

# Disruption of Lipid Bilayers of Stratum Corneum by Transdermal Delivery of Glipizide in Microemulsion and Binary Co-solvent Systems

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(Received: May 9, 2019; Accepted: May 12, 2020; Published (web): June 28, 2020)

**ABSTRACT:** The study was designed to develop and characterize structured vehicles for effective delivery of glipizide (GPZ) through excised stratum corneum (SC). Several mixed binary systems of ethanol or propylene glycol (PG) were prepared by homogeneous mixing with double distilled water. Different microemulsions were prepared by titration method and their physicochemical properties determined. Transdermal permeation of GPZ was studied *in-vitro* using modified Franz diffusion cells. Apart from the slight difference in their pH values, there was no significant difference in the physicochemical properties of the drug-loaded coconut oil-based microemulsions and their blank counterparts. Transdermal flux was highest in binary mixtures of 9:1 (v/v) aqueous ethanol ( $J_{ss}$   $30.25 \pm 5.75 \mu\text{g}/\text{cm}^2\text{h}$ ) and PG ( $J_{ss}$   $6.34 \pm 1.29 \mu\text{g}/\text{cm}^2\text{h}$ ) compared to their lower strengths. Transdermal GPZ flux,  $J_{ss}$ ,  $\mu\text{g}/\text{cm}^2\text{h}$  was higher in o/w ( $121.2 \pm 9.98$ ) compared to w/o ( $3.89 \pm 0.19$ ) microemulsions with enhanced permeation of  $\geq 23$  fold using patch size of  $10.45 \text{ cm}^2$ . Biophysical analysis of untreated and treated SC showed that GPZ permeation could depend on the extent of disruption of lipid and protein bilayers of SC by the vehicles. Cremophor RH 40/ethanol/coconut oil-based o/w microemulsion and 9:1 v/v mixed binary systems of ethanol or PG are promising vehicles for delivery of GPZ transdermally.

**Key words:** Microemulsion, co-solvents, glipizide, stratum corneum, transdermal, flux.

## INTRODUCTION

The research on transdermal drug delivery has been on the increase in the recent time due to the highly organized structure (non polar and rigid components) of the SC. This is as a result of the differences in SC lipid composition, water content, morphological characteristics (thickness, number of pores and follicles) and its barrier properties to penetrants.<sup>1</sup> More so, there is urgent need to improve the limited number of candidate drug that can be

administered through the percutaneous route.<sup>2</sup> The use of diffusion cells and SC from various source such as human skin (cadaver or surgical reduction), mice, rats, rabbits and pigs have been adopted for quantitative determination of permeants *in vitro*.<sup>2</sup> Formulation scientists have demonstrated various techniques of circumventing SC barrier properties in different drug candidates.<sup>3-5</sup> GPZ is a second generation sulphonylurea derivative with good prospects for transdermal delivery such as molecular weight of 445 Daltons, plasma half-life  $< 5 \text{ hc}$ , log P value of 2.5, pKa of 5.9, melting point  $208.5^\circ\text{C}$  and serum concentration of  $5 \text{ ng}/\text{ml}$ . Its delivery using different transdermal approaches has been

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Dhaka Univ. J. Pharm. Sci. 19(1): 29-35, 2020 (June)

DOI: <https://doi.org/10.3329/dujps.v19i1.47816>

reported.<sup>1,6,7</sup> Our previous study also demonstrated the usefulness of microemulsions and co-solvent systems for delivery of macromolecules.<sup>8</sup> As an adjuvant to diet for the control of hyperglycaemia and its associated symptomatology in patients with type-2 diabetes mellitus, GPZ stimulates the release of insulin from the pancreas, increase in insulin sensitivity and a decrease in hepatic glucose production.<sup>9</sup> At present, it appears to be the most prescribed antidiabetic agent for treatment of type-2 diabetes; however, oral GPZ causes bioavailability fluctuations due to erratic absorption, gastric disturbances and resulting in unpredictable hypoglycaemia.<sup>9</sup> We report an investigation aimed at by-passing the barrier properties of SC, circumventing associated gastrointestinal disturbances and reducing fluctuations of GPZ plasma levels during therapy using novel techniques of structured microemulsion and aqueous mixed binary systems by developing safe, flexible and suitable vehicles for its transdermal delivery.

