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STUDIES ON TRANSDERMAL DELIVERY OF IRBESARTAN: MICROEMULSION AS A DELIVERY SYSTEM

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ABSTRACT: The objective of the study was to investigate the effect of microemulsion on *in-vitro* skin permeation, percutaneous absorption of Irbesartan and to postulate probable mechanism of skin permeation. Microemulsion was prepared by aqueous phase titration method. Pseudoternary phase diagrams were constructed for the microemulsions. Thermodynamic stability study was carried out on selected microemulsions. Characterization of selected Irbesartan-loaded microemulsions was done by droplet size and polydispersity index determination. Physicochemical properties of Irbesartan-loaded microemulsions were determined using reported procedures. *In-vitro* skin permeation was determined using Franz cell. Skin permeation mechanism was assessed by Fourier Transform Infra - Red spectra analysis, Differential Scanning Calorimetry and activation energy measurement. A non-compartmental pharmacokinetic study was carried out to estimate the percutaneous absorption through the rat skin. The particle size and polydispersity index of the spherically shaped microemulsions were in the range of 83 - 86 nm and 0.270 - 0.290 respectively. *In-vitro* skin permeation study gave the permeability coefficient and steady state flux of Irbesartan to be 3.52 ($\times 10^{-3}$) cm/h and 17.59 ± 0.38 µg/cm^2/h for oil-in-water (O/W); 6.63 ($\times 10^{-3}$) cm/h and 33.16 ± 0.33 µg/cm^2/h for water-in-oil (W/O) respectively. Compared with the Irbesartan suspensions, pharmacokinetics of Irbesartan-loaded microemulsions in Wistar rats indicated higher plasma drug concentrations and larger area under the curve. The pharmacokinetic and *in-vitro* studies suggest that microemulsion could be an effective transdermal drug delivery system to improve bioavailability of Irbesartan. Disruption of lipid bilayer, protein denaturation and skin hydration are considered to be the probable skin permeation mechanism.

INTRODUCTION: The skin is a route to deliver active agents that are meant to act in the tissue (cutaneous delivery) and those active agents that have to cross the tissue and be absorbed into the systemic circulation in order to reach its site of action (transdermal delivery or percutaneous absorption) [1].

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Transdermal route minimizes gastrointestinal disorders, first-pass liver metabolism, inter and intra patient variability. It is advantageous for drugs with short half-lives and high potencies and also has flexible drug administration [2]. However, the stratum corneum (SC) of the skin creates barrier to this route of administration.

Therefore, overcoming the barrier becomes necessary to ensure an efficient delivery of drugs and other active compounds [3]. Stratum corneum differs in terms of lipid composition, water content and morphological characteristics such as thickness, number of pores and follicles [4]. A number of techniques have been developed and

employed to overcome this barrier to transdermal delivery and among them, microemulsions have been extensively investigated.

Microemulsions are clear or translucent, optically isotropic and thermodynamically stable systems (colloidal dispersions) generally composed of oil, surfactant, cosurfactant, and aqueous phase [5]. The system is of interest in pharmaceutical research because of its simplicity of manufacturing, spontaneous formation, high solubilization capacity for lipophilic solutes, and improved bioavailability of hydrophobic drugs [6, 7]. They have been reported as one of the most promising techniques as drug delivery vehicles for oral, transdermal, topical, nasal, intravenous, and other administration routes of poorly soluble drugs [8, 9, 10, 11, 12]. Microemulsion presents a small droplet size generally up to 150nm [13, 14]. The mechanism of skin permeation of some drugs has been studied by using microemulsion technique [15, 16] and this technique has been reported to enhance skin permeation of drugs effectively [17].

Irbesartan, 2-*n*-Butyl -4- spirocyclopentane- 1-[(2'-tetrazol-5-yl)biphenyl-4-yl)methyl]-2-imidazolin-5-one is a potent long acting AII receptor antagonist with high specificity for the AT1 subtype and clinically used for the treatment of hypertension [18-19]. Irbesartan was chosen as drug of choice in this investigation because a combination of its drawbacks and various advantages when compared to other antihypertensive agents including angiotensin II receptor blockers [20, 21, 22], makes it a good candidate for such investigation. Amongst such drawbacks are: (i) a very poorly water soluble drug (< 10 µg/ml), thus making bioavailability to be limited by dissolution rate, and (ii) its plasma level does not increase proportionally with dose thereby leading to potential inter and intra patient variability.

The advantages of the drug could be very beneficial to geriatric patients when administered by alternative route rather than the oral route that is currently the only route of administration. To overcome these drawbacks and exploit the advantages, there was a need to develop an alternate route. Transdermal route was considered the alternate route. As literature review has revealed that no previous study has dealt on transdermal delivery of Irbesartan, the present study therefore, considered it of interest to investigate the bioavailability enhancement of Irbesartan through transdermal route and the probable mechanism of skin permeation by using microemulsion as delivery system.

