Apixaban Ultrafine O/W Nano Emulsion Transdermal Drug Delivery System: Formulation, *In Vitro* and *Ex Vivo* Characterization

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ABSTRACT

Apixaban (APX) is a potent oral anticoagulant drug that directly inhibit coagulation factor Xa for prevention of venous thromboembolism (VTE) following total hip or knee replacement surgery. Orally APX has poor water solubility (0.028 mg/mL) and relative low bioavailability (50%). Transdermal APX delivery was utilized as a convenient alternative route to control oral limitations. This study designed to formulate ultrafine APX o/w nanoemulsion with self-permeation enhancing properties through skin barrier utilizing the ultrafine (> 50 nm) nanosized droplets as well as nanoemulsion components themselves to act as a permeation enhancer. Solubility study result in selecting triacetin oil, triton-x-100 and carbitol as oil phase, surfactant and cosurfactant respectively, while pseudoternary phase diagram construct nanoemulsion area for choosing formulations. Twenty-one o/w nanoemulsions prepared and characterized for droplet size, pH values, percent transmittance, electroconductivity, APX content, *in vitro* APX release, and *ex vivo* permeation through Albino Wistar rat abdominal skin to simulate human skin. Among formulations, ten preparations demonstrate ultrafine APX o/w nanoemulsions with high percent transmittance and electroconductivity, pH values appropriate for skin application, ultrafine droplet sizes (> 50 nm) and accepted APX content. *In vitro* release studies reveal significant (p ≤ 0.05) increase in APX dispersibility and diffusion through dialysis membrane. *Ex vivo* APX permeation through rat abdominal skin was significantly (p ≤ 0.05) increased in comparison with pure drug as assured by significant (p ≤ 0.05) enhancement in permeation parameters Jss, KP and ER with shorter T$_{lag}$, which could be attributed to permeation enhancing properties of nanoemulsion formulation itself.

Key words: Apixaban (APX); Ultrafine o/w nanoemulsion; transdermal drug delivery.

INTRODUCTION

Apixaban (APX) is a potent oral anticoagulant drug that selectively and directly inhibit coagulation factor Xa and used as a prophylactic therapy for the prevention of venous thromboembolism (VTE) following total hip or knee replacement surgery(1). It was approved by FDA on December 28, 2012, for the prevention of stroke and systemic embolism in patients with non-valvular atrial fibrillation (AF) and marketed by Bristol-Myers Squibb/Pfizer with trade name Eliquis. Unfortunately, APX has poor water solubility of 0.028 mg/mL at 24 °C and relative low bioavailability of about 50% after oral administration of a single 10 mg dose. This low bioavailability could be attributed to the incomplete absorption of APX in the gastrointestinal tract (GIT), and from the effect of first-pass metabolism in gut and liver(2). Additionally, tablet dosage form is the only available form for APX, which consider somewhat expensive for most patients(3), although APX treatment was the dominant strategy in the prevention of stroke and systemic embolism over warfarin therapy, as APX shown to be safer than warfarin with superior therapeutic activity in the prevention of stroke(4). Therefore, regarding the limitations of the marketed APX tablet in bioavailability and economic cost, the development of new APX formulation was of interest and important to obtain desirable pharmacokinetic properties, including increased bioavailability, and more cost-effective dosage form. Transdermal drug delivery has several advantages over the oral route, as transdermal route can overcome the limited APX absorption through intestine and evade first-pass metabolism problems in gut and liver, and hence, improved absorption via the skin and enhanced bioavailability(5). Consequently, transdermal APX administration through the skin was the most attractive dosage form. One of the most promising tools for transdermal drug administration is the lipid-based dispersion nanoemulsion, due to desired features of the nanoemulsion system that demonstrate enhanced solubility of lipophilic or poorly soluble drugs, good thermodynamic stability and improved dermal and transdermal drug delivery by permeation enhancing properties of its components through biologic membranes, excellent drug loading capacity and negligible or minimum skin irritation tendency(6, 7). Nanoemulsion is a transparent heterogenous system consisting of two immiscible liquids, water and oil, stabilized by an interfacial layer of surfactant / cosurfactant mixture, or namely Smix, forming isotropic system that contain or "hide" the drug molecules in solubilized form within the oil phase droplets(8). Nanoemulsion dispersion display uniform distribution with droplet size ranged from 20 - 200 nm, this permit high drug flux and penetration through the intracellular lipophilic pathway of the skin that allow the nano-sized droplets of less than 20 nm to permeate easily and creates a drug depot within the stratum corneum and epidermis(9). Recently, ultrafine nanoemulsion formulation developed as an advanced approach of emulsion system, which designate clear isotropic nanoemulsion with droplet size of below 50 nm. The key step for such nanoemulsion preparation is to find the appropriate blend of oil and surfactant that able to dissolve the required dose of

drug, which is 5 mg of apixaban in our study, and eventually form clear isotropic mixture of oil, water, surfactant and cosurfactant to form nanoemulsion system within ultrafine droplet size below 50 nm(10). Transdermal application of such ultrafine formulation offer several advantages over other traditional transdermal delivery systems due unique properties of the ultrafine nanoemulsion, including better spreading ability of ultrafine nano-sized drug particles over larger surface of the skin caused by increased effective area of available drug particles exposed to the skin; increased size to volume ratio of ultrafine sized particles (below 50 nm) and thereby decrease the amount of final formulation and increase its capacity for drug loading; in addition to the permeation enhancing properties of the components of nanoemulsion, the formulation itself of ultrafine nanoemulsion can act as a permeation enhancer without using any chemical or physical permeation facilitating technique(11, 12).

This study designed to prepare ultrafine oil in water (o/w) nanoemulsion formulations for transdermal delivery of apixaban (APX) as a novel technology with improved pharmaceutical physical properties of the drug, including increased solubility and decreased crystallinity, and to evaluate the permeation enhancing properties of the prepared ultrafine o/w nanoemulsion formulations for their impact as being themselves permeation enhancers using abdominal skin of Wistar Albino rat for the *ex vivo* permeation study.

MATERIALS AND METHODS

Instruments and Materials

Instruments used in this study include, Water Bath Shaker (Kottermann, type 3047, Hanigsen, Germany) for solubility study, Centrifuge (Fanem, 206-R Centrifuge, Brazil), UV VIS Spectrophotometer (Spectrumlab 752Pro, China), Vortex Mixer (Labinco L46, Netherland), Electrical Conductivity Meter (DDS-11A, China), Brookhaven instrument (Zeta Plus, Serial NO: 21521, USA), Intelligent Transdermal Diffusion Instrument (TP-6, China), Digital pH meter (BP 3001, Singapore), OriginLab 2018 software program was used to plot pseudo-ternary phase diagram, Dialysis Membrane (M.W 8000 - 14000 Da, USA).

Materials used include, Pure Apixaban obtained from ZHEJIANG CP CHEMICAL CO., LTD; Methanol and Ethanol Lab grade solvents (Sigma Aldrich, USA); Oils include oleic acid and triacetin (Hangzhou Hyper Chemicals Limited), castor, sesame, black seed, jojoba, argan, olive, coconut, avocado, anise, almond, funnel and wheat oils (NOW® CO., USA); Surfactants include cremophor EL, cremophor RH 40, tween 20, tween 80, span 20, span 80 and triton-X100 (Sigma Aldrich, USA); Cosurfactants include carbitol, methyl carbitol, glycerin, PEG 200, PEG 400 and propylene glycol (Sigma Aldrich, USA).

Preparation of Apixaban Loaded O/W Nanoemulsion Formulations

Apixaban Saturated Solubility Study

An excess amount of APX was mixed with 5 mL of oils, surfactants and cosurfactant each separately in screw stoppered 5 mL vials and vortex for 1 min, then place each sample in water bath shaker for 72 h at 32 ± 0.5 °C and 100 rpm followed by equilibrium for 24 hours. After reaching equilibrium, each mixture was centrifuged at 3500 rpm for 20 min to separate the excess of insoluble drug and then the supernatant was filtered through 0.45 μm membrane filters and diluted with methanol. The solubilized amount of APX was quantified spectrophotometrically using UV-Visible spectrophotometer at 278 nm (λ_{max} of APX in methanol) using methanol as blank(13, 14).

