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DEVELOPMENT AND OPTIMIZATION OF TRANSDERMAL MICROEMUSION FORMULATION OF BCS CLASS II ANTIDIABETIC DRUG BY DOE

Ashish Srivastava * and Naresh Kalra

Lords College of Pharmacy, Lords University, Chikani, Alwar - 301028, Rajasthan, India.

Keywords:

Transdermal drug delivery, Microemulsion, Simplex lattice design, Glibenclamide

Correspondence to Author:
Ashish Srivastava

Research scholar,
Lords College of Pharmacy,
Lords University, Chikani,
Alwar - 301028, Rajasthan, India.

E-mail: ashishs75@gmail.com

ABSTRACT: The main aim of present investigation was to examine the potential of a microemulsion formulation developed through simplex lattice design as well as desirability function approach for transdermal delivery of BCS class II anti diabetic drug Glibenclamide. Pseudo-ternary phase diagrams were plotted by water titration method for multiple microemulsion formulations using oleic acid (oil), tween 80 (surfactant) ethanol (cosurfactant) mixture and water. The area of Pseudo ternary phase diagram was the basis of selecting surfactant cosurfactant ratio (i.e. 1:2).Simplex lattice design for microemulsion preparation was used to identify the effect of independent variables oil concentration (X_1), scs concentration (X_2) and water (X_3) with response variables like percentage transmittance (Y_1) as well as cumulative % drug permeation (Y_2). Polynomial equations as well as model graphs obtained by performing simplex lattice design experiments were used to evaluate the effect of independent variables on responses. The concentration of surfactant cosurfactant mixture was directly related to transmittance and hydration of skin also affected the permeation. So there was significant effect on the responses ($p<0.05$). Using desirability function, the optimized formulation with desirability value (0.998) was developed with oleic acid (4.06%), surfactant cosurfactant(1:2) concentration (44.3%) and water 51.52% showed the transmittance value 99.87± 2.35% and permeation value 86.17±2.79%. The %permeation from optimized microemulsion was found 2.73 times more that of the control suspension formulation through excised rat skins in franz diffusion cell . The other parameters of optimized formulation like globule size was uniform, stable and showed same observed and predicted responses.

INTRODUCTION: NIDDM (Non-insulin-dependent diabetes mellitus) is a chronic disease in which the blood sugar concentration becomes high (hyperglycemia) due to insulin hormone deficiency as well as insulin resistance. People with hyperglycemia have a high risk of developing a form of eye disease, kidney disease and coronary artery disease.

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Uncontrolled diabetes is a major cause of blindness, kidney failure, heart attacks, stroke, and lower limb amputation. In 2019, diabetes was the ninth leading cause of death, with an estimated 1.5 million deaths directly caused by diabetes [1]. Sulphonylureas are a medicine used to enhance insulin release to control sugar levels in the normal range.

Glibenclamide (Glb) is one of the most potent second-generation sulphonyurea (dose 2.5-20 mg) for it. Glb is highly lipophiic (pka- 5.3) and BCS class II drug. This drug shows variable oral bioavailability due to poor drug solubility and dissolution. It also shows serious and sometimes fatal side effects like hypoglycemia attack and

gastric disturbances like heartburn, nausea, vomiting, anorexia or increased appetite during oral route drug therapy [2]. Since Glb is usually taken for a long period, the compliance of the patients is very important [3]. So, Transdermal drug delivery systems (TDDS) may be a preferred alternative to oral drug delivery system because it provides a controlled release pattern of the drugs, avoidance of dissolution-related issues, avoidance of first-pass metabolism effects, lesser systemic side effects and also improve the dosage efficiency by maintaining steady-state blood drug profiles throughout whole treatment [4]. Despite so many advantages, transdermal drug delivery through the skin has major limitation of poor drug permeation due to the presence of horny or keratinized skin barrier (stratum corneum), which resist the entry of any drug molecule from outside the body. This has created a significant hindrance to skin/ transdermal drug delivery [5].

To improve the drug permeability through the skin, the carrier system must help drug molecules cross stratum corneum. The Microemulsion formulation has potential as a drug carrier system for topical and transdermal drug delivery. The microemulsion is a thermo-dynamically stable, clear or transparent dispersion of two opposite-nature liquid phases, water, and oil; stabilized by a surfactant and co-surfactant mixture which forms the interfacial film between oil and water [6]. The microemulsion has various remarkable properties like enhanced solubilization ability of the poorly water-soluble drugs, high bioavailability and permeation ability due to ultra-low surface tension (10^{-4}mN/m) and very small droplet size less than 100 nm [7].

Due to its very small size, it cannot reflect visible light (400 to 800 nm) and cannot be viewed by the optical microscope, making the microemulsion system a transparent system [8]. Microemulsion can act as a carrier for both lipophilic and hydrophilic drugs [9]. Oil, water, surfactant, and cosurfactant mixture represent three edges of the pseudo ternary phase diagram, which characterize the microemulsion zone. The selection of excipients for the effective and stable microemulsion is very cumbersome which requires detailed knowledge of oil phase, surfactant and cosurfactants. The physicochemical properties of oil phase, like polarity, molecular volume, molecular weight and surface tension, considerably affect the spontaneous formation, globule size, and drug entrapment efficiency. The surfactant having high solubilization capacity and the ability to decrease surface tension at a low level should be selected. The surfactant having HLB (HLB>12) is a prerequisite for the instantaneous development of oil in water microemulsion [10]. The design of experiment (DoE) approach is widely applicable in pharmaceutical formulation development it is used for screening and optimization of formulation parameters and process variables to get the best formulations and processes. Simplex lattice design is also a type of DOE for mixture design and optimization [11]. In the present investigation O/W microemulsions containing Glb (pka-5.3) were developed after screening microemulsion components. A Simplex lattice design with a desirability function was used to design and optimize microemulsion formulation. Further, the effect of microemulsion components like oil (oleic acid), surfactant (tween 80), and cosurfactant (ethanol) on various response variables like % transmittance and % permeation was also evaluated.

EXPERIMENTAL:

MATERIAL: Glb was kindly provided by macloids ltd Mumbai India. All other chemicals were purchased from reputed manufacturers like Merck, Sigma and Qualigens (Mumbai India). All the reagents and excipients were used as received. Double distilled water was prepared freshly for the use.

METHOD:

Screening of Oil, Surfactant and Cosurfactant: The excipients (oil, surfactant and cosurfactant) were selected on the basis of their drug solubilization capacity. Various oils (oleic acid, castor oil, eucalyptus oil ethyl acetate, ethyl butyrate) surfactants (tween 80, cremophore EL, tween 40) and cosurfactants (ethanol, propylene glycol, n butanol, n propanol) by shake flask method. The solubility of Glibenclamide in different oils and surfactants and cosurfactants was determined by using shake flask method. Briefly, an excess of Glibenclamide was added individually to the oils (oleic acid, castor oil, eucalyptus oil, ethyl acetate, ethyl butyrate) surfactants (tween 80,cremophore EL, tween 40) and cosurfactants

(ethanol, propylene glycol, n butanol, n propanol) (5 ml each) in screw capped tubes. Water bath shaker was used to shake mixtures at 100 rpm for 24 h. The temperature was maintained at 37^0C. After end of 24 h, each suspension was centrifuged at 5000 rpm for 20 mins to separate undissolved amount of drug and placed it on standing for 12 hrs after that the clear supernatant liquid was decanted. The supernatant (1.0 ml) was diluted by methanol and the amount of Glibenclamide present in the supernatant was analyzed by uv vis spectrophotometer (UV-1700, Shimadzu corporation, Tokyo, Japan) against methanol as blank at 300 nm. The study was done in three times and their mean value was recorded [12].