MATERIALS AND METHODS:

Materials: Irbesartan (Bristol-Myers Squibb, USA), glycerol monocaprylocaprate (Inwitor 742 - Cremer Oleo GmbH and Co., Germany), poly-ethylene glycol 400 and polysorbate 80 (Sigma and Aldrich, USA) were used. All other chemicals and reagents purchased from Sigma and Aldrich (USA) were of analytical or high-performance liquid chromatography (HPLC) grade.

Preparation of the Microemulsion: Various microemulsions were prepared by aqueous phase titration method. Surfactant (polysorbate 80) and cosurfactant (polyethylene glycol 400) with specific weight ratios of 1:1, 1:2, 1:3, 1:4, 2:1, 3:1, 3:2 and 4:1 were vortex vigorously to make the surfactant mixture (Smix). The oil phase was mixed with each Smix in different volume ratios from 9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8 and 1:9 (v/v) in different glass vials. Water was added in aliquots to each combination of oil and Smix separately and mixed to obtain a clear and transparent micro-emulsion or milky or cloudy emulsion. The change in physical states from transparent to turbid and *vice-versa* were noted and recorded. A phase diagram was constructed using only microemulsion points for each Smix ratio. Different formulations were then made from the microemulsion region from each phase diagram constructed. For each percentage of oil selected, the formula that utilized the minimum concentration of Smix for its microemulsion was picked from the pseudo ternary phase diagram and used for the preparation of microemulsions that will be loaded with Irbesartan.

Preparation of Irbesartan-loaded Optimized Microemulsion: Optimized oil-in-water (O/W) microemulsion formulation of Irbesartan was prepared by dissolving 0.075 % w/w of Irbesartan in 20 % w/w oil and was followed by slow addition of 35 % w/w mixture of surfactant and co-surfactant (3:1) into oil phase. A 45 % w/w of distilled water was added to obtain the final preparation. For the water-in-oil (W/O) formulation, the percentage of oil was 45% w/w, while the

amount of distilled water added to make the final preparation was 20% w/w. The samples were physicochemical characterized and compared to their blank counterparts.

Thermodynamic Stability Measurement: Selected formulations were subjected to centrifugation, heating-cooling cycles and freeze-thaw cycles. Centrifugation was done at 3,000 rpm for 45 min. The formulations that failed to show any phase separation were subjected to heating and cooling cycle. This was done at temperature of 4 ºC and 45ºC with storage at each temperature for 48 h. They were cycled four times. Those formulations that were stable (lack of cracking, creaming or phase separation) further went for freeze-thaw test. Three freeze-thaw cycles were carried out between -21 ºC and + 25 ºC for 48 h.

Characterization of Microemulsion: Optimization of microemulsion was in terms of droplet size and polydispersity index.

The droplet size and polydispersity index of Irbesartan-loaded microemulsion were determined in triplicate by Photon Correlation spectroscopy [Zetasizer 1000 HS (Malvern Instruments, UK)].

Physicochemical Studies:

pH Measurement: The apparent pH of the formulations was measured in triplicate at 25 ºC using a pH meter with combination electrode (Eutech, Japan).

Refractive Index: The refractive index of the microemulsions was determined at 25 ºC using Abbe refractometer (Searchtech Instruments, England).

Viscosity Measurement: Viscosity of the samples was measured at 25 ºC using, a cone and plate viscometer (NDJ 5s viscometer, England). The test was carried out in triplicate.

Conductivity Measurement: The conductivity of the microemulsion was determined using conductivity meter (WTW LF90, Germany). The test was carried out in triplicate.

Determination of Irbesartan Content in Irbesartan-loaded Optimized Microemulsion: Irbesartan-loaded microemulsion was centrifuged at 10,000 rpm for 15 min. The drug concentration was analyzed spectrophotometrically (UV- Visible spectrophotometer, Shimadzu 1800, Japan) at maximum wavelength of 244 nm after appropriate dilution with methanol.

Preparation of Full Thickness Rat Skin: The approval to carry out the animal studies was obtained from Faculty of Veterinary Medicine, University of Nigeria, Nsukka Animal Ethics Committee. Care of the animals was in accordance with the institutional guidelines in Experimental methods in Pharmacology. Male Wister rats were sacrificed with prolonged exposure to chloroform. Hairs on the skin of animal were removed with electrical clipper and the abdominal skin of each rat was excised. The dermis side was wiped with isopropyl alcohol to remove residual adhering fat after the subcutaneous tissues were removed. The skin was washed with distilled water and the epidermis was prepared from it. The unused full thickness skin was wrapped in aluminium foil and stored at -20 °C till further use.