Development of Pseudoternary Phase Diagram
Pseudoternary phase of oil, surfactant/cosurfactant (Smix), and water was developed using aqueous titration method. Surfactant/cosurfactant weight ratios of (1:1, 1:2, 1:3, 1:4, 2:1, 3:1, 4:1) were screened for nanoemulsion formation, these ratios used for detailed study of phase diagram which reflect increasing concentrations of cosurfactant with respect to surfactant and increasing concentrations of surfactant with respect to cosurfactant(15). For each phase diagram, different weight ratios of oil and Smix were combined in a range from 1:9 to 9:1 in separated vials, where a homogenous mixture of oil and Smix was formed using vortex for 5 minutes, then aqueous phase of deionized water titrated slowly in a dropwise manner at 25 °C under continuous stirring and visual observation until first turbidity and clear transparent oil in water (o/w) nanoemulsion was obtained(16).

Criteria for Selection Nano emulsion Formulations Subjected for Thermodynamic Stability Testing
To make a selection of optimum formulae, different o/w nano emulsion formulations were prepared from each phase diagram within nano emulsion region for APX loading and to subject thermodynamic stability studies depending on the following criteria:

1. The selected dose of APX for incorporation into the oil phase was 5 mg for the preparation of 2 g oil in water (o/w) nano emulsion.
2. The oil concentration in the selected formulations should able to solubilize the used dose (single dose) of APX easily, which is 5 mg.
3. Different concentrations of oil were selected from each phase diagram in the nano emulsion region with a difference of 5%.
4. Minimum concentrations of surfactant and co-surfactant, hence Smix, and large percent of water were selected from each phase diagram for preparation of o/w nano emulsion formulations(17, 18).

Formulation of Apixaban Loaded O/W Nano emulsions
A series of nano emulsion formulations were selected to be prepared (Table 1.) using aqueous titration method, in which, the assigned dose of APX of 5 mg was added to the specified amount of oil phase and vortex until dissolving the drug, then surfactant and co-surfactant (S mix) were added

to the oil loaded drug with the aid of vortex mixing. Then, aqueous phase of deionized water was titrated gradually, drop by drop, with gentle mixing until isotropic clear nano emulsion was obtained(19). The preparation experiment was performed in triplicate.

Table 1. Composition (w/w %) of apixaban nanoemulsion formulations

F-code	Smix ratio	Triacetin oil %	Water %	F-code	Smix ratio	Triacetin oil %
F-1	1:1	5	55	F-12	4:1	15
F-2	1:1	10	50	F-13	1:2	5
F-3	1:1	15	45	F-14	1:2	10
F-4	2:1	5	55	F-15	1:2	15
F-5	2:1	10	50	F-16	1:3	5
F-6	2:1	15	45	F-17	1:3	10
F-7	3:1	5	50	F-18	1:3	15
F-8	3:1	10	45	F-19	1:4	5
F-9	3:1	15	40	F-20	1:4	10
F-10	4:1	5	50	F-21	1:4	15
F-11	4:1	10	45			

Thermodynamic Stability Study of the Prepared APX O/W Nano emulsion Formulations
Three thermodynamic stability tests used to assess physical stability of the prepared o/w nano emulsion formulations and include centrifugation test, where nano emulsions centrifuged at 3500 rpm for 30 min, followed by six heating-cooling cycles utilized by storing each formulation between refrigerator (4 °C) and heating (45 °C) temperatures for 48 h in each temperature. Finally, accelerated stability assessment by exposing the formulations to three freeze-thaw cycles between freezing (– 20 °C) and thawing at room (25 °C) temperatures for 24 h at each temperature. After each test, samples discarded if demonstrate phase separation, precipitation or cracking by visual check(20, 21).

Characterization Techniques of the Prepared APX O/W Nano emulsion Formulations
Droplet Size and Poly dispersity Index (PDI) Measurement
The average droplet size of the prepared o/w nano emulsions was measured by dynamic light scattering (DLS), which analyze fluctuations in light scattering at 25 °C and scattering angle of 90 ° caused by the Brownian motion of the particles using photon correlation spectrophotometer (PCS). Poly dispersity index (PDI) is a measure of homogeneity in droplet size which ranges from 0 to 1 and measured by electrophoretic light scattering technique(22).
pH Measurement
Digital pH meter used to measure pH values of APX o/w nano emulsions, the pH of final transdermal APX formulations is important for their compatibility with the pH of skin to avoid possible irritation(23). The experiment was performed in triplicate.

Transmittance Percent and Electrical Conductivity Measurement

To confirm the type of the prepared nano emulsion, transmittance percent of the prepared APX nano emulsions was measured for optical transparency using UV-Visible spectrophotometer at 650 nm, samples were not diluted while keeping distilled water as blank(24), while electro-conductivity (σ) measured using conductivity meter consisting of digital meter and two Pt / platinized electrodes. The metal electrodes immersed in 5 mL of each sample at 25 °C and then reading was recorded (25). The experiment was performed in triplicate.

Apixaban Content Measurement
Two grams of each APX nanoemulsion (supposed to contain 5 mg of APX) diluted with methanol and sonicated for 15 min for complete mixing, then filtered with 0.45 μm filter syringe and analyzed spectrophotometrically at 278 nm using methanol as a blank(26). The experiment was performed in triplicate.

In Vitro Apixaban Release Study of Ultrafine APX O/W Nano emulsions
In vitro APX release from ultrafine o/w nano emulsions was achieved using vertical Franz cell diffusional system with receptor part volume of 15 mL and donor part of 3 mL, and dialysis membrane (M.W 8000 - 14000 Da, USA), as diffusional barrier, mounted between donor and receptor parts of Franz cell with diffusional area of 1.77 cm^2. The dialysis membrane was first soaked in phosphate buffer saline (PBS) pH 7.4 for 24 h prior to use, in which PBS at 32 ± 0.5 °C (temperature of skin surface) containing 1 % Sodium Lauryl Sulphate (SLS) (to keep APX in solubilized form) under continuous stirring of 600 rpm (to simulate *in vivo* conditions) was used as releasing medium(27, 28).
Two grams of the prepared ultrafine o/w nano emulsions loaded with single dose APX of 5 mg, as well as 2 g of PBS pH 7.4 containing 5 mg of pure APX suspended in it as a control, were placed separately in donor compartments of

Franz cell instrument under experimental parameters mentioned above. Samples of 0.1 mL were withdrawn every 5 mins and replaced with equivalent volume of fresh PBS dissolution medium after each withdrawal. APX content of the samples was quantified spectrophotometrically at 280 nm using PBS pH 7.4 buffer as a blank and until complete release of APX was achieved, then the cumulative amount of APX released was calculated and plotted as a function of time(29, 30). The experiment was performed in triplicate.

Kinetics of APX *In Vitro* Release

Various mathematical models were applied for the plotting data obtained from *in vitro* release experiment to determine the kinetics and mechanism of APX release from each prepared nano emulsion, including zero order kinetic, first order kinetic, higuchi model and korsmeyer - peppas model using equations in Table 2.(31). The accuracy and predictability of the applied models were compared on the basis of plot linearity and calculated squared regression coefficient (R^2) constructed from graphs made for each model, in which the plot that is linear with a highest value of R^2 would consider the selected kinetic model for APX release from its formulation(32, 33).

Table 2. Kinetics Models for Apixaban In Vitro Release Study [1]

Model	Equation
Zero order	$Q_t - Q_0 = K_0 t$
First order	$Ln\ (Q_t / Q_0) = K_1 t$
Higuchi Model	$Q_t = K_H \sqrt{t}$
Hixson-Crowell Model	$(Q_0)^{\frac{1}{3}} - (Q_t)^{\frac{1}{3}} = K_{HC} t$

[1] K_0, K_1, K_H, K_{HC}: rate constant for respective model; Q_t: amount of APX released at time (t); Q_0: initial amount of APX in formulation

Ex Vivo Apixaban Permeation Study of Ultrafine APX O/W Nanoemulsions
Permeation Skin Preparation of Wister Rat Abdomen
Abdominal skin of Wister Albino rat was used as diffusional membrane for ultrafine APX o/w nanoemulsion permeation, as it demonstrates comparable stratum corneum thickness and water permeability to human skin(34). Albino male rats of 2-3 months age and each weighing 200 ± 10 g were scarified by ether inhalation and then the abdominal hair was removed with care to avoid accidental skin damage using electrical clipper. A fresh rat abdominal skin, with rectangular shape of few centimeters in each dimension, was excised from the animal using sharp surgical blade(35). To remove the adipose tissue from the skin, diethyl ether solvent was wiped on dermal side using cotton wool to solubilize the adipose tissue and easily get rid the muscles and blood vessels from the skin, followed by scrapping using a scalpel carefully and washed with normal saline solution for sterility and hygienic. The skin was then stabilized to ensure complete removal of UV-visible absorbing materials by placing it in water bath containing PBS pH 7.4 at 37 °C for 2-3 h with replacing the medium every 30 min until no UV-visible absorbance was observed (36, 37). The prepared sections of skin were then wrapped into aluminum foil and stored at − 20 °C until use, where prior to the experiment, the samples of the skin were thawed at room temperature for at least 30 min and wiped carefully with PBS of pH 7.4 using cotton wool balls.

