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Effect of Penetration Enhancer DMSO on *In-Vitro* Skin Permeation of Acyclovir Transdermal Microemulsion Formulation

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Abstract

The aim of this research was to enhance the flux of transdermal drug delivery by using penetration enhancers DMSO. Skin penetration enhancers have been used to improve bioavailability and increase the range of drugs for which topical and transdermal delivery is a viable option which penetrate into skin to reversibly decrease the barrier resistance. Penetration enhancing activity of dimethylsulphoxide (DMSO) at 5% w/w and 10% w/w concentration were determined in aqueous solution of ACV and in microemulsion formulations though calculation of transdermal flux of ACV with Keshary Chein Frenz Diffusion cell by using wistar albino rat skin. The transdermal flux of formulations PD, PD5D, PD10D, ME1 and ME10D was found to be 2.47, 50.7529, 119.7691, 238.1432 and 266.6721µg/cm^2/h. The flux of microemulsion formulation ME10D was found 266.6721± 8.49 µg/cm^2/h. Which showed highest value and skin flux of the drug could be enhanced up to 107 fold compared to its aqueous solution by preparing microemulsion ME10D. DMSO in microemulsion formulation is safe to the skin at 10% DMSO w/w.

Keywords: DMSO, Penetration enhancer, Ethanol, Transdermal Microemulsion, Acyclovir (ACV)

Introduction

The improvement of drug permeability though the skin is always a difficult problem because of barriers function of human skin epithelia to exogenous substances. Therefore, the major challenge in topical administration is to increase the drug penetration into the skin moreover the most of the pharmaceutical substances are lipophilic in nature. The clinical efficacy of such drugs is being impeded by their low aqueous solubility resulting in poor penetration and absorption mainly when they are designed for transdermal administration. Skin is a remarkably efficient barrier, designed to keep ‘‘our insides in and the outsides out’’. This barrier property causes difficulties for transdermal delivery of therapeutic agents. One long-standing approach to increase the range of drugs that can be effectively delivered via this route has been to use penetration enhancers [1]. The use of a microemulsion as vehicle may enhance transdermal penetration by various mechanism, many molecules or solubilized in microemulsion in addition microemulsion induce a change in the thermodynamic activity of the drug they contain, modifying their partition coefficient and thus favour penetration of the stratum corneum. Furthermore, their component surfactant reduces

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the functional barrier of stratum corneum. This latter function may be more or less important depending on the nature of the surfactant used [2].

In principle, microemulsion and its gel can be used to deliver drug to the patient via several routes, but the transdermal application of microemulsion has gained increasing interest. Microemulsion gel has improved the transdermal delivery of several drugs over the conventional preparation such as emulsion [3, 4].

Microemulsion

Microemulsion is defined as thermodynamically stable transparent, single optically isotropic liquid system of water oil and surfactants frequently in combination with suitable cosurfactants [5, 6]. The concept of micro-emulsion was fit introduced by Hoar and Schulman in 1940 [7] and the term microemulsion was first carried by schulman and coworkers in 1959 to described the clear fluid systems obtained by titration to the point of clarity of an ordinary milky emulsion (macroemulsion) by the addition of a medium chain alcohol such as pentanol and hexanol. The droplet size (100-600 nm) was much hence their transparent appearances and the adoption of the term microemulsion according to Schulman [8]**.** But according to some other scientist the microemulsion droplet size lies in the range of 10- 100nm Jang *et al*., 1992. These colloidal dispersions are formed at appropriate ratio of oil, water surfactants and cosurfactant. Unlike coarse emulsion micronized with external energy microemulsion are based on low interfacial tension. This is achieved by adding a cosurfactant which leads to spontaneous formation of a thermodynamically stable microemulsion. The droplet size in the dispersed phase is usually below 140 nm in diameter, which makes microemulsion transparent liquid [9]. Microemulsions are emulsions producing a transparent product that has a very small droplet size and does not have tendency to coalesce. A large amount of drug can in fact be incorporated in these formulations, Due to the high solubilising capacity of hydrophobic drugs [10].

Materials and Methods

Acyclovir was obtained as gift sample by Cipla Ltd, Mumbai. Castor Oil was purchased from Loba chemicals, Carbitol, Eucalyptus oils, Isopropyl meristate, Methanol, Olive oil, Oleic Acid, Propylene glycol, PEG 400, Span 80, Tween 80, Triacetin and Tween 20 purchased from CDH, Delhi, INDIA. Corbopol^{934} was purchased from CDH, Delhi. Ethanol (Qualigens fine chemicals, Mumbai) were obtained from, Departmental chemical store of B. U. Jhansi, India. All other chemicals used were of analytical reagent grade and used as received without further purification. Double-distilled water was used though out the study.

Determination of Drug Solubility

Drug solubility was determined in water by dissolving an excess amount of ACV was added to distilled water. This suspension was stirred at room temperature for 24 h with a magnetic stirrer. The sample was then filtered though a 0.45-µm membrane filter. The concentration of ACV was determined spectrophotometrically at 254 nm.