Construction of Phase Diagram: A water titration method was used to prepare the microemulsion. Briefly, a mixture of surfactant and cosurfactant (Km=1:1, 1:2, 1:3, 2:1 and 3:1 ratio) was prepared. After that, the oil and surfactant, and cosurfactant mixture (scs) were mixed homogenously at different ratio of (9:1,8:2,7:3, 6:4, 5:5, 4:6, 3:7, 2:8 1:9) into different vials and were diluted dropwise with double distilled water and the mixture was vortexed 3-4 min. It was allowed to equilibrate at room temperature for 30 mins. After equilibrium, the mixtures were examined for transmittance value, phase separation and the point at which phase separation started or became turbid was considered the end point of the titration.

On the basis of it, the concentration of components was recorded for the determination of the area of microemulsion as well as preparation of pseudo ternary phase diagram with the chemix software. The microemulsions were subjected to stand for 2 hours. Then, the transmittance value was determined at 650 nm by double beam UV Vis spectrophotometer (UV-1700, Shimadzu Corporation, Tokyo, Japan) using water as blank. On the basis of these diagrams, the concentrations range of materials were identified for the preparation of microemulsion. The ratio of surfactant and cosurfactant mixture showing largest microemulsion area was selected [13].

Simplex Lattice Experiment Design: A three-factor, three-level simplex lattice design was generated by Minitab 18 software. It was used to ascertain the effects of formulation-independent variables and their combined effects on response variables. It is a statistical approach to evaluate the main as well as interaction effects of the independent variables on dependent variables. In this experiment design, three factors were analyzed by changing their concentrations simultaneously but maintaining their total concentration constant. For a three-component system, the simplex lattice design is represented by an equilateral triangle with ten points in two-dimensional space **Fig. 1**. Total of ten batches was prepared: at each vertex point (A, B and C), at the halfway point between vertices (AB, BC and AC), next at the center point (ABC), at the halfway point from the centre (ABC) to vertex point (A, B and C). The concentrations of oil (X_1), surfactant cosurfactant mixture (scs) (X_2), and water (X_3) was chosen as the independent variables. The transmittance value (Y_1) and the cumulative % drug permeation of Glibenclamide through excised rat skins (Y_2) were taken as responses, as well as the goal of dependent variables given in **Table 1,** respectively. The Simplex lattice design equation (special cubic model) is described as follows:

$$Y = b_1X_1 + b_2X_2 + b_3X_3 + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{23}X_2X_3 + b_{123}X_1X_2X_3 \quad \text{.....Eq-1}$$

Here the dependent variable is Y and b_i is the estimated coefficient for the variables X_i. The (X_1, X_2 and X_3) were the main effects which shows the results of altering one variable at a time from its lower value to higher value and the interactions between two variables were X_1X_2, X_2X_3, X_1X_3 and between all variables $X_1X_2X_3$ model. It was used to optimize the formulation composition of microemulsions [14].

Preparation of Glibenclamide Loaded Microemulsion: After analyzing pseudoternary phase diagram, microemulsion zone was identified and the concentration range selected for various excipients was as follows: X_1–oil (05–15%), X_2–surfactant cosurfactant mixture (55–75%) and X_3–water (20–30%). The response variables were transmittance (Y_1), Cumulative % drug permeation (Y_2). The design layout and code value with range are both given in **Table 2**. Ten formulations were prepared incorporating 5 mg/gm of the formulation. The drug was dissolved in oil first, followed by the addition of a surfactant cosurfactant mixture. All the components were mixed well with vortex

shaker and were then warmed up to 37°C on a magnetic stirrer till a precise homogenized mixture was obtained. Then the water was added to it till it was acquired and maintained transparency. The formulation was placed at room temperature for further study.

TABLE 1: VARIABLES IN SIMPLEX LATTICE DESIGN

S. no.	Independent variables	Dependent variables	Goal for dependent variables
1	oil (X_1)	Transmittance (Y_1)	Maximize
2	surfactant co-surfactant mixture (scs) (X_2)	Cumulative % drug permeation(Y2)	Maximize
3	Water (X_3)		

TABLE 2: DESIGN LAYOUT OF SIMPLEX LATTICE DESIGN FOR PREPARED MICROEMULSIONS

Experimental run	Formulation code	Coded variable levels		
		Oil (X_1)	Scs (X_2)	Water (X_3)
1	Me1	66.667	16.667	16.667
2	Me2	16.667	66.667	16.667
3	Me3	33.333	33.333	33.333
4	Me4	16.667	16.667	66.667
5	Me5	0.000	50.000	50.000
6	Me6	100.000	0.000	0.000
7	Me7	0.000	0.000	100.000
8	Me8	0.000	100.000	0.000
9	Me9	50.000	0.000	50.000
10	Me10	50.000	50.000	0.000

Transformation of coded levels in actual units

Coded variable levels *	Low	Middle	High
	0	0.5	1.0
Amount of oil (X_1)	0.05	0.10	0.15
Amount of S-Cs (X_2)	0.55	0.65	0.75
Amount of water (X_3)	0.20	0.25	0.30

*Levels were screened on the basis of pseudoternary phase diagram for visual as well as %age transmittance values.

Evaluation of Prepared Microemulsions:

Percent Transmittance (%T): The prepared microemulsion was allowed to stand for 45 mins and the %T was analyzed at 650 nm by double beam UV-visible spectrophotometer [15]. The transmittance study was conducted in triplicate

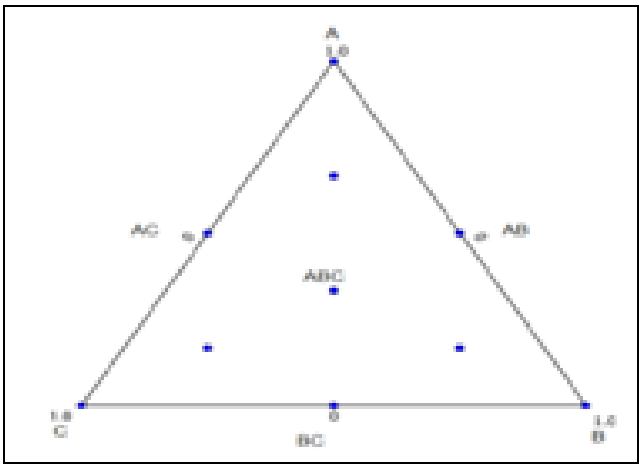


FIG. 1: SIMPLEX LATTICE DESIGN OF THREE COMPONENTS, THREE-LEVEL VARIABLES

In-vitro Permeation Experiments:

Preparation of Skin: *In-vitro* permeation studies were conducted using skin of albino rat weighing 120±5 g that had been previously sacrificed for other experimental purposes. The manner of experiment was not affecting the skin. The hairs were removed with the help of electric clipper and the subcutaneous fat was removed with the help of scalpel. The skins were washed carefully and examined for integrity, after that the skins were placed in a refrigerator at 4°C overnight and then permeation experiments were performed on that [16].

***In-vitro* Permeation Study:** *In-vitro* permeation studies were performed on franz diffusion cells having an effective diffusion area of 3.14cm^2. The skin samples were fitted carefully between the donor and receiver compartment on franz diffusion cells, with the stratum corneum facing toward the donor side. The test formulation was kept in the donor compartment. The receiver compartment contains 22 ml phosphate buffer pH 7.4 with ethanol 4:1 mixture to ensure sink condition, and the temperature was also maintained at 37±0.5°C with stirring at 100rpm in a magnetic stirrer throughout the experiment. 1ml sample of the receiver medium was taken at a predecided time interval. Then the volume was refilled immediately with an equal volume of fresh ethanol phosphate

buffer pH 7.4.mixture (4:1). Cellulose membrane filter having 0.45 µm pore size were used for filtration of all samples. Then the samples were analyzed spectrophotometrically at 300 nm using phosphate buffer pH 7.4 with ethanol mixture as blank.

