



## Research Article

## 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 through 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 266.6721  $\mu\text{g}/\text{cm}^2/\text{h}$ . The flux of microemulsion formulation ME10D was found 266.6721  $\pm$  8.49  $\mu\text{g}/\text{cm}^2/\text{h}$ . 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 through the skin is always a difficult problem because of barrier 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 outside 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 microemulsion was first introduced by Hoar and Schulman in 1940 [7] and the term microemulsion was first carried by Schulman and coworkers in 1959 to describe 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<sup>934</sup> 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 throughout 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 through a 0.45- $\mu$ m membrane filter. The concentration of ACV was determined spectrophotometrically at 254 nm.

To find out suitable oils that have good solubilizing 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 cosurfactants separately in 5ml capacity stoppered vials and mixed by continuously stirred for 72 h. The mixture vials were then kept at  $37 \pm 0.5$  °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  $\lambda_{\max}$  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 Oil(1:1)   | 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

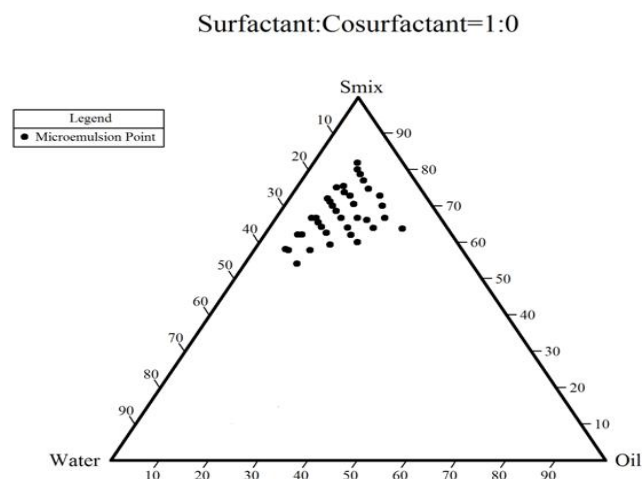
| S.No. | Surfactants / Cosurfactants | Solubility (mg/ml) |
|-------|-----------------------------|--------------------|
| 1.    | Tween80                     | 79.0 ± 5.00        |
| 2.    | Tween20                     | 1.37 ± 0.50        |
| 3.    | Span80                      | 30.00 ± 2.0        |
| 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 ( $S_{\text{mix}}$ ) were

mixed in different weight ratios (1:0, 0.5:1, 1:1, 2:1 and 3:1). These  $S_{\text{mix}}$  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  $S_{\text{mix}}$  were mixed well in different ratios. Sixteen different combinations of oil and  $S_{\text{mix}}$  (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  $S_{\text{mix}}$  and visual observation was used for transparent, clear and easily flowable microemulsion. The physical state of microemulsion was marked on a pseudo three 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  $S_{\text{mix}}$  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  $S_{\text{mix}}=1:0$