To find out suitable oils that have good solublizing capacity of ACV as well as ability to yield system with larger microemulsion existed area, solubility of ACV in various oils (oleic acid , olive oil, IPM, castor oil, triacetin, capmul oil and combination of these oils) surfactants(tween20, tween80, span80) and cosurfactants (carbitol, ethanol, propylene glycol, PEG400) were determined by dissolving excess amount of ACV in 2 ml of each selected oils, surfactants and cosurfactnts separately in 5ml capacity stoppered vials and mixed by continuously stirred for 72 h. The mixture vials were then kept at 37 ± 0.5 °C in an isothermal shaker (Jyoti instrument Industry M.P., India) for 72 h to get equilibrium. Then equilibrated samples were removed from shaker and centrifuged at 10,000 rpm for 10 min. The

supernatant of ACV was separated, filtered and after appropriate dilution with methanol, solubility was determined by UV spectrophotometer at λ_{max} 254 nm. Solubility of ACV in oils phase and in surfactant and cosurfactant was shown in Table. 1. And Table.2: respectively.

Table.1: Solubility of Acyclovir in different oils.

S. No.	Oils	Solubility (mg/ml)
1.	Olive oil	3.48 ± 0.20
2.	Oleic Acid	9.73 ± 1.50
3.	Oleic Acid+IPM(1:1)	8.57 ± 2.00
4.	Oleic Acid+Olive Oil1:1)	3.48 ± 1.00
5.	Oleic Acid+Castor oil(3:1)	45.53 ±1.50
6.	Oleic Acid+ Castor oil(1:2)	35.00 ±2.00
7.	Oleic acid+Castor oil(1:4)	40.53 ±2.10
8.	OA+OO+Castor oil (1:1:1)	11.28 ±1.00
9.	Capmul oil	1.37 ±0.50
10.	Triacetin	0.33 ±0.10
11.	IPM	1.95 ±0.05
12.	Castor oil+ IPM(3:1)	3.75 ±2.0

Table .2: Solubility of Acyclovir in different surfactants and cosurfactant

S.No.	Surfactants / Cosurfactants	Solubility (mg/ml)
1.	Tween80	79.0±5.00
2.	Tween20	1.37±0.50
3.	Span80	30.00±2.0
4.	Carbitol	88.21±5.00
5.	Propylene Glycol	11.28±2.00
6.	PEG400	3.19±0.50
7.	Ethanol	92.32±6.20

Construction of pseudoternary phase diagrams

On the basis of solubility studies, combination of oleic acid and castor oil (3:1) was selected as the oil phase. Tween 80 and Ethanol were chosen as surfactant and cosurfactant respectively on the basis of optimization by constructing phase diagram and analyzed the result. Double distilled water was used as an aqueous phase. For the determination of existence zone of microemulsion, pseudoternary phase diagrams were constructed using water aqueous titration method. Surfactant and cosurfactant (S_{mix}) were mixed in different weight ratios (1:0, 0.5:1, 1:1, 2:1 and 3:1). These S_{mix} were chosen in increasing concentration of surfactant with respect to cosurfactant for detailed study of the phase diagrams.

For each phase diagram, oil and specific S_{mix} were mixed well in different ratios. Sixteen different combinations of oil and S_{mix} (1:9, 1:8, 1:7, 1:6, 1:5 1:4, 1:3.5, 1:3, 1:2.3, 1:2, 1:1.5 1:1, 6:4, 7:3, 8:2 and 9:1) were made so that maximum ratio could be covered for the study to delineate the boundaries of the phases formed precisely in the phase diagrams. Slow titration with aqueous phase was done for each weight ratio of oil and S_{mix} and visual observation was used for transparent, clear and easily flowable microemulsion. The physical state of microemulsion was marked on a pseudo thee component phase diagram with one axis representing the aqueous phase, one representing oil and the third representing a mixture of surfactant and cosurfactant. For each S_{mix} ratio separate phase diagram was constructed (Fig.1-5). Pseudoternary phase diagrams were constructed with Ternary Phase Diagram Software (Chemix School Ver.3.50 software USA.)