Calculation of the *In-vitro* Data: The cumulative amount permeated (*Qn*, $mgcm^{-2}$) through excised mouse skins was determined based on following equation (Eq-2).

n=1

Cumulative amount of drug permeated (Qn, mg/cm^2) = Cn x V0 + $\sum_{i=1}$ Ci x Vi / S….(Eq-2)

Where *Cn* is used for the drug concentration in the receiver medium at each sampling time, *Ci* stands for the *i*th sample drug concentration and *Vi and V*0 stand for the volumes of the ethanolic phosphate buffer pH 7.4 solution of the sample and receptor compartment, respectively, *S* stand for the effective diffusion area [17]. The Percentage permeation was calculated by

$$\% \text{ permeation} = Qn / 5 \times 100$$

Formulation Optimization by Desirability Function: In this study, all the responses (%transmittance and Cumulative % drug permeation) were studied simultaneously to optimize formulation by response optimizer in Minitab software [18]. In this a specific target was assigned to each response as per given in **Table 1**. A partial desirability function is linked with a single response where 0 is associated with an unacceptable/ undesirable response while the value from 0 to 1 is acceptable. The higher value from 0 to 1 indicates the closeness of the response to its most accepted or target value. Therefore the desirability function helps to find out most favorable and suitable points in the design space that fulfill the set goals for responses [18].

Evaluation of Optimized Formulation: Optimized formulation was prepared on the basis of desirability function and was assessed for the parameters/response variables (% transmittance and Cumulative % drug permeation) and compared with that of predicted values. The other parameters like globule size, pdi value, zeta potential, tem analysis, staining study, and thermodynamic stability were also performed to evaluate microemulsion characteristics.

Globule size, Polydispersity Index (PDI) and zeta Potential Determination: The optimized formulation globule size, zeta potential as well as polydispersity Index (PDI) were evaluated by DLS method by Anton Paar Litesizer 500 (Anton Paar GmbH, Austria) at 25°C. . The Litesizer 500 measured globule size by analyzing the random intensity fluctuations in scattered light due to the Brownian motion of the globules [19].

TEM Analysis: The morphology of Glibenclamide microemulsion was determined by transmission electron microscopy (CM 12 TEM, Philips, Amsterdam, Netherlands). Negative staining of the sample was done with 1 % phosphotungstic acid (PTA) aqueous solution. Carbon coated copper grid was used for drying of the microemulsion. After drying, the sample morphology was scanned under microscope [20].

Staining Test: The staining test was performed by using water soluble dye and methylene blue to check the prepared formulation as O/W or W/O type. The dye was dispersed into the prepared optimized formulation, and after 5 min the formulation was visually inspected [21].

Thermodynamic Stability: Thermodynamic stability is determined in three phases. Phase I includes the heating and cooling cycle where optimized microemulsion formulation withstands six heating and cooling cycles, performed at a high temperature 40 °C and low temperature 4 °C, and was stored for at least 48 h at every temperature. After completion of phase I, formulation faced the next Phase II centrifugation at 5000 rpm for the time period of 30 minutes. After that, the microemulsion was checked for its physical instability like any phase separation, cracking or creaming [22]. In Phase III, the formulation was subjected to three freeze-thaw cycles,in which the formulation was freezed at − 04°C for 48 h, subsequently thawing it at 40 °C for 48 h [23].

Comparison of % Permeation Profile of Optimized Microemulsion Formulation with Suspension Formulation: The cumulative % drug permeation profile of optimized formulation was compared with the control (suspension)

formulation. The suspension was prepared by suspending the 5.0 mg drug in 1.0 gm of double distilled water. The optimized formulation, as well as control formulation permeation, was evaluated for 12 hrs, respectively. The whole study was performed in triplicate, and the mean value was used for comparison

RESULTS AND DISCUSSION:

Components Screening for Microemulsion: In analyzed four oils, the solubility of Glibenclamide was found highest in oleic acid (3.56mg/ml), followed by Eucalyptus oil (1.73mg/ml) and the least solubility was found in ethyl butyrate (0.56 mg/ml) **Fig. 2A**. Literature study showed that the oil having the maximal solubilizing potential will be selected to achieve optimum drug loading [24].

It was also found that oleic acid was a powerful enhancer for dermal delivery since it could increase the fluidity of the stratum corneum's lipid portion, resulting in better permeation [25]. So, oleic acid was chosen as oil.

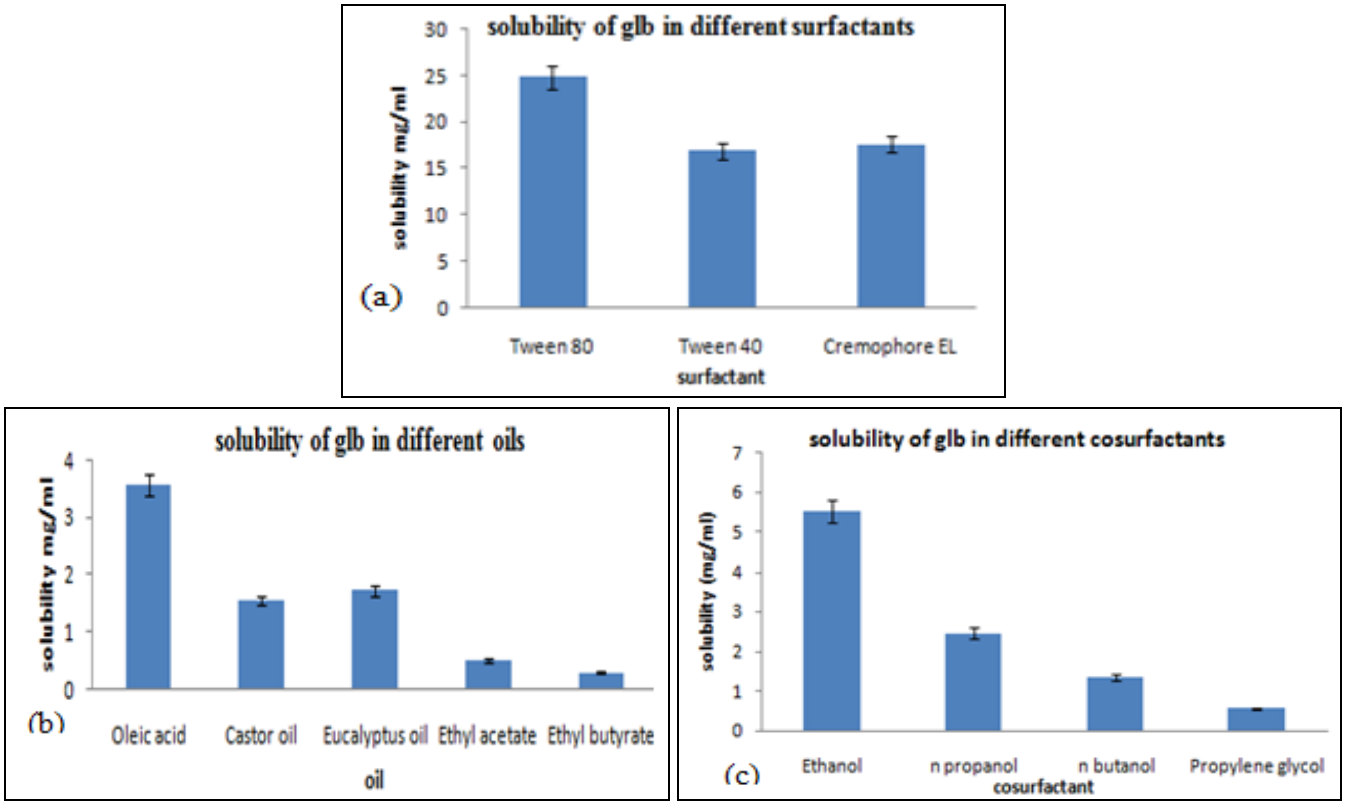
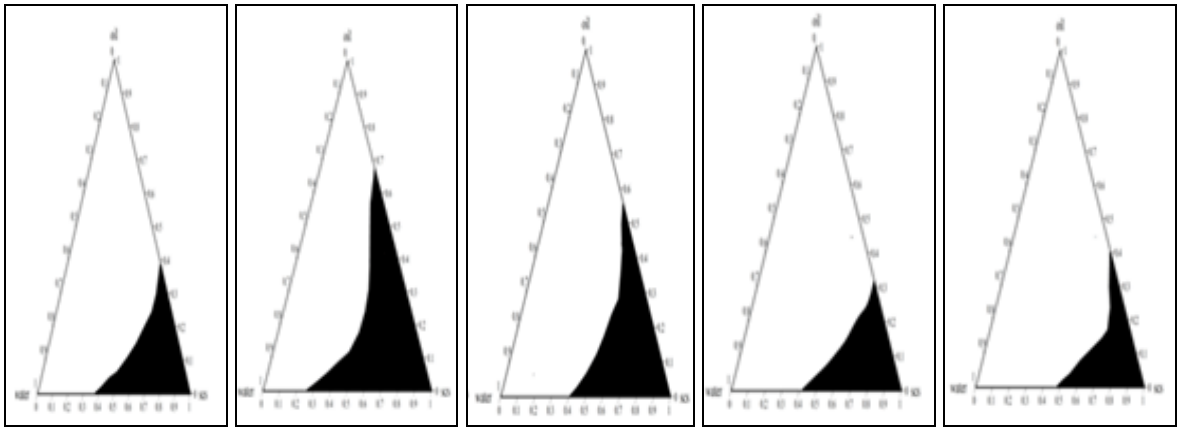