## MATERIALS AND METHODS

**Chemicals and reagents.** GPZ (Generics Ltd, UK), cremophor RH 40 (BASF, Germany), coconut oil (purified, Nigeria), Ethanol, acetonitrile, Sodium chloride, Potassium chloride, Disodium hydrogen phosphate dihydrate, Potassium dihydrogen phosphate, Sodium azide, Propylene glycol, Sodium hydroxide and trypsin (Sigma-Aldrich, USA). All other chemicals and reagents were purchased from Sigma-Aldrich (USA) and were used as received.

### Methods

**Preparation of mixed binary systems.** Ethanol or PG and double distilled water were homogeneously mixed in different ratios to obtain the binary co-solvent systems of 9:1, 8:2 or 7:3 v/v, respectively.

**Preparation of microemulsions.** Different microemulsion systems were prepared by aqueous titration method<sup>10</sup> and a pseudo ternary phase diagram constructed using the *SigmaPlot* 13.0 Exact Graphs and Data Analysis software. After obtaining

the microemulsion regions in the phase diagram (Figure 1), formulations were selected at ratio of the surfactant (Su) to co-surfactant (CoSu) mixture of 1:1 which were likely to produce both o/w and w/o microemulsions.

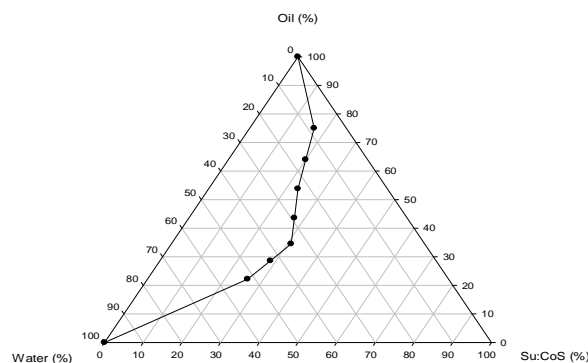


Figure 1. Pseudo ternary phase diagram of 1:1 Su:CoSu.

The choice of a 1:1 ratio phase diagram was based on large area of micro-emulsification, stability profile and physicochemical characteristics of the microemulsions observed when compared to other phase diagram ratios of Su:CoSu 1 : 2, 2 : 1, 1 : 3 and 3 : 1 similarly constructed with 20% (w/w) of cremophor RH 40 (Su) and ethanol (CoSu). The w/o (M1) and o/w (M2) microemulsions were formulated with coconut oil (60%) and water (20%) and coconut oil (20%) and water (60%) respectively. GPZ was spiked at 5% (w/w) into M1 and M2 without breaking the microemulsion structures.

**Physicochemical characterization of micro-emulsions.** The apparent pH was measured by a HI 98129 pH/conductivity/TDS tester (Hanna, USA) at 298 K without re-calibration. Droplet size distributions were determined by high resolution, high sensitive photomicrograph (Moticam, Japan) attached to light microscope and PC. Briefly, the diluted sample was filtered through 0.22  $\mu\text{m}$  membrane and placed on slide for droplet size analysis. Polydispersity index was estimated from the ratio of standard deviation to the mean droplet size. Viscosity was measured at 298 K using a high torque, 13.3 rpm cone and plate viscometer (Brookfield, USA). Surface tension was measured at 298 K using Du Noüy ring tensiometer (Krüss GmbH, Germany) and stability monitored on the laboratory shelf for 21

days while observing them for phase separation and/or creaming.

**Preparation of rat SC.** The approval to use animals for this study was obtained from the University of Nigeria, Nsukka Animal Ethics Committee. Male Wistar rats were sacrificed with prolonged chloroform anesthetics and the epidermis was prepared by heat separation technique<sup>8</sup>. The epidermis was wrapped with aluminum foil and stored at  $-20^{\circ}\text{C}$  until used. Prior to the permeability studies, the stored epidermis was allowed to thaw, cut into  $4.5 \times 4.5 \text{ cm}^2$  pieces and hydrated by placing in phosphate buffered saline (PBS) overnight.<sup>11</sup>

