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DEVELOPMENT AND CHARACTERIZATION OF TRANSDERMAL MICROEMULSION GEL FOR AN ANTIVIRAL DRUG

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ABSTRACT

The objective of this study was to design and develop o/w microemulsion for transdermal delivery of poorly water soluble acyclovir by aqueous titration method. Oleic acid: castor oil (3:1), tween 80, and ethanol were selected as oily phase, surfactant and cosurfactant respectively. The Pseudoternary phase diagrams were constructed by aqueous titration method. The cosurfactant affect the shape and extant of microemulsion regions. Ethanol (cosurfactant) is expected to disorder the interfacial film gave extended microemulsion zones by destabilizing the liquid crystalline phase. Largest Microemulsion single phase region was found at S_{mix} (2:1) than the system at other S_{mix}. Characterization of microemulsion were done for droplet Shape and size, refractive index, pH, Viscosity, drug loading capacity. The mean droplet size of microemulsion was found below 50 nm. The maximum solubility of ACV in microemulsion system was found to be 47.4 mg/ml. The ex-vivo skin permeation studies were done using skin of Wistar albino rat by Franz diffusion cell, and microemulsion formulation MEC1 exhibited highest flux, was found to be 238.1±4.87 µg/cm^2/hr, while flux of MEGel, aqueous solution and conventional emulsion of ACV were found to be 230.40±6.23 µg/cm^2/hr, 2.47±0.76 µg/cm^2/hr and 8.65 ±1.21 µg/cm^2/hr respectively. The pharmacokinetic parameters of MEGel after topical application to the Wistar albino rat skin were significantly different from those of ACV in aqueous solution (PD) and conventional emulsion (CE). It can be concluded that microemulsion of ACV prepared with Oleic acid: castor oil (3:1) as oily phase, tween80 as surfactant, and ethanol as cosurfactant can be used as transdermal drug carrier for this and other poorly water soluble drug.

INTRODUCTION: The programmed released capability of the conventional dosage form like oral and parenteral are limited and undesired side effects may occurs of their applications. These problems may be overcome by using transdermal drug delivery system. TDDS are aimed for systemic action, controlling the rate of delivery and modulating distribution of drug in the systemic circulation [1]. The fundamental of a successful formulation are to deliver the active substance at target organ with minimal discomfort and side effect. In this respect, transdermal route excels because of avoidance of hepatic first pass metabolism, typical peak trough plasma profile, easy of application etc. [2].

Microemulsion appears to have the ability of deliver larger amount of topically applied agents into the mucosa than the traditional gel & creams. Microemulsions are defined as thermodynamically stable transparent, single optically isotropic liquid system of water, oil and surfactants frequently in combination with suitable cosurfactants [3, 4]. Microemulsions are known to enhance the bioavailability of drugs via topical and systemic routes [5]**.** The use of a microemulsion gel as vehicle may enhance transdermal penetration by various mechanism, many molecules or solubilised 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 the functional barrier of stratum corneum. This latter function may be more or less important depending on the nature of the surfactant used [6].

Acyclovir absorption in the gastrointestinal tract is slow, variable, and incomplete [7]. The bioavailability of acyclovir after oral administration ranges from 10% to 20% [8.] Approximately 80% of an oral dose is never absorbed and is excreted through the faeces. The main excretory organ for acyclovir is the kidney. The plasma half-life of oral Acyclovir on average is 3 hours in adults with normal renal function Acyclovir is categorized as a class III drug according to the Biopharmaceutical Classification System (BCS) because of its high solubility and low permeability [9].

The objectives of this study was to formulate transdermal delivery system of poorly water soluble ACV without chemical modification and to enhance its flux through rat skin. In present study, we selected the oleic acid: castor oil (3:1), tween 80 and ethanol as oily phase, surfactant and cosurfactant respectively. The effects of cosurfactant were evaluated on flux through. Characterization of microemulsion was done for droplet size, pH, and viscosity determination. Pharmacokinetic parameters of ACV, conventional emulsion and microemulsion loaded with ACV after topical administration were also evaluated.

MATERIALS AND METHODS: Acyclovir was obtained as gift sample from Cipla Ltd. Mumbai, India. Oleic acid, castor oil, Tween 80, Isopropyl meristate, Methanol, Triacetin, Tween 20, Transcutol P, Acetonitile, octanol, Corbitol, Olive oil was purchased from CDH, Delhi, Ethanol (Qualigens fine chemicals) was obtained from department chemical store Bundelkhand University Jhansi (U.P). Corbopol[934] from Lova, New Delhi, DMSO from CDH, Mumbai & Rankem, New Delhi. Wistar albino rat skins were obtained from Animal

House of Institute of Pharmacy, Bundelkhand University, Jhansi (UP), 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. Acyclovir was analyzed using FTIR spectrophotometer (Perkin Elmer BX, UK). Surface morphology of Microemulsion was performed using Transmission Electron Microscopy (TEM) (Morgangni 268D SEI, USA). Microemulsion droplet size was determined using Malvern Zetasizer (1000 HS, Malvern Instruments, UK), the deionised water was used as dilution media. Franz diffusion cell (Rama scientific work New Delhi, India), the λ_{max} was determined using UV 1700 (Shimadzu UV 1700, Japan).

Screening of Oils, Surfactant and Cosurfactants: To find out appropriate oils that have good solubilising capacity of ACV and, thus, can be used as the oil phase in microemulsion, the solubility of ACV in various oils was measured. The solubility of ACV in various oily phase (oleic acid, castor oil, triacetine, olive oil, Sun flower oil, capmul oil, IPM, and mixture of the above oils), surfactants (tween 80, tween 20, span 80, and cosurfactants (Corbitol, Ethanol, propylene glycol, PEG 400) was determined by dissolving excess amount of ACV in 2.0 ml of each of selected oils, surfactants and cosurfactants in 5ml capacity stoppered vials separately and mixed by continuously stirred for 72 hr. The mixture vials were then kept at 37 ± 0.5 ^{0}C in an isothermal shaker (Jyoti Instrument Industry M.P., India) for 72 hr 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 at λ_{max} 254 nm. Solubility of ACV in oils phase and surfactant and cosurfactant was shown in Table 1 & 2 respectively.

