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Pharmacokinetics Studies of Mirtazapine Loaded Nanoemulsion and Its Evaluation as Transdermal Delivery System

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ABSTRACT

The in vitro study of skin permeability plays a vital role in the selection of candidates for the development of transdermal dosage forms. In this work, mirtazapine nanoemulsions were developed, characterized and the pharmacokinetics were studied. Nanoemulsions were prepared using the spontaneous emulsification mechanism. Surfactant (sodium lauryl sulfate) and cosurfactant (propylene glycol) were mixed in different volume ratios. Pseudoternary phase diagrams were developed using the aqueous titration method. Nanoemulsions formed were characterized by particle size, polydispersity index, and zeta potential. Analysis of variance (ANOVA) of data was evaluated and optimized nanoemulsions with Smix ratio of (3:1) were obtained. The zeta potentials of the optimized nanoemulsions were –94.000±0.001 and -92.900±0.004 mV for nanoemulsion formulation 16 (NE16) and nanoemulsion formulation 17 (NE 17) respectively, while mean droplet size (photon correlation microscopy) for the same optimized formulations were 56.00±0.01, 24.00±0.01 nm respectively. There was good correlation between mean droplet size result obtained from PCS and the values obtained using transmission electron microscope (46.0 ± 12.15 and 38.2 ± 7.62 nm respectively). The in vitro skin permeation study using excised rat skin showed the permeability coefficient values for the optimized nanoemulsions to be 0.0284±0.005 and 0.0332±0.002 cm/h for formulations NE 16 and NE 17 respectively, while that of the control (mirtazapine suspension in distilled water) was 0.0019±0.003 cm/h. The relative bioavailability was observed to be 79.17 % and 44.38 % compared to the calculated 16.80 % for the oral suspension thus making the developed Nanoemulsion more potent than previous oral formulations.

Keywords: Nanoemulsion; Transdermal delivery; Mirtazapine; Thermodynamic stability

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INTRODUCTION

Drug molecules prior to been absorbed into the systemic blood circulation, have to spend some time in both aqueous solutions of the gastrointestinal tract and lipid phase of the biological membrane. For this process to occur efficiently, aqueous and lipid solubility of the drug are required. However, literature has shown that about 90 % of new drugs and many existing drugs are poorly water soluble and therefore tend to exhibit very poor bioavailability [1]. To improve the bioavailability of these lipophilic drugs, various recent techniques utilizing lipid based systems including nanoemulsions have been reported [2,3,4,5]. Nanoemulsion, a thermodynamically stable transparent or translucent formulation is prepared by the spontaneous emulsification method that involves mixing oil, water, surfactant, and cosurfactant, in the correct proportion, with mild agitation. A number of studies have found

nanoemulsions to be one of the most promising techniques for enhancement of transdermal permeation and bioavailability of poorly soluble drugs [6,7,8]. The size range has been reported to be between 10-1000nm. Transdermal delivery has been found to be an attractive alternative to oral and delivery of drugs [9, 10, 11]. When compared to oral route of drug delivery, it is used to avoid significant first-pass effect of the liver, is non-invasive and can be self-administered, improves patient compliance, avoids pains experienced during parenteral administration and is generally inexpensive. Mirtazapine (±) – 2 –methyl -1,2 ,3 ,4 ,10 ,14 b – hexahydropyrazino [2, 1 -a] pyrido [2 ,3–e] benzazopine , whose chemical structure is presented in Figure 1, is a tetracyclic antidepressant drug clinically used for the treatment of moderate to severe depression and anxiety.

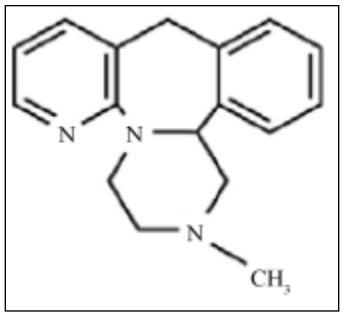


Figure 1: Chemical structure of mirtazapine

Mirtazapine is practically insoluble in water and highly hydrophobic (log partition coefficient in Octanol-water is 2.9). Mirtazapine is available only as tablets in doses of 15 mg, 30 mg, and 45 mg respectively. The drug has bioavailability of 50 % due to first-pass metabolism. It has high protein binding (80 %) and very high half-life (20 – 40 h). The withdrawal symptoms of the drug such as diarrhea, nausea, anxiety, aggression, irritability, internal restlessness, hostility, deep depression limit its clinical use. This creates a need for an alternative route of administration that bypasses these drawbacks. Transdermal route is considered such an alternative. This route of choice is also substantiated by the drug properties namely low potency and first-pass metabolism. Literature review has revealed little or no report on the transdermal delivery of mirtazapine. Therefore, the purpose of the present study was to investigate the *in vitro* skin permeability of mirtazapine using nanoemulsion formulation as a delivery system, in order to ascertain its potential in the development of a transdermal therapeutic system for mirtazapine.

