeation Parameters

The cumulative amount of minoxidil permeated (Qt) per unit of rat skin surface area(S) was plotted as a function of time. The permeation rate of minoxidil at steady-state (Jss, μg/cm^2/hr) was calculated by linear regression analysis by interpolation of the cumulative amount permeated through rat skin per unit area versus time.

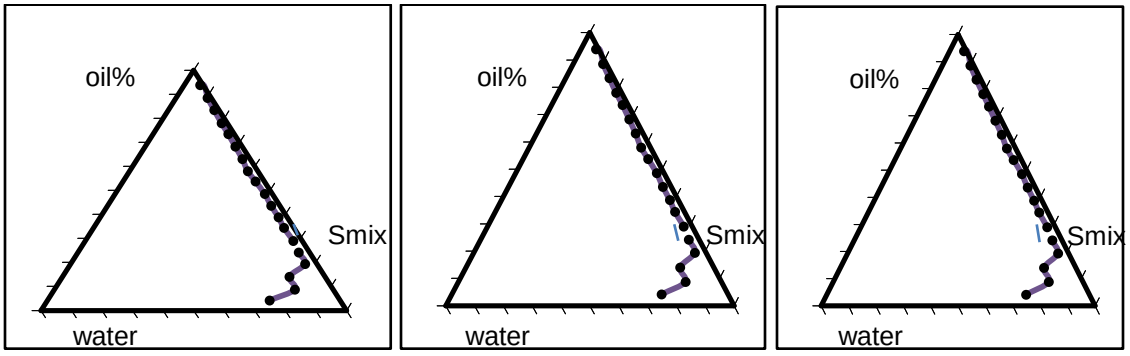

Figure 1 a **Figure 1 b** **Figure 1 c**

Figure 1. Pseudo ternary phase diagram of oil (oleic acid), Smix (tween 80 and PEG 200) in the ratios 1:1, 1:2, and 1:3 along with the water phase.

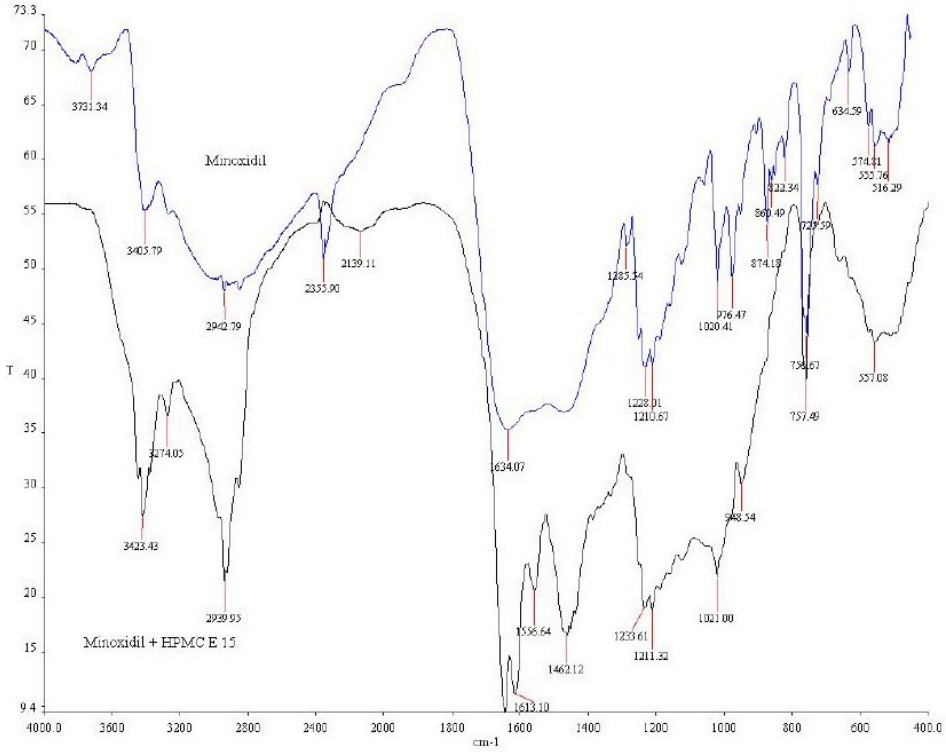

Figure 2. Overlay IR spectra of the pure drug minoxidil and drug+polymer mixture.

$$Jss = \Delta Qt/S.\Delta t \; .. \; (1)$$

The permeability coefficient (Kp, cm/hr) was calculated according to the equation (2) ... (2)

$$Kp = slope \; X \; Vd/S$$

Where

Vd = Volume of the donor compartment (30 mL)

S = Surface area (2.6 cm^2)

It is assumed that under sink conditions the drug concentration in the receiver compartment is negligible compared to that in the donor compartment. All skin permeation experiments were repeated three times and data were expressed as mean of three experiments ± standard deviation (S.D).