Franz Cell - *Ex Vivo* Permeation Study through Rat Abdominal Skin
According to the approval of animal ethical committee of Baghdad University / College of Pharmacy, *ex vivo* skin permeation study was performed utilizing the abdominal skin of adult Wister Albino male rats.

Franz cells – water bath system device used for the *ex vivo* skin permeation study consisting of six Franz diffusion cells and each diffusion cell consist lower receptor and upper donor compartments. Skin cuts mounted onto receptor compartment of Franz cells with effective diffusional area of 1.77 cm^2 of skin and 15 mL capacity of receiver chamber. The dermal side of the skin was exposed to the receptor medium consisting of PBS pH 7.4 containing 1 % Sodium Lauryl Sulphate (SLS), while the epidermal side was faced to donor part of Franz cell under ambient conditions and fastened with an O-ring(38). Prior to experiment, the skin was equilibrated with the experimental temperature of 32 ± 0.5 °C to mimic skin surface temperature by filling both compartments with receptor medium with magnetic stirring at 600 rpm for 30 min to get rid of any air bubbles(39). Then 2 g of APX loaded ultrafine nanoemulsions each containing 5 mg of APX, was weighed and applied to the surface of skin in the donor compartment. The receptor medium of PBS pH 7.4 / 1 % Sodium Lauryl Sulphate (SLS) was agitated using magnetic stirrer within the device at 600 rpm and the temperature was kept at 32 ± 0.5 °C throughout the experiment to mimic *in vivo* conditions(29, 40). Samples of 0.1 mL were then withdrawn periodically from the sampling port in the side of receptor cell part at predetermined time intervals, which replaced with equal volume of fresh PBS pH 7.4 media to maintain sink condition, until complete permeation of APX achieved using filtered syringe with pore size of 0.45 μm. Samples analyzed using UV-Visible spectrophotometer at 280 nm and the cumulative amount of drug permeated was calculated and plotted against time(41). The experiment was made in triplicate.

Ex Vivo Apixaban Permeation Data Analysis
Permeation profile obtained by plotting the cumulative amount (Q, μg/cm^2) of APX permeated across the rat skin

on Y-axis as a function of time (t, min) on X-axis. This profile used to calculate permeation parameters, including permeation rate or transdermal APX flux at the steady state (Jss, mg/cm²/h), which obtained from the slope of straight linear portion of the regression line, Lag time (T_{lag}), which was determined from the intercept of regression line. Permeability coefficient (KP, cm/h), calculated from the ratio of APX flux (Jss) divided by the initial concentration (C_0) of APX placed in donor compartment, while enhancement ratio (ER), calculated by dividing flux (Jss) of APX from tested formulation by control (pure APX) flux(42, 43).

RESULTS AND DISCUSSION

Preparation of Apixaban Loaded O/W Nanoemulsion Formulations
Solubility study
Among tested oils, triacetin exhibit higher solubilizing capacity (31.870 mg/mL), followed by oleic acid and sesame oils with saturated solubility of 21.672 and 15.301 mg/mL respectively, while lowest solubility obtained in wheat oil (1.533 mg/mL). Therefore, triacetin oil was selected as oil phase in preparation and further investigation of nanoemulsion formulations. Triacetin has beneficial properties for construction in transdermal preparations, since it act as skin penetration enhancer through stratum corneum, for example in Oxytrol® patch indicated for overactive bladder which is available commercially and contain triacetin as penetration enhancer(44). Triacetin also display good miscibility with other components during nanoemulsion formulation that enable facile preparation of homogenous one phase system and good loading properties. In surfactants, highest solubility of APX was achieved in triton-X-100 (9.256 mg/mL) and cremophor EL (8.725 mg/mL), while span 20 displayed lowest saturated solubility of APX (3.264 mg/mL). Therefore, triton-X-100 was selected as a surfactant for nanoemulsion formulation, it is non-ionic hydrophilic surfactant with hydrophilic lipophilic balance (HLB) value of 13.4, which is appropriate for facile o/w nanoemulsion formation with low toxicity(45). Triton-X-100 also has membrane permeabilization properties for living cells bio-membranes which rely on lipid composition, triton-X-100 concentration and the ease to intercalate between lipids(46). Carbitol demonstrate highest solubilizing capability to APX (159.959 mg/mL), while

glycerol demonstrates lowest APX solubility (6.772 mg/mL), therefore carbitol selected to mix with the surfactant triton-X-100 to prepare Smix. It is frequently applied in the formulation of transdermal nanoemulsion drug delivery system, as carbitol spreads easily over the skin without streaking, high skin biocompatibility and safety, its ability to solubilize large number of drugs and most importantly, it has skin penetration enhancing properties(47).

Pseudoternary Phase Diagram
Seven pseudoternary phase diagrams (Figure 1.) were constructed by aqueous titration method using triacetin as oil phase, triton-X-100 and carbitol as surfactant and cosurfactant respectively at different ratios and deionized water as aqueous phase. The results showed nearly same nanoemulsion regions (shaded area) for all Smix ratios with biggest nanoemulsion region at surfactant / cosurfactant ratio 1:1, this may be caused by the greater penetration of the oil phase into the hydrophobic tail region of the surfactant triggered by the presence of hydrophobic cosurfactant carbitol(48). Additionally, the increased entropy of nanosystem would expect to force oil molecules with enhanced penetration to interfacial surfactant layer due to their smaller size molecule compared to surfactant molecule used(49). Concerning the effect of surfactant/cosurfactant ratio, there was slight increase in nanoemulsion area with the increase in surfactant concentration with respect to cosurfactant, and therefore diagrams with Smix ratios 2:1, 3:1 and 4:1 have wider nanoemulsion area than 1:2, 1:3 and 1:4 Smix diagrams, this increase in nanoemulsion area with Smix ratio could be attributed to the increase in HLB value of nanoemulsion system caused by increment of hydrophilic surfactant triton-X-100 (HLB 13.4) and hence, increased hydrophilicity of system with improved micelle formation, enhanced solubilizing capacity of nanoemulsion and eventually optimized aqueous miscibility(50).
Cosurfactant carbitol increase nanoemulsion solubility by their insertion into void spaces between the surfactant molecules and hence aid in the reduction of interfacial tension and increase fluidity(51), it also improves APX dispersibility in the system due to hydrophobic nature of carbitol with HLB value of 4, therefore triton-x-100 surfactant and carbitol cosurfactant were able to form a stable oil in water (o/w) nanoemulsion system with HLB value above10 indicating hydrophilic surfactant(52).

| (a) | (b) | (c) |
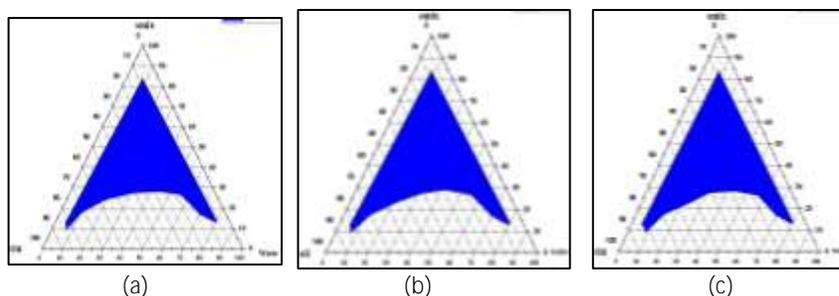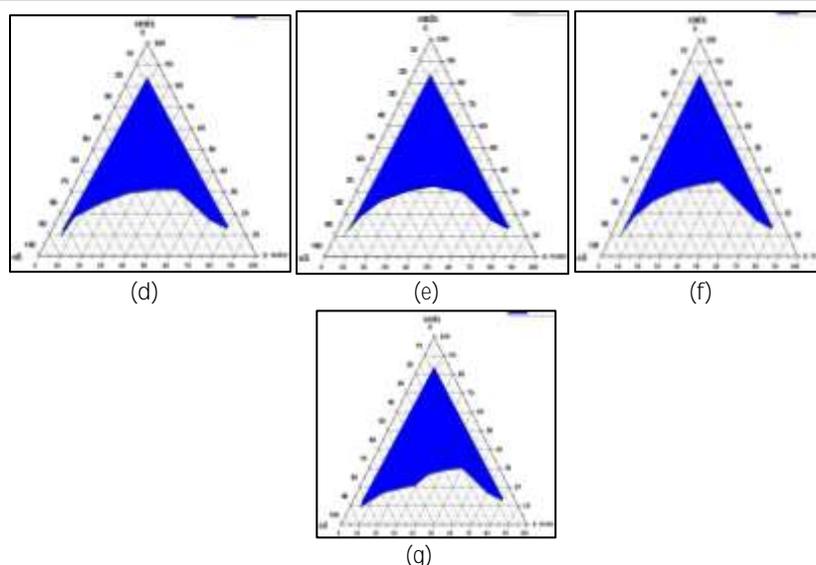
Figure 1. Pseudo-ternary phase diagrams of triacetin, triton-X-100 and carbitol at different Smix ratios of (a) 1:2, (b) 1:3, (c) 1:4, (d) 2:1, (e) 3:1, (f) 4:1 and (g) 1:1, Shaded area represent (o/w) clear nano emulsion regions.