TABLE 1: SOLUBILITY OF ACYCLOVIR IN DIFFERENT OILS

OILS	SOLUBILITY(MG/ML)
Olive oil	3.48 ± 0.20
Oleic Acid	9.73 ± 1.50
Oleic Acid + IPM(1:1)	8.57 ± 2.00
Oleic Acid + Olive Oil1:1)	3.48 ± 1.00
Oleic Acid + Castor oil(3:1)	45.53 ±1.50
Oleic Acid + Castor oil(1:2)	35.00 ±2.00
Oleic acid + Castor oil(1:4)	40.53 ±2.10
OA+OO + Castor oil(1:1:1)	11.28 ±1.00
Oleic Acid +DMSO(5-6drops)	26.07 ±2.00
Capmul oil	1.37 ±0.50
Triacetin	0.33 ±0.10
Triacetin + IPM(1:1)	1.10 ±0.50
IPM	1.95 ±0.05
Castor oil+ IPM(3:1)	3.75 ±2.0
Sun flower oil	15.26 ±2.40
Sun flower oil + Oleic Acid(1:1)	8.76 ±1.30
Castor oil + SFO(1:1)	4.00 ±0.40
Castor oil + SFO(2:1)	8.96 ± 1.81

TABLE 2: SOLUBILITY OF ACYCLOVIR IN DIFFERENT SURFACTANTS AND COSURFACTANTS

SURFACTANTS	SOLUBILITY (MG/ML)
Tween80	79.0±5.00
Tween20	1.37±0.50
Span80	30.00±2.0
Corbitol	88.21±5.00
Propylene Glycol	11.28±2.00
PEG400	3.19±0.50

Construction of Phase Diagrams: Pseudoternary phase diagrams were constructed to examine the formation of oil in

water microemulsions using four components: oil, surfactant, cosurfactant, and aqueous phase. The four component system consisted of (1) mixture of oleic acid and castor oil in ratio (3:1) as oil phase, (2) tween 80 as surfactant (3) ethanol as cosurfactant and (4) double distilled water as aqueous phase. The pseudoternary phase diagrams were constructed by instillation of homogenous liquid mixtures of oil, surfactant, and cosurfactant, with water at ambient temperature**.** At desired S_{mix} (1:0, 1:1, 1:2, 2:1, 3:1), oily mixtures of oil, surfactant and cosurfactant were prepared. The ratio of oil to the mixture of surfactant and cosurfactant was varied from 9:1 to 1:9. Water was added drop by drop under gentle stirring to each liquid mixture [10]. If turbidity appeared followed by a phase separation, the samples were considered to be biphasic. If clear and transparent mixtures were visualized after stirring, the samples were considered monophasic. The samples were marked as points in the phase diagram. The area covered by these points was considered to be the microemulsion region of existence [7].

Preparation of Microemulsion Formulation: Microemulsions were prepared by dissolved Acyclovir in mixture of oleic acid: castor oil (3:1), and then it mixed with the mixture of tween 80 and ethanol, 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. The final concentration of acyclovir in the microemulsions was 5%.

Formulation Selection: For each phase diagram constructed, different formulations were selected from microemulsion region so that ACV could be incorporated into oil phase 5% w/v of ACV was dissolved oil phase of microemulsion formulation, which was kept constant in all the selected formulation. The selected formulations were subjected to thermodynamic stability studies [11].

Preparation of Conventional Emulsion and Aqueous solution of ACV: Weighed required amount of oil (castor oil), water, and gum acacia (4:2:1) for primary emulsion. Transferred weighed amount of oil in dry mortar and added calculated quantity of gum acacia powder and calculated amount of ACV triturated rapidly to form a uniform mixture. Added required quantity of water and triturate vigorously till a clicking sound is produced and product becomes white and then added remaining water to produce the required volume.

Formulation of Optimized Microemulsion into Microemulsion Gel: On the basis of drug permeation studies, the optimized microemulsion formulation which showed the highest permeation profile, was selected and formulated into gel by the use of 1% w/w Carbopol^{934} which shows better consistency, weighed amount of Carbopol^{934} was soaked in the microemulsion, stirred to dispersed Carbopol^{934} in the microemulsion and left over night to obtained a gel of desirable viscosity with high thickening capability and compatibility with the microemulsion. $Carbopol_{934}$ did not caused any precipitation or alter the microemulsion droplet size.

Physical Stability Studies: To overcome the problem of metastable formulation, thermodynamic stability tests were performed.

Selected formulations loaded with drug were centrifuged at 3500 rpm for 30 minutes [11]. 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 45°C with storage at each temperature of not less than 48 hr were done. The formulations, which were stable at these temperatures, were subjected to a freeze-thaw cycle test. Three 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 [12]**.** Composition of these formulations is given in table 3.

TABLE 3: COMPOSITION OF SELECTED MICROEMULSION FORMULATION

MICROEMULSION	OLEIC ACID + CASTOR OIL (3:1) (% w/w)	TWEEN 80 + ETHANOL (S_{MIX}) (% w/w)	SURFACTANT/COSURFACTANT (S_{MIX})	DD WATER (% w/w)	DRUG (ACV) (mg)
MEA1	8.33	75.00	1:0	16.67	50
MEA2	9.43	75.47	1:0	15.09	50
MEB1	6.06	54.55	1:1	39.39	50
MEB2	6.12	48.93	1:1	44.95	50
MEC1	5.00	45.00	1:2	50.00	50
MEC2	6.12	48.93	1:2	44.95	50
MED1	5.00	45.00	2:1	45.00	50
MED2	4.55	40.90	2:1	54.55	50
MEE1	6.06	54.55	3:1	39.39	50
MEE2	5.56	50.00	3:1	44.44	50

Characterization of Microemulsion:

Droplet size determination: The droplet size of microemulsion formulations were determined by Photon correlation spectroscopy that analyzed the fluctuations in light scattering due to Brownian motion of the particles [13] using a Zetasizer (1000 HS, Malvern Instruments, UK). The microemulsion formulation sample were diluted to 1:2000 and prepared in filtered deionised water in dilution range of 1:2000. Few drop of diluted sample was then added to a glass cuvette containing filtered deionised water, positioned between the laser light and detector for checking light intensity in the range of 50, 000 to 1, 00, 0000 of the dispersed particles. Light scattering was monitored at 25 °C at a 23.0°, 30.1°, 62.5° and 90° angle. Because intensity of light is angle dependent since particles scatter light to different angle with different intensities [14].

Refractive Index: Refractive index of Microemulsion was determined using an Abbes type refractometer (Rajat Scientific Work, Moradabad U.P.) at 25±0.5°C. Refractive index of MEs is shown in Table 4.

pH: The pH of microemulsion formulations were measured by digital pH meter (VSI-IB, VSI electronics, India)in triplicate at 25±1°C.The pH of microemulsion formulations of MEs are shown in Table 4.