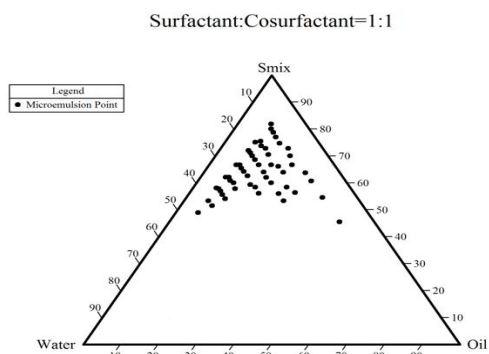


Figure 2. ME region at  $S_{mix}=1:1$

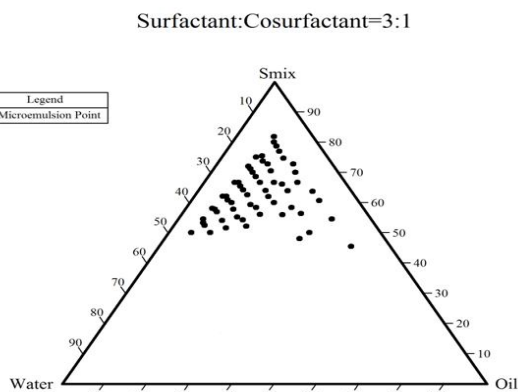


Figure 5. ME region at  $S_{mix}=3:1$

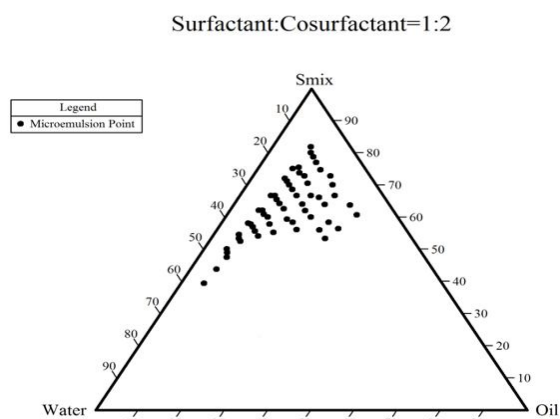


Figure 3. ME region at  $S_{mix}=1:2$

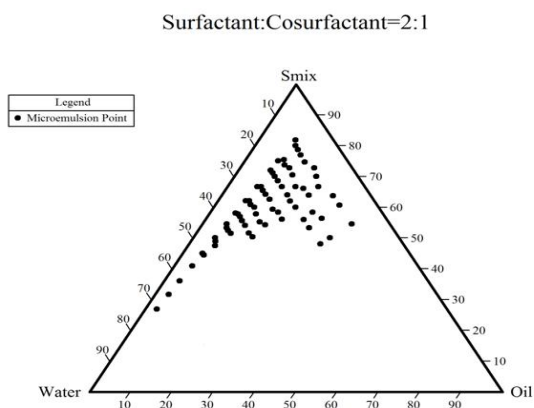


Figure 4. ME region at  $S_{mix}=2:1$

### 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 (4°C) and temperature 45 °C 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 -21°C and 25°C. 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 25°C at 23°, 30.1°, 62.5° and 90° 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 through excised rat skin ( $Q$ ,  $\mu\text{g}/\text{cm}^2$ ) 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

( $J_s$ ,  $\mu\text{g}/\text{cm}^2/\text{h}$ ) was calculated as the slope divided by the skin surface area. The intercept on the X-axis was taken as the lag time ( $TL$ , h).

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

Flux ( $J_s$ ) = slope of the steady state portion of the plot between cumulative amount of the drug permeated Vs time. ( $J_s$ ,  $\mu\text{g}/\text{cm}^2/\text{h}$ )

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

$C_{\text{donor}}$  = Concentration of drug in donor compartment

### 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  $\text{cm}^2$  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 compartment and whole assembly was maintained at  $37 \pm 0.5$  °C and magnetically stirred at 600 rpm [15]. 0.5ml of aliquot was collected from the sampling arm from the receptor compartment 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  $37 \pm 0.5$  °C. After appropriate dilution, samples were filtered using 0.45 $\mu$ m membrane filter and the amount of drug in the receptor fluid was analyzed using UV spectrophotometer at  $\lambda_{\text{max}}$  254 nm. The cumulative amounts of drug permeated in  $\mu\text{g}/\text{cm}^2$  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 Diego USA), Graphpad InStat Softwares (Inc, San Diego, 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 ( $88.21 \pm 5.00$  mg/ml) and ethanol ( $92.32 \pm 6.20$  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  $S_{\text{mix}}$ , 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  $S_{\text{mix}}$  and visual

observation was used for the transparent, clear and easily flowable microemulsion. The physical state of microemulsion was marked on a pseudo three 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**

| Formulations 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 zetasizer size distribution report was also found 49.53nm shown in Figure 7.