Formulation of Apixaban Loaded O/W Nano emulsions
Pseudo ternary phase diagram can be used for demonstration of lower and greater weight percent of oil and Smix for the preparation of o/w nano emulsion formulations(53). Twenty-one o/w nano emulsion formulations (Table 1.) prepared using aqueous titration method, constructed by selecting three formulations from each phase diagram of different Smix ratio. Two criteria were dependent for the preparation of the selected formulations; first, the amount of oil was selected at 5 % weight interval selecting 5, 10 and 15 % of triacetin oil ratio concentration so that largest number of formulations could be selected to cover the nano emulsion area for each phase diagram(54). The second criterion based on selecting formulations with minimum concentrations of Smix, as mentioned previously, to avoid possible skin irritation by surfactant application(55). Hence, nano emulsion formulations taken from each phase diagram for each selected percent of triacetin (5%, 10% and 15 %) were only those having the minimum concentrations of Smix, which were detected as 30 % and 40 % for Smix ratios 1:1, 1:2, 1:3, 1:4 and 2:1; while for Smix ratios 3:1 and 4:1, minimum concentrations were detected at 35 % and 45 % for emulsification. Additionally, the selected percentages of triacetin oil (5, 10 and 15%) were able to completely solubilize single dose of APX (5 mg) depending on the results of solubility study. There was no obvious change in visual appearance during formulation, i.e. phase separation, turbidity or color change, as well as no drug precipitation of APX was observed during deionized water addition.

Thermodynamic Stability Study of the Prepared APX O/W Nano emulsion Formulations
Thermodynamic stability tests were applied for all twenty-one formulations and results demonstrate stability of all formulations and therefore flocculation, aggregation, phase separation, creaming, cracking or coalescence not take place when prepared at particular proportions of surfactant, co-surfactant, oil and water. The inherited high stability of the prepared nano emulsions could be attributed to the stearic stabilization of nonionic surfactant triton-X-100(56). This thermodynamic stability confers long shelf life to nano emulsions as compared to ordinary emulsions(57).

Characterization Techniques of the Prepared APX O/W Nano emulsion Formulations
Droplet Size and Poly dispersity Index (PDI)
Average droplet size was measured for the entire prepared o/w nano emulsions as all pass thermodynamic stability tests successfully. Results presented in Table 3. and demonstrate o/w nano emulsions formation with droplet sizes ranged from 11.47 nm to 691.32 nm. The wide variation in droplet size caused by using different ratios of o/w nano emulsion components of Smix, oil and water, as well as by changing Smix ratios of surfactant and co-surfactant (58), which affect the surface curvature of the film by changing its flexibility and consequently influences droplet size.
Among tested formulations, ten APX o/w nano emulsions demonstrate ultrafine droplet size of less than 50 nm(10, 59) including all three prepared formulations with Smix ratio of 3:1 (F-7, F-8 and F-9) with smallest size of 11.47 nm by F-7. Although higher triton-x-100 surfactant concentration, only one formulation with Smix ratio of 4:1 (F-10) display ultrafine droplets with average size of 15.42 nm, the same was observed at Smix ratios of 1:1, 1:2, 1:3 and 1:4 each demonstrating one formulation within ultrafine nano-sized droplets including F-1, F-13, F-16 and F-21 with average sizes of 26.41, 13.12, 27.53 and 38.42 nm respectively, while Smix ratio 2:1 showed two ultrafine sized formulations, F-5 and F-6 with average droplet size of 27.67 nm and 26.08 nm respectively. According to the results, a decrease in droplet size was demonstrated with the increase in surfactant concentration, and consequently in Smix ratio, as smaller sizes obtained at Smix ratios of 4:1 and 3:1, the reason for

the surfactant impact on the particle size could be attributed to the increased HLB value and hence, hydrophilicity of surfactant mixture which facilitate reduction in the curvature of the triacetin oil interface which present with fairly high solubility and therefore leading to droplet size reduction(60). Additionally, stabilization effect of the triacetin oil droplets by localization of triton-x-100 surfactant molecules at the oil/water interface resulting in higher stability and smaller droplet size(61).

PDI was measured for all formulations and values ranged between (0.206 – 0.431) as displayed in Table 3. The majority of the prepared o/w nano emulsion formulations

presented with PDI of 0.3 or below, which considered to be acceptable, indicating the formation of homogenous monodispersed nano emulsions with good stability and very narrow poly dispersity with good uniformity in droplet size distribution upon dilution. Other formulations presented with higher PDI of larger than 0.3 values, indicating the formation of poly disperse emulsion systems with reduced stability (62, 63). It is obvious from the results that, larger droplet size distribution demonstrates higher PDI and therefore reduced stability of droplets within their o/w nano emulsion formulation and lesser uniformity of droplets distribution or poly disperse system.

Table 3. Results of mean particle size distribution (PSD); poly dispersity index (PDI); pH values; electrical conductivity; transmittance percent; percent of APX content; for the prepared APX o/w nano emulsion formulations F-1 to F-21, (mean ± SD, n = 3).

F-code	PSD	PDI	pH	Electrical conductivity (µs/cm)	Transmittance %	% APX content
F-1	26.41	0.296	5.68 ± 0.07	174.52 ± 0.93	98.38 ± 0.02	99.72 ± 0.15 %
F-2	343.31	0.263	5.53 ± 0.01	162.65 ± 1.24	99.62 ± 0.02	97.06 ± 0.22 %
F-3	127.49	0.412	5.47 ± 0.04	144.38 ± 0.88	99.98 ± 0.03	97.55 ± 0.1 %
F-4	482.62	0.312	5.64 ± 0.03	173.06 ± 0.67	98.15 ± 0.04	96.91 ± 0.12 %
F-5	27.67	0.328	5.52 ± 0.07	165.23 ± 0.38	99.87 ± 0.03	99.03 ± 0.11 %
F-6	26.08	0.259	5.44 ± 0.04	141.86 ± 0.46	97.88 ± 0.06	99.78 ± 0.23 %
F-7	11.47	0.206	5.73 ± 0.02	161.56 ± 0.58	98.89 ± 0.04	99.95 ± 0.25 %
F-8	15.89	0.251	5.62 ± 0.07	149.66 ± 1.03	97.80 ± 0.03	99.04 ± 0.19 %
F-9	46.06	0.352	5.48 ± 0.03	132.55 ± 0.76	98.89 ± 0.01	99.71 ± 0.28 %
F-10	15.42	0.268	5.76 ± 0.04	159.83 ± 0.96	97.04 ± 0.01	98.99 ± 0.22 %
F-11	92.46	0.423	5.65 ± 0.06	136.22 ± 1.17	97.92 ± 0.01	97.38 ± 0.17 %
F-12	355.58	0.405	5.59 ± 0.02	128.45 ± 0.73	99.46 ± 0.04	96.92 ± 0.16 %
F-13	13.12	0.276	5.57 ± 0.02	182.26 ± 1.42	97.07 ± 0.02	99.78 ± 0.12 %
F-14	184.77	0.421	5.51 ± 0.04	169.06 ± 1.01	99.99 ± 0.01	98.38 ± 0.27 %
F-15	316.59	0.394	5.43 ± 0.05	155.71 ± 0.07	97.28 ± 0.05	97.27 ± 0.22 %
F-16	27.53	0.317	5.46 ± 0.07	175.48 ± 0.97	99.62 ± 0.02	99.48 ± 0.26 %
F-17	142.90	0.431	5.34 ± 0.01	162.88 ± 0.69	98.18 ± 0.06	97.08 ± 0.13 %
F-18	260.72	0.356	5.22 ± 0.03	144.83 ± 0.83	97.34 ± 0.01	97.16 ± 0.2 %
F-19	691.32	0.399	5.46 ± 0.05	183.45 ± 0.48	98.86 ± 0.02	96.84 ± 0.24 %
F-20	150.62	0.431	5.33 ± 0.06	168.84 ± 1.04	98.78 ± 0.06	97.38 ± 0.28 %
F-21	38.42	0.334	5.21 ± 0.03	143.98 ± 0.98	99.08 ± 0.04	99.82 ± 0.16 %

PH Measurement

The pH measurements summarized in Table 3. and reveal pH range for APX o/w nano emulsions of (5.21 – 5.76) for F-21 and F-10 respectively, these values were suitable for topical application due to comparable values with skin pH which ranges from 4.5 to 6.5 and therefore evade skin irritation and/or sensitivity(64). pH values obtained slightly reduced with the increase in triacetin oil concentration within APX nano emulsions, this could be attributed to the increased acetic acid release caused by partial triacetin hydrolysis into acetic acid and glycerol in the aqueous phase of nano emulsion (65). Apixaban is a neutral compound that does not ionize at physiologic pH range (1.2 – 6.8), therefore it does not ionize at pH range (4.67 – 5.76) of the prepared o/w nano emulsions (66).