EXPERIMENTAL SECTION

Materials

Mirtazapine (Mylan Pharmaceuticals,USA). Propylene glycol, sodium lauryl sulphate (Sigma-Aldrich, USA), methanol (Fisher Scientific USA), purified oils (Arachis, Soya Bean, Castor, Sunflower, Olive etc.), other chemicals were of analytical reagent grade.

Methods

The method applied in the research and the materials used are explained as follows:

Solubility study

The mirtazapine powder was added to vials containing various oils and distilled water respectively. The vials were capped and shaken on mechanical shaker for 24hours at 25^{o}C. Samples with drug crystals present were considered to have reached equilibrium. The samples were then filtered through 0.45 µm filter and the drug in the supernatant was determined using UV/VIS spectrophotometric method (Perkin Elmer Lambda 35 UV-VIS spectrophotometer) at 290 nm. Determination was done in triplicate.

Construction of pseudo phase ternary diagram

Based on the results of solubility of mirtazapine in oil, sunflower oil was used as the oil phase; sodium lauryl sulphate was selected as surfactant and propylene glycol as co-surfactant. The water titration method [12, 13] was used to obtain the pseudo-ternary phase diagram consisting of oil, surfactant mix and water.

The surfactant mix (S_{mix}) ratio consisting of 1:0, 1:1, 1:2, 1:3, 2:1, 3:1 and 4:1 was chosen in increasing concentration of co-surfactant and vice versa. Sixteen different nanoemulsions were made at each S_{mix} using the

following oil and S_{mix} volume ratio: 1:9, 1:8, 1:7, 1:6, 1:5, 1:4, 1:3.5, 1:3, 1:2.3,1:2, 1:1.5, 1:1, 1:0.7, 1:0.43, 1:0.25, 1:0.1. Titration was carried out by drop-wise addition of water followed by homogenization. Pseudoternary phase diagrams were drawn with Sigma plot 11.0, USA. Nanoemulsion regions were identified from which different nanoemulsion formulations were made.

Preparation of mirtazapine-loaded nanoemulsion

Formulations with desired component ratios were prepared from the identified nanoemulsion regions. Accurately weighed amount of mirtazapine to give 1.5 mg/ml of formulation was dissolved in the oil phase in a beaker and warmed at 37^{o}Con a water-bath and the required weight of S_{mix} was added followed by slow titration with aqueous phase till a clear solution was obtained by homogenization.

Thermodynamic stability studies

The test involves centrifuging all formulations for 30 minutes and those formulations that failed to show any phase separations were stored at 5^{o}C and 40^{o}C respectively for 48 hours through three cycles. Stable formulation samples at the end of the test were further subjected to freeze thaw cycles. Three freeze-thaw cycles were done for the formulations between –21^{o}C and +25^{o}C. The formulations that survived thermodynamic stability tests underwent characterization, physicochemical tests and assay of drug content.

Determination of droplet size, polydispersity index and zeta potential

Photon correlation spectroscopy:

A 0.2 % v/v dilution of the nanoemulsion was uniformly mixed in the flask prior to the test. The mean droplet size, zeta-potential and polydispersity index were determined by dynamic light scattering (DLS) technique using a photon correlation spectrophotometer (Malvern Nano ZS.ZS 290.UK).

Scanning electron microscopy (SEM) of nanoemulsion:

The morphology and size of nanoemulsion were studied using scanning electron microscope. A drop of 1% v/v dilution of nanoemulsion was allowed to dry on a silicon mesh, followed by a drop of 0.15 %w/v of sudan black reagent and was left to dry. The shape and size of droplets were determined.

Transmission electron microscopy (TEM) of nanoemulsion:

Further optimization of the morphology and size of the nanoemulsion was done by transmission electron microscopy. A drop of 1% v/v dilution of nanoemulsion was deposited on a cupper grid, allowed to dry and sealed with a monolayer of gold. After drying, the sample was photographed by transmission electron microscope.

Physicochemical tests

pH measurement:

The apparent pH of the formulation was determined at 25^{o}C using a pH meter with combination electrode (Eutech, Japan). The pH test was done in triplicate at 25^{o}C.

Viscosity determination:

The viscosity of nanoemulsion was determined using Brookfield cone and plate viscometer (Brookfield Eng. Lab. Inc, USA) at 25±0.5^{o}C . Viscosity was done in triplicate at 25^{o}C.