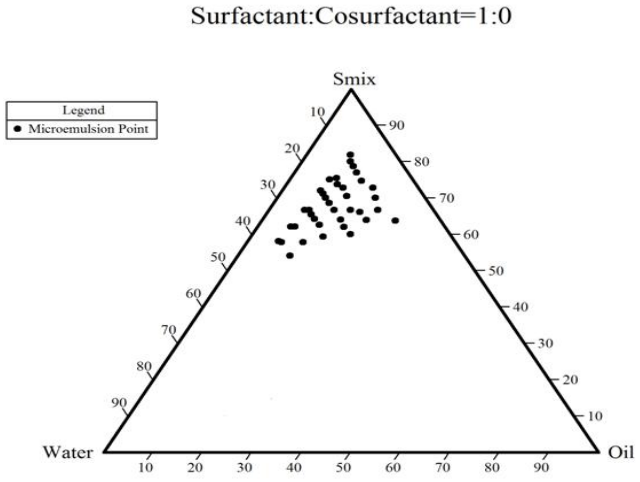

Figure 1. ME region at S_{mix}=1:0

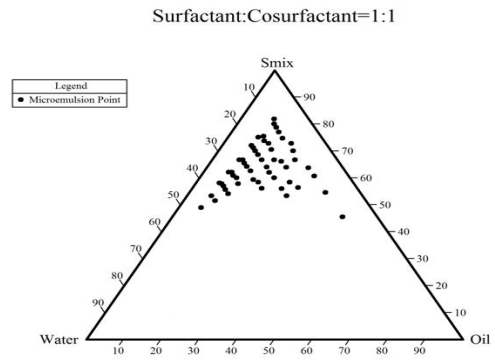

Figure 2. ME region at S_{mix}=1:1

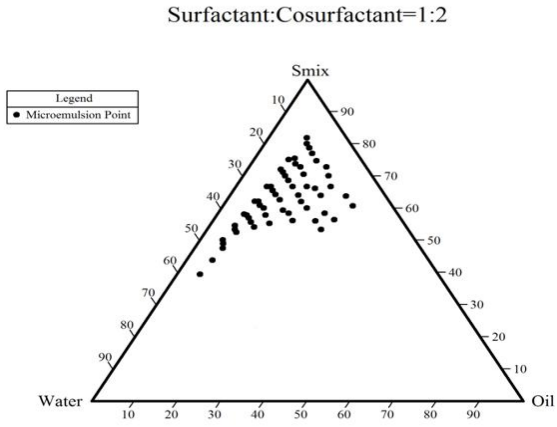

Figure 3. ME region at S_{mix}=1:2

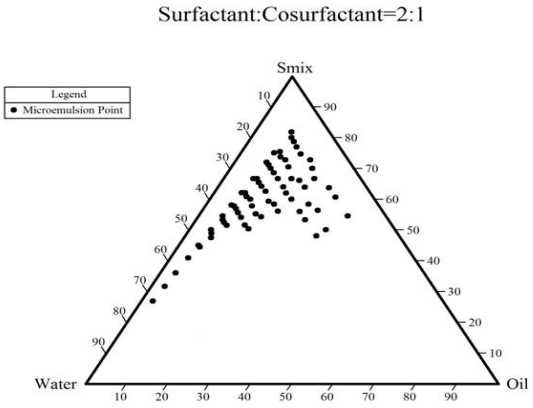

Figure 4. ME region at S_{mix}=2:1

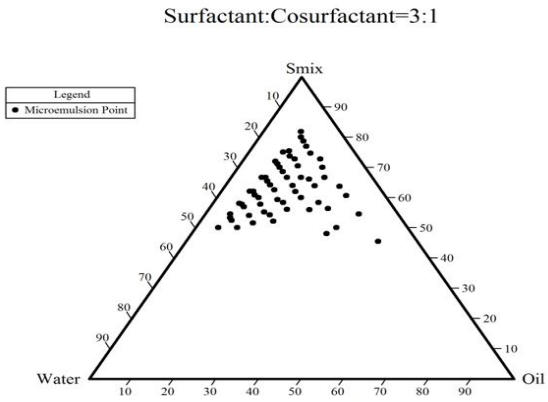

***Figure 5.* ME region at S_{mix}=3:1**

Preparation of Aqueous solution of ACV

For preparation of aqueous solution of ACV, 5 % w/w ACV were weighed and dissolved in distilled water for control and having 5 % and 10 % (w/w). DMSO for comparative studies. Then solutions were then filtered with whatmann filter paper.

Preparation of Microemulsion Formulations

Oil -in- Water (O/ W) liquid microemulsions were prepared by dissolving tween80 (surfactant) in ethanol (co-surfactant). Mixture of tween80 and ethanol were then mixed in the mixture oleic acid: castor oil (3:1) (oil) followed by gentle mixing with double distilled water at room temperature until the system was transparent under vortexing. The monophasic formulations were formed spontaneously at room temperature.

Formulation of drug loaded microemulsions

For the preparation of drug loaded microemulsions, required amount of Acyclovir was dissolved in the oil phase. The required amount of mixture of surfactant and cosurfactant (in case of cosurfactant) was added and double distilled water was then added drop wise drop till a clear and transparent liquid was obtained. Then added 5 % and 10 % (w/w) DMSO separately. The prepared microemulsions were stored in tightly in the suitable container at ambient temperature.