FIG. 2: SOLUBILITY OF GLIBENCLAMIDE IN SURFACTANTS (A) OIL (B) AND COSURFACTANTS (C)

In this study, Tween 80 (24.72 ± 0.26 mg/ml) showed a better solubility for Glibenclamide than cremophore el (17.57 ± 0.41mg/ml) despite high HLB values of both surfactants 15 and 13.5 respectively. **Fig 2B** crempohore el was not selected due to its cutaneous side effects (pruritus, flushing, or rashes) [46]. Cosurfactants help the surfactants in the solubilization of hydrophobic drugs by decreasing the surface tension upto ultra low level [27]. Among the cosurfactants, ethanol was chosen as it showed the maximum solubility potential for Glibenclamide **Fig. 2C** and worked as a penetration enhancer. The mechanism of permeation enhancement of ethanol is not perfectly known but it is well established that ethanol partitions well into the skin and enhances the permeation of both polar and nonpolar drug molecules; there are several proposed mechanisms ranging from lipid extraction from the skin lipid bilayer layer, fluidization of the lipid bilayer, alteration of SC protein conformation, co-permeation of drug with alcohol (pull effect) and enhancement of the drug miscibility in the SC lipids. The relative existence of each mechanism depends upon the ethanol concentration used in the donor solution/formulation and on the lipophilicity of the drug/actives [28].

Construction of Pseudo-ternary Diagrams: Based on drug solubility in microemulsion

components, Oleic acid (oil), tween 80 (surfactant), and ethanol (cosurfactant) were selected and used to construct the pseudo-ternary phase diagrams with double distilled water. **Fig. 3A-E** represents five microemulsion formulations having different scs Km values (1:1, 1:2, 1:3, 2:1 and 3:1 ratio). The shaded area represents the microemulsion zone, and rest unshaded area represents a biphasic system or poor formulation. On comparing all the five systems, system (b), having Km value 1:2 ratio, showed the broader microemulsion zone. On analyzing all five systems, it was concluded that the area region of microemulsions became higher as the Km value decreased, reaching the maximum value at Km of 1:2. Based on the phase diagram of the microemulsion system (b) the concentration range of oil, scs mix, and water was determined and found as 05-15%, 55-75% and 20-30% respectively. These values were selected for optimization of the formulation.



(a) Km=1:1 **(b) km-1:2** **(c) Km=1:3** **(d) km= 2:1** **(e) Km= 3:1**

FIG. 3: PSEUDO-TERNARY PHASE DIAGRAMS OF MICROEMULSIONS AT VARIOUS KM VALUES

Experimental Design: In the present investigation, a simplex lattice design was selected to analyze the consequences of three independent variables (microemulsion components) on dependent variables. The constraints of all variables (independent and dependent) are shown in **Tables 1** and **2**. As per simplex lattice design, 10 formulations were formulated and evaluated for their response variables i.e., transmittance value (Y_1) and cumulative % drug permeation (Y_2). All the data were separately fitted to a special cubic mixture equation, and the significance of the model was evaluated by ANOVA, lack of fit test, and multiple correlation coefficient (R^2). To be fitted well in a special cubic mixture model, the p-value should be less than 0.05 (level of significance). The data variation is analyzed by lack of fit and should be insignificant (p value >0.05) compared to the pure error. The multiple correlation coefficient values of (R^2) should be close to 1 [29].

TABLE 3: OBSERVED RESPONSES FROM THE RANDOM RUN OF THE SIMPLEX LATTICE DESIGN

Formulation code	%Transmittance	Cumulative% drug Permeation
Me1	98.15± 0.21	82.12±2.31
Me2	99.41±0.15	85.20±1.86
Me3	98.39±0.32	84.12± 2.43
Me4	98.25±0.48	88.34± 2.03
Me5	99.12±0.24	91.65± 1.78
Me6	97.68±1.54	74.66± 2.65
Me7	97.91±0.23	93.02± 1.87
Me8	100.31±0.51	78.36±3.25
Me9	98.05±0.17	83.02± 2.82
Me10	99.01±0.25	89.57±3.05

Evaluation of Microemulsion Developed as Per Simplex Lattice Design:

% Transmittance of Microemulsion: The transmittance value of formulations in **Table 2** was evaluated. The transmittance value depends upon the scattering of radiations, the microemulsion that scatters more incident radiations results in lower transmittance. The transmittance result of

Glibenclamide microemulsion was found to range between 97.68% to 100.31% **Fig. 5A** at 650 nm **Table 3**. The available results showed a considerable increase in transmittance with an increased concentration of surfactant cosurfactant mixture and decreased concentration of oil **Fig. 4A, B, C**.

Lower transmittance value microemulsion showed a slightly hazy nature, but there was no phase separation or creaming. Based on the simplex lattice design, the combinations of independent factor oleic acid (X_1), Tween 80 ethanol mix (scs) (X_2), and water (X_3) resulted in the response variable (Y_1) for % transmittance. The mathematical relationship of a special cubic equation for the measured response Y_1 (transmittance), is mentioned below.

$$Y_1 = 0.97683X_1 + 1.00311X_2 + 0.97900 - X_3 - 0.00001X_1X_2 + 0.00010X_1X_3 - 0.00001X_2X_3 - 0.00001X_1X_2X_3 \quad \text{(Eq.3)}$$

The above equation shows the quantitative effect of independent variables (X_1, X_2 and X_3) and their interactions X_1X_2, X_1X_3, X_2X_3 and $X1X2X3$ on the response variable Y. The p-value for response (Y_1) being less than 0.05 of the coefficient indicated that the independent variables, i.e., oil concentration (X_1), the concentration of Scs mix(X_2), and water (X_3), significantly affect the responses (Y)., **Table 4**.