Conductivity measurement:

The electrical conductivity of nanoemulsion was determined using an S70 Seven Multi^{TM} conductivity meter (Mettler Toledo, Columbus, USA). The conductivity meter was fitted with an Inlab$^{(R)}$ 730 conductance electrode having a cell constance of 0.58cm^{-1}. The test was done in triplicate at 25^{o}C.

Drug content:

Drug contents of stable nanoemulsions were estimated by dissolving in methanol and filtering through 0.45 µm filter membrane. Drug content in methanol filtrate was analysed at 290 nm using Perkin Elmer Spectrophotometer against standard solvent solution of mirtazapine.

Skin permeation analysis

Preparation of rat abdominal skin:

The animal studies were performed after an approval was obtained from Faculty of Veterinary Medicine, University of Nigeria, Nsukka Institutional Animal care and Use committee's guidelines. (IACUC/UNN 2015/PG046). Male Wister rats were sacrificed with prolonged exposure to chloroform. Abdominal hairs on the animal were removed with electrical clipper. Full thickness skin was surgically removed from each rat. The epidermis was prepared from the full thickness skin by soaking entire abdominal skin in water at 60^{o}C for 1 minute [14]. The epidermis was cut into 4.5 × 4.5 cm^2 pieces and used for the permeation study. The unused epidermis was wrapped with aluminum foil and stored at −20˚C until required. Before use, the stored epidermis was allowed to thaw, cut into 4.5 × 4.5 cm^2 pieces and hydrated by placing in phosphate buffer saline (PBS) overnight before use [15].

***In-vitro* permeation studies:**

The studies were carried out using modified Franz diffusion cell. The diffusion area of the cells was 2.54 cm^2. The cell was immersed in a circulating water bath and the temperature was controlled at 37˚C. The receiver fluid (methanolic phosphate buffer saline, PBS (30:70), pH 7.4) was placed in the receiver compartment that has a volume of 15 ml and was stirred by an externally driven Teflon-coated magnetic bar. The prepared epidermis was sandwiched between the receiver compartment and the donor compartment with the stratum corneum facing upwards. The donor compartment was clamped. A 1 ml of nanoemulsion containing 1.5 mg of mirtazapine was added to the donor compartment that was covered with a glass lid. The sampling arm of the donor compartment was sealed with aluminum foil. At suitable time interval (0, 1, 2, 4, 6, 8, 10, 12 and 24 hours), 1 ml of sample was withdrawn from the center of the receiver compartment with a syringe connected with a needle. An equal volume of fresh PBS (37˚C) was replenished immediately. The amount of drug in the receiver fluid was determined by UV spectrophotometry at 290 nm.

Spectrophotometric assay:

The concentrations of mirtazapine permeated to the receiver fluids were quantitatively determined by Perkin Elmer Lambda 35 UV-VIS spectrophotometer. The samples were analyzed at maximum wavelength of 290 nm. The calibration curve (absorbance versus drug concentration) was constructed by measuring standard solutions of the drug in PBS in the concentration range of 1 - 5 µg/ml both intra-day and inter-day.

Data analysis:

The skin permeation data were measured as the cumulative drug permeation per unit of skin surface Qt/S (S =2.54 cm^2). The cumulative drug permeation (Qt) was calculated from Equation (1)

$$Q_t = V_r C_t + \sum_{i=0}^{t=1} V_s C_i \quad (1)$$

Where:

C_t is the drug concentration of the receiver fluid at each sampling time,

C_i is the drug concentration of the ith sample,

V_r and V_s are the volumes of the receiver fluid and sample respectively.

The steady-state fluxes (J_{ss}) were determined as the slope of the linear portion of the graph obtained by plotting cumulative drug permeation per unit of skin surface versus time.

One-way analysis of variance (ANOVA) was used to compare fluxes of nanoemulsions and the control at a *p*-value of 0.05. The penetration enhancing effect of each nanoemulsion was calculated in terms of enhancement ratio (ER) using Equation (2):

$$ER = \frac{K_p \text{ (test vehicle)}}{K_p \text{ (control)}} \quad (2)$$

Preparation of stratum corneum (sc) for biophysical analysis

The rat stratum corneum samples were prepared by soaking freshly prepared epidermis membrane in a 0.1 % trypsin solution for 12hours. The SC samples were cleaned by washing with distilled water and blotted dry before utilizing it for FTIR [16] studies.