Thermodynamic stability testing of drug loaded microemulsions

To overcome the problem of metastable formulation, thermodynamic stability tests were performed. Selected formulations loaded with drug were centrifuged at 5000 rpm for 30 minutes. The formulations that did not show any phase separations were taken for the heating and cooling cycle. Six cycles between refrigerator temperature (4ºC) and temperature 45 ºC with storage at each temperature of not less than 48 h were done. The formulations, which were stable at these temperatures, were subjected to a freeze-thaw cycle test. The freeze thaw cycles were done for the formulation between –21ºC and 25ºC. The formulations that survived thermodynamic stability tests were selected for further study [11].

Determination of droplet shape and size in microemulsion

The surface morphology of microemulsion droplets were evaluated by the transmission electron microscopy, TEM (Morgangni 268D SEI, USA). Operating at 200 KV and capable of 0.18 nm of point to point resolutions. Combination of bright field imaging at increasing magnification and of diffraction modes were used to reveal the shape and size of the microemulsion. In order to perform the TEM observation the diluted microemulsion was placed on the holey film grid and observed after drying.

The size of oil droplets in microemulsion was determined using a Zetasizer ((1000 HS, Malvern Instruments, UK) at 25ºC at 23º, 30.1º, 62.5º and 90º angle. Because intensity of light is angle dependent since particles scatter light to different angle with different intensities [12].

Data analysis

The cumulative amount of Acyclovir permeated though excised rat skin (Q, µg/ cm^2) was plotted as a function of time. The slope and intercept of the linear portion of the plot was derived by regression. The permeation rate at steady-state (*J*s, µg/ cm^2/h) was calculated as the slope divided by the skin surface area. The intercept on the X-axis was taken as the lag time (*T*L, h).

$$\text{Cumulative amount of drug permeated} = \frac{\text{Concentration (µg/ml)} \times \text{Dilution factor}}{\text{Skin area (cm}^2\text{)}}$$

Flux (*J*s) = slope of the steady state portion of the plot between cumulative amount of the drug permeated Vs time. (*J*s, µg/ cm^2/h)

$$\text{Permeability coefficient} = \frac{Js}{C_{donor}}$$

C_{donor} = Concentration of drug in donor comportment

Enhancement ratio:

ER = Transdermal flux from Microemulsion formulation / Transdermal flux from control

***In-Vitro* skin permeation studies**

In-Vitro skin permeation studies were carried out using Keshary-Chien Franz diffusion cells (Rama Scientific works, Delhi, India) with a diffusional surface area of 0.385 cm^2 and 5.0 ml of receptor cell volume, placed in heating stirring module. The full-thickness rat skin was excised from the abdominal region, and hair was removed with an electric clipper. The subcutaneous tissue was removed surgically, and the dermis side was wiped with isopropyl alcohol to remove adhering fat. The cleaned skin was washed with distilled water and stored in the deep freezer at –21°C until further use. The skin pieces of wistar albino rat [13], were mounted over diffusion cells with the dermal-side in contact with receptor phase and then air bubbles were removed then equilibrated for 2 h. The receptor compartments were filled with phosphate buffered saline (PBS) pH 7.4, containing 10% w/wof DMSO [14]. The receiver fluid was filled in the assembled apparatus the Keshary-Chien Franz diffusion and stirred with a magnetic stirred at a speed of 600 rpm, and the temperature maintained at 37±0.5°C. All the PBS was replaced every 30 minutes to stabilize the skin. It was found that the receiver fluid showed negligible absorbance after 4.5 hours and beyond, indicating complete stabilization of the skin. After complete

stabilization of the skin, 3 g microemulsion was applied on the skin surface in the donor comportment and whole assembly was maintained at 37±0.5 °C and magnetically stirred at 600 rpm [15]. 0.5ml of aliquot was collected from the sampling arm from the receptor comportment at designated time intervals (i.e. 0, 1, 2, 3, 4, 5, 6, 8, 10, 12, and 24 h) for 24 h period and replaced immediately with an equal volume of fresh phosphate buffer saline equilibrated at 37±0.5°C. After appropriate dilution, samples were filtered using 0.45µm membrane filter and the amount of drug in the receptor fluid was analyzed using UV spectrophotometer at λ_{max} 254 nm. The cumulative amounts of drug permeated in µg/cm^2 and the percentage of the cumulative amount permeated after 24 h were calculated. Then flux of every formulation and plain drug ACV was calculated and given in the Table 4.

Statistical Analysis

The data were analyzed statistically by the Repeated Measures Analysis of Variance ANOVA test followed by the least significant difference procedure. This statistical analysis was carried out using GraphPad Prism5.0 Softwares (Inc, San Digeo USA), Graphpad InStat Softwares (Inc, San Digeo, USA). The data from different formulations were compared for statistical significance by Repeated Measures Analysis of variance (ANOVA) (Dunnet multiple comparison test). Differences were considered to be statistically significant when P*< .05, P**< .01. P***< .001, each set of experiments were performed at least three times (n=3).