Transmittance Percent and Electrical Conductivity Measurement

All prepared formulations tested for their %T (Table 3.) and results ranged from 97.04 % – 99.99 % for F-10 and F-14 respectively, indicating transport light easily, optically clear, transparent and nanosized droplets(51). Conductometer was employed for all prepared APX o/w nano emulsions and readings ranged from 128.45 – 183.45 µs/cm for F-12 and F-16 respectively as presented in Table 3. which revealed the formulation of o/w nano emulsions with high degree of electrical conductivity as water represents the external phase and can conduct electrical current(67).

Apixaban Content Measurement

APX content within o/w nano emulsions was measured for all formulations and range from 96.84 % to 99.95 % for F-19

and F-7 respectively, and hence, set within the official range (85 % - 115 %) accepted according to the united states pharmacopeia (USP) as presented in Table 3. APX was therefore loaded successfully within all the prepared o/w nano emulsions without any precipitation or degradation of the drug.

In Vitro Apixaban Release Study
Ten formulations of ultrafine apixaban o/w nano emulsions (droplet size less than 50 nm) selected for APX *in vitro* release estimation as displayed in Figures 2 and 3, and demonstrate variable time durations for complete APX release, in which F-1, F-5, F-6 and F-16 ultrafine formulations release APX completely after 45 min, while F-7, F-10 and F-13 formulations take 35 min for complete APX release, and eventually F-8, F-9 and F-21 demonstrate nearly 100% release after 40, 55 and 50 min. Pure APX suspended in phosphate buffer saline (PBS) of pH 7.4 was also included for *in vitro* study as a control and demonstrate significantly ($p \leq 0.05$) slower release profile than other tested samples of APX after formulation as ultrafine o/w nano emulsion, in which only 16.12 % and 25.88 % of APX released after 35 and 55 min respectively. APX release from each ultrafine o/w nano emulsion was highly dependent on droplet size, as faster APX release of 35 min was reported by o/w nano emulsion F-7, F-10 and F-13 formulations with droplet sizes below 20 nm, while longer release duration of 55 min was displayed by o/w nano emulsion F- 9 formulation with droplet size 46.06 nm. The cumulative amount of APX released from F-7, F-10 and F-13 after 35 min, and F-9 after 55 min, was significantly ($p \leq 0.05$) higher than plain APX suspended in PBS with about 6.203 and 3.863 folds respectively. This influence of droplet size on APX release could be attributed to the pronounced increase

in the effective interfacial area of APX particles exposed to PBS dissolution media and hence, higher dissolution rate and faster drug release(68, 69). Although no significant (p>0.05) difference observed in the *in vitro* release rate profile between ultrafine APX loaded formulations with nearly same droplet size, they differ in the proportion of each component of Smix, triacetin oil and water, as well as in the surfactant / co-surfactant ratio for Smix used in each ultrafine formulation. Regarding the effect of Smix ratio within each ultrafine o/w nano emulsion, it was observed that formulations with higher Smix ratios (higher triton-x-100 surfactant concentration) demonstrate faster APX release profile, the reason for this effect caused by solubilizing efficacy and hydrophilicity enhancement of APX induced by using triton-x-100 surfactant in high concentration and hence, present APX in dissolved form that is the only form can cross the membrane(70, 71). Additionally, smaller droplet size of ultrafine nano emulsions produced by the increase in Smix ratio was accompanied with the increase in APX release rate(72).

Kinetics of Apixaban *In Vitro* Release
The *in vitro* release kinetics reveal pure APX in PBS pH 7.4 and all studied ten formulations of ultrafine APX o/w nano emulsions with highest correlation coefficient (R^2) fit with zero order kinetic model, indicating concentration independent APX release kinetic. This fitting of APX release with zero order kinetics could be attributed to the APX flow retardation effect produced by dialysis membrane, that regulate and control APX release rate rather than slowing it(73). While for pure APX, as drug demonstrate low solubility in PBS pH 7.4, then APX solubility control its release as reported by Noyes Whitney equation for solid dissolution(74).
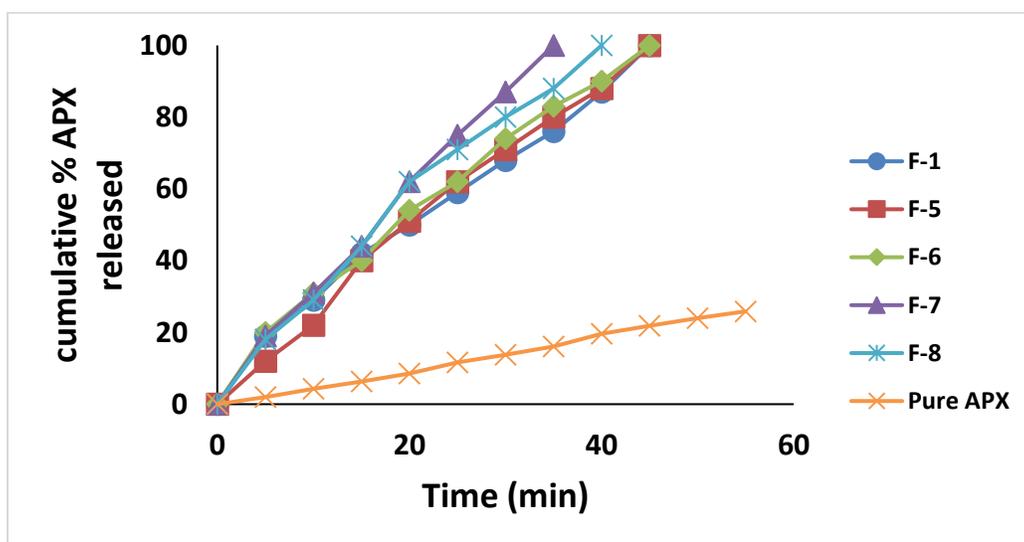
Figure 2. Comparative *in vitro* release study of ultrafine apixaban o/w nano emulsion formulation F-1, F-5, F-6, F-7 and F-8 with pure APX in phosphate buffer saline pH 7.4.
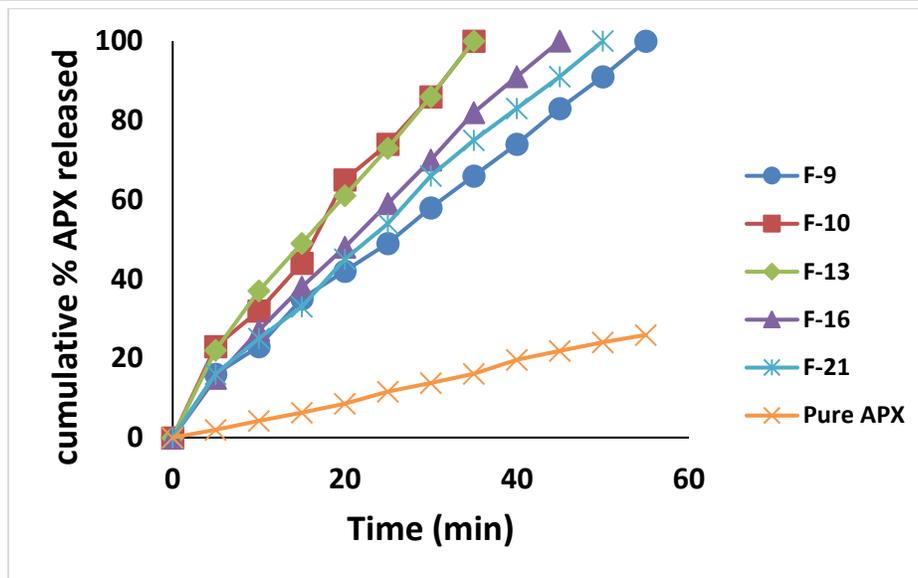
Figure 3. Comparative *in vitro* release study of ultrafine apixaban o/w nano emulsion formulation F-9, F-10, F-13, F-16 and F-21 with pure APX (control) in phosphate buffer saline pH 7.4.