TABLE 4: CHARACTERIZATION PARAMETERS OF MICROEMULSION FORMULATIONS

FORMULATION	REFRACTIVE INDEX	PH	VISCOSITY (mP)	DROPLET SIZE (NM)
MEA1	1.454 ±0.11	6.87±0.02	40.21±3.63	56.72-2.48
MEA2	1.435 ±0.12	6.5 ±0.08	41.01±2.14	60.3-76.16
MEB1	1.402 ±0.07	6.26±0.20	38.21±2.32	60.00 - 83.0
MEB2	1.403 ±0.09	6.67±0.03	38.98±3.98	46.29 - 57.1
MEC1	1.397± 0.02	6.29±0.02	34.37±4.23	41.91- 52.79
MEC2	1.401 ±0.11	6.59±0.02	33.28±2.18	53.48 - 81.07
MED1	1.420 ±0.04	5.91±0.05	35.21±7.23	59.58 - 89.80
MED2	1.442 ±0.06	6.12±.004	35.54±5.03	15.90 - 26.22
MEE1	1.452 ±0.05	5.87±0.02	39.06±3.88	43.71 - 48.83
MEE2	1.402 ±0.04	5.94±0.06	39.14±6.17	46.75 - 65.25

Viscosity: The viscosities of microemulsion formulations were determined by using Brookfield DV III Ultra V6.0 RV cone and plate rheometer (Brookfield engineering laboratories, Inc., Middleboro, MA) using spindle at 25±0.5^{o}C. The software used for the viscosity calculations was Rheocalc V2.6. Experiments were performed in triplicate for each sample, and results were presented as average ± standard deviation. The viscosity of microemulsion formulations of MEs were shown in Table 4.

Solubility ACV in optimized Microemulsion: Excess amount of ACV was added to 10 ml of the microemulsion, and left at 37°C in the dark with magnetic stirring for 163 hr. At 24, 48, 72, 96 and 163 hr, we took an aliquot of the microemulsion and centrifuged it for 20 min at 5000 rpm. The supernatant was filtered through 0.45 µm polyvinyl difluoride filters (to remove drug in suspension), and was then diluted with an appropriate volume of methanol. ACV was quantified in the filtrate by spectrophotometer (Shimadzu 1700, UV) at λ_{max} 254 nm. For optimized microemulsion, three replicate assays were performed.

Data analysis: The cumulative amount of Acyclovir permeated through 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/hr) 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, hr).

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

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/hr),

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

C_{donor} = Concentration of drug in donor comportment

$$\text{ER} = \frac{\text{Transdermal flux from Microemulsion formulation}}{\text{Transdermal flux from plain drug}}$$

***In-Vitro* Skin Permeation Studies:** Ex vivo permeation studies were carried out using

jacketed vertical Keshary- Chien Franz diffusion cells with a diffusion surface area of 0.385 cm^2 and 5.0 ml of receptor cell volume, placed in heating stirring module. The receptor compartments were filled with phosphate buffered saline (PBS) pH 7.4, containing 10% v/v of DMSO. The receptor phase was maintained at 37±0.5°C. The skin pieces of wistar albino rat were mounted over diffusion cells with the dermal side in contact with receptor phase [15]. Then air bubbles were removed then equilibrated for 2 hr [16]**.** 50 mg of ACV in 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 [17]**.**

0.5 ml 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 hr) for 24 hr 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 0.45 µm membrane filter and the amount of drug in the receptor fluid was analyzed using UV at 252 nm. The cumulative amounts of drug permeated in mg/cm^2 and the percentage of the cumulative amount permeated after 24 hr were calculated. The data were analyzed statistically by the one-way ANOVA test followed by the least significant difference procedure. This statistical analysis was carried out using GraphPad Prism 5.0 Softwares (Inc, San Digeo USA), Graphpad InStat Softwares (Inc, San Digeo, USA).

Skin irritation studies: The skin irritancy test was performed to confirm the safety of transdermal formulation. A single dose of 15 µl of microemulsion gel (MEC1) was applied to the left ear of the rats, and controls to the right ear of the rats. The development of erythema, which is a manifestation of cutaneous vascular dilation, was monitored daily for 7 days using the Van-Abbe method [18]**.**

Pharmacokinetic Data Analysis: *In vivo* study of optimized microemulsion formulation was performed on Wistar albino rats. 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/08/002)** 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. Male albino rats 7-9 weeks old and weighing 200-250 gm was used for the studies.

The animal were kept under standard laboratories conditions (temp: 25±2 ^oC; relative humidity 55 ± 5%). The animals were housed in polypropylene cage, with free access to standard laboratory diet and water *ad libitum*. For transdermal application, the rats were anaesthetized by i. v. injection of combination of ketamine hydrochloride (75 mg/kg) and xylazine (5 mg/kg) [19]**.** The hair on dorsal skin was trimmed off and washed gently with distilled water. For *in-vivo* permeation studies, three groups each containing six rats were formed. Three formulations: plain drug solution (PD), drug-in-conventional emulsion (CE), and drug in microemulsion were applied on back of the rat skin surface (2.0 cm^2) in open containers glued to the skin by silicon rubber and ACV

concentration in the blood was determined for 24 hr. The blood samples (200 µL) were collected from tail vein at 0, 0.5, 1.0, 2.0, 3.0, 4.0, 6.0, 8.0, 12.0 and 24.0 hr. The blood samples of controls and test groups were collected into a heparinised microcentrifuge tubes, and mixed and centrifuged at 5000 rpm for 20 min. The plasma was separated and stored at -21^{o}C until drug analysis was carried out using HPLC (Dionex Ultimate 3000, Japan). For transdermal administration, the area under the drug concentration-time curve from 0 to 24 hr (AUC) t_{max}, and C_{max} were calculated by GraphPad Prism5.0 Softwares, (Inc, San Digeo, USA), for pharmacokinetic analysis.

Statistical Analysis: The data from different formulations were compared for statistical significance by one-way analysis of variance (ANOVA). Differences were considered to be statistically significant when P*< .01, P**< .001.

RESULT AND DISCUSSION:

Screening of oils, Surfactant and Cosurfactants: 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 [20]. The choice of oily phase is often a compromise between its ability to solubilise the drug and its ability 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 Oleic acid and Caster oil (3:1) (45.53 mg/ml). Therefore Oleic acid: Castor oil (3:1) was chosen as the oil phase.

The use of Oleic Acid is advantageous because it increase skin permeability by two mechanistic scenarios of the enhancer; (a) lipid fluidization, and (b) lipid phase separation [21], OA is a model skin permeation enhancer [22], the screening of surfactant and cosurfactants on the basis of solubility is difficult because all surfactant and cosurfactant cannot solubilised all type of oil phase. On the basis of constructed pseudoternary phase, using tween 80 and corbitol, tween 20 and corbitol, span 80 and corbitol, 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 region, therefore tween 80 was selected as surfactant. 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 amphiphilic nature of ethanol enables formulation of microemulsion or with a variety oily phase and

surfactant [23]. Titrations were done by using tween 80 as surfactant with different cosurfactants (Ethanol, Corbitol, Propylene Glycol, and PEG 400). 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.