The positive coefficient indicates the response is favored, but the negative value shows an inverse relationship between the factor and the response. If the coefficient value is large, it also confirms their substantial effect on the response.

The large value of scs mixture coefficient than the oil and water coefficient represents the more pronounced effect of scs mixture than oil and water.

All responses were studied well in the special cubic mixture model. The model efficiency was verified by ANOVA and correlation value (R^2). The result of the special cubic model is shown in **Table 3**.

The p values and R2 values were found to be 0.007 and 99.92% for Y_1. Due to the observed p values (< 0.05) and R^2 value (close to 1) concluded, the significant effect of independent variables in predicting the response (Y_1) (Rsq(pred)=98.38 %). In addition, the multi-collinearity of the independent factors was also assessed by a variance inflation factor (VIF).

It was concluded that there was no multi-collinearity amid the independent variables (X_1-X_3) in the special cubic model, as the value VIF was 2.47 (VIFs greater than 10 indicate multi-collinearity. As a general rule, VIFs less than 10 are tolerable). The result of contour plot, and mixture surface response plot are illustrated in **Fig. 4A, 4B, 4C** and **Fig. 5A**.

The increase in transmittance of microemulsion with the increase in scs and decrease in the quantity of oil was observed. It may be due to the presence of a higher quantity of scs acting as an emulsifier. The scs emulsified the oil with water effectively. Due to the better emulsification process, very fine-sized and stable globules were formed, and the transmittance value related to globule size was nearly transparent [30].

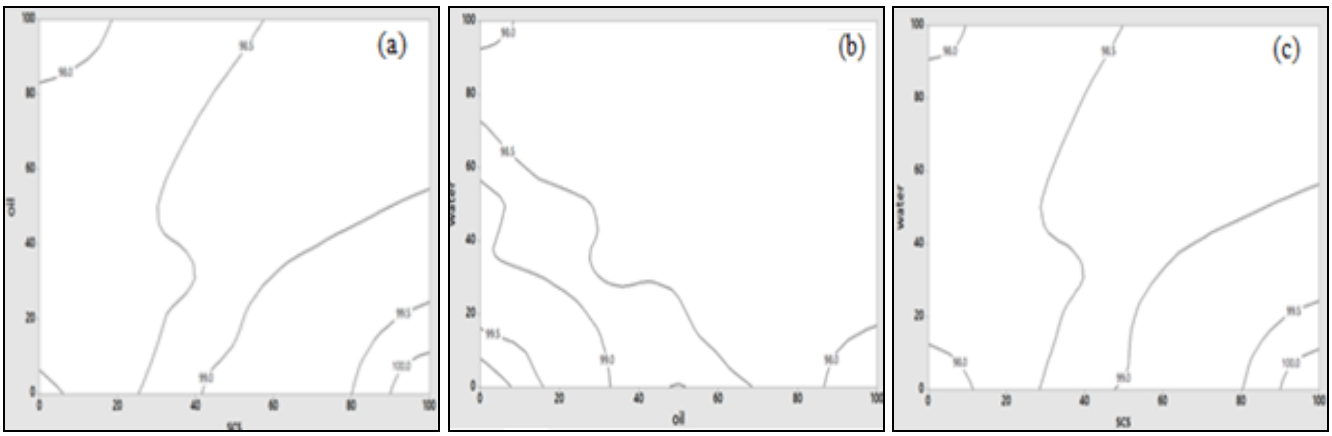


FIG. 4: CONTOUR PLOTS OF %TRANSMITTANCE WITH INDEPENDENT VARIABLES (A) OIL SCS (B) WATER OIL AND (C) WATER SCS OF SIMPLEX LATTICE DESIGN

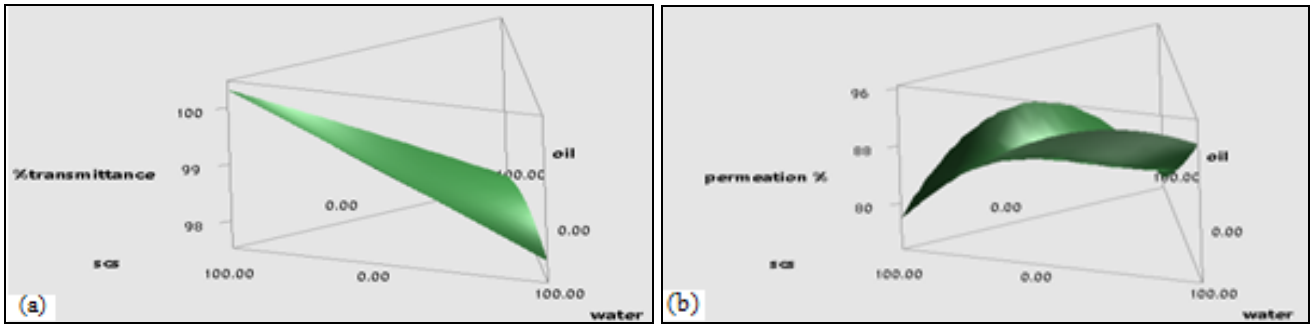


FIG. 5: MIXTURE SURFACE RESPONSE PLOT OF % TRANSMITTANCE (A) AND CUMULATIVE % DRUG PERMEATION (B) OF SIMPLEX LATTICE DESIGN

Cumulative % Drug Permeation Study: the permeation of drug of different formulations developed as simplex lattice design **Table 2** was performed through rat skin in Franz diffusion cell and the results **Fig. 8** were analyzed by Minitab 18 Software, the results of analysis is shown below -

$$Y_2 = 0.74809X_1 + 0.77901X_2 + 0.92989X_3 + 0.00530X_1X_2 - 0.00029X_1X_3 + 0.00243X_2X_3 - 0.00016X_1X_2X_3 \quad \text{Eq. 4}$$

As per transmittance value, the positive coefficient has a positive impact on permeation and the negative coefficient reduces the permeation. This equation can be applied to calculate the predicted values for other developed formulations because of the observed response p value was 0.001 <0.05) and R-sq = 99.88% (close to 1) and (R-sq (pred) = 95.65) for Y_2 (Cumulative % drug permeation). The response surface plot of drug skin permeation showed that a moderate level of S/CS, high level of water, and a moderate level of oil ratio resulted in a pronounced increase in the permeation of the drug by the microemulsion formulations. The drug delivery through microemulsion to skin depends upon two processes: drug release from microemulsion and drug permeation into skin. The solubility of Glibenclamide in scs was much higher than in oleic acid and water, so the use of a high level of scs mix in the preparation of microemulsion hindered the release of drug from the microemulsion or generation of partition towards the skin, [31] and the drug was not able to leave the vehicle after application at the surface of skin, and consequently low permeability was observed. The formulation ME8 showed the least permeation (78.36±3.25 %) due to a very high concentration of scs. Literature studies also suggested that using surfactants as penetration enhancers in microemulsion [32] generally increased the skin permeability but not every time [33]. In General, increasing drug delivery to the skin depends upon the leaving nature of the drug from the vehicle or formulation. The high oil level also supported that because the drug has good solubility despite its penetration-enhancing ability in formulation ME6 (74.66±2.65%) [34]. The permeability of the drug through the skin also depends upon various other factors, especially skin hydration. This appeared in higher drug permeation of microemulsion formulation ME7 (93.02± 1.87%) having higher water content than ME6 (74.66± 2.65%) containing low water content as per simplex lattice design run. The reason may be due to better dispersion of oil globules in microemulsion and the drug present in oil globules may connect with skin easily and release the drug molecules better [35].