Results and Discussion

Determination of Drug Solubility and Construction of pseudo ternary phase diagrams

Selection of an appropriate oily phase is very important as it influences the selection of other ingredients and microemulsion, mainly in case of o/w microemulsion. Usually the oil, which has maximum solubilising potential for the selected drug candidates, is selected as oily phase for the formulation of microemulsion. This help to achieve the maximum drug loading in the microemulsion. At the same time ability of the selected oil to yield system with larger microemulsion existed area is also important. It is difficult for the single oily component to amalgamate both the requirements [16].

The choice of oily phase is often a compromise between its ability to solubilise the drug and to facilitate formation of microemulsion of desired characteristics. In certain case, mixture of oils is also used to meet both requirements. After performing solubility study in different oils and its combinations, it was found that Acyclovir exhibited maximum solubility in mixture of oils Oleic acid and Castor oil (3:1) (45.53 mg/ml), carbitol (88.21±5.00 mg/ml) and ethanol (92.32±6.20 mg/ml) shown in Table 1. Therefore Oleic acid:Castor oil (3:1) was chosen as the oil phase, carbitol as surfactant and ethanol as cosurfactant respectively.

Screening of surfactants

Choice of surfactant is critical for the formulation of microemulsion. The surfactant should favour microemulsification of the oil phase and should also possess good solubilising potential for the drug. It should be noted that the surfactants are not innocuous. The factor must be considered while choosing a type & concentration of surfactant. Generally, surfactants of natural origin are preferred over synthetic surfactants. Low HLB surfactant such as sorbitan monostearate (HLB, 4.3) are preferred for w/o microemulsion where as high HLB surfactant such as tween 80 (HLB, 15.0), tween 20 (HLB, 16.7) are preferred for o/w microemulsion. Taking carbitol as cosurfactant different surfactants (tween 20, tween 80, span 80) were titrated with water in sixteen different ratios (1:9, 1:8, 1:7, 1:6, 1:5 1:4, 1:3.5, 1:3, 1:2.3, 1:2, 1:1.5, 1:1, 6:4, 7:3, 8:2 and 9:1) of oil to S_{mix}, so that maximum area could be covered for the study to delineate the boundaries of the phases formed precisely in the phase diagram. Slow titration with aqueous phase was done for the each weight ratio of oil and S_{mix} and visual

observation was used for the transparent, clear and easily flowable microemulsion. The physical state of microemulsion was marked on a pseudo thee component phase diagram with one axis representing the aqueous phase, second representing oil phase and third representing a mixture of surfactant and cosurfactant at fixed weight ratio (S_{mix} ratio). On the basis of constructed pseudoternary phase diagram using tween 80 and carbitol, tween 20 and carbitol and span 80 and carbitol, it was clear that microemulsion region in the pseudoternary phase diagram were decreasing in order respectively, because tween 80 had greater oil mixture (oleic acid: castor oil) solubilising power than tween 20 and span 80. It was clear after phase diagram study that phase diagram having tween 80 showed largest microemulsion area, therefore tween 80 was selected as surfactant

Screening of Cosurfactants

Most of the times, surfactant alone cannot lower the oil-water interfacial tension sufficiently to yield a microemulsion which was necessities addition of an amphiphilic short chain molecules or cosurfactant, bring about the surface tension close to zero. Short chain length ranging from C_2 – C_{10} & amphiphilic nature of these agents enables them to interact with surfactant monolayer at the interface thereby affecting their packing. Liquid crystalline phase are formed when the surfactant film is too rigid.
Cosurfactants penetrate into surfactant monolayer providing additional fluidity to interfacial film and thus disrupting the liquid crystalline phases. Furthermore cosurfactants also distributed themselves between aqueous and oily phase, thereby altering the chemical composition & hence relative hydro/lipophilicity of the system.

Short chain amphiphilic nature of ethanol enables formulation of microemulsion or with a variety oily phase and surfactant [17]. Titration was done by using tween 80 as surfactant with different cosurfactants (Ethanol, Carbitol, Propylene Glycol, and PEG 400). The ratio of surfactant and cosurfactant were kept constant 1:1.while oil to S_{mix} ratio was 1:9, 1:8, 1:7, 1:6, 1:5 1:4, 1:3.5, 1:3, 1:2.3, 1:2, 1:1.5, 1:1, 6:4, 7:3, 8:2 and 9:1 by considering that the higher concentration of surfactant or S_{mix} is favourable for maximum microemulsion formation . After studying the result of pseudoternary phase diagram microemulsion region (covered area), it was found that maximum microemulsion region or points were obtained with ethanol and hence ethanol was chosen as cosurfactant for microemulsion formulation. It is advantageous to select ethanol as cosurfactant is that ethanol is a cosolvent. .