Preparation of Epidermis: The epidermis was prepared from the full thickness skin by heat separation technique [23]. It involved soaking entire abdominal skin in water at 60 ºC for 1 min. Distilled water was used to clean the epidermal sheet and dried under vacuum. It was then was cut into 4.5 × 4.5 cm^2 pieces and used for the permeation study. The unused epidermis was wrapped with aluminium foil and stored at −20 ˚C until needed.

***In-vitro* Skin Permeability Studies:** Prior to being used in the *in-vitro* skin permeability studies, the stored epidermis was allowed to thaw to room temperature, cut into 4.5 × 4.5 cm^2 pieces and hydrated by placing in phosphate buffer saline (PBS, pH = 7.4) overnight before use [24]. The studies were done using Franz diffusion cells. The diffusional area of the cells was 3.14 cm^2. The diffusion cells were connected with a circulating water bath and at a controlled temperature of 37 ºC. Phosphate buffer saline (PBS) used as a receiver fluid was placed in the receiver compartment that has a volume of 15 ml. The prepared epidermis was sandwiched between the receiver compartment and the donor compartment with the stratum corneum facing upwards. The donor compartment was clamped.

One ml (5 mg) of drug-loaded microemulsion was applied onto skin surface facing the donor compartment that was covered with a glass lid. The sampling arm of the donor compartment was also covered with aluminium foil. While the receiver fluid was being stirred, a 0.5 ml of each sample was withdrawn at suitable time interval (0, 1, 2, 3, 4, 6, 8, 10, 12 h) from the center of the receiver compartment with a syringe connected with a needle. An equal volume of fresh PBS (37 ºC) was immediately used to replace the withdrawn fluid. The amount of Irbesartan in the receiver fluid was determined spectrophotometrically at a maximum wavelength of 244 nm.

Data Analysis: The skin permeation data were measured as the cumulative drug permeation per unit of skin surface (Qt/S). The cumulative drug permeation (Qt) was calculated from Equation (1):

$$Q_t = V_t C_t + {}_{t-o}^{t-i}\Sigma V_s C_i \quad \text{..... Eq. 1}$$

Where, C_t is the drug concentration of the receiver fluid at each sampling time t, C_i is the drug concentration of the nth sample, V_t and V_s are the volumes of the receiver fluid and sample respectively. Graphing cumulative drug permeation per unit of skin surface versus time gave a linear plot. A linear regression analysis was used to determine the steady-state flux and lag time of the drug. The slope of the linear portion of the graph provided the steady-state flux (Jss, µg/cm/h). The lag-time was obtained by extrapolating the linear portion of the graph to the x-axis [25]. The permeability coefficient (P) was evaluated as

$$P = J_{ss} / C \quad \text{.....Eq. 2}$$

Where, C is the drug concentration in donor compartment.

One-way analysis of variance (ANOVA) was used to compare the flux obtained. A *p*-value of 0.05 was considered to be statistically significant. The penetration enhancing effect of each micro-emulsion was calculated in terms of enhancement ratio (ER) using Equation 3:

$$ER = K_p \text{ (microemulsion / } K_p \text{ (control)} \quad \text{.....Eq. 3}$$

Preparation of Stratum Corneum (SC) for Biophysical Analysis: To obtain the rat stratum corneum sample, freshly prepared epidermis membrane was soaked in a 0.1% trypsin solution for 12 h. Distilled water was used to clean the SC samples and blotted dry before been used for FTIR [26] and DSC [27] analysis respectively.

Fourier Transform Infrared (FTIR) Analysis: The analysis was performed on both completely dried samples of untreated and treated rat stratum corneum (SC) using FTIR instrument (Shimadzu 8400S, Japan). The microemulsion-treated and untreated (control) SC samples respectively were vacuum-dried at 25 ºC ± 1 ºC for 48 h and stored in desiccators to remove residual solvent. The control samples were treated in phosphate buffer saline (PBS).

Differential Scanning Calorimetry (DSC) Studies of Microemulsion Treated and Untreated Rat Skin: Saturated potassium sulphate solution was used to hydrate freshly prepared SC for 3 days and then blotted. The hydrated SC sample was dipped into microoemulsion formulation dissolved in 20 ml of methanolic PBS (pH 7.4) (30:70) and kept for 24 h at 37 ± 2 °C. Stratum corneum was removed and blotted after treatment. DSC analysis (Mettler Star SW 1300 Nietzsche DSC 200PC, USA) was carried out on a very small portion that was cut out, sealed in aluminium pan and equilibrated for 1 h. Scanning was done at the rate of 10 °C/min over the temperature range of 60 to 300 °C.