Enhancement ratio (ER) was used to evaluate the effect of permeation enhancer on diffusion and permeation of selected drug molecules. It is calculated by using the formula below:

$$ER = \frac{\text{Permeability coefficient of drug with enhancer}}{\text{Permeability coefficient of drug alone}}$$

2.4.8. Kinetic Analysis of Drug Release

To analyze the mechanism of drug release from minoxidil micro-emulsion, micro-emulsion based gel, and the *in vitro* dissolution data were fitted to zero order, first order, Higuchi release and Korsemeyer - Peppas model and the model

with higher correlation coefficient was considered to be the best fitted model.

2.5. Skin Irritation Test

The test was performed on healthy albino rat weighing around 175 g. Aqueous solution of formalin 0.8% was used as the standard irritant. The hair was removed on the hind limbs. The animals were divided into 2 groups [Ethical Committee Reg. No: 1548/PO/a/11/CPCSEA]. The optimized micro-emulsion formulations gel were applied to dorsal right limb and the left dorsal limb was used as control, after 24 h the gel was removed with the help of an alcohol swab. The skin of the animals was examined for erythema or edema.

2.6. Stability Studies

2.6.1. Centrifuge Stress Test

The optimized microemulsion of minoxidil was evaluated for any signs of phase separation by subjecting to centrifugation at a 2000 rpm for a period of 4 h and then it was examined macroscopically for any signs of instability indicated by separation of phase [24].

2.6.2. Droplet Size and Polydispersity

Droplet size and polydispersity of the best formulation were determined using a laser scattering particle size analyzer (Malvern Zeta-Sizer ZEN3600, UK). Approximately 100µL of the microemulsion was diluted to 10 mL with double distilled water to prepare the sample for study.

3. Results and Discussion

3.1. Pseudo Ternary Phase Diagram

A phase behavior investigation to develop microemulsion system is the suitable approach of determining the water phase, oil phase, and surfactant/ co-surfactant concentrations. Therefore pseudo ternary phase diagrams were

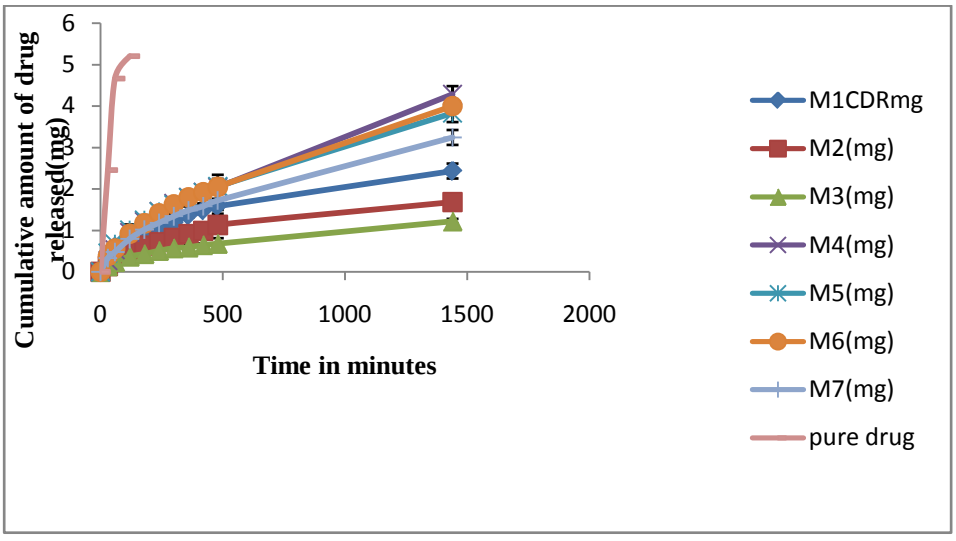

Figure 3. *In vitro* drug release profiles of minoxidil formulations F1-F7(mean±SD,n=3).