Ex Vivo Apixaban Permeation Study

Rat abdominal skin used as permeation barrier for *ex vivo* evaluation of ultrafine APX o/w nano emulsions using Franz cell diffusional system. Among ten ultrafine APX nano emulsions, five formulations (F-1, F-6, F-7, F-13 and F-21) were selected for *ex vivo* studies evaluation. The results, as shown in Figure 4., indicate significantly ($p \leq 0.05$) enhanced permeability of ultrafine APX nano emulsions in comparison with that of pure APX, in which complete permeation was observed after 7, 6, 5, 6 and 8 hours for F-1, F-6, F-7, F-13 and F-21 respectively, while pure APX demonstrate permeation percent of 13.32, 17.94, 24.93 and 28.07 after 5, 6, 7 and 8 hours respectively. This significant increase in permeation of APX form ultrafine nano emulsions could be attributed to several factors, first, the ultrafine droplets (< 50 nm) of nano emulsion significantly increase the rate of permeation, as the nanosized droplets enable drug transfer through the skin barrier and therefore easily reach stratum corneum (75, 76). Secondly, APX present or hidden within triacetin droplets in solubilized form, which was aided by the presence of co-surfactant carbitol in Smix and hence, can permeate through lipophilic skin layers more efficiently(77). Third, as this study was aimed, the formulation itself of ultrafine nano emulsion act as permeation enhancer due to its components of triacetin oil and triton-x-100 surfactant which already demonstrate permeation enhancing properties according to previous literatures(78, 79).
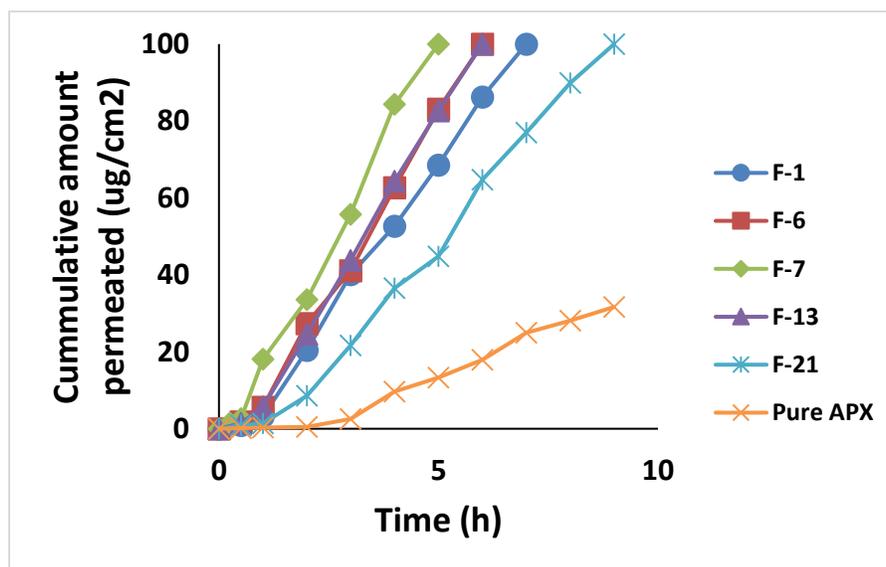
Figure 4. Comparative *En vitro* permeation study of ultrafine apixaban o/w nano emulsion formulation F-1, F-6, F-7, F-13 and F-21 with pure APX (control) through rat abdominal skin.

Ex Vivo Apixaban Permeation Data Analysis

Permeation parameters J_{ss}, K_P and ER were calculated and their values increased significantly ($p \leq 0.05$) in comparison with pure APX control as shown in Table 4. The lag time for starting permeation (T_{lag}) also assure faster permeation of APX nano emulsions than pure drug, which were 0.5 – 1.33 h, while it was found 2.5 h for pure APX. These outcomes encourage APX formulation for transdermal delivery of drug with more efficient and safer use than marketed tablet form(80).

Table 4. *Ex vivo* permeability parameters of ultrafine APX o/w nano emulsion formulations and pure APX (control)

Parameter / F-code	Flux ($\mu g/cm^2.h$)	KP (cm/h) × 10^{-3}	T_{lag} (h)	ER
F – 1	16.091	6.436	1	3.413
F – 6	18.874	7.550	0.83	4.003
F – 7	21.743	8.697	0.5	4.612
F – 13	19.045	7.618	0.75	4.040
F – 21	13.444	5.378	1.33	2.852
Pure APX	4.7561	1.886	2.5	1

CONCLUSION

As a conclusion from this work, apixaban was formulated successfully into o/w nano emulsion with ultrafine sized droplets (< 50 nm) with preferential properties for transdermal application. Apixaban oral administration drawbacks of limited bioavailability and poor solubility were evaded by the optimized ultrafine APX o/w nano emulsion as a novel delivery system for transdermal application of the drug. Additionally, characterization techniques, *in vitro* release studies and *ex vivo* permeation testing of the prepared APX formulations approve their relevance for effective, safe and practical transdermal application over the skin without using chemical or physical permeation enhancing techniques.

CONFLICT OF INTEREST

Authors declare no conflict of interest was reported.

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REFERENCES

1. JAIN HK, NIKAM VK. Formulation development and stability indicating HPLC assay of tablets of apixaban. Int J Pharm Pharm Sci. 2017;9(10):24-32.
2. Byon W, Garonzik S, Boyd RA, Frost CE. Apixaban: A Clinical Pharmacokinetic and Pharmacodynamic Review. Clinical pharmacokinetics. 2019;58(10):1265-79.
3. Ofek F, Barchel D, Mahajna A, Filipovich-Rimon T, Garach-Jehoshua O, Berlin M, et al. International normalized ratio as a screening test for assessment of anticoagulant activity for patients treated with rivaroxaban or apixaban: A pilot study. Frontiers in pharmacology. 2019;10(2019):1-14.
4. Altawalbeh SM, Alshogran OY, Smith KJ. Cost-Utility Analysis of Apixaban versus Warfarin in Atrial Fibrillation Patients with Chronic Kidney Disease. Value in Health. 2018;21(12):1365-72.
5. Maurya VB, Kumar V, Kumar R. An Overview on Transdermal Drug Delivery System. Journal of Drug Delivery and Therapeutics. 2019;9(4-A):773-8.
6. Majeed A, Bashir R, Farooq S, Maqbool M. Preparation, Characterization and Applications of Nanoemulsions: An Insight. Journal of Drug Delivery and Therapeutics. 2019;9(2):520-7.
7. Shaker DS, Ishak RA, Ghoneim A, Elhuoni MA. Nanoemulsion: A Review on Mechanisms for the Transdermal Delivery of Hydrophobic and Hydrophilic Drugs. Scientia Pharmaceutica. 2019;87(3):1-34.
8. Pavoni L, Perinelli DR, Bonacucina G, Cespi M, Palmieri GF. An Overview of Micro-and Nanoemulsions as Vehicles for Essential Oils: Formulation, Preparation and Stability. Nanomaterials. 2020;10(1):1-24.
9. Sharadha M, Gowda D, Gupta V, Akhila A. An overview on topical drug delivery system–Updated review. International Journal of Research in Pharmaceutical Sciences. 2020;11(1):368-85.
10. Badran MM, Taha EI, Tayel MM, Al-Suwayeh SA. Ultra-fine self nanoemulsifying drug delivery system for transdermal delivery of meloxicam: dependency on the type of surfactants. Journal of Molecular Liquids. 2014;190(2014):16-22.
11. Kanke PK, Pathan IB, Jadhav A, Usman MRM. Formulation and evaluation of febuxostat nanoemulsion for transdermal drug delivery. Journal of Pharmaceutical and BioSciences 2019;7(1):1-7.
12. Rai VK, Mishra N, Yadav KS, Yadav NP. Nanoemulsion as pharmaceutical carrier for dermal and transdermal drug delivery: Formulation development, stability issues, basic considerations and

applications. Journal of controlled release. 2018;270(2018):203-25.

13. Ullio-Gamboa G, Udobi KC, Dezard S, Perna MK, Miles KN, Costa N, et al. Dodecyl creatine ester-loaded nanoemulsion as a promising therapy for creatine transporter deficiency. Nanomedicine. 2019;14(12):1579-93.