Preparation of Microemulsion Formulations

For the preparation of drug loaded microemulsions, required amount of Acyclovir was dissolved in the oil phase. The required amount of mixture of surfactant and cosurfactant (in case of cosurfactant) was added and double distilled water was then added drop wise drop till a clear and transparent liquid was obtained. Then added 5% and 10% (w/w) DMSO separately. The prepared microemulsions were stored in tightly in the suitable container at ambient temperature. The composition of aqueous solution of ACV and ACV microemulsion without DMSO or with DMSO were shown in Table.3.

Table.3: Compositions of the Selected Microemulsion Formulations

Formul-ations Code	Oleic acid + Castor oil (3:1) (% w/w)	Tween 80 + Ethanol (S_{mix}) (% w/w)	Double Distilled Water (%w/w)	Drug (ACV) (%w/w)	DMSO (%w/w)
PD	-	-	95	5	-
PD5D	-	-	90	5	5
PD10D	-	-	85	5	10
ME1	4.96	45.01	50.03	5	-
ME2	6.12	48.93	44.95	5	-
ME3	7.49	53.43	40.08	5	-
ME4	4.55	40.90	54.55	5	-
ME5	3.51	31.58	64.91	5	-
ME6	6.67	53.33	40.00	5	-
ME10D	4.96	45.01	40.03	5	10

Thermodynamic stability testing of drug loaded microemulsions

Thermodynamic stability testing was done to overcome the problems to identify the deference

between stable microemulsions and metastable microemulsion. Freeze thaw cycles were done to check the stability at low temperature and centrifugation studies to check stability at higher shear. All microemulsion formulations subjected to the above studies should returned to their original form when subjected to freeze thaw cycle and did not show any sign of turbidity and phase separation on high speed centrifugation. All selected microemulsions did not show any phase separation, turbidity, and change in colour & drug precipitation. Selected thermodynamically stable microemulsion formulations were carried out for further study of *in-vitro* permeation study.

Determination of droplet shape and size in microemulsion

The surface morphology of microemulsion droplets were evaluated by the transmission electron microscopy which was shown in the Figure.6. Microemulsion was a colloidal dispersion .The drug loaded microemulsion oily phase droplets shapes were found to be spherical. The mean droplet size of oil phase loaded with ACV of the optimized microemulsion formulation was found to be in range of ME1 46.29 to 57.10 nm, where as macroemulsion droplet size was found to be in range of 659 to 781nm. The droplet size increased with the increase in concentration of oil in the formulations [18]. The droplet size was also depending on the concentration of S_{mix}, water content, viscosity of the microemulsion**.** Lower the viscosity of microemulsion smaller the droplet size and this is due to effect of S_{mix} because it lower the interfacial tension closed to zero, smaller the droplet size containing microemulsion have a greater stability and transdermal flux.

The droplet size distribution of microemulsion was determined by Malvern Zetasizer(1000 HS, Malvern Instruments, UK). The mean size of droplet of microemulsion ME1 was shown also in Malvern zetasiser size distribution report was also found 49.53nm shown in Figure 7.

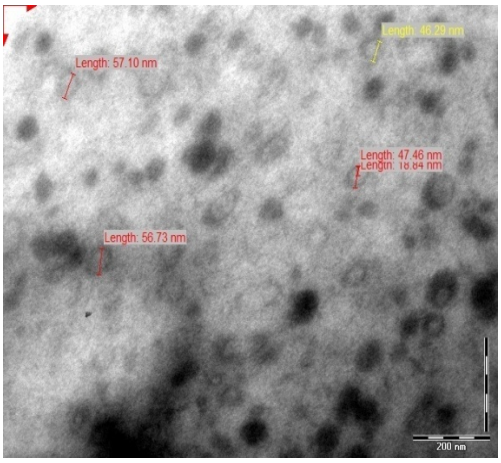

Figure 6: TEM image of Microemulsion

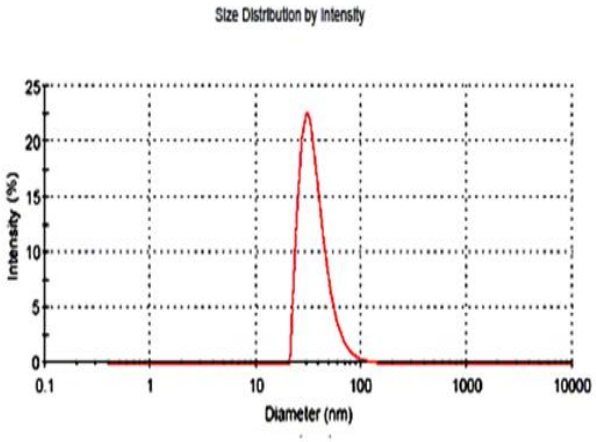

Figure 7.Zetasizer droplet size analysis report

***In-Vitro* skin permeation studies**

All experiments and protocols described in this study were approved by the Institutional Animal Ethics Committee of Bundelkhand University, Jhansi (Approval No. BU/ Pharm/ IAEC/ 2008/ 002) and are in accordance with the Committee for Purpose of Control and Supervision of Experiments on Animals, Ministry of Social Justice and Empowerment, Government of India. The permeation capability of the selected microemulsion formulations and aqueous solution of ACV were evaluated by conducting the in-vitro skin permeation experiments with keshary chein frenz diffusion cell. The permeation parameters, transdermal flux, permeability coefficients and cumulative amount of ACV permeated of selected microemulsion and control formulations were