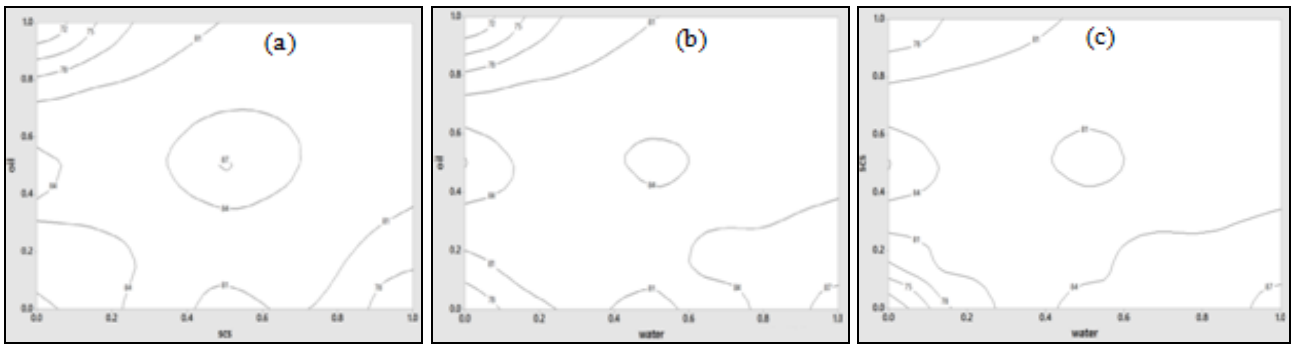


FIG. 6: CONTOUR PLOTS OF CUMULATIVE %DRUG PERMEATION WITH INDEPENDENT VARIABLES (A) OIL, SCS (B) OIL WATER AND (C) WATER.SCS

Optimization and Evaluation of Optimized Formulation by Desirability Function Approach: As per result of % transmittance and cumulative % drug permeation of the developed microemulsions as per simplex lattice design run, the microemulsion formulation was optimized by numerical optimization technique, which was based upon the desirability function. Equal weight (1) was given to both responses, and target values and range were also specified in it. In the desirability function method, every desirability function was calculated to combine all the responses in one solution. This will facilitate the optimum level of the independent variables. Based on the desired criteria, one best-optimized formulation solution was predicted by the Minitab 18 software, and the desirability value of 0.987 was found in the optimized formulation. The predicted optimized microemulsion contained X_1 = 4.57% oil, X_2 = 47.47% Scs, and X_3 = 47.95% water.

TABLE 4: OBSERVED AND PREDICTED RESPONSE OF OPTIMIZED FORMULATION

Response (%)	Predicted value	Observed value	Percent bias
%Transmittance	98.99	99.87± 2.35	0.881 %
% Permeation	89.96	86.17±1.87	- 4.398 %

The optimized predicted microemulsion was prepared in triplicate and evaluated for response variables as observed values. The comparison between the observed and predicted value was done to validate the model and find its suitability. The percentage biasness was found between - 4.398 to 0.881 % **Table 4**.

Characterization of Optimized Formulation:

Globule size zeta Potential and Pdi Analysis: The globule size of the optimized formulation was measured by DLS method using Anton Paar Litesizer 500 (Anton Paar GmbH, Austria) at 25°C. The globule size of the microemulsion was found 15.77 nm at the highest weightage **Fig. 7**.

The material refractive index (RI) was set to 1.48 with absorption set to 0.001. Dispersant viscosity and refractive index were set to that of water (i.e., 0.00089 Pa·s and 1.330 at 25°C). Measurement was performed at 173°, 7 runs with 10 sec/run. The Z-Average size, (intensity weighted distribution) PdI were obtained. The optimal measurement was determined using the "auto-seek" function for micro-cuvette. PDI can determine the uniformity of globule size and less than 1 value is desirable. A higher polydispersity index value represents non-uniformity of the droplet size in the formulation. The pdi of the optimized formulation was found 0.182, so the formulation globules were monodisperse [36]. The zeta potential helps to determine the physical stability of any emulsion. The value between ±0-10 mv is highly unstable, ±10-20 mv moderately stable, and ±20-30 mv is highly stable. The optimized zeta potential value was found 30.6 ±1.5 mv, so the stability of formulation was very good [37].

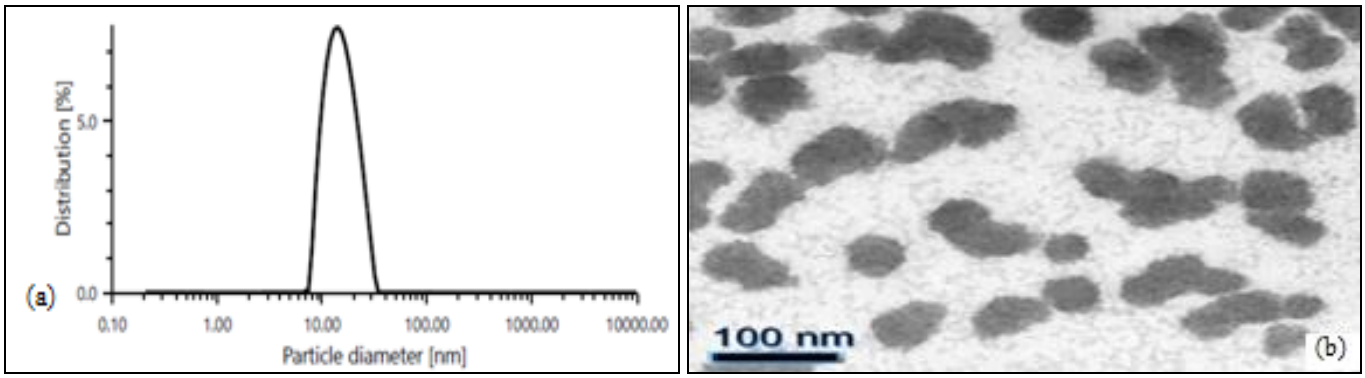


FIG. 7: GLOBULE SIZE DISTRIBUTION (A) AND TEM RESULT (B) OF OPTIMIZED FORMULATION

Tem Analysis: Fig. 7B showed the morphology of the optimized formulation, which was evaluated by transmission electron microscopy. As per the details of Tem, the globules are spherical.

Staining Study: The dye solubility test revealed that a water-soluble dye (methyl orange) distributed uniformly throughout the ME system, which implied that the formulated ME was of O/W type (data not shown).

Thermodynamic Stability: The optimized formulation was assessed by all three phases for thermodynamic stability. The result demonstrated

no sign of instability (like creaming, phase separation, precipitation etc. So the optimized formulation confirmed the stability (data not shown).

Comparison of Transdermal Permeation between Optimized Microemulsions and Suspension: The results of cumulative % drug permeation of both suspension and O/W microemulsion were also evaluated and shown in **Fig. 9**. The optimized microemulsions and suspension showed 86.17±1.87% and 31.56±1.21 % permeation respectively (p<0.05). The optimized microemulsion formulation enhanced the permeation nearly 2.73 times more than the suspension formulation.

The microemulsion could improve the permeation as stated earlier; due to the solubilization of the drug, the microemulsion was able to dissolve the drug very effective in comparison to suspension and solubilization helps in the permeation of the drug furthermore, the components of microemulsion oleic acid, surfactants may affect the structure of barrier layer *i.e.*, stratum corneum and reduce the diffusional barrier by acting as a permeation enhancer [38, 39, 40].