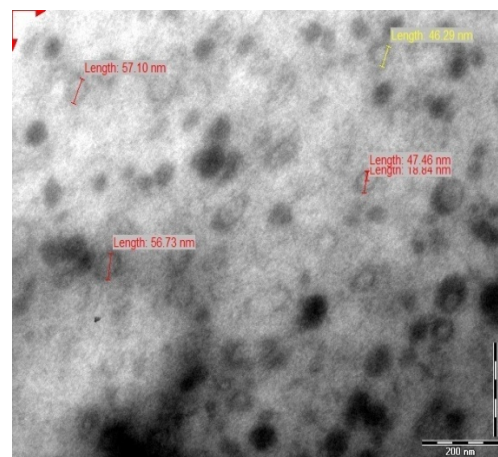


Figure 6: TEM image of Microemulsion

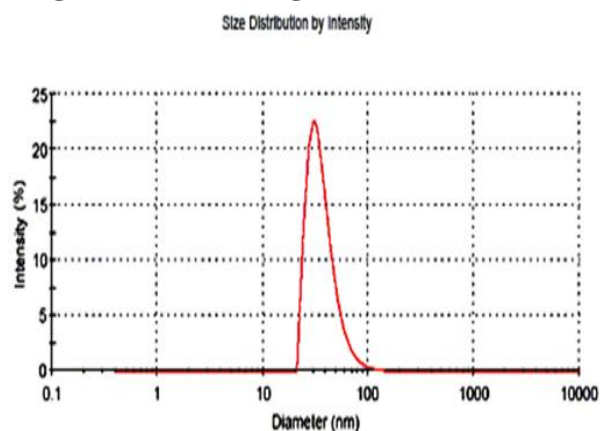


Figure 7. Zetasizer droplet size analysis report

#### *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  $\mu\text{g}/\text{cm}^2/\text{h}$  which was greater than two time more transdermal flux at 5 % DMSO concentration PD5D 50.75  $\mu\text{g}/\text{cm}^2/\text{h}$ . 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],

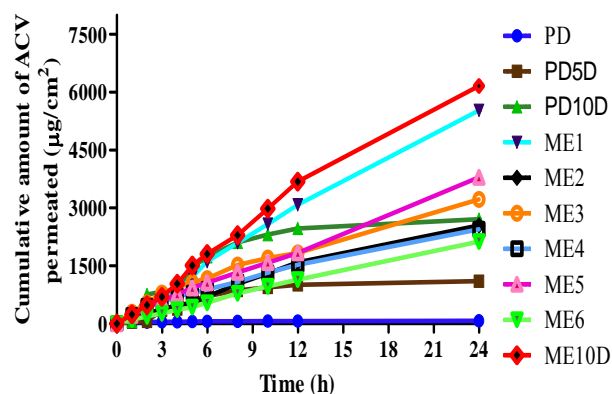
Oleic acid is a model skin permeation enhancer [20], Oleic acid facilitates penetration into the skin by disrupting the fluidity of the stratum corneum [21]. The thermodynamic activity of drug in the formulation is a significant driving force for the release and penetration of the drug into skin [22]. 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  $\mu\text{g}/\text{cm}^2/\text{h}$ , In comparison with ME1 (238.1432  $\mu\text{g}/\text{cm}^2/\text{h}$ ) (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.**

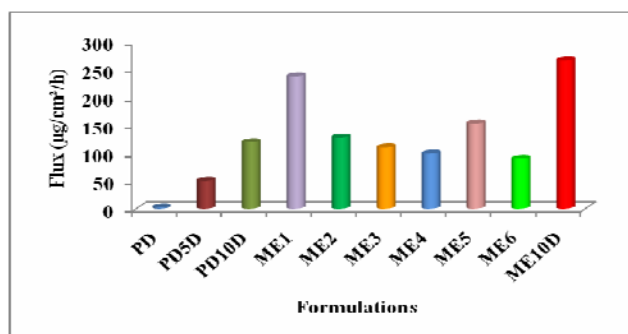
| Formulation Code | Flux (J) ( $\mu\text{g}/\text{cm}^2/\text{h}$ ) | Permeability Coefficient ( $K_p$ ) $\times 10^{-2}$ (cm/ h) | Enhancement ratio with PD |
|------------------|---|---|---------------------------|
| PD               | 2.47 $\pm$ 0.76                                 | 0.014   | -----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

Values are mean  $\pm$  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 through rat skin. Ethanol is model penetration enhancer. Ethanol can exert its permeation enhancing activity through 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 through 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 over 40 years ago on healthy volunteers painted with 90% DMSO twice daily for 3 weeks resulted in erythema, scaling, contact urticaria, 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 conformation, from a helical to  $\beta$  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  $266.6721 \pm 8.49 \mu\text{g}/\text{cm}^2/\text{h}$  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.

### Conclusion

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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