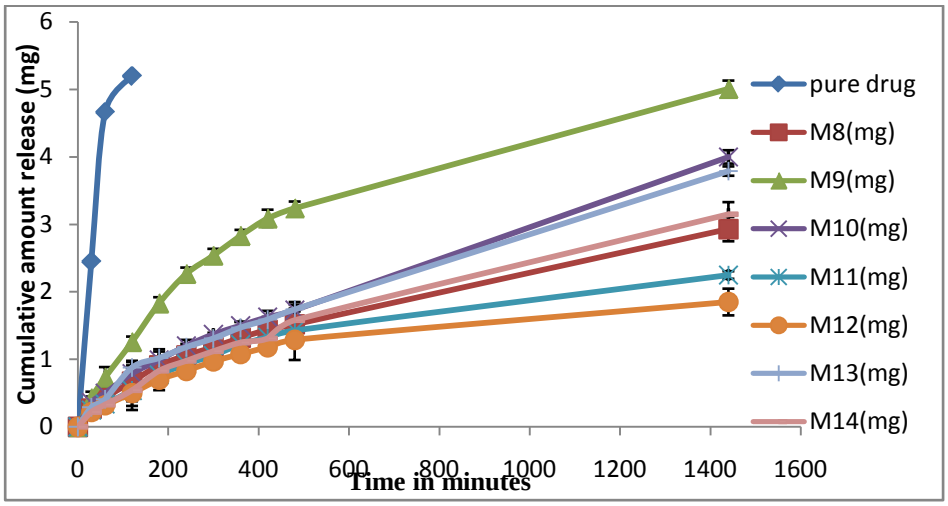

Figure 4. *In vitro* drug release profiles of minoxidil formulations F8-F14(mean±SD,n=3).

constructed to determine the composition of the prepared microemulsions. The microemulsion regions in pseudo ternary phase diagrams to determine the composition of oil phase consisting of oleic acid, Tween 80 as surfactant and polyethylene glycol 200 as cosurfactant in the ratios of (1:1, 1:2 and 1:3 v/v) for the formulation of minoxidil microemulsion at room temperature were and fourteen formulas were selected for the development of minoxidil microemulsions (Table 1, Figure 1). Using the composition of selected microemulsion, minoxidil microemulsions were formulated and investigated.

3.2. Drug Content

The drug content for the minoxidil microemulsions was ranging from 84.48 ± 2.12 % to 92.85 ± 1.59 % respectively. Viscosity of the microemulsion of minoxidil was ranging from 89.12 ± 1.801 to 144.24 ± 0.95 cps (Table 2).

3.3. Investigation of Physicochemical Compatibility of Drug and Polymer

The IR spectral analysis of minoxidil alone showed the principal peaks at wave numbers 3449.95, 2936.63, 1643.57, 1612.90, 1557.2 and 1449.90 cm^{-1} . The peaks of IR spectra were in accordance with previous report and confirming the purity of the drug (Figure 2). The IR spectra of the physical mixture of drug and polymer used showed peaks at 3423.43, 2939.95, 2039.11, 1643.95, 1613.10, 1556.54, and 1460.12 cm^{-1} which were found to be similar with the standard IR spectra peaks given in British Pharmacopoeia [25]. These results suggest that there was no physical interaction between drug and polymer used in the present study as none of the characteristic peak altered.

3.4. In Vitro Permeation Studies

The various minoxidil microemulsions were investigated for *in vitro* permeation studies through dialysis membrane with a cut off of molecular weight of 12000 D. The *in vitro* permeation studies were performed for all the prepared formulations including the marketed topical solution. At the end of the 24 h the drug release of all the formulations is ranging between 32 ± 3.26 to 99 ± 3.78 percentage of drug release. The results of *in vitro* drug release studies from microemulsions are depicted in Figure 3 and 4. The marketed formulation showed immediate release and highest drug permeation when compared to the microemulsion and microemulsion based hydrogel formulation. The *in vitro* release data obtained were treated for different kinetics models like zero-order, first-order, Higuchi and Korsmeyer-Peppas models to assess the mechanism of drug release. The results of the curve fitting into these above mentioned models indicate that the release of drug is by diffusion (R^2=0.978 to 0.997) over 24 h as the best fit amongst all other investigated models. The results also indicated that the drug permeation from these minoxidil microemulsions followed the non-Fickian anomalous mechanism (Table 4).