Construction of Phase Diagrams: On the basis of selected surfactant and cosurfactant, pseudoternary phase diagrams were constructed by phase titration in order to define the extent and nature of microemulsion region and surrounding two and three phase domains. The construction of pseudoternary phase diagrams was started using surfactant i.e. tween 80, Cosurfactant ethanol in different ratios viz. 1:0, 1:1, 1:2, 2:1, 3:1. (Fig 1-5). The microemulsion region in the pseudoternary phase diagram was highest in 2:1 ratio of tween 80/ethanol (Fig. 4) because ethanol penetrate into surfactant monolayer, providing additional fluidity to interfacial film and thus disrupting the liquid crystalline phases. Furthermore ethanol also distributed itself between aqueous and oily phase, thereby altering the chemical composition and hence relative hydro/ lipophilicity of the system. Microemulsion of tween 80/ ethanol at ratio 2:1 formed a larger single phase region than the systems at other S/CoS ratios. It was reported that at the optimum S/CoS value, the cosurfactant was insert into the cavities between the surfactant molecules exactly, and the formed microemulsion had the maximum solubilisation capacity [24]. The emulsified area was low at S/CoS ratio 3:1, it was because ethanol is a polar solvent with the tendency to highly incorporate into water, and the relatively lower ethanol content in the microemulsion systems decreased the hydrophilicity of the mix-surfactant, so the area of O/W microemulsion was small. In contrast, at the ratio of S/CoS 1:3, the low concentration of surfactant reduced the amount of micelle, which consequently decreased the solubilisation capacity of microemulsion and few microemulsion points were obtained with 1:4 ratio of tween80/ethanol. After studied the result it was found that with increase of S/CoS ratio the microemulsion gets increased up to certain limit and vice versa.

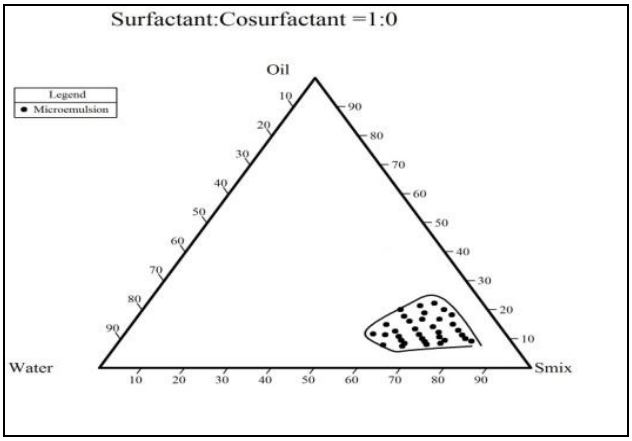

FIG. 1: ME REGION AT S_{MIX} 1:0

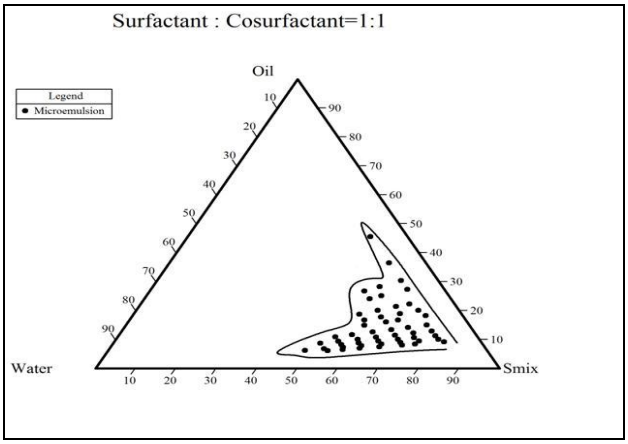

FIG. 2: ME REGION AT S_{MIX} 1:1

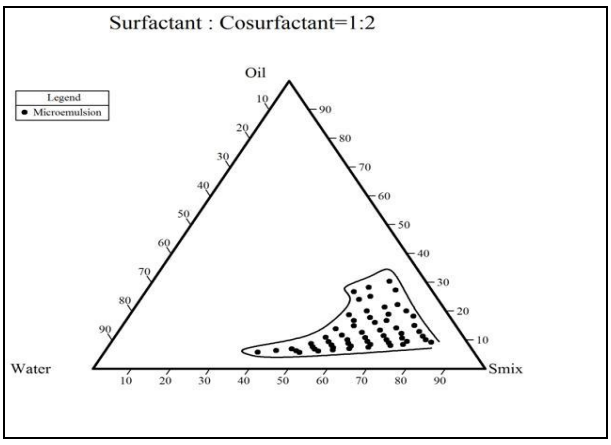

FIG.3: ME REGION AT S_{MIX} 1:2

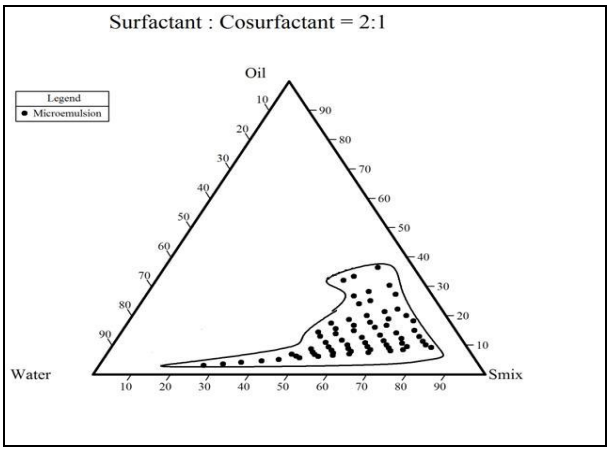

FIG.4: ME REGION AT S_{MIX} 2:1

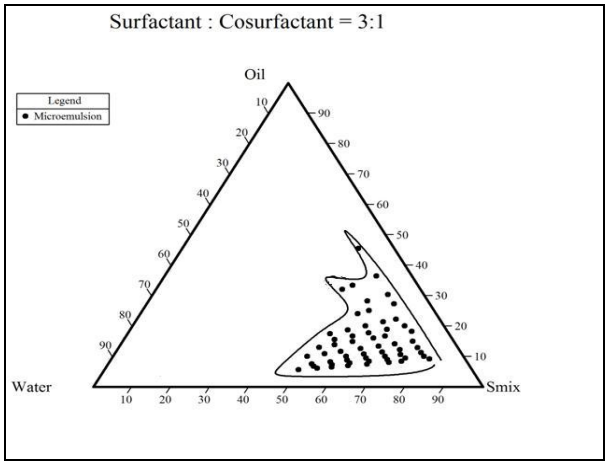

FIG.5: ME REGION AT S_{MIX} 3:1

Characterization of Microemulsion: The optimized microemulsion formulation were characterized for different parameters like surface morphology, droplet size and its distribution, refractive index, pH, viscosity, solubility of ACV in optimized formulation. The surface morphology of microemulsion droplets were evaluated by the transmission electron microscopy, TEM **(Morgangni 268D SEI, USA)** which was shown in the Fig. 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 formulations were found to be in range of MEA1 (56.72-2.48 nm), MEA2 (60.3-76.16 nm), MEB1 (60 to 83.0 nm), MEB2 (46.29 to 57.1 nm), MEC1(41.91 to 52.79 nm), MEC2 (53.48 to 81.07 nm), MEED1 (59.58 to 89.80 nm) and MED2 (15.90 to 26.22 nm), MEE1 (43.71 to 48.83 nm), MEE2 (46.75-65.25nm), whereas macroemulsion droplet size were found to be in range of 659 to 781nm.