Fourier Transform Infrared (FTIR) analysis

The infrared analysis was carried out on both untreated and treated rat stratum corneum. The nanoemulsion-treated and untreated (control) SC samples respectively were vacuum-dried at 25˚C ± 1˚C for 48 h and stored in desiccators to remove traces of solvent. The control samples were treated in phosphate buffer saline (PBS). All sample were treated for 24hours prior to been vacuum-dried and subjected to FTIR (Shimadzu 8400S, Japan) analysis. Peaks that occurred near 2850 cm−1 and 2920 cm−1 that were due to symmetric and asymmetric C-H stretching vibrations respectively as well as those at 1550 cm−1 and 1650 cm−1 indicating amides (C=O) stretching vibrations were characterized.

Determination of activation energy

The activation energy of mirtazapine across the rat skin was calculated by using data from *in vitro* skin permeation study at 25, 37 and 50°C. The procedure has been described under *in vitro* skin permeation studies. The permeability coefficient was calculated at each temperature and the activation energy of mirtazapine was obtained from Arrhenius relationship: [17].

$$P = Po\ e^{-Ea/RT} \quad (3)$$

Where, Ea is the activation energy,
R is gas constant (8.3145J/mol),
T is absolute temperature in K,
P is the permeability coefficient, and Po is the Arrhenius factor.

Pharmacokinetics study of mirtazapine nanoemulsion

Protocol:

The optimized nanoemulsion formulations NE 17 and NE 16 were subjected to invivo pharmacokinetic study. A total number of 15 wistar rats of both sexes (200 – 250 g), were housed in three groups of five, in a 12 hour light/dark cycle at room temperature and were fasted for 24hours with free access to water before the experiment. The animals were randomly divided into three groups of five animals each. Group 1 received 10 mg/kg (control) of mirtazapine per oral suspension. Group 2 received 2.5 mg/kg mirtazapine in nanoemulsion formulation (NE 17), while group 3 received 2.5 mg/kg of mirtazapine in nanoemulsion formulation (NE 16). Administration of nanoemulsion was performed by placing the dose across a marked surface area of the rat that was devoid of hair. The drug suspension was given by oral intubation. Blood samples (0.3 ml) were collected at intervals of 0, 0.5, 0.75, 1, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, and 24 hours via a modified needle cannula in the animal right jugular vein. Samples were placed in the collection tubes containing heparin. The blood samples were centrifuged at 3000 rpm for 10 min and the plasma obtained were stored at -20oC until use. The frozen plasma was thawed at room temperature just prior to extraction. The plasma protein in the plasma was precipitated in acetonitrile by mixing carefully for 10 minutes. After centrifuging at 3000 rpm for 10 min, the acetonitrile layer was re-extracted in 2.0 ml of chloroform, mixed for 5 min and evaporated to dryness on a warm water- bath (40^oC). The dry sample was reconstituted in 2.0 ml of analytical methanol and plasma mirtazapine concentration determined with Perkin Elmer Lambda 35 UV-VIS spectrophotometer at 290 nm. Data obtained were used to establish the plasma concentration- time curve and for the determination of pharmacokinetic parameters.

Pharmacokinetic analysis

The plasma concentration against time curve was analyzed using Sigma plot 11.0 ™ software. Data from plasma concentration-time curve within 24hours of drug administration were used to estimate the necessary pharmacokinetic parameters. Student's t-test was used for statistical analysis at $p < 0.05$. Values are reported as mean ± SD and the data were considered statistically significant at $p < 0.05$.The % relative bioavailability was also evaluated.

RESULTS AND DISCUSSION

The result of the solubility of mirtazapine is given in Figure 2. Among the oils investigated, the solubility of mirtazapine was found to be highest in sunflower oil (64.8 ± 2.0 mg/ml). Drug solubility in oil is very important, as it determines the extent the drug will remain in solubilized form within the nanoemulsion.

The pseudoternary phase diagram (Figure 3) of the optimized nanoemulsion, showed that oil could be solubilized up to the extent of 18 % vol/vol. Thus, from the phase diagram a different concentration of oil that formed a

nanoemulsion was selected covering the nanoemulsion area of the phase diagram. Selection of percentage of oil from the phase diagram was based on formulations that used a minimum concentration of S_{mix}. No change was found in the phase behavior of the pseudoternary phase diagram when mirtrazapine was incorporated into nanoemulsion depicting that the production and stability of the nanoemulsion were never affected by the physicochemical properties of the drug. The compositions of these formulations are given in Table 1.