presented in Table.4 and Figure.8 respectively. The transdermal flux of plain ACV PD was too less, this may be due to large content of drug may have substantially reduced the partition coefficient between the skin and the vehicle for the drug, which can counteract the benefit of the increased concentration gradients effect, and thereby actually decrease the transdermal flux [4]. Aqueous solution of ACV having 10% DMSO PD10D, shown the transdermal flux 119.76 µg/cm^2/h which was greater than two time more transdermal flux at 5 % DMSO concentration PD5D 50.75 µg/cm^2/h. Oleic Acid is advantageous to use as oily phase because it increase skin permeability by two mechanistic scenarios of the enhancer; (a) lipid fluidization, and (b) lipid phase separation [19],

Oleic acid is a model skin permeation enhancer [20], Oleic acid facilitates penetration into the skin by disrupting the fluidity of the stratum corneum [21]. The thermodynamic activity of drug in the formulation is a significant driving force for the release and penetration of the drug into skin [22].
Many different theories concerning the mechanism of action of penetrates have appeared in the literature. One of them attributes the penetrant effects of DMSO, dimethylformamide, and dimethylacetamide to their hygroscopic properties. Organic solvents like benzene, alcohol, and ether, which have been shown to enhance the penetration rate of both water-soluble and lipid-soluble substances, may act by removing the lipids from the stratum corneum. However, the action of hydrogen-bonding solvents like DMSO, dimethylformamide, and dimethylacetamide is attributed to membrane expansion and uniform increase in media diffusivity and thus enhances the transdermal flux**.** The increased flux of ME1+10%DMSO (ME10D) was found to be 266.6721 µg/cm^2/h, In comparison with ME1 (238.1432 µg/cm^2/h) (Fig. 9).Which is reported that DMSO enhances the transdermal flux of poorly water soluble drugs[4]. Short-chain alkanols are widely used as permeation enhancers. Ethanol is very common among transdermal formulations and its addition is known to enhance the flux of several drugs. Sometimes when using ethanol-water-based vehicles, the effect of ethanol is concentration-dependent and therefore, under certain conditions it can even decrease the permeation. Various mechanisms have been suggested for the enhancing activity of ethanol. It can increase the drug solubility in the vehicle or it can alter the structure of the membrane and increase the permeability of the drug. Another mechanism is based on the fact that ethanol is volatilized from the applied formulation and, consequently, increases the drug concentration to a supersaturated state with a greater driving force for permeation. In addition, ethanol may extract some of the lipid fraction from the stratum corneum and, thus can improve the drug flux though it [23].

Table.4*:* In-vitro parameters of Aqueous solution of plain drug ACV, Aqueous solution of plain drug ACV+5% (w/w) DMSO (PD5D), Aqueous solution of plain drug ACV+10% (w/w) DMSO (PD10D) microemulsion (ME), ME+10% (w/w) DMSO (ME10D) Formulation.

Formul- ation Code	Flux (J) (µg/cm^2/h)	Perme-ability Coefficient (K_p) $\times 10^{-2}$ (cm/ h)	Enhancement ratio with PD
PD	2.47±0.76	0.014	--------
PD5D	50.7529±1.21	0.203	20.4814
PD10D	119.7691±5.42***	0.718	48.3329
ME1	238.1432±4.82***	1.428	96.1029
ME2	127.6±2.82*	0.7656	51.4931
ME3	110.0 ±4.14***	0.66	44.3906
ME4	98.65±3.37**	0.5919	39.8103
ME5	153.2±4.81***	0.9192	61.8240
ME6	90.01±4.26	0.54006	36.3236
ME10D	266.6721±8.49***	1.600	107.6159

Values are mean ± SD, n=3, P* <0.05, P** <0.001, P*** <0.0001, As compared to control (PD), Repeated Measures Analysis of Variance ANOVA (Dunnet multiple comparison test).