The drug loaded droplet size of the optimized microemulsion formulation MEC1 was found to be in the range of 41.91 to 52.79 nm. The droplet size increased with the increase in concentration of oil in the formulations [12]. 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.

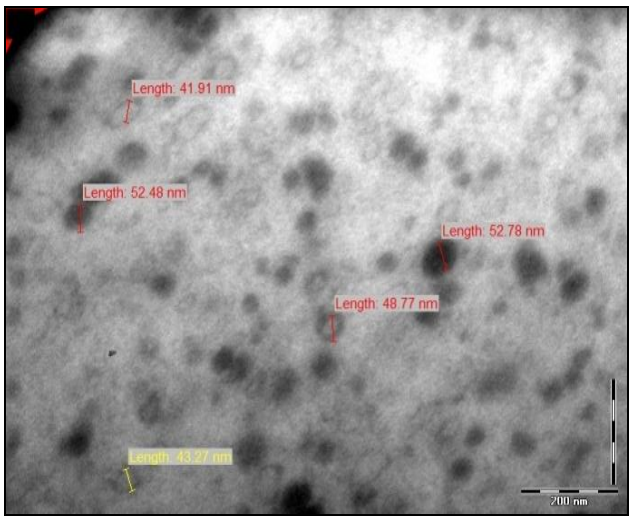

FIG. 6: TEM IMAGE OF OPTIMIZED MICROEMULSION FORMULATION MEC1

The droplet size distribution of microemulsion was determined by Malvern Zetasizer (1000 HS, Malvern Instruments, and U.K.). The mean size of droplet of microemulsion MEC1 was shown also in Malvern Zetasizer size distribution report was also found 49.53 nm (Fig. 7). The mean values of the refractive index of drug-loaded formulations are given in Table 4: Refractive index of drug-loaded optimized microemulsion formulation MEC1 was found to be 1.397.

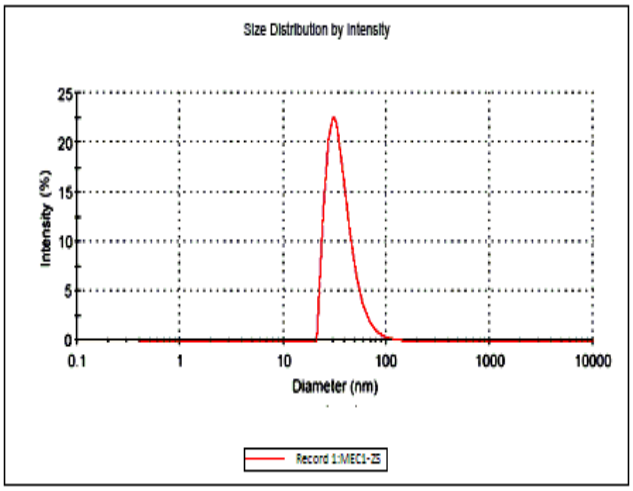

FIG.7: DROPLET SIZE REPORT

When the refractive index values for formulations were compared with those of the placebo microemulsion; it was found that there were no significant differences between the values. Therefore, it can be concluded that the microemulsion formulations were not only thermodynamically stable but also chemically stable and remained isotropic; thus, there were no interactions between microemulsion excipients and drug [12]. The pH value for the optimized ACV loaded Microemulsion (MEC1) formulation was recorded on digital pH meter which was 6.29 and is suitable for topical as well as transdermal application because the pH of skin in the range of 5.5 to 7.0.

The viscosity values of all samples were low and relatively constant at 33.28 to 41.01 mP. All samples exhibited Newtonian flow behaviour, as expected from microemulsions. It could be noted that the viscosity values tended to increase slightly when the water concentrations increased or when the system turned into oil/water type because oil/water microemulsions have higher viscosities than those of water/oil systems. The slight increment of viscosity might be the result of interaction of microemulsion droplets in oil/water systems. High concentration of S_{mix} (Cosurfactant & surfactant) also decreases the viscosity by enhancing fluidity of microemulsion.

***In- Vitro* skin permeation studies:** The permeation capability of the selected microemulsion formulations were evaluated by conducting the *in-vitro* skin permeation experiments. The permeation parameters (cumulative amount of ACV permeated, transdermal flux, permeability coefficients) of selected microemulsion and control

formulations were presented in table 5. In these formulations content of oil phase was varied as 4.55 to 9.43%, while the content of S_{mix} was varied from 40.90 to 75.47 %. The effect of the content of oil and S_{mix} on the skin permeation of ACV was evaluated. On the basis of permeation studies, it was found that the microemulsion formulation MEC1 consisting of 5% oil phase (oleic acid : castor oil; 3:1), 15 % tween 80, 30 % Ethanol and 50 % distilled water (from the phase diagram of S/CoS ratio 1:2) exhibited highest permeation profile. The cumulative amount of ACV from different microemulsion formulation is given in Fig. 8. The cumulative amount of ACV from MEC1 was 63.87 % at the end of 24 hr and skin permeation flux, permeability coefficient of MEC1 formulation was 238.1 µg/cm^2/hr and 1.428×10^{-2} (cm/hr) respectively, while all the other microemulsion exhibited lesser percentage of drug permeation and flux. The aqueous solution of (PD) ACV and its conventional emulsion(CE) exhibited 2.47 µg/cm^2/hr and 8.65 µg/cm^2/hr transdermal flux respectively through rat skin having permeability coefficient (Kp) (0.014×10^{-2} cm/hr) and (0.051 ×10^{-2} ^{cm}/hr) respectively and comparison was shown in fig. 9. The transdermal flux of microemulsion MEC1 was 96.39 fold greater than the aqueous solution (PD) of plain ACV, 27.52 fold greater than the conventional emulsion (CE) of ACV.

TABLE 5*: IN-VITRO* PARAMETERS OF CONTROL, CONVENTIONAL EMULSION AND MICROEMULSION FORMULATION

FORMULATION CODE	FLUX (µG/CM^2/HR)	PERMEABILITY COEFFICIENT K_P ×10^{-2} (CM/ HR)	ENHANCEMENT RATIO WITH PD	ENHANCEMENT RATIO WITH CE
PD	2.47±0.76	0.014	----`	----
CE	8.65±1.21	0.051	3.50	----
MEA1	58.21±5.42	0.349	23.56	6.72
MEA2	55.40±4.82	0.332	22.42	6.40
MEB1	191.90±8.49**	1.151	77.69	22.18
MEB2	123.80±6.85*	0.742	50.12	14.31
MEC1	**238.1±4.87*** **	**1.428**	**96.39**	**27.53**
MEC2	110.0±4.14*	0.660	44.53	12.72
MED1	105.02±2.42	0.630	42.51	12.14
MED2	98.65±3.37	0.591	39.93	11.4
MEE1	69.31±4.43	0.415	28.06	8.01
MEE2	109.0±3.93	0.654	44.12	12.60
MEGel	230.40±6.23***	1.380	93.27	26.64