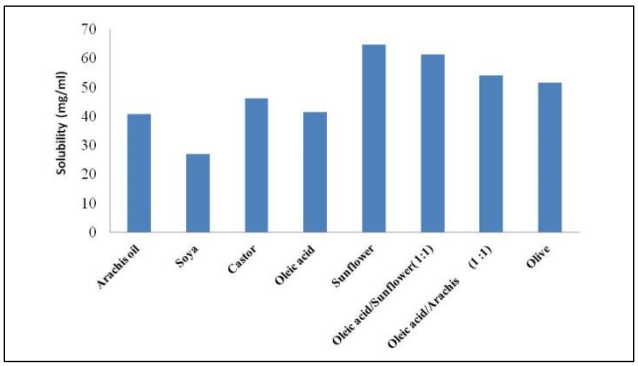


Figure 2: Solubility of mirtarzapine in oils

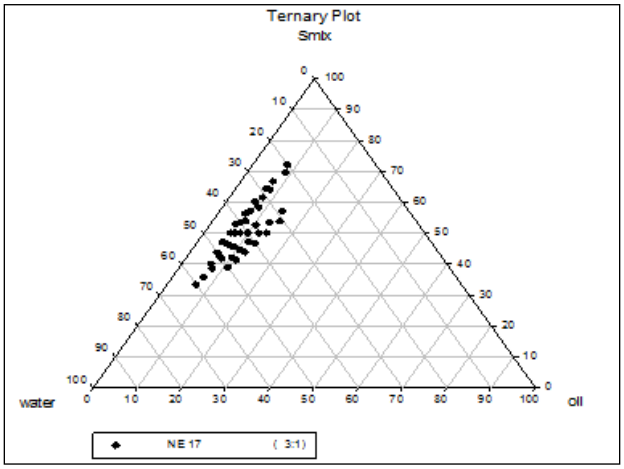


Figure 3: Pseudoternary phase diagram for nanoemulsion (NE 17)

Table 1: Percent composition of mirtazapine loaded- nanoemulsion formulations

Ingredients	Formulations					
	NE 15	NE 16	NE 17	NE 18	NE 19	NE 20
Mirtazapine	1.5	1.5	1.5	1.5	1.5	1.5
Sunflower oil	11	6.35	8.05	9.2	10.12	11.06
Smix	63.33	44.32	45.71	52.08	57.36	62.37
Distilled water	24.17	47.83	44.74	37.22	31.02	25.07

The results of the thermodynamic stability studies helped: stable nanoemulsions to be identified, avoid metastable formulations and invariably will prevent frequent tests to be performed during storage. Survival of this thermodynamic stability tests could be attributed to low interfacial tension between oil and water at this weight ratio of surfactant with respect to the co-surfactant and also the position of the droplet size could have been responsible for the observed stability. Analysis of drug content for stable nanoemulsions showed no evidence of drug degradation. The percent contents are given in Table 2. Two formulations (designated NE 16 and NE17 respectively) out of the formulations that passed the thermodynamic stability test produced acceptable characterization values (Table 2). The zeta potential of NE16 and NE17 are - 94 ±0.001 and -92.9 ± 0.004 mV respectively. The mean droplet size (photon correlation microscopy analysis, Figure 4) for NE16 and NE17 are 56.0 ± 0.01, 24.0 ± 0.01 nm respectively.

Table 2: Droplet size, zeta potential, polydispersity index (PDI) and assay of mirtazapine loaded-nanoemulsion

Formulation	Droplet size (nm)	Zeta potential (mV)	PDI	Assay (%)
NE 15	159.0 ± 0.02	-75.6 ± 0.28	0.825 ± 0.01	92.13 ± 0.3
NE 16	56.0 ± 0.01	-94.7 ± 0.01	0.578 ± 0.02	97.05 ± 0.5
NE 17	24.0 ± 0.01	-92.9 ± 0.04	0.486 ± 0.01	99.85 ± 0.8
NE 18	180.0 ± 0.02	-81.3 ± 0.01	0.638 ± 0.02	95.00 ± 0.1
NE 19	195.0 ± 0.02	-89.0 ± 0.01	0.689 ± 0.01	94.15 ± 0.2
NE 20	200.0 ± 0.40	-84.0 ± 0.01	0.720 ± 0.01	90.18 ± 0.1

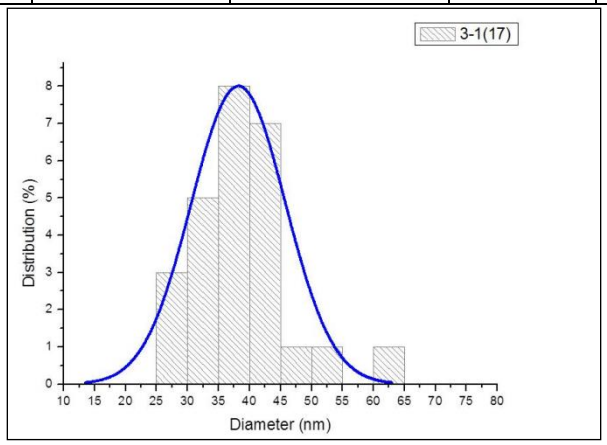


Figure 4: Droplet size analyses by transmission electron microscopy (NE 17)

The results of transmission electron microscopy revealed spherical, discrete but homogeneous droplets which were formed in the size range of less than 60 nm, complementing the result from photon correlation microscopy. Photograph of the transmission electron microscopy (TEM) of the optimized nanoemulsion formulation NE17 is shown in Figure 5.

