DOI: https://doi.org/10.31351/vol29iss2pp214-222

Preparation, Characterization and *Ex vivo* Permeability Study of Transdermal Apixaban O/W Nanoemulsion Based Gel

Mustafa R. Abdulbaqi[*,1] and Nawal A.Rajab[**]

[*]Department of Pharmaceutics, College of Pharmacy, University of Al-Bayan, Baghdad, Iraq
[**]Department of Pharmaceutics, College of Pharmacy, University of Baghdad, Baghdad, Iraq

Abstract

This study designed to prepare ultrafine apixaban (APX) o/w nanoemulsion (NE) based gel with droplet size below 50 nm as a good method for transdermal APX delivery without using permeation enhancer, alternatively, the formulation components itself act as permeation enhancer. APX, a potent oral anticoagulant drug that selectively and directly inhibit coagulation factor Xa, was selected as a good candidate for transdermal delivery as it displays poor water solubility (0.028 mg/mL) and low bioavailability (50%). APX-NE gel was prepared using triacetin, triton-x-100 and carbitol as oil phase, surfactant and cosurfactant respectively, while Carbopol 940 used as a gelling agent. *Ex vivo* permeation of APX-NE gel through human stratum corneum reveal significant ($p \leq 0.05$) enhancement in permeation parameters (Jss, PC and Er, and shorter T_{lag}) in comparison with the prepared pure APX gel.

Key words: Apixaban, Nanoemulsion, *Ex vivo* permeation.

تحضير ، تشخيص و دراسة النفاذية لجل مستحلب دواء الابكسابان النانوي عن طريق الجلد

مصطفى رعد عبد الباقي[*،١] و نوال عياش رجب[**]

[*]فرع الصيدلانيات، كلية الصيدلة، جامعة البيان ، بغداد ،العراق.
[**]فرع الصيدلانيات، كلية الصيدلة، جامعة بغداد، العراق.

الخلاصة

صممت هذه الدراسة لتحضير جل لمستحلب الابكسابان النانوي المتناهي الصغر بحجم قطيرة أقل من ٥٠ نانومتر لتسليم الابكسابان عبر الجلد دون استخدام مُحسِّن النفاذية ، بدلاً من ذلك ، تعمل مكونات الصياغة تعمل على تعزيز النفاذية . تم اختيار الابكسابان ، وهو دواء قوي مضاد للتخثر عن طريق الفم يعمل بشكل انتقائي على تثبيط عامل التخثر Xa ، كمرشح جيد للتسليم عبر الجلد و ذلك لذوبانيته القليلة في الماء (٠,٠٢٨ مجم / مل) وتوافر حيوي منخفض (٥٠٪). تم تحضير جل مستحلب الابكسابان النانوي باستخدام ثلاثي الأسيتات ، تريتون اكس١٠٠ والكاربيتول كطور زيتي ، خافضة للتوتر السطحي وخافض توتر مساعد، بينما استخدم كاربوبول ٩٤٠ كعامل جل. كشفت دراسة النفاذية (*ex vivo*) لـجل مستحلب الابكسابان النانوي عن زيادة معاملات النفاذية خلال الطبقة القرنية البشرية بشكل ملحوظ ($p \leq 0.05$) في Jss و PC و Er ، وتم الحصول على وقت ضائع (T_{lag}) أقصر مقارنةً مع جل الابكسابان النقي.

الكلمات المفتاحية: ابكسابان ، مستحلب نانوي ، النفاذية خلال الجسم الحي.

Introduction

Transdermal drug delivery provides a suitable alternative to circumvent limitations associated with oral drug administration. It avoids hepatic first pass metabolism, a painless method and enable self-application by patients. However, the main objective of transdermal route is the large globule size and substantial barrier of skin to drug penetration, particularly stratum corneum in epidermis layer. Nanoemulsion (NE) reflect one of the most promising techniques for transdermal carriage of drugs, as it improved the dispersibility of poorly soluble drugs and hence, improve their permeation, accepted thermodynamic stability, high loading capacity of both lipophilic and hydrophilic drugs, and efficient permeating properties of its components through biologic membranes[1, 2].

Nanoemulsion is isotropic mixture of water and oil stabilized by Smix (surfactant and cosurfactant blended in different ratios)[3]. Its droplets uniformly distributed with average size range of 20 - 200 nm allowing higher drug flux through intracellular lipophilic pathway of skin and crafts drug depot within epidermis and stratum corneum[4]. For more convenience application, ultrafine nanoemulsion formulation developed with droplet size below 50 nm that prepared by selecting appropriate oil and surfactant blend to dissolve the intended dose of drug. Such system demonstrates better spreading properties caused by higher area to volume ratio of ultrafine nano-sized droplets; high drug loading capacity; ultrafine nanoemulsion formulation itself behave as penetration enhancer in absence using any chemical or physical permeation facilitating technique[5].