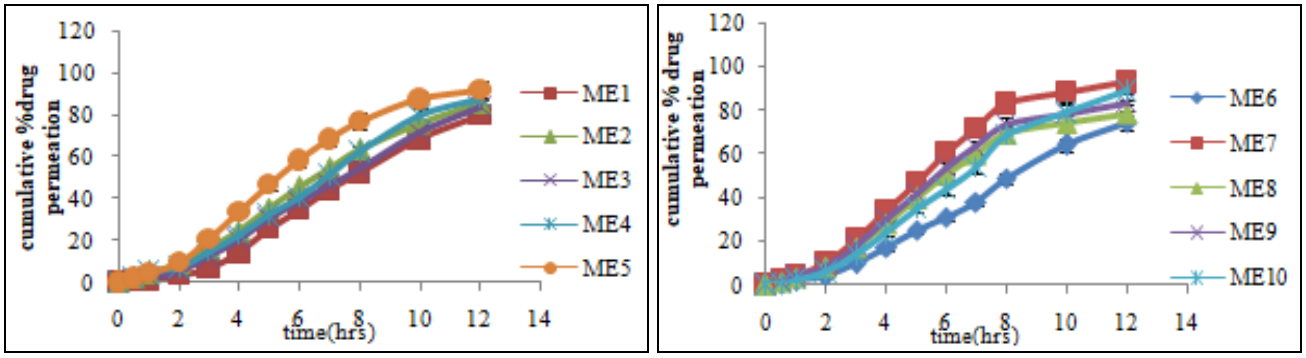


FIG. 8: CUMULATIVE % DRUG RELEASE FROM DIFFERENT MICROEMULSIONS PREPARED AS PER SIMPLEX LATTICE RUN. (MEAN ± S.D, N =3)

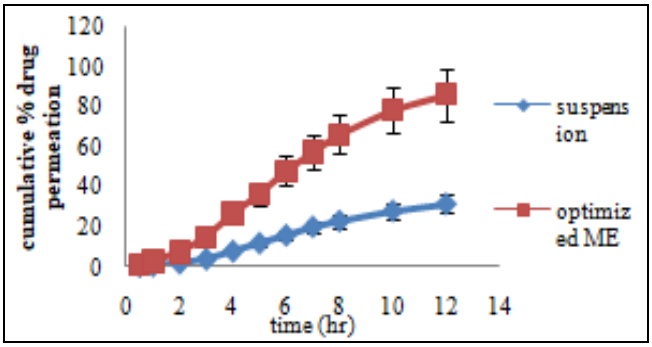


FIG. 9: CUMULATIVE % DRUG PERMEATION PROFILE OF THE OPTIMIZED MICROEMULSION AND THE CONTROL SUSPENSION FORMULATION (MEAN ± S.D, N =3) (P < 0.05)

CONCLUSION: In this study, the usefulness of microemulsion for transdermal delivery of Glibenclamide was investigated, and a simplex lattice design approach with desirability function was applied to optimize the formulation. The obtained results proved the permeation-improving effect of the microemulsion system. Compared with Glibenclamide suspension, the skin permeation ability of Glibenclamide was significantly improved by microemulsion system, which may be due to the unique properties of microemulsion formulation. So it is the promising alternative to reduce the variation of bioavailability as well as reduction in serious, acute and sometimes fatal hypoglycemia side effect caused by unpredictable dissolution profile through oral route as well as it can maintain the steady state drug concentration by controlled drug delivery through skin. Further investigations are required for *in-vivo* study.

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REFERENCES:

1. WHO- Global report on diabetes; WHO Press, World Health Organization, 2020 Avenue Appia, 1211 Geneva 27, Switzerland. https://www.who.int/publications/i/item/9789241565257.
2. Rui Li, Yi-an Bi, Vildhede Anna, Scialis Renato J, Mathialagan Sumathy, Yang Xin, Marroquin Lisa D, Jian Lin and Manthena Varma VS: Transporter-Mediated

Disposition, Clinical Pharmacokinetics and Cholestatic Potential of Glyburide and Its Primary Active Metabolites, Drug Metabolism and Disposition 2017, 45 (7) 737-747; DOI: https://doi.org/10.1124/dmd.116.074815.