3.5. Ex Vivo Permeability Studies

The permeation ability of the selected microemulsion formulation and the microemulsion hydrogel was evaluated by the *ex vivo* permeation experiments. The optimized formulation F4 was also studied for *ex vivo* permeation study (Figure 5). The hydrogel based microemulsion released the drug slowly and was

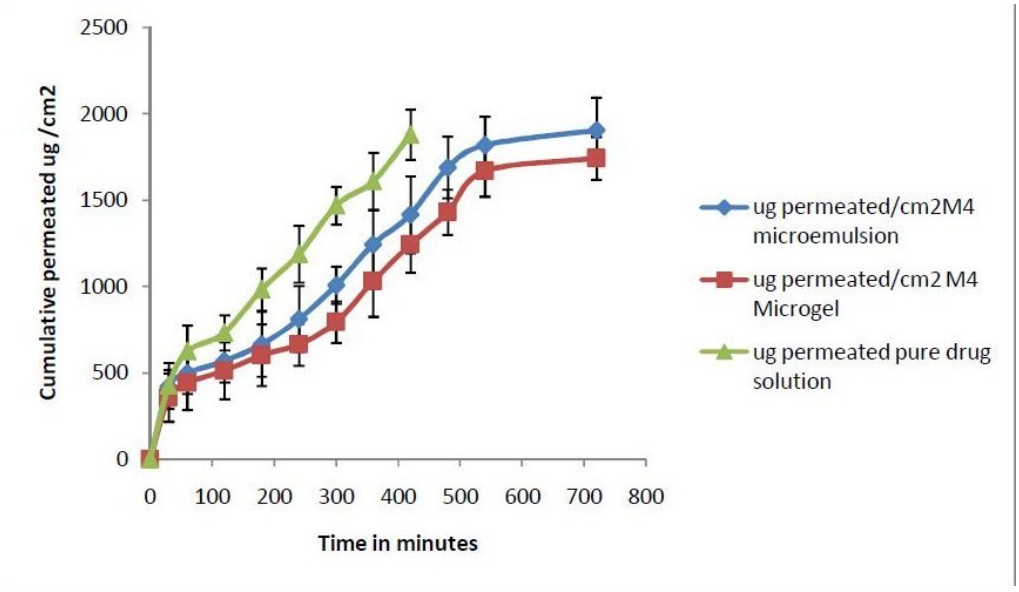

Figure 5. *Ex vivo* release profiles of the optimized microemulsion and microemulsion based gel(mean±SD,n=3).

Table 3. Flux and permeability data of selected micro-emulsions and microemulsion based gel of minoxidil.

S.No	Formulation code	Flux (µg/cm2/hr)	KP(cm/hr)	ER
I	F4 microemulsion	90.26 ± 11.46	27.18± 6.69	0.60
II	F4 microgel	70.11 ± 10.81	30.21 ±5.16	0.66
III	Pure Drug	113.37 ±14.42	45.09 ± 9.14	1

comparable with the plain microemulsion. The values of transdermal flux for the microemulsion, hydrogel and the control were 70.11 ± 10.81, 90.26 ± 11.46 and 88.75 ± 14.42 (µg/cm^2/hr) and permeation coefficient is ranging from 27.18 ± 6.69, 30.21 ± 5.16 and 45.09 ± 9.14 (cm/hr). The results demonstrated that the permeation rate and permeation coefficient of the formulations through rat skin are significantly less for the microemulsion based gel when compared to the pure drug solution. It was also apparent from the results that the minoxidil permeation increased with the increase in the oil and surfactant content. This could be attributed to skin permeation enhancement capacity of the used surfactant, as surfactants loosen or fluidize the lipid matrix of the *stratum corneum* which is the principal diffusion barrier of the skin and act as skin permeation enhancer. But, the enhancement ratio of minoxidil microemulsions and minoxidil microemulsion hydrogels were less when compared to the pure drug solution indicating the sustained release of the minoxidil from the prepared formulations (Table 3). Minoxidil microemulsion hydrogels was found to have high ER when compared to simple microemulsions of the optimized formulation.

Table 4. Curve fitting of the *in vitro* permeation data of various minoxidil gels.

Minoxidil micro-emulsions and gels	Zero order	First order	Higuchi	Peppa's	
Formulation code	**R^2**	**R^2**	**R^2**	**R^2**	**K**
F1	0.805	0.902	0.978	0.958	0.662
F2	0.816	0.880	0.982	0.988	0.559
F3	0.876	0.894	0.995	0.985	0.629
F4	0.938	0.985	0.991	0.997	0.531
F5	0.909	0.997	0.997	0.995	0.595
F6	0.925	0.995	0.995	0.978	0.586
F7	0.916	0.982	0.997	0.996	0.603
F8	0.920	0.977	0.997	0.980	0.686
F9	0.805	0.995	0.996	0.993	0.584
F10	0.970	0.982	0.966	0.989	0.582
F11	0.841	0.894	0.983	0.989	0.619
F12	0.788	0.831	0.966	0.981	0.600
F13	0.957	0.989	0.978	0.988	0.658
F14	0.952	0. 992	0.983	0.994	0.694