[1]Corresponding author E-mail**:** drmustafa1986@yahoo.com
Received: 21/3 /2020
Accepted: 19/7 /2020

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[6]. It was selected as a suitable candidate for incorporation into ultrafine transdermal o/w nanoemulsion, as APX display relatively low molecular mass (459.497 g/mol); poor water solubility of 0.028 mg/mL at 24 °C; and low bioavailability of about 50% after oral administration, 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[7], low dose (2.5 or 5 mg) and suitable balanced partition coefficient of 44.7 (log p 1.65). However, the low viscosity of nanoemulsion made it is inconvenient to use transdermally, due to low retention at application site, therefore the ultrafine APX-NE was included into gel system.

The present study designed to explore the feasibility of ultrafine with particle < 50 nm o/w nanoemulsion based gel as a carrier of poorly water-soluble drug apixaban for systemic transdermal delivery, and to investigate the potential of the prepared formulation to act itself as penetration enhancer through human stratum corneum without using physical or chemical permeation enhancing techniques.

Materials and Methods

Materials

Materials used include, Pure Apixaban obtained from ZHEJIANG CP CHEMICAL CO., LTD; Methanol Lab grade solvent (Sigma Aldrich, USA); Triacetin (Hangzhou Hyper Chemicals Limited); Triton-X100 (Sigma Aldrich, USA); Carbitol (Sigma Aldrich, USA).

Methods

Preparation of APX-NE

According to previous related study[8], triacetin oil, triton-X-100 and carbitol selected as oil phase, surfactant and cosurfactant respectively for apixaban (APX) o/w nanoemulsion (NE) preparation. APX-NE formulations prepared at Smix weight ratios of 1:1, 2:1, 3:1, 4:1, 1:2, 1:3 and 1:4. In which, the assigned dose of 5 mg APX dissolved in triacetin oil, and then add Smix (surfactant/cosurfactant) with continuous vortex mixing. Finally, deionized water was titrated gradually until clear isotopic APX-NE formed[9].

Thermodynamic stability study

Three thermodynamic stability tests used to assess physical stability of prepared APX-NE formulations. These tests include centrifugation test, where nanoemulsions centrifuged at 3500 rpm for 30 min, followed by six heating-cooling cycles at 45 and 4 °C for 48 h in each temperature and eventually, three freeze-thaw cycles at – 20 and 25 ˚C for 24 h at each temperature. After each test, samples discarded if demonstrate phase separation, precipitation or cracking by visual check[10].

Characterization of APX-NE formulations

Droplet size and polydispersity index (PDI) measurement

Photon correlation spectrophotometer (Brookhaven instrument, Zeta Plus, USA) used to measure average droplet size of prepared APX-NE by dynamic light scattering (DLS) technique, which analyze fluctuations in the light of scattering at 25 °C and scattering angle of 90°. Polydispersity index (PDI) is a measure of homogeneity in droplet size which ranges from 0 to 1 and measured by electrophoretic light scattering technique[11].

Transmittance percent and electrical conductivity measurement

Transmittance percent (T%) measured for optical transparency of prepared APX-NE using UV-Visible spectrophotometer (Spectrumlab 752Pro, China) at 650 nm keeping distilled water as blank[12], while electroconductivity measured using conductivity meter (DDS-11A, China). These tests performed to confirm the type of prepared APX-NE and performed in triplicate.

pH and APX content determination of APX-NE

Digital pH meter (BP 3001, Singapore) used to measure pH values of APX-NE formulations. While APX content detected after desired dilution of 2 g sample from each APX-NE with methanol. Samples then filtered via 0.45 µm filter syringe and analyzed spectrophotometrically at 278 nm (λ_{max} of APX in methanol)[13]. The experiments performed in triplicate.

Selection of optimum APX-NE formula for gel preparation

Due to low viscosity of nanoemulsion, and thereby easily washout upon dermal application, one APX-NE formulation selected for further fabrication into APX-NE based gel for more convenient transdermal use. The selection of most optimum formula made depending on characterization techniques of APX-NE.

APX-NE gel preparation

One gram of Carbopol 940 dispersed in small amount of distilled water and then volume completed to 100 mL and left in dark place for 24 h to ensure complete swelling of Carbopol 940 and allow escape of entrapped air bubbles**.** Then, pre-prepared APX-NE added slowly to the aqueous solution of Carbopol 940 (1% w/v) in a ratio of 1:1 with continuous stirring at 250 rpm. Afterward, 0.5 g triethanolamine added to neutralize the gel resulting in clear homogenous viscus gel of APX-NE with strength of 1 mg APX in each 1 g of gel[14].

APX-excipient compatibility studies

Fourier Transform Infrared (FTIR)

The purity and compatibility of APX with other excipient in NE and NE-gel was assessed using FTIR spectroscopy (Shimadzu, Japan) at the

range 4000 – 400 cm $^{-1}$ to check possible chemical interaction between their functional groups[15]. FTIR performed for pure APX, APX-NE and APX-NE gel each separately.

X-ray Diffraction (XRD)

Lattice nature and drug – excipients possible interactions characterized using XRD diffractometer (XRD-6000, Shimadzu, Japan) operated at voltage 40 kV, current 30 Ma, Cu-Kα radiation at λ = 1.5406 nm, scanning speed of 5°/ min and 2θ range of 0 – 60 degree for pure APX and APX-NE gel each separately[16].