Values are mean ± SD, n=3, P* <0.05, P** <0.01, P*** <0.001.As compared to control (PD), One-way ANOVA (Dunnet multiple comparison test)

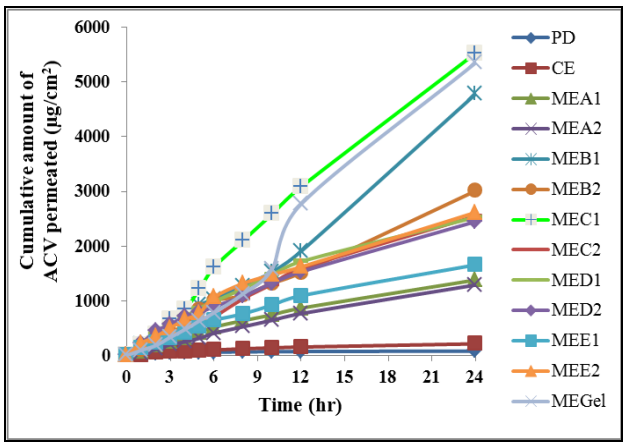

FIG. 8: IN-VITRO SKIN PERMEATION STUDIES FROM VARIOUS MICROEMULSION FORMULATIONS

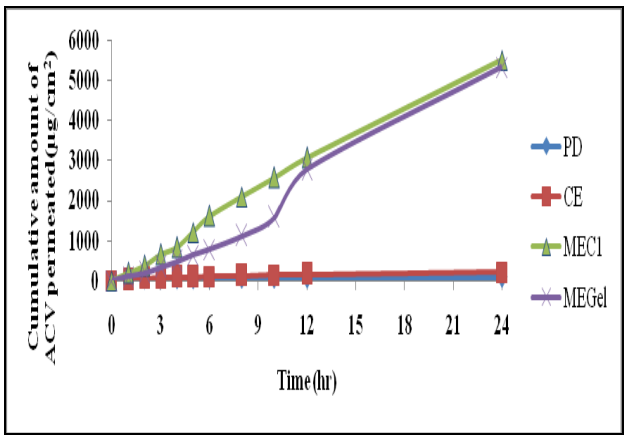

FIG. 9: COMPARATIVE *IN-VITRO* SKIN PERMEATION PROFILE OF MEC1, MEGEL CONVENTIONAL EMULSION (CE), AQUEOUS SOLUTION (PD) OF ACV

The transdermal flux of plain ACV 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 [25]. The increased *in-vitro* parameters of microemulsion formulation MEC1 is due to the following reasons i.e. Oil phase, surfactants cosurfactants, penetration properties of oil and cosurfactants and water contents. Some previous studies have founded that the drug release from microemulsion is dependent on their proportional composition, and notably on their proportional water content [26, 27]. The content of oil also played an important role in microemulsion formulation and it affected the skin permeation rate directly. Skin permeation rate would increase with the decreasing oil content, (9.43 to 4.55%), the transdermal flux was increased from 55.40 to 238.1 µg/cm^2/hr, which was 4.29 fold greater than microemulsion having 9.43% oil phase. It's because the water in microemulsion could hydrate skin and caused the corneous cell to swell thus made drug channels wide, therefore with the increasing amount of the water content in the system, the cumulative permeation amount was improved [10].

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 [21], Oleic acid is a model skin permeation enhancer [22]. Oleic acid facilitates penetration into the skin by disrupting the fluidity of the stratum corneum [27]. The thermodynamic activity of drug in the formulation is a significant driving force for the release and penetration of the drug into skin [29]. Formulation containing a lower amount of S/CoS provides higher flux than formulation containing higher amount of S/CoS .The content of S_{mix} in microemulsions affected the skin permeation flux of the drug significantly. This may be due to an increased thermodynamics activity of the drug in microemulsion at the lower concentration of the surfactant and cosurfactant [30]. 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 through it [31].

It is known that with an increase in the aqueous phase fraction, the droplet size increases [32]. Due to the small droplet size, droplets settled down to close contact with the skin and a large amount of inner Oleic acid (OA) in microemulsions might penetrate into skins. OA and ethanol as permeation enhancers had strong permeation enhancing effect. They could enhance the solubility of ACV in the skin and partition coefficient might not be necessary to decrease with increasing the solubility of drug in the formulation. Then *P* could be increased due to permeation enhancers [33, 34]. After studying the *in vitro* permeation studies, it was found that the flux of ACV increases from MEA (55.40 to 58.21), MEE (69.31 to 109.0), MED (98.65 to 105.0), MEB (123.80 to 191.90), MEC (110.0 to 238.1), with the change in S_{mix} ratio from 1:0 to 3:1 to 2:1 to 1:1 and 1:2 respectively. The highest flux was found for the S_{mix} ratio 1:2, because ethanol was used as cosurfactant which was two times greater than tween 80. 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 [30].

Further, permeation of ethanol into the stratum corneum can alter the solubility properties of the tissue with a consequent improvement for drug partitioning into the membrane [36]. Additionally, it is also feasible that the rapid permeation of ethanol or evaporative loss of this volatile solvent, from the donor phase modifies the thermodynamic activity of the drug within the formulation. Such an effect is most apparent when applying a finite dose of a formulation onto the skin surface before evaporation of the alcohol; as ethanol is lost, drug concentration can increase beyond saturated solubility providing a supersaturated state with a greater driving force for permeation. Such a mechanism may operate for transdermal delivery from patches and gel where ethanol, typically included to solubilise the drug or to apply the adhesive, may traverse the stratum corneum rapidly leaving behind a metastable supersaturated permeant which is inhibited from crystallising by polymers that are typically incorporated into formulation.

A further potential mechanism of action arising as a consequence of rapid ethanol permeation across the skin has been reported; solvent 'drag' may carry permeant into the tissue as ethanol traverses, although such a mechanism has been discounted for morphine hydrochloride permeation from ethanol and methanol containing formulations. In addition, ethanol as a volatile solvent may extract some of the lipid fraction from within the stratum corneum when used at high concentration for prolonged times; though not an 'enhancing' effect, such a mechanism would clearly improve drug flux through skin [37].

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 [38]. The overall flux enhancements were observed for microemulsion formulation MEC.