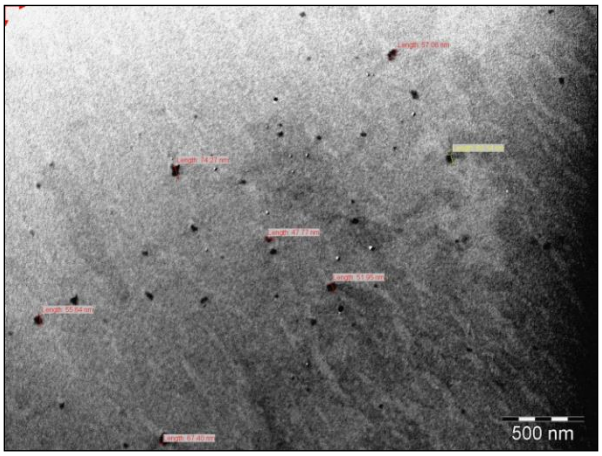


Figure 5 Transmission electron microscopy image for mirtazapine loaded- nanoemulsion formulation NE 17

There was good correlation between mean droplet size result got from photon correlation microscopy analysis and the values (46.0 ± 12.15 and 38.2 ± 7.62 nm respectively) obtained using transmission electron microscopy. All the droplets were found in the nanometer range suggesting the suitability of both formulations for transdermal drug delivery. The polydispersity values of the formulations NE16 and NE17 were found to be 0.578 and 0.486 for NE16 and NE17 respectively, which signified uniformity of droplet size within the formulations.

Table 3: Physicochemical properties of mirtazapine loaded-nanoemulsion

Formulation	Conductivity (s/cm)	pH	Refractive index	% Transmittance	Viscosity (cP)
Blank	0.632±0.001	9.03±0.01	1.330±0.001	100	0.8860±0.001
NE 15	0.635±0.001	9.03±0.01	1.340±0.001	100	0.8872±0.001
Blank	0.618±0.002	8.04±0.01	1.330±0.002	100	0.8860±0.001

NE 16	0.619±0.001	8.04±0.01	1.330±0.002	100	0.8872±0.002
Blank	0.629±0.002	8.01±0.01	1.330 ±0.001	100	0.8861±0.002
NE 17	0.631±0.002	8.01±0.01	1.334±0.001	100	0.8872±0.001
Blank	0.652±0.001	9.03±0.01	1.330±0.002	100	0.8868±0.001
NE 18	0.654±0.001	9.04±0.01	1.338±0.002	100	0.8872±0.002
Blank	0.725±0.002	9.02±0.01	1.330±0.001	100	0.8868±0.002
NE 19	0.727±0.002	9.02±0.01	1.338±0.001	100	0.8872±0.001
Blank	0.753±0.001	9.01±0.01	1.330±0.002	100	0.8868±0.001
NE 20	0.754±0.001	9.02±0.01	1.348±0.002	100	0.8872±0.001

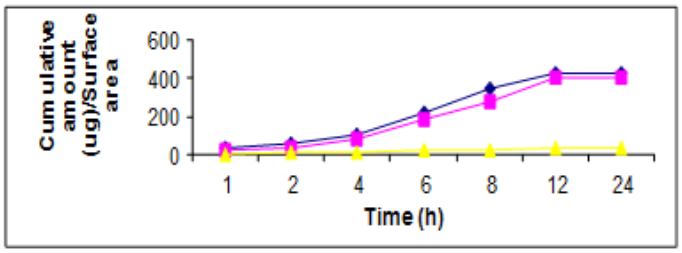
As shown in Table 3, the results of the physicochemical properties of mirtazapine-loaded nanoemulsions compared to the blank counterparts indicated that nanoemulsions properties were not altered after the drug was incorporated. However, slight changes in pH observed could be due to the intrinsic properties of the drug.