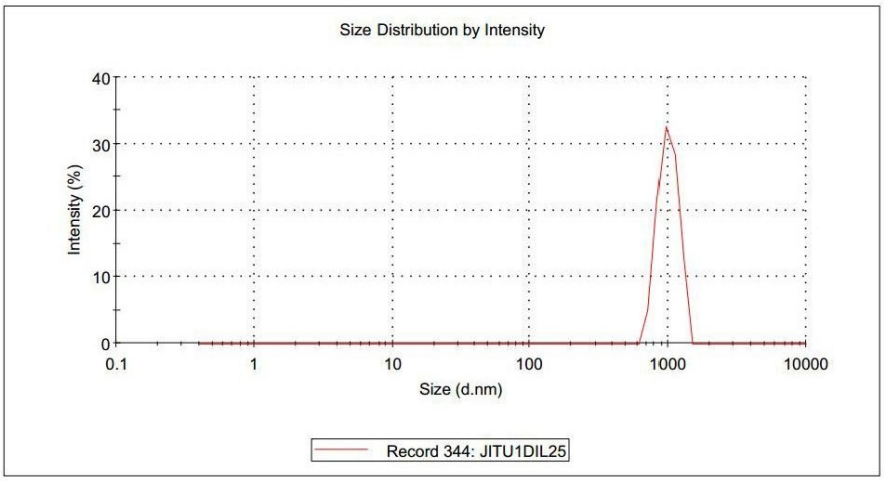

Figure 6. Particle size data of the best formulation.

This could be related to the hydration caused by the polymer used in the preparation of the gel.

6 and 7). From the results it can be concluded that the particle size is in microns and the

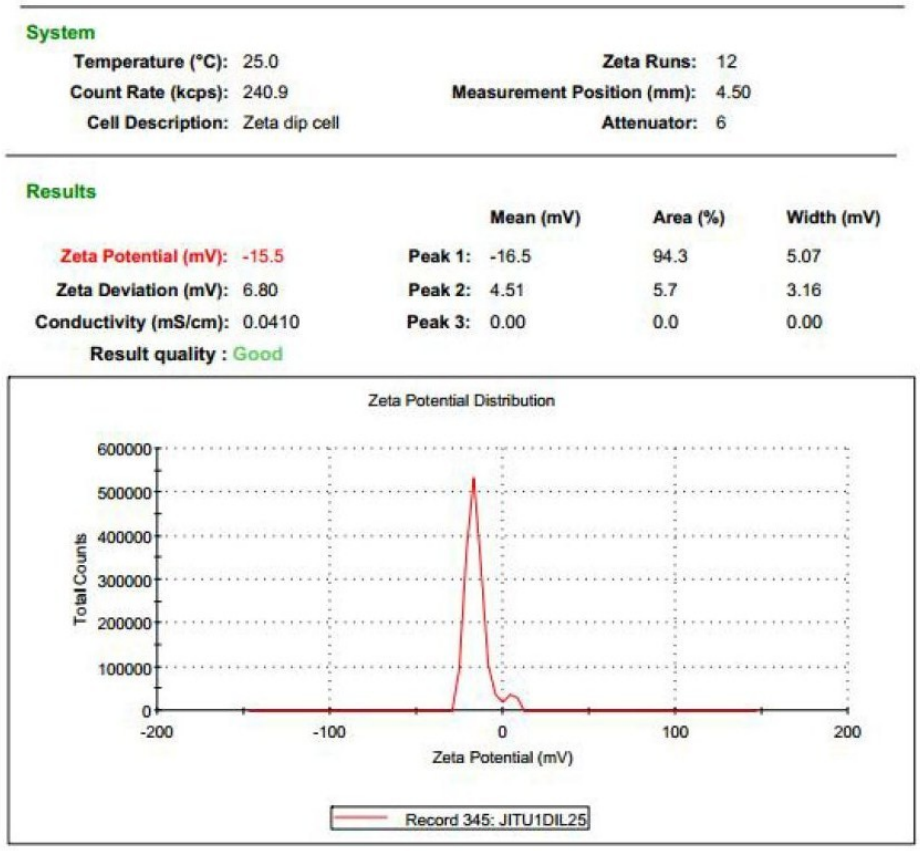

Figure 7. Zeta Potential data of the best formulation.

suitable for the transdermal application with sustained release of the drug, thus may overcome the drawback of minoxidil topical solutions by enhancing the contact time of the drug with the scalp and may reduce the frequency of applications. Further work is necessary to support efficacy claims by long term pharmacokinetic and pharmacodynamic studies in human beings.

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