However, when microemulsion MEC1, MEC2 were compared, the flux was decreased, when the S_{mix} concentration was increased but not proportionally, because oil content and water content also play important role in drug permeation across rat skin. The maximum flux (238.1µg/cm^2/hr) was obtained from MEC1, at 5 % oil phase, and 45 % S_{mix} (surfactant 15 % and co-surfactant 30 %). Observation also agrees with the earlier study where high flux values were obtained at low oil phase and S/CoS concentration [10]. The microemulsion gel MEGel exhibited transdermal flux (230. 41 µg/cm^2/hr) was assessed by *in-vivo* study. The flux of microemulsion Gel of ME Gel was found 230.41µg/cm^2/hr which showed high value, but slightly less than the microemulsion MEC1, but significantly very higher than the controls (PD & CE), the decrease in the flux (compared with microemulsion MEC1) might be due to change in the water content, viscosity of the microemulsion gel formulation. The skin permeation profile of microemulsion MEC1 & MEGel was shown significant, when compared with that of PD and CE (P^{***}<0.001). The significant difference in Acyclovir permeation between microemulsion formulations, PD and CE was probably due to the mean size of internal phase droplets, which were significantly smaller in microemulsions.

The skin irritancy was performed on the Wistar albino rats for safety purpose when administrated topically. The optimized formulation microemulsion gel (MEGel) was applied and erythema occurrence was found to be 0.238. Van abbe & Draize mentioned that if indices lie between 0-9. It indicates that the applied formulation probably will cause irritation to the human skin. From these studies it can be concluded that the prepared microemulsion was safe to be used as transdermal drug delivery system.

Pharmacokinetic Data: Pharmacokinetic parameters obtained with following transdermal administration of aqueous solution of ACV (PD), ACV loaded conventional emulsion (CE) and microemulsion Gel (MEGel) to six rats (mean value ± S.D.; n=6).

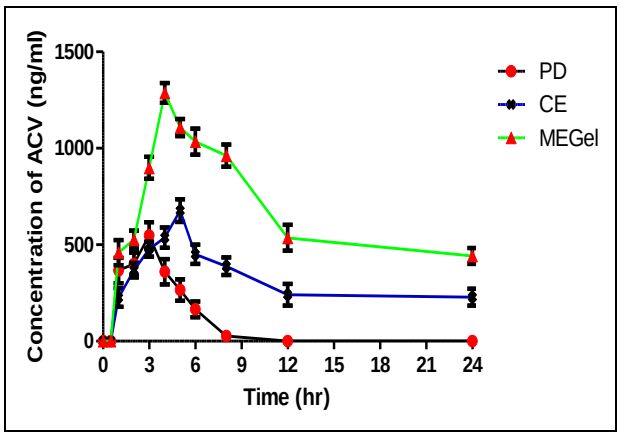

FIG. 10: ACV PLASMA CONCENTRATION

Fig. 10; Shows the pharmacokinetic profiles of Acyclovir after topical application of plain drug aqueous solution (PD), conventional emulsion (CE), and microemulsion gel formulation (MEGel) The calculated parameters of pharmacokinetics were given in table 6**.** The AUC $_{0\text{-}t}$ of topically applied microemulsion gel formulation was approximately 7 fold and 3 fold higher compared to topically applied aqueous solution of ACV(PD) and conventional emulsion respectively, and statistic calculation provided a significance difference between above two values (PD & MEGel) is P**<0.01, P***<0.001.

TABLE 6: PHARMACOKINETIC PARAMETERS

PARAMETERS	AQUEOUS SOLUTION OF ACV (PD)	CONVENTIONAL EMULSION (CE)	MICROEMULSION GEL (MEGEL)
C $_{max}$(µg/ml)	0.54±0.0185	0.676 ± 0.08	1.2873 ± 0.26**
t $_{max}$ (hr)	3.0 ± 0.2	5.2 ± 0.5	4.2 ± 0.5
$AUC_{0\text{-}t}$ (µg/ml/hr)	2.17 ± 0.32	7.36 ± 0.9	15.53 ± 1.89***

Values are mean±SD, n=6, P* <0.05, P** <0.01, P*** <0.001.As compared to control (PD), One-way ANOVA (Dunnet multiple comparison test)

$AUC_{0\text{-}t}$, MEGel Significantly higher than control (PD) of ACV (P*** < 0.001)

After topical application of aqueous solution of ACV (PD) and conventional emulsion (CE), the plasma level of ACV reached a peak of 0.54 ± 0.0185 µg/ml at 3.0 ± 0.2 hr and 0.67 ± 0.08µg/ml at 5.2 ±0.5 hr respectively. While microemulsion formulation reached peak 1.2873 ± 0.26 at 4.2 ± 0.5 hr. The effective drug concentration (> 500 ng/ml in plasma) was maintained for at least 14 hr through transdermal administration .Microemulsion showed the high C_{max} and prolonged t_{max} which was due to the barrier properties of the skin and slow portioning of the drug into the skin from microemulsion gel. The *in-vivo* data, which have demonstrated significantly higher bioavailability of ACV after transdermal application through the microemulsion, (compared to aqueous solution and conventional emulsion of ACV) might be due to altered partition coefficient between skin and drug and the thermodynamic activity of drug in the formulation is a significant driving force for the release and penetration of the drug into skin [29]**.** Thus Transdermal administration of Acyclovir (ACV) through Oleic acid: castor oil/tween 80 /ethanol/water microemulsion had a sustained and enhanced absorption.

CONCLUSION: The study demonstrates that the microemulsion formulation can be employed to improve the transdermal flux hence bioavailability of a poorly water soluble drug. The ratio of oleic acid: castor oil, tween80, ethanol and water played a major role in formulating the microemulsion. The optimum microemulsion formulation contained oleic acid: castor oil (5%), tween80 (15%), ethanol (30%), and water (50%), which was a transparent and less viscous system.

After topical administration on the skin of rats, the optimized microemulsion (MEC1) showed an enhanced transdermal flux 96.39 fold greater and 27.52 fold greater than aqueous solution of ACV and Conventional emulsion (CE) respectively and microemulsion gel formulation (MEGel) showed an enhanced transdermal flux 93.29 fold greater and 26.64 fold greater than aqueous solution of ACV and Conventional emulsion (CE) respectively. And microemulsion formulation was found to be safe for transdermal application.

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REFERENCES:

1 Changez M, Chander J, Dina AK: Transdermal Permeation of tetracain hydrochloride by lecithin microemulsion in-vivo colloid and surface B: Biointerface 2006; 48: 58-66.
2 Chien H, Hang X, Weng T, Bhao X, Gao Z, Yang Y, Xu H, Yang X: A Study of microemulsion for transdermal delivery of triptolid. J. control Rel 1989; 98: 427-436.
3 Dannielsson I, Lindman B: The definition of microemulsion; Colloids and Surfaces 19981; 3:391-392.
4 El-laithy HM, El-shoboury KMF: The developmental cutina lipogel and gel microemulsion for topical administration of fluconazole. AAPS. Pharm. Sci. Tech 2002; 3:35, 1-9.
5 Nandi I, Barri M, Joshi H: (2003). Study of IPM microemulsion system containing cyclodextrin to improve the solubility of 2 model hydrophobic drug. AAPS Pharm. Sci. Tech.2003; 4: 10, 1-9.
6 Delgado-Charro MB, Iglesias-Vilas G, Blanco-Me´ndez J: Delivery of a hydrophilic solute through the skin from novel microemulsion systems. Eur. J. Pharm. Biopharm1997; 43: 37-42.
7 Ghosh PK, Majithiya RJ, Umrethia ML, Murthy RSR: Design and Development of Microemulsion Drug Delivery System of Acyclovir for Improvement of Oral Bioavailability. AAPS Pharm SciTech 2006; 7:77.
8 Khandelwal A, Bahadduri PM, Chang C, Polli JE, Swaan PW, Eskins S: Computational Models to Assign Biopharmaceutics Drug Disposition Classification from Molecular Structure. Pharmaceutical Research 2007; 12:2249-2262.
9 Yuan, Y., Li, S-M., Mo, F. K., Da-fang, Z. D. F., (2007) Investigation of Microemulsion System for Transdermal Delivery of Meloxicam; International Journal of Pharmaceutics., 1-26.
10 Baboota S, Al-Azaki A, Kohali K, Ali J, Dixit A, Shakeel F:(2007). Development and Evaluation of Microemulsion Formulation for Transdermal Delivery of Terbinafine. PDA Journal of pharmaceutical Science and technology 2007; 4:276-285.
11 Shakeel F, Baboot S, Ahuja A, Khar RK, Ali M, Shafiq S: Nanoemulsions as Vehicles for Transdermal Delivery of Aceclofenac. AAPS Pharm SciTech 2007; 8:104, E1-E9.
12 Frantzen CB, Iingebrigtsen L, Sakar M, Brandl M: Assessing the Accuracy of routine Photon correlation spectroscopy Analysis of Heterogeneous Size Distribution. AAPS SciTech 2003; 4:36, 1-9.
13 Gaitonde CD, Gui Z, Qu A, Anik S, Flack M, Vengroff: Merthod Validation for particle size distribution of nanoemulsion using Photon Correlation Spectroscopy; *Nano Bio Corporation*2008; 27: 1-5.
14 Ammar HO, Ghorab M., El-Nahhas SA, Kamel R: Evaluation of chemical penetration enhancers for transdermal delivery of aspirin. Asian Journal of Pharmaceutical Science 2007; 2: 96-105.
15 Panchagnula R, Narishetty STK: Transdermal delivery of zidovudine: effect of terpenes and their mechanism of action. Journal of Controlled Release2003*;* 95: 367– 379.
16 Rhee YS, Coi J, Park E, and Chi S: Transdermal delivery of ketoprpfen using microemulsion. Int. J. Pharm2001; 228: 161-170.
17 Van-Abbe NJ, Nicholas P, Boon E: Exaggerated exposure in topical irritancy and sensitization testing. J Soc. Cosmet Chem.1075; 26: 173-187.
18 Sintov AC, Botner S: Transdermal drug delivery using microemulsion and aqueous system. Influence of skin storage condition on the *in vivo* permeability of diclofenac from aqueous vehicle system. Int. J. Pharm *2006;* 311: 55-62.
19 Malcolmson C, Shindhu A, Satra C, Kantaria S, Lawrence MJ: Effect of nature of oil on the incorporation of testosterone propionate into non ionic oil-in-water microemulsion. *J. Pharm. Sci 1998.*87: 109-116.
20 Naik A, Pechtold LARM, Potts RO, Guy RH: Mechanism of oleic acid –induced skin penetration enhancement in vivo

in humans. Journal of Controlled Release1995; 37: 299-306.
21 Tanojo H, Boelsma E, Junginger HE, Ponec M, Bodde HE: In vivo human skin permeability enhancement by oleic acid: a laser Doppler velocimetry study. Journal of controlled Release1998; 58: 97-104.
22 Nagarsenker MS, Date AA: Parenteral microemulsion: An overview. Journal of pharmaceutics2008; 355:19-30.
23 Kawakami K, Tojo K: Skin Irritation in Transdermal Drug Delivery Systems: A Strategy for its Reduction. Pharmaceutical Research 2002; 24: 399-404.
24 .Kreilgaard M: Influence of microemulsions on cutaneous drug delivery. Advanced Drug Delivery Reviews 2002; 54: S77–S98.
25 .Osborn DU, Ward AJI, O'neill KJ: Microemulsions as Topical Drug Delivery Vehicles. I. Characterization of a Model System. Drug development and industrial pharmacy1988*;* 14(9): 1203-1219.
26 Alvarez-Figuero MJ, Blanco-MJ: Transdermal delivery of methotrexate: iontophoretic delivery from hydrogels and passive delivery from microemulsions; International Journal of Pharmaceutics2001; 215: 57–65.
27 Aboofazeli R, Zia H, and Needham TE: Transdermal Delivery of Nicardipine: An Approach to In Vitro Permeation Enhancement; Drug Delivery 2002; 9:239–247.
28 Walters KA, Brain KR, Green DM, James VG, Watkinson AC, Sands RH: Comparison of the transdermal delivery of estradiol from two gel formulations. Maturitas. Adv. Drug Deliv. Rev.1998; 29: 189–195.
29 Yang JH, Kim YI, Kim KM: Preparation and evaluation of aceclofenac microemulsion for transdermal drug delivery. Arch. Pharm. Res 2002; 25:534-540.
30 Kogan A, Garti N: Microemulsions as transdermal drug delivery vehicles; Advances in Colloid and Interface Science2006; 123: 369–385.
31 Attwood: Microemulsions. Colloidal Drug Delivery Systems, Dekker, J. Kreutzer (Ed.), New York, 31–71.
32 Peltola S, Saarinen-SP, Kiesvaara J, Suhonen TM, Urtti A: Microemulsions for topical delivery of estradiol. International Journal of Pharmaceutics *2003*; 254, 99–107.
33 Chen H, Chang X, Du D, Li j, Xu H, Yang X: Microemulsion-based hydrogel formulation of ibuprofen for topical delivery. International Journal of Pharmaceutics2007*;* 315: 52–58.
34 Pershing LK, Lambert LD, Knutson K: Mechanism of a ethanol-enhanced estradiol permeation across human skin in vivo. Pharm. Res.1990; 7: 170– 175.
35 Megrab NA, Williams AC, and Barry B.W: Oestradiol permeation across human skin, silastic and snake skin membranes: the effects of ethanol/water co-solvent systems. Int. J. Pharm *1995;* 116: 101– 112.
36 Morimoto H, Wada Y, Seki T, Sugibayashi K: *In-vitro* skin permeation of morphine hydrochloride during the finite application of penetration-enhancing system containing water, ethanol and L-menthol. Biological. Pharm. Bulletin 2002; 25: 134–136.
37 Williams AC, Barry BW: Penetration enhancers. Advanced Drug Delivery Reviews 2004; 56: 603-618.
38 Thevenin MA, Grossiord JL, Poelman MC: Sucrose esters/ cosurfactant microemulsion systems for transdermal delivery: assessment of bicontinuous structures. International Journal of Pharmaceutics 1996; 137: 177-186.