In vitro permeation studies

The results of the permeation studies of mirtazapine through the rat skin are given in Table 4. The results indicate that formation NE 17 showed higher permeation than formulation NE 16. This could be probably due to its smaller droplet size and higher oil composition when compared to formulation NE16. The lag time (Figure 6) for both formulations was found to be 1 h. The permeability coefficients are 0.0158.0±0.0005 and 0.0187±0.0004 cm/h for formulations NE 16 and NE17 respectively. The steady-state flux was found to be 23.70± 0.44 for formulation NE 16 and 28.01± 0.57µg/cm^2/h for formulation NE 17 respectively. The slight difference in the steady-state flux of the two formulations might be due to varying influences of the formulations on the biophysical properties of the stratum corneum and differences in thermodynamic activity of the drug. The enhancement ratio data for permeability coefficient are 21-fold and 25-fold increase for NE 16 and NE 17 respectively when compared with the control.

Table 4: Skin permeation parameters of mirtazapine through rat stratum corneum

Formulation	Permeability coefficient (×10^{3}cm/h)	Flux (µg/cm^2/h)	Enhancement ratio
Control	1.9±0.3	-	-
NE 16	28.4.±0.5	42.60±0.36	14.95
NE 17	33.2± 0.2	49.80.01±0.41	17.47



□---------□ **NE 17**
□---------□ **NE 16**
Δ--------Δ **Control**

Figure 6: Plot of cumulative of mirtazapine released versus time

The FTIR spectra of untreated (control) and treated SC were obtained in the range of 400-4000 cm^{-1}. The spectra showed various peaks due to molecular vibration of proteins and lipids present in the SC. For untreated SC, the absorption band seen at the wave number of 3147.9cm^{-1} might be due to the C-H stretching of the alkyl groups present in both proteins and lipids. The bands at 2924 cm^{-1} and 2847 cm^{-1} were assigned to the asymmetric -CH_2 and symmetric -CH_2 vibrations of long chain hydrocarbons of lipids respectively. The bands at 2955 cm-1 and 2870 cm^{-1} were due to the asymmetric and symmetric CH_3 vibrations respectively [18]. These narrow bands were attributed to the long alkyl chains of fatty acids, ceramides and cholesterol which are the major components of the SC lipids.

The two bands observed at 1604.83 cm-1 and 1496.81 cm-1) could be due to the amide I and amide II stretching vibrations of SC proteins arising from C = O stretching vibration and C-N bending vibration respectively. A difference in the FTIR spectra of untreated and nanoemulsion treated SC was observed with noticeable decrease in asymmetric and symmetric CH- stretching of peak height and area. This hydrogen bonding of lipid (ceramides) bilayers of SC is responsible for the stability and strength of lipid bilayers and thus imparts barrier property to SC [19]. It has been reported that when skin is treated with nanoemulsion formulations or other skin delivery vehicles, hydrogen bond networks get broken at the head of ceramides due to penetration of nanoemulsions or other skin

delivery vehicles into the lipid bilayers of SC [20]. This disruption of the lipid bilayers by the optimized nanoemulsions seemed to be responsible for the permeation of mirtazapine through the excised rat skin. The results are given in Table 5

Table 5: FTIR spectral data of nanoemulsion treated and untreated rat skin

Vehicle	Asymmetric C-H stretching		Symmetric C-H Stretching		Amide I stretching Vibration		Amide II bending Vibration	
	Peak height	% decrease in peak height	Peak height	% decrease in peak height	Peak height	% decrease in peak height	Peak height	% decrease in peak height
Untreated	0.2519	-	0.3203	-	0.3423	-	0.2255	-
NE 16	0.1892	24.89	0.239	25.37	0.2311	32.48	0.1692	24.97
NE17	0.1821	27.71	0.2272	29 .08	0.1816	46.93	0.1351	40.07

The Arrhenius plot between logarithms of permeability coefficient (log p) and reciprocal of absolute temperature (1/T) was observed to be linear within the temperature range between 25–50°C. The linearity implies that there was no significant structural or phase transition changes within the skin membrane. The activation energy value for permeation of mirtazapine across the rat skin was calculated from the slopes of Arrhenius plots to be 1.7860Kcal/mol and 1.8158 Kcal/mol for NE16 and NE 17 respectively. The correlation coefficients (r^2) obtained from the linear graphs were-0.9594 and -0.9661 for NE16 and NE 17 respectively. The Arrhenius plot for is shown in Figure 7. The decrease in activation energy (Ea) when compared with reported value [21, 22] of 4.1Kcal/mol for ion transport across human epidermis suggest that the SC lipid bilayers have been disrupted.