14. Derawi D, Azman NAZ, Raman IA, Jantan I. Formulation Screening of Palm-based Nanoemulsion for an Oral Drug Vehicle of Phyllanthin. malaysian journal of chemistry (MJChem). 2019;21(2):43-9.

15. Zeng L, Liu Y, Pan J, Liu X. Formulation and evaluation of norcanthridin nanoemulsions against the Plutella xylostella (Lepidotera: Plutellidae). BMC biotechnology. 2019;19(1):1-11.

16. Moura MCP, Neto AAD, Peres AEC, Neitzke PRM, de Castro Dantas TN. Microemulsions and nanoemulsions applied to iron ore flotation. Journal of Materials Research and Technology. 2019;8(3):2978-86.

17. Puppala RK, A VL. Optimization and solubilization study of nanoemulsion budesonide and constructing pseudoternary phase diagram. Asian J Pharm Clin Res. 2019;12(1):551-3.

18. Kumar R, Soni GC, Prajapati S. Formulation development and evaluation of Telmisartan Nanoemulsion. International Journal of Research and Development in Pharmacy and Life Sciences. 2017;6(4):2711-9.

19. Leitner S, Solans C, García-Celma M, Calderó G. Low-energy nano-emulsification approach as a simple strategy to prepare positively charged ethylcellulose nanoparticles. Carbohydrate polymers. 2019;205:117-24.

20. Lala R, Patel P. Nanoemulsion for improved permeability of Centella asiatica extract: formulation, ex-vivo and in-vivo evaluation. Int J Pharm Sci & Res. 2019;10(4):1711-18.

21. Elbardisy B, Galal S, Abdelmonsif DA, Boraie N. Intranasal Tadalafil nanoemulsions: formulation, characterization and pharmacodynamic evaluation. Pharmaceutical Development and Technology. 2019;2019(1):1-12.

22. Tatiya A, Bhavsar S, Mahajan H, Surana S. Experimental Design and Characterization of Nanoemulsion Based Topical Herbal Gel Developed for Site-Specific Activity of Glycyrrhiza Glabra Extract: In Vitro And Ex Vivo Studies. Biosc Biotech Res Comm. 2019;12(2):1-12.

23. Cao Y, Gao H, Xia H, Zhu X, Li B, Zhou X, et al. Development and Evaluation of a Water-in-oil Microemulsion Formulation for the Transdermal Drug Delivery of Teriflunomide (A771726). Chemical and Pharmaceutical Bulletin. 2019;67(8):786-94.

24. Katsouli M, Tzia C. Effect of lipid type, dispersed phase volume fraction and emulsifier on the physicochemical properties of nanoemulsions fortified with conjugated linoleic acid (CLA): Process optimization and stability assessment during storage conditions. Journal of Molecular Liquids. 2019;292(2019):1-12.

25. Calderó G, Leitner S, García-Celma MJ, Soláns C. Modulating size and surface charge of ethylcellulose nanoparticles through the use of cationic nano-emulsion templates. Carbohydrate polymers. 2019;225(2019):1-10.

26. Ghareeb MM. Design and in vitro characterization of a topical nanoemulsion-enriched hydrogel of econazole nitrate. Journal of Applied Pharmaceutical Science. 2019;9(1):51-7.

27. Londhe VY, Bhasin B. Transdermal lipid vesicular delivery of iloperidone: formulation, in vitro and in vivo evaluation. Colloids and Surfaces B: Biointerfaces. 2019;183(2019):1-12.

28. Gokhale JP, Mahajan HS, Surana SS. Quercetin loaded nanoemulsion-based gel for rheumatoid arthritis: In vivo and in vitro studies. Biomedicine & Pharmacotherapy. 2019;112(2019):1-11.

29. Kaur R, Ajitha M. Transdermal delivery of fluvastatin loaded nanoemulsion gel: Preparation, characterization and in vivo anti-osteoporosis activity. European Journal of Pharmaceutical Sciences. 2019;136(2019):1-10.

30. Mishra VV, Bhanja S, Panigrahi B. Development and Evaluation of Nanoemulsion gel for transdermal delivery of Valdecoxib. Research Journal of Pharmacy and Technology. 2019;12(2):600-10.

31. Huanbutta K, Sangnim T. Design and development of zero-order drug release gastroretentive floating tablets fabricated by 3d printing technology. Journal of Drug Delivery Science and Technology. 2019;52(2019):831-7.

32. Lisik A, Musiał W. Conductomeric Evaluation of the Release Kinetics of Active Substances from Pharmaceutical Preparations Containing Iron Ions. Materials. 2019;12(5):1-9.

33. Kanwal T, Kawish M, Maharjan R, Ghaffar I, Ali HS, Imran M, et al. Design and development of permeation enhancer containing self-nanoemulsifying drug delivery system (SNEDDS) for ceftriaxone sodium improved oral pharmacokinetics. Journal of Molecular Liquids. 2019;289(2019):1-11.

34. Todo H. Transdermal permeation of drugs in various animal species. Pharmaceutics. 2017;9(33):1-11.

35. AHMED AB, DAS G. Effect of Menthol on the Transdermal Permeation of Aceclofenac from Microemulsion Formulation. Int J App Pharm. 2019;11(2):117-22.

36. Kaur G. TPGS Loaded Topical Nanoemulgel of Mefenamic Acid for the Treatment of Rheumatoid Arthritis. International Journal of Pharmacy & Pharmaceutical Research. 2019;15(1):64-107.

37. Omar MM, Hasan OA, El Sisi AM. Preparation and optimization of lidocaine transferosomal gel containing permeation enhancers: a promising approach for enhancement of skin permeation. International journal of nanomedicine. 2019;14:1551-62.

38. Viswanad V, Anju P, Kumar GS, Nair SG. Formulation development and in-vitro characterisation of ethosomes for the enhanced transdermal delivery of clotrimazole. International Journal of Research in Pharmaceutical Sciences. 2019;10(2):874-82.

39. Alavi T, Rezvanian M, Ahmad N, Mohamad N, Ng S-F. Pluronic-F127 composite film loaded with erythromycin for wound application: formulation, physicomechanical and in vitro evaluations. Drug delivery and translational research. 2019;9(2):508-19.

40. Kaur R, Ajitha M. Formulation of transdermal nanoemulsion gel drug delivery system of lovastatin and its in vivo characterization in glucocorticoid induced osteoporosis rat model. Journal of Drug Delivery Science and Technology. 2019;52(2019):968-78.

41. da Silva JD, Gomes MV, Cabral LM, de Sousa VP. Evaluation of the in vitro release and permeation of Cordia verbenacea DC essential oil from topical dosage forms. Journal of Drug Delivery Science and Technology. 2019;53(2019):1-32.

42. Yang Q, Liu S, Gu Y, Tang X, Wang T, Wu J, et al. Development of sulconazole-loaded nanoemulsions for enhancement of transdermal permeation and antifungal activity. International Journal of Nanomedicine. 2019;14:3955–66.

43. Abdel-Messih HA, Ishak RA, Geneidi AS, Mansour S. Tailoring novel soft nano-vesicles 'Flexosomes' for enhanced transdermal drug delivery: Optimization, characterization and comprehensive ex vivo–in vivo evaluation. International journal of pharmaceutics. 2019;560(2019):101-15.

44. Puri A, Bhattaccharjee SA, Zhang W, Clark M, Singh O, Doncel GF, et al. Development of a Transdermal Delivery System for Tenofovir Alafenamide, a Prodrug of Tenofovir with Potent Antiviral Activity Against HIV and HBV. Pharmaceutics. 2019;11(4):1-29.

45. Silva SIJBe, Ferreira GMD, de Lemos LR, Rodrigues GD, Mageste AB. Liquid–Liquid Equilibrium of Aqueous Two-Phase Systems Composed of Nonionic Surfactant (Triton X-100, Triton X-165, or Triton X-305) and Choline Chloride. Journal of Chemical & Engineering Data. 2019;64(4):1632-9.

46. Mattei B, Lira RB, Perez KR, Riske KA. Membrane permeabilization induced by Triton X-100: The role of membrane phase state and edge tension. Chemistry and physics of lipids. 2017;202(2017):28-37.

47. Hernandez C, Jain P, Sharma H, Lam S, Sonti S. Investigating the effect of transcutol on the physical properties of an O/W cream. Journal of Dispersion Science and Technology. 2019(2019):1-7.

48. da Silva JD, Gomes MV, Cabral LM, de Sousa VP. Evaluation of the in vitro release and permeation of Cordia verbenacea DC essential oil from topical dosage forms. Journal of Drug Delivery Science and Technology. 2019;53(2019):1-12.