Determination of Activation Energy: *In-vitro* skin permeation study of Irbesartan across rat skin was also carried out at 25 °C, 37 °C and 50 °C respectively in the methanolic PBS pH 7.4 (30:70) as previously described. Permeability coefficient was calculated at each temperature and activation energy of Irbesartan was calculated from Arrhenius relationship.

$$P = P_o\, e^{-(Ea/RT)} \text{ or } \log P = Ea/2.303\, RT + log P_o \quad \text{.. Eq. 4}$$

Where, Ea is the activation energy, R is gas constant (8.143 kJ/mol), T is absolute temperature in K, P is the permeability coefficient, and Po is the Arrhenius factor.

Pharmacokinetic Studies: An approval to carry out pharmacokinetic studies was got from Faculty of Veterinary Medicine, University of Nigeria, Nsukka. Animal Ethics Committee Guidelines of the ethics committee were followed for the studies.

Pharmacokinetic studies were performed on optimized Irbesartan-loaded microemulsions and commercial tablet. Albino male rats weighing between 200-210 g were used for the study. The rats were kept under standard laboratory conditions (temperature 25 ± 2 °C and relative humidity of 55 ± 5%) and housed in polypropylene cages (six per cage). They had free access to standard laboratory diet and water *ad libitum*. About 5 cm^2 of skin was shaved on the abdominal side of rats in each group for transdermal administration except group treated with commercial tablet.

The rats were shared into 3 groups (n = 6). Group I received Irbesartan-loaded microemulsion (o/w) transdermally, Group II received Irbesartan-loaded microemulsion (w/o) transdermally while group III received commercial tablet suspension orally. Irbesartan dose was 30 mg/kg of body weight. Chloroform was used to anaesthetize the rats and blood samples (1.0 ml) were withdrawn from the retro orbital pleux of rat at 0 (pre-dose), 0.5, 1, 2, 4, 8, 12, 24 h in EDTA bottles. The blood collected after proper mixing with the EDTA was centrifuged at 5000 rpm for 20 min. The plasma was separated and stored at -21 °C until drug analysis was carried out using UV/Vis spectro-photometer. Plasma protein after the plasma sample has thawed and attained room temperature was precipitated from drug sample by adding acetonitrile : methanol mixture (4:1) and centrifuging at high speed (5000 rpm) for sufficient time. The separated clear supernatant liquid was analyzed spectrophotometrically. The concentration of unknown in plasma samples was calculated from the previously constructed calibration curve plotted between absorbance *vs.* Irbesartan concentrations.

Pharmacokinetic Analysis: The plasma concentration of Irbesartan at different time intervals was subjected to pharmacokinetic (PK) analysis to determine various pharmacokinetic parameters. The values of maximum plasma concentration (C_{max}) and time to reach the C_{max} (T_{max}) were read directly from the plot of time versus plasma concentration of Irbesartan. The area under curve (AUC) was calculated by using the linear trapezoidal method. The relative bio-availability of Irbesartan after the transdermal administration versus the oral administration was calculated using the following relationship:

$$\%F = \frac{[AUC]samplel}{[AUC]oral} \times \frac{[Dose]oral}{[Dose]sample} \times 100$$

One-way analysis of variance (ANOVA) was used to compare the pharmacokinetic data between different formulations and was compared for statistical significance.

RESULTS:

Pseudoternary Phase Diagrams: The compositions of optimized microemulsions are given in **Table 1**. Out of various pseudo ternary phase diagrams constructed, it was observed that the area of microemulsion decreased as the ratio of co-surfactant increased for specific oil to Smix ratio. One of the plots obtained from microemulsion formulations using 35% w/w mixture of surfactant: cosurfactant ratios are shown in **Fig. 1**. In the **Fig.**, the enclosed area marks the microemulsion region. Two representative microemulsion formulations referred to as O/W and W/O respectively, were identified from the micro-emulsion region and designated as MEa (O/W) and MEb (W/O) for surfactant: cosurfactant in the ratio of 3:1.

TABLE 1: COMPOSITIONS OF MICROEMULSIONS

Ingredients	MEa (O/W)	MEb (W/O)
Irbesartan (% w/w)	0.75	0.75
Imwitor 742 (%w/w)	20	45
Smix	35	35
Distilled water to (% w/w)	45	20

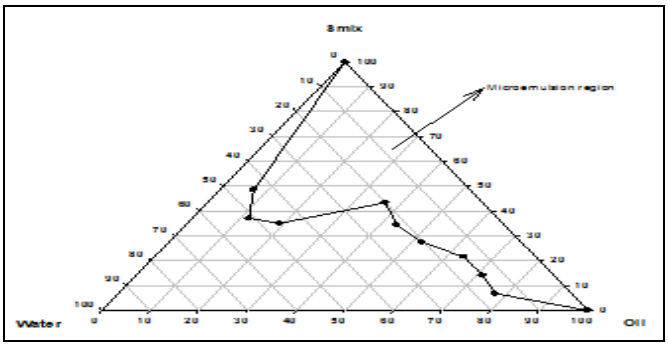

FIG. 1: PSEUDOTERNARY PHASE DIAGRAM OF Smix RATIO 3:1

Thermodynamic Studies: The results of thermodynamic stability studies showed no signs of cloudiness, phase separation or precipitation and therefore enabled very stable formulations to be selected for further optimization studies.