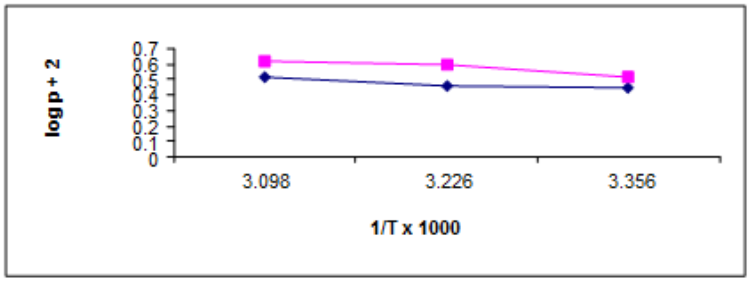


Figure 7: Plot of logarithm of permeability coefficient versus reciprocal of absolute temperature

Results of pharmacokinetic study

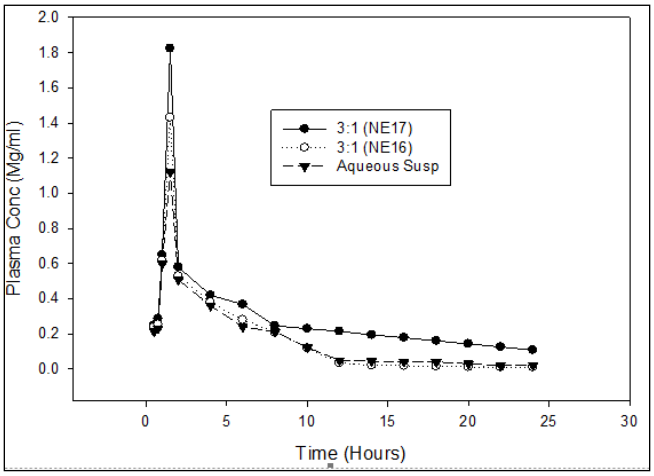


Figure 8: Mirtazapine plasma concentration versus time profiles after administration of aqueous suspension of tablet and transdermal nanoemulsion formulation NE17 and NE16 in Rats (n=5)

Table 6: Pharmacokinetic parameter after the oral administration of mirtazapine suspension and transdermal delivery of mirtazapine nano-emulsion formulation NE16 & NE17 in Rats (n=5)

	Nanoemulsion formulations		Tablet suspension
	NE 16	NE 17	
C_{max} (mg/ml)	1.45	1.824	1.12
T_{max} (h)	1.55	1.5	1.59
$AUC_0 - \alpha$(mg.h/ml)	2.134	3.806	0.809
Bioavailability	44.38	79.17	16.8

*P < 0.05 by the student t-test compared to the tablet suspension

It was observed that the maximum concentration (Cmax) of mirtazapine loaded nanoemulsion formulations NE17&NE16 delivered through transdermal route were 1.824 mg/ml, and 1.450 mg/ml respectively compared to the oral suspension of mirtazapine which was observed to be 1.120 mg/ml and this difference were quite significant (P<0.05). It was also observed that the AUC $_{(0-\alpha)}$ for transdermaly delivered formulation NE17&NE16 were 3.8060 and 2.1342 mg.h/ml respectively, the difference was quite significant when compared to 0.8090 mg.h/ml for the oral aqueous suspension. There was no significant difference in the T_{max} for both formulations (NE17&NE16) when compared to that of oral suspension. The relative bioavailability was observed to be 79.17 % and 44.38 % compared to the calculated 16.80 % for the oral suspension.

Nanoemulsion formulation consists mainly of oil in water system mixed with a perfect blend of surfactants and co-surfactants. The co-surfactant (propylene glycol) is a known permeation enhancer and has helped in the transdermal delivery of mirtazapine across membranes and into the system for an improved bioavailability, also the nanometer size droplets when exposed to the surface area of the stratum conium were able to permeate faster with precise delivery of active pharmaceutical principle to reach the maximum plasma concentration within the specified time and were comparatively better than the oral suspension (tablet) delivery of mirtazapine. The mirtazapine loaded nanoemulsion (transdermal delivery) was compared with the oral suspension of commercial tablet in wistar rats. The bioavailability of mirtazapine nanoemulsions were significantly enhanced compared with that of tablet (oral) (p<0.05).

CONCLUSION

Mirtazapine nanoemulsion formulations were successfully prepared by the spontaneous emulsification method (titration method). The FTIR spectra of skin treated with nanoemulsion indicated that permeation occurred due to the disruption of SC lipids by nanoemulsion. The decrease in activation energy for mirtazapine permeation across rat skin also substantiated the disruption of the SC lipid bilayers. The characterized values for optimized nanoemulsions were considered to be adequate. Finally, the results suggest that both nanoemulsion formulations can be successfully used as vehicles to enhance skin permeation of mirtazapine. In this research, the aqueous solubility and potency of mirtazapine was enhanced as the work provides the only information on utilization of nanoemulsion for the transdermal delivery of mirtazapine.

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