**Evaluation of *in-vitro* skin permeability.** The study was performed using modified Franz diffusion cells (SES GmbH, Germany) with diffusional area of  $2.54 \text{ cm}^2$  and the receiver compartment volume of 25 ml. The diffusion cells were connected with a circulating water bath and the temperature was controlled at  $37 \pm 0.5^{\circ}\text{C}$ . PBS, pH 7.4 was used as a receiver fluid and stirred by an externally driven Teflon-coated magnetic bar (Thomas Scientific, USA). The prepared epidermis was placed on the receiver compartment with the SC facing upwards. The donor compartment was then connected with a clamp. A 1 ml of co-solvent system or microemulsion containing 5 mg of GPZ was applied unto skin surface of the donor compartment. The donor compartment was covered with a glass lid and the sampling arm was covered with aluminum foil. At suitable time intervals (0, 1, 2, 4, 6, 8, 10, 12 and 24 hrs), an accurate amount (1 ml) of each sample was withdrawn from the centre of the receiver compartment with a syringe connected with a needle. An equal volume of fresh PBS ( $37 \pm 0.5^{\circ}\text{C}$ ) was replenished immediately. The amount of drug in the receiver fluid was determined chromatographically at 276 nm.

**Chromatographic assay.** The concentrations of GPZ permeated into the receiver fluids were quantitatively determined by chromatographic assay on LC-4000 series integrated HPLC (Jasco AS-2055, Japan) with intelligent autosampler, variable UV/Vis detector and ChromoNav 2.0 software. Mobile phase

consisted of freshly prepared acetonitrile (70) and distilled water (30) both of HPLC grade filtered through a  $0.45 \mu\text{m}$  Millipore vacuum filter system and degassed by sonication. All assays were performed at room temperature by continuously pumping mobile phase at flow rate of 0.5 ml/min and effluent quantified at 276 nm. Validation of the method was performed to ensure that the calibration curve between 1 and  $20 \mu\text{g/ml}$  was in the linearity range of the assay and the coefficients of variation were less than 2% both intra-day and inter-day.

**Statistical and permeation analysis of data.** The skin permeation data were expressed as the cumulative drug permeation per skin surface area. The steady-state fluxes ( $J_{ss(8-24 \text{ h})}$ ,  $\mu\text{g/cm}^2\cdot\text{h}$ ) were calculated by linear regression interpolation of the experimental data at steady-state (between 8 - 24 hrs,  $\delta T$ ). The penetration enhancing effect of each vehicle was calculated in terms of enhancement ratio (ER) using the permeability coefficient ( $K_p$ ) estimated from the Fick's first law of diffusion.<sup>12</sup>

The patch size of the formulation required to deliver transdermally the effective plasma concentration of GPZ was estimated from the input rate.<sup>3</sup> The  $J_{ss}$  and  $K_p$  were analyzed using the Statistical Package for the Social Sciences (SPSS Inc. Chicago), v. 15.0 and GraphPad Prism v. 6.01.2012 (GraphPad Software Inc., San Diego, CA, USA) software and data were expressed as a mean  $\pm$  standard error of the mean (SEM) ( $n=3$ ). One way analysis of variance (ANOVA) was done to test for the significant difference between the means of treatments and control at  $p < 0.05$  by post-hoc using 2-sided Dunnett's test. In all cases, a  $p < 0.05$  was considered to be significant

**Biophysical analysis of SC.** The rat SC were prepared by floating freshly prepared epidermis membrane on a 0.1 % trypsin solution for 12 hrs. The SC sheets were cleaned by washing with distilled water and blotted dry and used for the Fourier transform infrared (FTIR) spectrophotometric and differential scanning calorimetric (DSC) studies.<sup>13,14</sup> The SC was treated with the relevant vehicles for 24 hrs and vacuum-dried at 298 K for 48 hrs. The dried

samples were subjected to FTIR (Shimadzu 8400S, Japan), focusing on characterizing the occurrence of peaks near 2850  $\text{cm}^{-1}$  and 2920  $\text{cm}^{-1}$  which were due to symmetric and asymmetric C-H stretching vibrations respectively. The frequencies at 1550 and 1650  $\text{cm}^{-1}$  indicate amide I (C=O stretching vibrations). The control samples were treated with PBS. The SC was also assessed by DSC (NETZSCH204 FI Kent Phoenix, Germany) at -90 - 600°C in terms of phase transition temperature of lipid components. The samples were scanned at 5 °C/min over the temperature range of 0 - 350 °C.

## RESULTS AND DISCUSSION

Both M1 and M2 were confirmed as w/o and o/w microemulsions respectively by aqueous dilution method. All the components of the mixed binary co-solvents and microemulsions were also confirmed safe by modified Draize skin irritation tests<sup>15</sup> using their primary irritancy indices (PII) of <2 compared to standard skin irritant (formaldehyde) PII of 5.33 as acceptable.