Characterization of Microemulsions: Micro-emulsions prepared from microemulsion regions of **Fig. 1** for each percentage of oil selected, while utilizing the minimum concentration of Smix from

the pseudo ternary phase diagrams were characterized in terms of droplet size and polydispersity index to obtain the optimized microemulsions of the type O/W and W/O respectively. The results are presented in **Table 2**. The low droplet size values indicated uniformity of droplets within the formulations. The morphology of Irbesartan-loaded microemulsions in the photomicroscopy photographs showed that the two formulations were nearly spherical in shape.

TABLE 2: CHARACTERIZATION, PHYSICOCHEMICAL PROPERTIES AND DRUG CONTENT OF IRBESARTAN-LOADED MICROEMULSIONS

Test	Blank (O/W)	MEa (O/W)	Blank (W/O)	MEb (W/O)
Droplet size nm	84.5 ± 0.26	83.4 ± 0.32	85.6 ± 0.46	85.8 ± 0.51
PDI	0.279 ± 0.006	0.281 ± 0.003	0.286 ± 0.004	0.293 ± 0.007
pH	4.75 ± 0.06	4.62 ± 0.02	4.94 ± 0.04	4.68 ± 0.05
Refractive index	1.4198 ± 0.0006	1.4222 ± 0.0007	1.4406 ± 0.0007	1.4427 ± .0003
Conductivity (µs/cm)	28.2 ± 0.21	29.1 ± 0.25	14.3 ± 0.22	14.1 ± 0.21
Viscosity (mpas)	245.8 ± 0.70	246.4 ± 0.75	246.9 ± 0.55	247.1 ± 0.27
Assay		97.9 ± 0.5		98.6 ± 0.2

Drug Content Analysis: The results of drug content of Irbesartan-loaded microemulsions are presented in **Table 2**. The results indicate very significant recovery of Irbesartan from the formulations.

Physicochemical Studies: The results of the physicochemical properties of Irbesartan-loaded microemulsions are presented in **Table 2**. When compared to the blank microemulsion, the results indicated that the types of polysorbate 80-based microemulsions were not altered after 0.75% w/w of the drug was incorporated.

***In-vitro* Permeation Studies:** The results of the permeability study are given in **Table 3** and **Fig. 2** respectively. The Lag time for the drug permeation through the stratum corneum from the control vehicle (distilled water) and the formulations is approximately 1 h. With the formulations, it was found that the W/O microemulsion showed higher flux for Irbesartan than the O/W microemulsion.

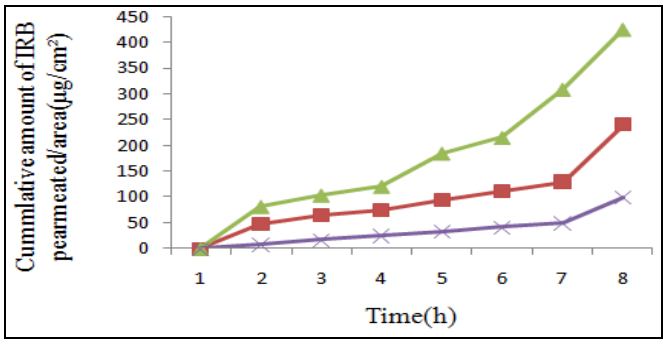

FIG. 2: *IN-VITRO* PERMEATION OF IRBESARTAN-LOADED MICROEMULSIONS THROUGH THE RAT SKIN AT 37 ºC

× -----× - Aqueous suspension of Irbesartan
□----- □ - Oil in water microemulsion (MEa)
Δ -----Δ - Water in oil microemulsion (MEb)

TABLE 3: *IN-VITRO* PERMEATION OF IRBESARTAN -LOADED MICROEMULSION THROUGH THE RAT SKIN AT 37 ºC

Parameter	Control	ME a (O/W)	MEb (W/O)
Permeability coefficient ($\times 10^3$cm/h)	2.03	3.92	7.32
Steady-state flux (µg/cm^2/h)	10.16 ± 0.09	19.6 ± 0.38	36.6 ± 0.33
Enhancement ratio		1.93	3.61

FTIR Analysis: The results of the FTIR analysis are shown in **Table 4**. Various peaks arising from molecular vibration of lipids and proteins found in the stratum corneum (SC) are seen in the FTIR spectrum of untreated SC (control, **Fig. 3**).