49. Bhattacharjee K. Importance of Surface Energy in Nanoemulsion. Nanoemulsions-Properties, Fabrications and Applications: IntechOpen; 2019.

50. Karami Z, Khoshkam M, Hamidi M. Optimization of olive oil-based nanoemulsion preparation for intravenous drug delivery. Drug research. 2019;69(5):256-64.

51. Kumar Sahoo S, Sankar Dash G, Biswal S, Kumar Biswal P, Chandra Senapati P. Fabrication and evaluation of self-nanoemulsifying oil formulations (SNEOFs) of Efavirenz. Journal of Dispersion Science and Technology. 2019;40(3):464-75.

52. Karami Z, Zanjani MRS, Hamidi M. Nanoemulsions in CNS drug delivery: recent developments, impacts and challenges. Drug discovery today. 2019;24(5):1104-15.

53. Ahmad N, Ahmad R, Al-Qudaihi A, Alaseel SE, Fita IZ, Khalid MS, et al. Preparation of a novel curcumin nanoemulsion by ultrasonication and its comparative effects in wound healing and the treatment of inflammation. RSC Advances. 2019;9(35):20192-206.

54. 54. Elmataeeshy ME, Sokar MS, Bahey-El-Din M, Shaker DS. Enhanced transdermal permeability of Terbinafine through novel nanoemulgel formulation; Development, in vitro and in vivo characterization. Future Journal of Pharmaceutical Sciences. 2018;4(1):18-28.

55. Saani SM, Abdolalizadeh J, Heris SZ. Ultrasonic/sonochemical synthesis and evaluation of nanostructured oil in water emulsions for topical delivery of protein drugs. Ultrasonics sonochemistry. 2019;55(2019):86-95.

56. Das D, Sahu P, Chaurasia A, Mishra VK, Kashaw SK. Nanoemulsion: The Emerging Contrivance in the Field of Nanotechnology. Nanoscience & Nanotechnology-Asia. 2018;8(2):146-71.

57. Janani S, Kumar RS, Karthika C, Vasanthi C, Kapoor SR. Study on Formulation development and shelf life indicating stability of Artesunate loaded Self-nanoemulsifying Drug Delivery System. Research Journal of Pharmacy and Technology. 2019;12(7):3228-36.

58. Mobarak D, Salah S, Ghorab M. Improvement of dissolution of a class II poorly water-soluble drug, by developing a five-component self-nanoemulsifying drug delivery system. Journal of Drug Delivery Science and Technology. 2019;50(2019):99-106.

59. Taha EI, Ak-Suwayeh S, Tayel MM, Badran MM. Fast ultra-fine self-nanoemulsifying drug delivery system for improving in vitro gastric dissolution of poor water soluble drug. Acta Pol Pharm. 2015;72(1):171-78.

60. Poh Y, Ng S, Ho K. Formulation and characterisation of 1-ethyl-3-methylimidazolium acetate-in-oil microemulsions as the potential vehicle for drug delivery across the skin barrier. Journal of Molecular Liquids. 2019;273(2019):339-45.

61. Li L, Zhou CH, Xu ZP. Self-Nanoemulsifying Drug-Delivery System and Solidified Self-Nanoemulsifying Drug-Delivery System. Nanocarriers for Drug Delivery. 2019: Elsevier; 2019. p. 421-49.

62. Izham M, Nadiah M, Hussin Y, Aziz MNM, Yeap SK, Rahman HS, et al. Preparation and Characterization of Self Nano-Emulsifying Drug Delivery System Loaded

with Citraland Its Antiproliferative Effect on Colorectal Cells In Vitro. Nanomaterials. 2019;9(7):1-18.

63. Velho MC, de Oliveira DA, da Silva Gündel S, Favarin FR, Santos RCV, Ourique AF. Nanoemulsions containing mancozeb and eugenol: development, characterization, and antifungal activity against Glomerella cingulata. Applied Nanoscience. 2019;9(2):233-41.

64. Alishlah T, Mun'im A, Jufri M. Optimization of Urea-glycerin Based NADES-UAE for Oxyresveratrol Extraction from Morus alba Roots for Preparation of Skin Whitening Lotion. Journal of Young Pharmacists. 2019;11(2):155-60.

65. Jiang Y, Li X, Zhao H, Hou Z. Esterification of glycerol with acetic acid over SO3H-functionalized phenolic resin. Fuel. 2019;255(2019):1-8.

66. Brew CT, Blake JF, Mistry A, Liu F, Carreno D, Madsen D, et al. Use of QSPR Modeling to Characterize In Vitro Binding of Drugs to a Gut-Restricted Polymer. Pharmaceutical research. 2018;35(4):1-10.

67. Azhar SNAS, Ashari SE, Salim N. Development of a kojic monooleate-enriched oil-in-water nanoemulsion as a potential carrier for hyperpigmentation treatment. International journal of nanomedicine. 2018;13(2018):6465-79.

68. Elsegaie D, Teaima M, Tadrous MI, Louis D. Formulation and In-vitro Characterization of Self Nano-emulsifying Drug Delivery System (SNEDDS) for enhanced Solubility of Candesartan Cilexetil. Research Journal of Pharmacy and Technology. 2019;12(6):2628-36.

69. Kim DH, Kim JY, Kim RM, Maharjan P, Ji Y-G, Jang D-J, et al. Orlistat-loaded solid sNeDDs for the enhanced solubility, dissolution, and in vivo performance. International journal of nanomedicine. 2018;13(2018):7095-106.

70. Hajjar B, Zier K-I, Khalid N, Azarmi S, Löbenberg R. Evaluation of a microemulsion-based gel formulation for topical drug delivery of diclofenac sodium. Journal of Pharmaceutical Investigation. 2018;48(3):351-62.

71. Chaudhari KS, Akamanchi KG. Novel bicephalous heterolipid based self-microemulsifying drug delivery system for solubility and bioavailability enhancement of efavirenz. International journal of pharmaceutics. 2019;560(2019):205-18.

72. Chikte AA, Umekar MJ, Borkhade MN, Raut NS. Effect of trans-esterification of oil on nanoemulsion gel for topical drug delivery. International journal of pharmaceutical sciences and research. 2018;9(2):589-98.

73. Ali HH, Hussein AA. Oral nanoemulsions of candesartan cilexetil: Formulation, characterization and in vitro drug release studies. Aaps Open. 2017;3(1):1-16.

74. Yokoyama R, Kimura G, Schlepütz CM, Huwyler J, Puchkov M. Modeling of Disintegration and Dissolution Behavior of Mefenamic Acid Formulation Using Numeric Solution of Noyes-Whitney Equation with Cellular Automata on Microtomographic and Algorithmically Generated Surfaces. Pharmaceutics. 2018;10(4):1-17.

75. Sarheed O, Shouqair D, Ramesh K, Khaleel T, Amin M, Boateng J, et al. Formation of stable nanoemulsions by ultrasound-assisted two-step emulsification process for topical drug delivery: effect of oil phase composition and surfactant concentration and loratadine as ripening inhibitor. International Journal of Pharmaceutics. 2020;576:1-34.

76. Luesakul U, Puthong S, Sansanaphongpricha K, Muangsin N. Quaternized chitosan-coated nanoemulsions: A novel platform for improving the stability, anti-inflammatory, anti-cancer and transdermal properties of Plai extract. Carbohydrate Polymers. 2020;230(2020):1-11.

77. Zhang Y, Zhang K, Wang Z, Hu H, Jing Q, Li Y, et al. Transcutol® P/Cremophor® EL/Ethyl Oleate–Formulated Microemulsion Loaded into Hyaluronic Acid–Based Hydrogel for Improved Transdermal Delivery and Biosafety of Ibuprofen. AAPS PharmSciTech. 2020;21(1):1-10.

78. Altamimi M, Haq N, Alshehri S, Qamar W, Shakeel F. Enhanced Skin Permeation of Hydrocortisone Using Nanoemulsion as Potential Vehicle. ChemistrySelect. 2019;4(34):10084-91.

79. Anand K, Ray S, Rahman M, Shaharyar A, Bhowmik R, Bera R, et al. Nano-emulgel: Emerging as a Smarter Topical Lipidic Emulsion-based Nanocarrier for Skin Healthcare Applications. Recent patents on anti-infective drug discovery. 2019;14(1):16-35.

80. Yang C, Daoping Z, Xiaoping X, Jing L, Chenglong Z. Magnesium oil enriched transdermal nanogel of methotrexate for improved arthritic joint mobility, repair, and reduced inflammation. Journal of Microencapsulation. 2020;37(1):77-90.