The physicochemical properties of M1 and M2 and their blank counterparts revealed no significant change in physical stability and colour, or other properties as shown in Table 1; however, slight differences in pH could be attributed to the intrinsic properties of GPZ which affected the pH M1 and M2.

The apparent viscosity was comparably low with correlation coefficient ( $R_{xy}$ ) between shear rate ( $x$ ) and shear stress ( $y$ )  $\geq 0.9$  showing Newtonian flow behaviour<sup>16</sup>. The mean droplet sizes of the optimized microemulsions were within the acceptable range of microemulsion for transdermal delivery with low PDI indicating uniformity of droplets of the microemulsions. The pH was observed to be higher than the isoelectric point of keratin (3.5) which was also required for transdermal delivery.

The results of *in vitro* permeability studies showed that 9 : 1 (v/v) binary co-solvent systems of ethanol-water and PG-water produced higher permeation fluxes ( $J_{ss}$ ) when compared to 8:2 and 7:3 co-solvent systems (Figure 2).

**Table 1. Physicochemical properties of the microemulsions.**

Formulations	pH	$\delta$ (nm)	PDI	$\gamma$	$\eta$	$R_{xy}$
M1	7.6±0.1	85.8±0.2	0.0098±0.0002	83.4±1.2	150±20	0.97
Blank	7.5±0.5	85.7±0.1	0.0098±0.0001	83.4±1.2	151±17	0.95
M2	7.2±0.3	89.9±0.3	0.0023±0.0001	65.2±1.1	145±12	0.98
Blank	7.4±0.2	89.8±0.6	0.0032±0.0002	65.2±1.1	148±14	0.99

Data expressed as mean  $\pm$  SEM (n = 5), surface tension ( $\gamma$ ), droplet size ( $\delta$ ), polydispersity index (PDI), viscosity ( $\eta$ ), correlation coefficient (R), shear rate (x), shear stress (y).

**Table 2. Permeation parameters of GPZ through SC from various vehicles.**

Vehicles	$J_{ss}$ ( $\mu\text{g}/\text{cm}^2\text{h}$ )	$K_p \times 10^{-3}$ ( $\text{cm}^2\text{h}$ )	ER	$R^2$	EPS ( $\text{cm}^2$ )
Control	5.25±0.34	1.05±0.11	-	0.9993	-
7:3 v/v ethanol	8.70±1.65 <sup>a</sup>	1.74±0.49	1.66	0.9998	145.517
8:2 v/v ethanol	12.10±3.25 <sup>a</sup>	2.42±0.81 <sup>a</sup>	2.31	0.9994	104.628
9:1 v/v ethanol	30.25±5.78 <sup>a</sup>	6.05±1.34 <sup>a</sup>	5.77	0.9986	*41.851
7:3 v/v PG	1.60±0.07	0.32±0.10	0.30	0.9979	791.250
8:2 v/v PG	5.38±0.49	1.08±0.39	1.03	0.9979	235.316
9:1 v/v PG	6.34±1.29 <sup>a</sup>	1.27±0.94 <sup>a</sup>	1.20	0.9977	199.316
M1	3.89±0.19	0.78±0.11	0.74	0.9946	160.051
M2	121.2±9.98 <sup>a</sup>	60.62±5.29 <sup>a</sup>	23.09	0.9968	*10.446

Data expressed as mean  $\pm$  SEM (n = 3), EPS = expected patch size, \*practically significant patch size (patch size < 100  $\text{cm}^2$ ).  
<sup>a</sup>p < 0.05 as compared with respective control values.