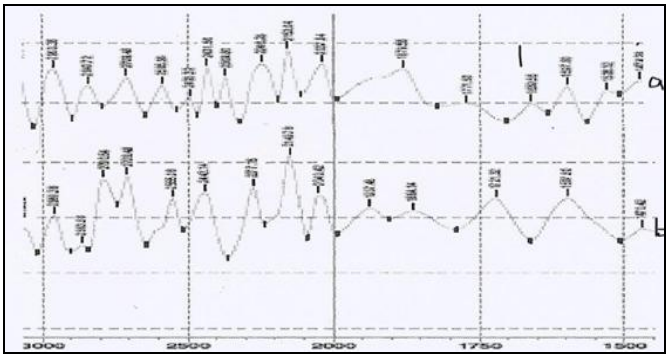

FIG. 3: FTIR PLOT OF (a) UNTREATED AND (b) TREATED STRATUM CORNEUM

The asymmetric -CH_2 and symmetric -CH_2 vibrations of long chain hydrocarbons of lipids occurred at vibration frequencies of 2960 cm^{-1} and 2840 cm^{-1} respectively. The amide I and amide II bands emanating from C = O stretching vibration and C-N bending vibration of SC proteins were seen to occur at vibration frequencies of 1659 cm^{-1} and 1597 cm^{-1} respectively. The assignment of these vibration bands was based on previous report [28]. Observable difference in the FTIR spectra of

untreated and microemulsion-treated SC occurred. Significant decrease in asymmetric and symmetric CH_2- stretching of absorbance intensities of microemulsion-treated SC was observed when compared to untreated SC.

The same observation was made for amide I, and amide II stretching vibrations. The peak positions were however, not altered in both treated and untreated stratum corneum.

TABLE 4: FTIR SPECTRAL DATA OF MICROEMULSION TREATED AND UNTREATED RAT SKIN

Vehicle	Asymmetric C-H stretching		Symmetric C-H stretching		Amide I stretching vibration		Amide II bending vibration	
	Absorbance intensity	% decrease in intensity	Absorbance intensity	% decrease in intensity	Absorbance intensity	% decrease in intensity	Absorbance intensity	% decrease in intensity
Untreated	2.5629	-	2.2883	-	1.9863	-	2.2757	-
ME a	1.5272	40.39	1.1253	50.82	1.7655	11.12	1.7689	22.27
MEb	1.4124	44.97	1.0076	55.96	1.6531	16.77	1.4749	35.14

DSC Studies: The endotherm at 110 ºC was used to evaluate the skin permeation mechanism of Irbesartan. This endotherm was observed to decrease by the microemulsion to lower melting point (105 ºC)

Determination of Activation Energy: The results of the activation energy determination are shown in **Fig. 4**. The results indicate that microemulsion decreased the activation energy of permeation through the rat skin when compared to aqueous suspension of Irbesartan.

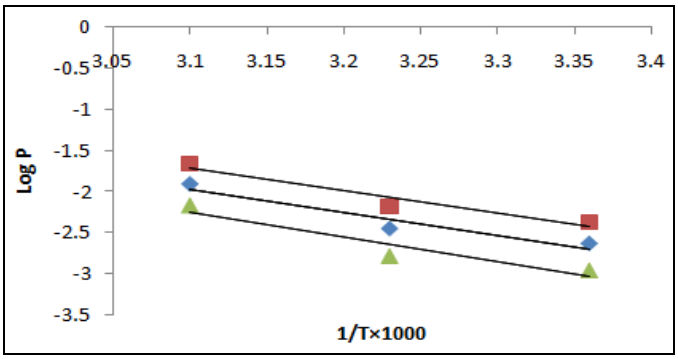

FIG. 4: ARRHENIUS PLOT BETWEEN LOGARITHMS OF PERMEABILITY COEFFICIENT *VERSUS* RECIPROCAL OF ABSOLUTE TEMPERATURE

◊ ----- ◊ - Oil in water microemulsion (MEa)
Δ -----Δ - Aqueous suspension of Irbesartan
□----- □ - Water in oil microemulsion (MEb)

Arrhenius plot **Fig. 4** between logarithms of permeability coefficient versus reciprocal of absolute temperature was observed to be linear in the temperature range between 25-50 °C. The value of Ea for Irbesartan permeability across rat skin was calculated from the slope of Arrhenius plot and was found to be 57.98 kJ/mol, 53.31 kJ/mol and 52.24 kJ/mol for aqueous suspension of Irbesartan, O/W micro-emulsion and W/O microemulsion respectively.