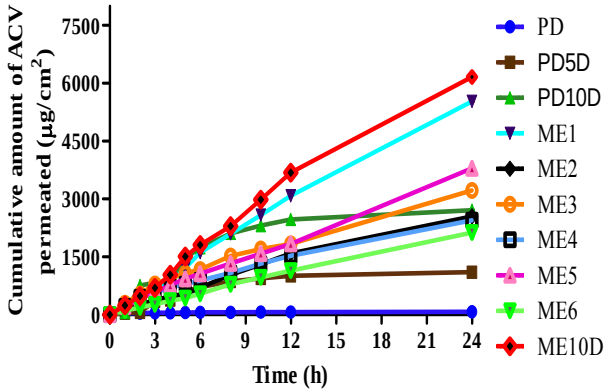

Fig 8. Cumulative amount of ACV permeated from microemulsion formulations through rat skin

Ethanol plays an important role in permeation of ACV though rat skin. Ethanol is model penetration enhancer. Ethanol can exert its permeation enhancing activity though various mechanisms. Firstly, as a solvent, it can increase the solubility of the drug in the vehicle, although at steady state the flux of a permeant from any saturated, non-enhancing, vehicle should be equivalent. However, for poorly soluble permeants that are prone to depletion within the donor during a steady state permeation study, then ethanol can increase permeant solubility in the donor phase [24].Liquid crystalline phase are formed when the surfactant film is too rigid. Ethanol penetrates into surfactant monolayer providing additional fluidity to interfacial film and thus disrupting the liquid crystalline phases. Furthermore, cosurfactants also distributed themselves between aqueous and oily phase, thereby altering the chemical composition & hence relative hydro/ lipophilicity of the system. It has been used to enhance the flux, drug like levonorgestrel, estradiol, hydrocortisone and 5-fluorouracil though rat skin [1].

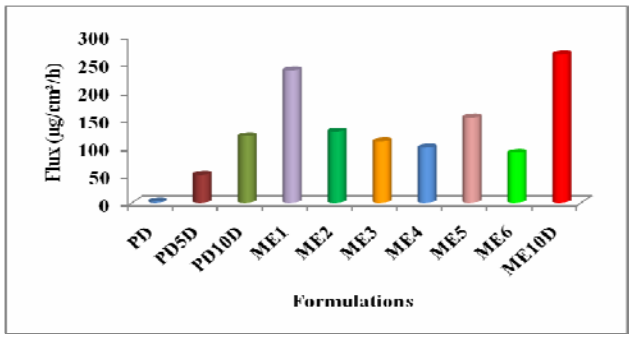

Figure 9. Flux of different formulations

Dimethylsulphoxide (DMSO) is one of the earliest and most widely studied penetration enhancers. It is a powerful aprotic solvent which hydrogen bonds with itself rather than with water; it is often used in many areas of pharmaceutical sciences as a ‘‘universal solvent’’. DMSO is used as a co-solvent in a vehicle for a commercial preparation of idoxuridine, used to treat severe herpetic infections of the skin, particularly those caused by herpes simplex. DMSO alone has also been applied topically to treat systemic inflammation, although currently it is used only to treat animals. A vast array of literature describes the penetration enhancing activities of DMSO, and studies have shown it to be effective in promoting both hydrophilic and lipophilic permeants. Thus, it has been shown to promote the permeation of, for example, antiviral agents, steroids and antibiotics. DMSO works rapidly as a penetration enhancer-spillage of the material onto the skin can be tasted in the mouth within seconds. Although DMSO is an excellent accelerant it does create problems. The effects of the enhancer are concentration dependent. However, at >60% relatively high concentrations DMSO can cause erythema and wheals of the stratum corneum and may denature some proteins. Studies performed over40 years ago on healthy volunteers painted with 90%DMSO twice daily for 3 weeks resulted in erythema, scaling, contact uticaria, stinging and burning sensations and several volunteers developed systemic symptoms [25].

DMSO is widely used to denature proteins and on application to human skin has been shown to change the intercellular keratin confirmation, from a helical to β sheet [26, 27]. As well as an effect on the proteins, DMSO has also been shown to interact with the intercellular lipid domains of human stratum corneum. Considering the small highly polar nature of this molecule it is feasible that DMSO interacts with the head groups of some bilayer lipids to distort to the packing geometry. Further, DMSO within skin membranes may facilitate drug partitioning from a formulation into this ‘‘universal solvent’’ within the tissue.

The flux of DMSO having microemulsion ME10D was found 266.6721±8.49µg/cm^2/h which showed highest value, and significantly very higher than the controls (PD & PD10D), the decrease in the flux of PD (compared with microemulsion ME10D) might be due to change in the water content, viscosity of aqueous solution of ACV.

The skin permeation profile of microemulsion PD10D, ME1 and ME10D was significantly different when compared with that of PD, P* <0.05, P** <0.002 and P** <0.002 respectively.

The significant difference in acyclovir permeation between microemulsion formulations, PD and ME10D was probably due to the mean size of internal phase droplets, which were significantly smaller in microemulsions and effect of DMSO penetration enhancer.

Conlusion

We have shown that DMSO and Ethanol acts as a penetration enhancer for the transdermal delivery of ACV and skin flux of the drug could be enhanced up to 107 fold compared to its aqueous solution. Increased percentage of DMSO 10% as compared to 5% in aqueous solution enhanced transdermal flux 2.36 fold greater. The skin flux of the drug is dependent on the concentration of water, DMSO and ethanol in microemulsion. Microemulsion is safe to the skin at 10% DMSO w/w.

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