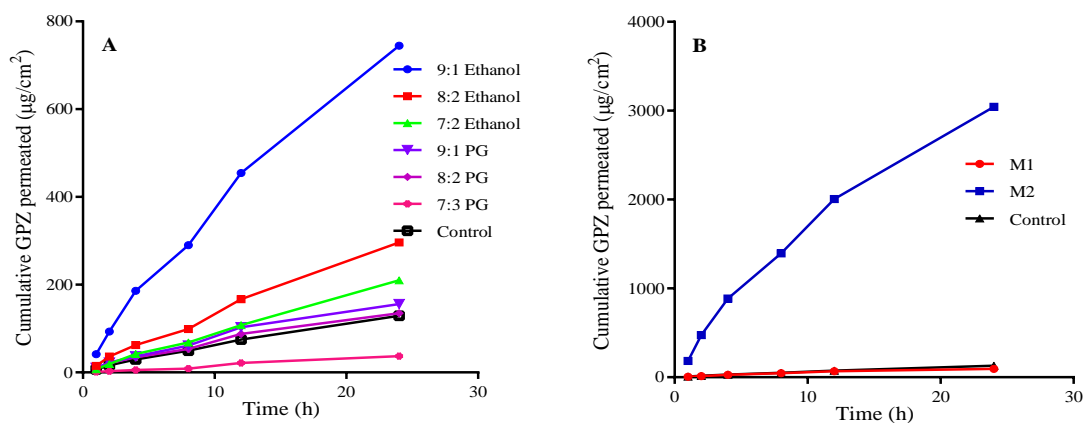


Figure 2. Permeation profiles of GPZ in (A) binary ethanol and PG co-solvent systems and (B) microemulsions compared with control.

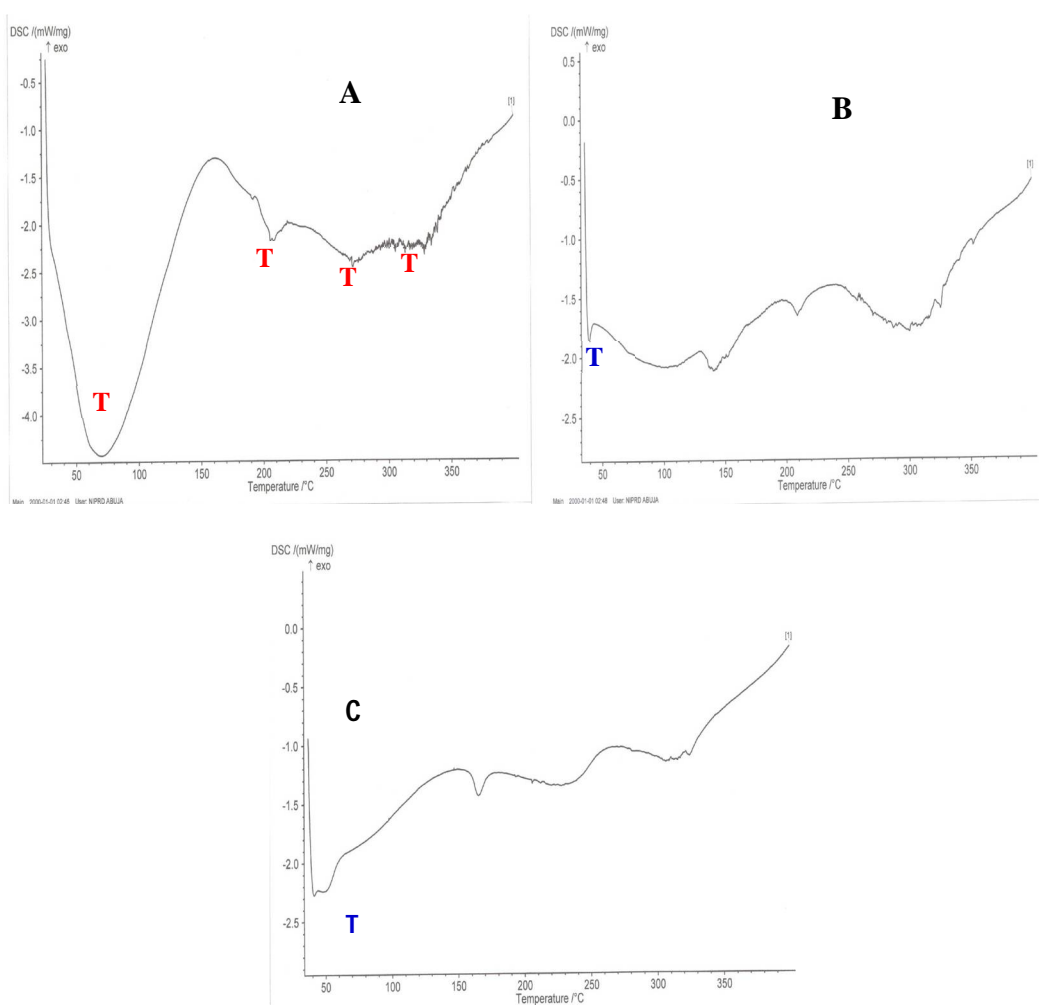


Figure 3. DSC thermograms of: (A) untreated (B) M2- and (C) 9:1 ethanol co-solvent-treated SC.