Pharmacokinetic Studies: Plasma concentration of Irbesartan from microemulsion formulations and tablet at various time intervals was determined by spectrophotometric method. The results are given in **Table 5** and **Fig. 5** respectively. The pharmacokinetic data (collected for a period of 24 h), for Irbesartan tablet formulation are 1160.18 ±5.86 µgh/ml, 0.0258 ± 0.0028 L/h/kg, 1.1416 ± 0.0234 L/kg, 44.25 ± 1.15 h, 0.0226 ± 0.0014 h^{-1} and 30.7 ± 0.41 h for area under curve at infinity ($AUC_{0\rightarrow\infty}$), total clearance (CL_{total}) , volume of distribution at a steady state (Vss), mean residence time (MRT), elimination rate constant (Ke) and elimination half-life ($t½$) respectively.

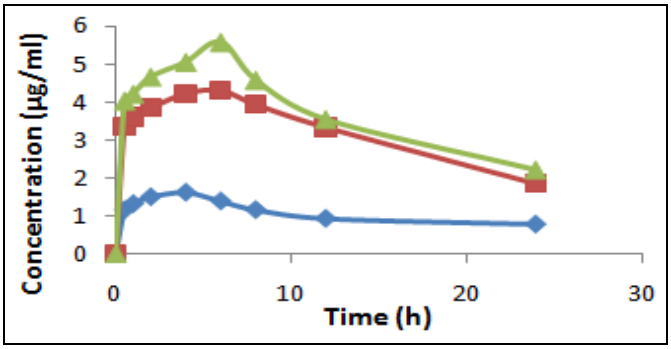

FIG. 5: THE PLOT OF IRBESARTAN PLASMA CONCENTRATION *VERSUS* TIME

◊ ----- ◊ - Aqueous suspension of Irbesartan
Δ -----Δ - Water in oil microemulsion (MEb)
□----- □ -Oil in water microemulsion (MEa)

TABLE 5: PHARMACOKINETIC PARAMETERS OF IRBESARTAN AFTER ORAL ADMINISTRATION OF REFERENCE TABLET AND MICROEMULSION

Parameter	ME a (O/W)	ME b (W/O)	Tablet formulation
C_{max} (µg/ml)	4.29 ± 0.06	5.58 ± 0.05	1.62 ± 0.03
T_{max} (h)	6	6	4
AUC→24 h (µg.h/ml)	49.07 ± 0.11	88.74 ± 0.45	25.44 ± 0.28

The Irbesartan-loaded microemulsion was found to enhance the bioavailability of Irbesartan through transdermal delivery by 1.9- fold increase for O/W microemulsion and 3.5- fold increase for W/O microemulsion respectively, when compared with the oral tablet. The significant ($p<0.05$) $AUC_{0\rightarrow24h}$ values observed with Irbesartan-loaded micro-emulsion depicted increased bioavailability of the Irbesartan from the formulation in comparison with oral tablet formulation.

DISCUSSION: The calibration graph was found to be linear within the concentration range of 2.0 – 10.0 µg/ml suggesting that Beer's law was obeyed. Regression equation defining the observed linearity was:

$$A = 0.0496C - 0.0461 \quad (r = 0.9944).$$

Pseudoternary Phase Diagrams: Pseudoternary phase diagrams with large microemulsion areas indicate how efficient polyethylene glycol 400 is as a cosurfactant in increasing the flexibility of the interfacial film. The phase behavior of the pseudo ternary phase diagram was not altered when Irbesartan was incorporated into microemulsion which implies that the formation and stability of the microemulsion were never influenced by the physicochemical properties of the drug.

Thermodynamic Studies: The observed results in thermodynamic study could have arisen from low interfacial tension between oil and water at the weight ratio of surfactant with respect to the co-surfactant as well as the position of the droplet size.

Characterization of Microemulsions: Charac-terization results suggest that the droplet size obtained could provide larger surface area available for transdermal Irbesartan absorption. Previous reports have shown that microemulsion with a droplet size less than 100 nm would enhance solubility, increase membrane permeability, and guarantee efficient absorption of drug [29, 30].

Drug Content Analysis: The results of the drug content analysis, suggests that irbesartan is completely solubilized in the microemulsion.

Physicochemical Studies: Any slight changes in the physicochemical properties of irbesartan-loaded microemulsions could arise from the intrinsic properties of the drug. The results suggest that the degree of permeation of Irbesartan in this investigation could be strictly controlled by the nature (W/O or O/W) of the microemulsion, since there was apparent similarities in the physico-chemical properties of the blank and drug-loaded microemulsions. These similarities imply that the constituents (oil, water and surfactant/co-surfactant) of the microemulsion, which control the physicochemical properties of microemulsion, have similar effects on the permeation of Irbesartan through the excised rat skin.