3. Nosheen Anwar, Syed Umer Jan and Gull Rehman: Formulation And Evaluation Of Glibenclamide Gel For Transdermal Drug Delivery, Int J Curr Pharm Res 2020; 12(5): 35-39.
4. Jeong WY, Kwon M and Choi HE: Recent advances in transdermal drug delivery systems: a review. Biomater Res 2021; 25(24): 1-15 (). https://doi.org/10.1186/s40824-021-00226-6.
5. Nastiti C, Ponto T, Abd E, Grice JE, Benson HAE and Roberts MS: Topical nano and microemulsions for skin delivery, Pharmaceutics 2017; 9(4): 1-25 DOI: 10.3390/pharmaceutics9040037.
6. Neubert RHH, Sommer E, Schölzel M, Tuchscherer B, Mrestani Y and Wohlrab J: Dermal peptide delivery using enhancer moleculs and colloidal carrier systems Part II: Tetrapeptide PKEK, Eur. J. Pharm. Biopharm 2018; 124: 28–33. DOI: 10.1016/j.ejpb.2017.12.004.
7. Shukla T, Upmanyu N, Agrawal M, Saraf S and Alexander A: Biomedical applications of microemulsion through dermal and transdermal route. Biomedicine & Pharmacotherapy 2018; 108: 1477–1494. https://doi.org/10.1016/j.biopha.2018.10.021.
8. Alexander A, Ajazuddin RJ, Patel S and Saraf S: Recent expansion of pharmaceutical nanotechnologies and targeting strategies in the field of phytopharmaceuticals for the delivery of herbal extracts and bioactives. J Control Release 2016; 241: 110–124. DOI: 10.1016/j.jconrel.2016.09.017.
9. Agrawal M, Saraf S, Saraf S, Antimisiaris SG, Chougule M, Shoyele SA, Gupta U and Ajazuddin A: Alexander, Recent advancements in the field of nanotechnology for the delivery of anti-Alzheimer drug in the brain region, Expert Opin. Drug Deliv 2018; 15(6) 589–617. DOI: 10.1080/17425247.2018.1471058.
10. Chauhan Neeraj Kumar and Upadhyay Prashant: A Review on Micro Emulsion Drug Delivery System as Bioavailability Enhancement Tool Using Solubilisations Technique International Journal of Scientific Development and Research (IJSDR) 2020; 5(12); 234-241.
11. Qing Hu, Han Lin, Yanfang Wang, Xiaoqin Wang, Jiayi Yao, Xiaoling Fu and Xiangbin Yu: Design, optimization and evaluation of a microemulsion-based hydrogel with high malleability for enhanced transdermal delivery of levamisole. International Journal of Pharmaceutics 2021; 605; 1-51, https:/ /doi.org/ 10.1016/j.ijpharm2021. 120829.
12. Maddiboyina B, Jhawat V, Nakkala RK, Desu PK and Gandhi S: Design expert assisted formulation, characterization and optimization of microemulsion based solid lipid nanoparticles of repaglinide. Prog Biomater. 2021; 10(4): 309-320. doi: 10.1007/s40204-021-00174-3.
13. Agubata CO, Nzekwe IT, Obitte NC, Ugwu CE, Attama AA and Onunkwo GC: Effect of Oil, Surfactant and Co-Surfactant Concentrations on the Phase Behavior, Physicochemical Properties and Drug Release from Self-Emulsifying Drug Delivery Systems. J Drug Discov Develop and Deliv 2014; 1(1): 7-14.
14. Agrawal Vikas, Patel Rashmin, Patel Mrunali, Thanki Kaushik and Mishra Sandip: Design and evaluation of microemulsion-based efinaconazole formulations for targeted treatment of onychomycosis through transungual route: *Ex-vivo* and nail clipping studies, Colloids and Surfaces B: Biointerfaces 2021; 201: 1-9 https://doi.org/10.1016/j.colsurfb.2021.111652.
15. Patel, Meenakshi, Shaikh Farhatjahan, Patel Vandana B. and Surti Naazneen Imtiaz: "Optimization of Glipizide Floating Matrix Tablet Using Simplex Lattice Design." Indian Journal of Pharmaceutical Sciences 2021.
16. Terzopoulou Z, Michopoulou A, Palamidi A, Koliakou E and Bikiaris D: Preparation and Evaluation of Collagen-Based Patches as Curcumin Carriers. Polymers (Basel) 2020 Oct 17; 12(10):2393. doi: 10.3390/polym12102393.
17. Todo H: Transdermal Permeation of Drugs in Various Animal Species. Pharmaceutics 2017; 9(3):33. DOI: 10.3390/pharmaceutics9030033.
18. Yadav P, Rastogi V & Verma A: Application of Box–Behnken design and desirability function in the development and optimization of self-nanoemulsifying drug delivery system for enhanced dissolution of ezetimibe. Futur J Pharm Sci 2020; 6(7). https://doi.org/10.1186/s43094-020-00023-3.
19. Dhaval M, Panjwani M, Parmar R. Soniwala MM, Dudhat K and Chavda J: Application of Simple Lattice Design and Desirability Function for Formulating and Optimizing SMEDDS of Clofazimine; Journal of Pharmaceutical Innovation 2020; https://doi.org/10.1007/s12247-020-09468-8.
20. Prajapati B and Dharamsi A: Characterization and pharmacodynamic evaluation of developed microemulsion for nasal drug delivery. IJPSR 2020; 11(8): 4017-4028. DOI: 10.13040/IJPSR.0975-8232.11 (8).4017-28.
21. Kuropakornpong P, Itharat A, Ooraikul B, Loebenberg R and Davies NM: Development and optimization of Benjakul microemulsion formulations for enhancing topical anti-inflammatory effect and delivery. Res Pharm Sci 2022; 17(2): 111-122. doi: 10.4103/1735-5362.335170.
22. Chakraborty Rajarshi and Bhowmik Ratul: Preparation and Evaluation of Topical Microemulsion System Containing Metronidazole. Int J Pharm Sci Rev Res 2021; 70(2): 149-155.
23. Wen Ming Ming, Ismail Noha Ismail Khamis, Maha MA. El-Kamel Nasra & Hassan Amal: Repurposing ibuprofen-loaded microemulsion for the management of Alzheimer's disease: evidence of potential intranasal brain targeting, Drug Delivery 2021; 28: 1, 1188-1203, DOI: 10.1080/10717544.2021.1937383.
24. Trivedi HR, Siriah TM and Puranik PK: Experimental design approach for development of novel microemulsion system and immediate release self microemulsifying tablet of nebivolol hcl; Braz J Pharm Sci 2020; 56: e18070. https://doi.org/10.1590/s2175-97902019000418070.
25. Bassant Atef, Rania AH. Ishak, Sabry S. Badawy and Rihab Osman: Exploring the potential of oleic acid in nanotechnology-mediated dermal drug delivery: An up-to-date review. Journal of Drug Delivery Science and Technology 2022; 67: https://doi.org/10.1016/j.jddst.2021.103032.
26. Meyler's: Side Effects of Drugs: The International Encyclopedia of Adverse Drug Reactions and Interactions, Sixteenth Edition 2016 edited by J. K. Aronson, Elsevier Science. ISBN9780444537164.
27. Sisak Abdul, Asri Muhammad, Ramli and Suria: Study on the effect of oil phase and co-surfactant on microemulsion systems. Malaysian Journal of Analytical Sciences 2017; 21: 1409-1416. 10.17576/mjas-2017-2106-23.
28. Heard Charles M, Kung Denise and Thomas Chris P: Skin penetration enhancement of mefenamic acid by ethanol and 1,8-cineole can be explained by the 'pull' effect,

International Journal of Pharmaceutics 2006; 321: 1–2, 167-170. doi: 10.1016/j.ijpharm.2006.05.018.

29. Mori Dhaval, Jatin Devani, Ramesh Parmar, M.M. Soniwala and Jayant Chavda: Formulation and optimization of microemulsion based sparfloxacin *in-situ* gel for ocular delivery: *In-vitro* and ex-vivo characterization, Journal of Drug Delivery Science and technology 2020, 55, https:// doi. Org //10.1016/j.jddst.2019.101373.
30. Rao S, Barot T and Rajesh KS: Formulation, optimization and evaluation of microemulsion based gel of Butenafine Hydrochloride for topical delivery by using simplex lattice mixture design. Journal of Pharmaceutical Investigation 2016; 46: 1–12. Christofori MR, Nastiti R, Thellie Ponto, Eman Abd, Jeffrey E. Grice, Heather AE and Roberts Michael S: Topical Nano and Microemulsions for Skin Delivery, Pharmaceutics 2017; 9(37): 1-25 doi:10.3390
31. Mori Dhaval, Jatin Devani, Ramesh Parmar, Soniwala MM and Jayant Chavda: Formulation and optimization of microemulsion based sparfloxacin in-situ gel for ocular delivery: *In-vitro* and *ex-vivo* characterization, Journal of Drug Delivery Science and Technology 2020; 55: 1-19. DOI:10.1016/j.jddst.2019.101373.
32. Baroli B, López-Quintela MA, Delgado-Charro MB, Fadda AM and Blanco-Méndez J: Microemulsions for topical delivery of 8-methoxsalen. J Control Release 2020; 69: 209–218. DOI: 10.1016/s0168-3659(00)00309-6.
33. Kim B, Cho HE and Moon SH: Transdermal delivery systems in cosmetics. Biomed dermatol 2020; 4; 10. https://doi.org/10.1186/s41702-020-0058-7.
34. Froelich A, Tomasz Osmałek, Barbara Jadach, Puri Vinam and Bozena Michniak-Kohn: Microemulsion-Based Media in Nose-to-Brain Drug Delivery Pharmaceutics; 2021, 13, 201. https://doi.org/10.3390/pharmaceutics13020201
35. Danae M, Dehghankhold M, Khorasani S and Mozafari MR: Impact of Particle Size and Polydispersity Index on the Clinical Applications of Lipidic Nanocarrier Systems Pharmaceutics 2018; 10: 57; doi:10.3390/pharmaceutics10020057
36. Samimi Shabnam, Maghsoudnia Niloufar, Eftekhari Baradaran Reza and Dorkoosh Farid: Lipid-Based nanoparticles for drug delivery systems. in micro and nano technologies, characterization and biology of nanomaterials for drug delivery. Elsevier 2019; 47-76, https://doi.org/10.1016/B978-0-12-814031-4.00003-9.
37. Rajpoot Kuldeep and Tekade Rakesh K: Ch 10 - Microemulsion as drug and gene delivery vehicle: an inside story, In Advances in Pharmaceutical Product Development and Research, Drug Delivery Systems, Academic Press 2019; 455-520, https://doi.org/10.1016/B978-0-12-814487-9.00010-7.
38. Kevin Ita: Microemulsions, Transdermal Drug Delivery, Academic Press, 2020, 97-122, https:// doi.org/ 10.1016 / B978-0-12-822550-9.00006-5.

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