The permeation coefficients of 9:1 v/v co-solvents of ethanol and PG were  $(6.05 \pm 1.34) \times 10^{-3}$  and  $(1.27 \pm 0.94) \times 10^{-3} \text{ cm}^2\text{h}$  respectively compared to  $(1.74 \pm 0.49) \times 10^{-3}$  and  $(0.32 \pm 0.10) \times 10^{-3} \text{ cm}^2\text{h}$  observed in 7:3 v/v mixed binary solvents respectively (Table 2). The  $J_{ss}$  of GPZ in M2, all ethanol and 9:1 v/v PG co-solvent systems showed statistically significant difference ( $p < 0.05$ ) with control. Similar observation was evident in  $K_p$  of GPZ with the exception of 7:3 v/v ethanol co-solvents when compared with control.

These differences could be attributed to various influences of ethanol and PG on the barrier properties of SC such as the disruption of the organized network of lipids and proteins in the SC domain.<sup>17</sup> The M2 produced higher  $K_p$  of  $(60.62 \pm 5.29) \times 10^{-3}$  with enhancement ratio  $>23$  compared to  $(0.78 \pm 0.11) \times 10^{-3}$  and 0.74 respectively of M1. This could be attributed to combined effects of lipophilic and hydrophilic domains of M2 that favour partitioning of GPZ into SC, destabilizing its lipid bilayer structure and increases permeability.<sup>17</sup>

The DSC study characterized the thermotropic phase transition of the lipid bilayers and conformational stability of the proteins<sup>18</sup>. The endothermic peaks obtained in the DSC study are shown in Figure 3.

A control thermogram (Figure 3A) of SC is characterized by four endotherms; T1 (34°C), T2 (82 °C), T3 (103 °C) and T4 (114°C). T1 represents sebaceous and minor structural rearrangement of lipid bilayer<sup>18</sup>. T2 and T3 could be due to the melting of SC lipids while the T4 denotes the denaturation of intracellular keratin.<sup>18</sup> Different treatments of the SC (Figures 3B and 3C) caused significant thermotropic transition in T1 to lower endotherm compared to the control suggesting that the skin permeation enhancement of GPZ by ethanol, PG or M2 might be due to disruption of lipid bilayers and keratin denaturation.<sup>18</sup> Similarly, peaks suggestive of molecular vibrations of SC lipids and proteins were also observed in FTIR spectrum of untreated SC (control). In SC FTIR, absorption bands (2929 and

2859  $\text{cm}^{-1}$ ) could be attributed to the asymmetric and symmetric  $-\text{CH}_2$  vibrations of long chain hydrocarbons of lipids while bands (1650 and 1550  $\text{cm}^{-1}$ ) were due to the amide I/II ( $-\text{C}=\text{O}$ ) stretching vibrations of proteins. The significant shifts in the asymmetric ( $>40\%$ ) and symmetric ( $>70\%$ ) C-H stretching and amides I/II ( $>20\%$ ) peak positions by various treatments on SC compared with controls further supported previous findings on the implications of peak height/area reductions of the amides I/II and symmetrical and unsymmetrical C-H stretching vibrations as per the SC FTIR data (spectra not shown).<sup>8,19,20</sup> The significant decrease in peak height with 9:1 v/v binary mixtures of ethanol and PG respectively, and M1 and M2 suggested also disruption of lipid bilayer in the SC.<sup>19</sup>

## CONCLUSION

Microemulsions (o/w or w/o) and aqueous mixed binary systems of ethanol and PG affect transdermal permeation of GPZ through the SC by disruption of lipid bilayer network and/or denaturation of the keratin components. By combination of in-vitro FTIR and DSC analysis of SC of rat, both o/w microemulsions and ethanol (9:1) co-solvent can efficiently deliver effective plasma GPZ concentration using sizeable patch.

## ACKNOWLEDGEMENT

Authors are grateful to Prof. Anthony Amaechi Attama of the Department of Pharmaceutics, University of Nigeria for the gift of *SigmaPlot 13.0* software used in constructing the ternary phase diagrams.

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