***In-vitro* Permeation Studies:** The *in-vitro* permeation study results demonstrated the lipophilicity of the drug. As microemulsion formulations and the drug suspension exhibited the same lag time, their comparison could be justified.

FTIR Analysis: Studies [31, 32] have shown that the rate limiting step for transdermal drug delivery is lipophilic part of SC in which lipids are tightly packed as bilayers due to the high degree of hydrogen bonding which imparts barrier property. The observed changes in the structure of the microemulsion treated-stratum corneum when compared to the untreated stratum corneum following FTIR analysis might be responsible for enhanced skin permeation of Irbesartan. Such permeation enhancement might be due to increase in drug diffusivity arising from any of these reported permeation mechanisms namely: small dispersed phase size [33, 34], disruption of the lipid structure of the stratum corneum [35, 36], increasing the partition coefficient of the drug between the skin and the vehicle [37] and an increase in skin hydration [38]. Previously, it has been reported that W/O emulsions enhanced transdermal delivery by hydration of the skin epidermis [39, 40].

DSC Studies: The endotherm at 110 ºC was attributed to the fourth endotherm of the untreated stratum corneum. Its decrease to lower melting point by microemulsion, suggests keratin denaturation as one of possible mechanisms of permeation enhancement of Irbesartan. Previous reports [41, 42] stated that the DSC thermogram of hydrated untreated human stratum corneum could show endothermic transitions at the following temperature ranges: 35-40 °C (T1), 65-80 °C (T2), 80-90 °C (T3) and 95-115 °C (T4) respectively. The first endotherm which showed the lowest

enthalpy was attributed to sebaceous lipids and other structural rearrangement of lipid bilayer. The second and third endotherms (T2 and T3) arose from the melting of SC lipid chain of the bilayer structure. The fourth endotherm (T4) was considered to arise from intracellular keratin denaturation.

Determination of Activation Energy: Route of diffusion and physicochemical properties drug control the activation energy (Ea) for diffusion of a drug molecule across skin. The activation energy for transdermal permeation of Irbesartan was found to be lower for the microemulsions when compared to aqueous suspension of Irbesartan indicating existence of a lower energy barrier for the micro-emulsions. The results also indicate significant change on SC lipid bilayer's morphological characteristics by the microemulsion thereby creating pathways in the bilayers that allowed transdermal permeation of Irbesartan to be enhanced. Previous studies have reported that colloidal dispersions can change Ea value to greater extent by their action on SC lipids [43, 44].

Pharmacokinetic Studies: Interferences was considered not occur with the spectrophotometric method employed in the pharmacokinetic study, because previous report [22] has shown that the only metabolite of Irbesartan (Irbesartan glucuronide) that could be found in plasma is at very low concentration. This polar metabolite (not observed in the UV spectrum) when compared to Irbesartan is expected to show maximum absorption of ultraviolet photon at a longer wavelength than Irbesartan. Furthermore, literature has shown that acetonitrile: methanol ratio (4:1) is very effective in precipitating plasma proteins.

A non-compartmental pharmacokinetic analysis was used to evaluate the rate and extent of irbesartan permeation through the rat skin into systemic circulation. The maximum plasma concentration of Irbesartan (C_{max}) and the time to reach Cmax (T_{max}) were obtained from the plot of plasma drug concentration versus time. The area under the drug concentration-time curve and mean residence time (MRT) were calculated based on the trapezoidal rule. Total clearance (CL_{total}) was estimated as dose (30 mg/kg)/$AUC_{0\rightarrow\infty}$ while volume of distribution at a steady state (Vss) as CL_{total} × MRT. The elimination half-life ($t½$) was evaluated from the division of 0.693 by the elimination-rate constant Ke (CL_{total}/Vss) respectively. The observed increased bioavailability from microemulsion formulations could be as a result of increase in irbesartan solubility and enhanced skin permeation.

CONCLUSION: The characterization data confirmed the suitability of the microemulsion formulation method. Thermodynamic stability data enabled meta stable formulations to be eliminated. FTIR spectra, DSC thermograms and activation energy data analyses suggest that permeation could have occurred by the disruption of SC lipids, denaturation of SC proteins and skin hydration. The pharmacokinetic studies indicated that significantly greater absorption of Irbesartan was obtained with microemulsion formulations than the oral tablet formulation ($p<0.05$). The results of the present study suggest that microemulsion can be successfully used for enhancement of skin permeation as well as bioavailability of Irbesartan and therefore a potential transdermal delivery system for Irbesartan.

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