Microscopic morphology studies

Transmission Electron Microscopy (TEM)

Surface images and approximate particle size of APX-NE and APX-NE gel studied using TEM microscope (CM 120, Philips, USA) with accelerating voltage of 100 kV by placing each sample separately on carbon coated copper grid and then left to dry and form thin film at room temperature for 60 sec. The excess was wiped out using Whatman filter paper[17].

Ex vivo APX human skin permeation study

To evaluate permeation efficiency of APX-NE gel through human stratum corneum, abdominal skin of human female utilized, from which outer most layer of epidermis, stratum corneum, was isolated and used as diffusional barrier.

Preparation of human skin

An abdominal skin of human female (age: 44 years old; weight: 96.75 Kg) obtained after routine plastic surgery for cutting sagging skin from abdomen of adult female in Baghdad educational hospital, which then transferred into laboratory using ice bag at 4 ˚C within 2-3 hours. Skin specimens first cooled to 4 ˚C and washed with phosphate buffer saline pH 7.4 solution with the aid surgical scalpel to get rid of the traces of blood, adipose tissue and subcutaneous fats remained after excision. Afterward, the epidermis layer was separated from the dermis layer of skin using heat separation method, in which the whole skin piece placed in water bath at 60 ^{0}C ± 0.5 ^{0}C for 60 - 90 sec and then epidermis peeled off carefully after its separation using anatomical forceps[18]. Finally, the skin was wrapped in aluminum foil and stored at -20 ^{0}C until use.

At time of experiment, specimens of epidermal skin thawed and washed with phosphate buffer saline pH 7.4 solution. For isolation of stratum corneum layer from epidermis, skin pieces of epidermis layer were placed in a petri dish and soaked in 1% trypsin in PBS solution of pH 7.4 for 24 h incubation period at 4 ˚C followed by 1 h at 37 ˚C. The soaking step repeated using fresh 1% trypsin solution until stratum corneum separated and floated as a transparent thin layer over the solution. Ultimately, stratum corneum transferred to another petri dish using anatomical forceps and rinsed with PBS of pH 7.4 twice to wash out any trypsin traces left. The unused stratum corneum was placed flat on aluminum foil and stored at -20 ^{0}C and used within three months[19].

Franz cell - Ex vivo permeation study through human skin

The use of abdominal skin of adult human female was reviewed and approved by ethical committee of Baghdad University / College of Pharmacy for its application in the *ex vivo* permeation study experiment for pure APX gel and ultrafine APX-NE based gel.

Human stratum corneum isolated from adult female abdominal skin was mounted between donor and acceptor parts of Franz cell at 32 ± 0.5 ˚C; 600 rpm stirring magnitude and diffusional area (1.77 cm^2); PBS containing 1% SLS as dissolution medium. Then, samples of 5 g APX-NE gel and pure APX gel placed above the skin in the donor part of Franz cell for permeation evaluation[20]. Samples of 0.1 mL withdrawn with filtered syringe of 0.45 µm at predetermined time intervals of 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 h, and substituted with same volume of fresh PBS pH 7.4 / 1% SLS medium. The amount of APX permeated per unit area quantified spectrophotometrically at 280 nm and plotted against sampling time[21]. The experiment performed in triplicate.

Ex vivo permeation data analysis

Permeation parameters including permeation flux (Jss), lag time (T_{lag}), permeability coefficient (PC) and enhancement ratio (Er) were calculated exploiting permeation profile plotted from the cumulative amount of APX permeated per unit area (Q, µg/cm^2) via the stratum corneum part of human skin on Y-axis against time (t, h) of experimental sampling on X-axis[22].

Statistical analysis

SPSS statistical program (version 16, USA) used for data analysis in this study research with the adoption of difference or P value ≤ 0.05 as statistically significant.

Results

Preparation of APX-NE

Twenty-one APX-NE formulations prepared (Table 1) by selecting three formulations of different Smix ratio. Two factors dependent for the preparation; first, amount of triacetin oil selected at 5 % weight interval (5, 10 and 15 %)[23]; secondly, selecting formulations with minimum Smix concentrations to avoid possible skin irritation by surfactant application[24]. No obvious change noticed during formulation, i.e. phase separation, turbidity or color change, as well as no drug precipitation of APX observed during deionized water addition.

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

F-code	Smix ratio	Smix%	Triacetin oil%	Water%	F-code	Smix ratio	Smix%	Triacetin oil%	Water%
F-1	1:1	30	5	65	**F-12**	4:1	35	15	50
F-2	1:1	30	10	60	**F-13**	1:2	30	5	65
F-3	1:1	30	15	55	**F-14**	1:2	30	10	60
F-4	2:1	30	5	65	**F-15**	1:2	30	15	55
F-5	2:1	30	10	60	**F-16**	1:3	30	5	65
F-6	2:1	30	15	55	**F-17**	1:3	30	10	60
F-7	3:1	35	5	60	**F-18**	1:3	30	15	55
F-8	3:1	35	10	55	**F-19**	1:4	30	5	65
F-9	3:1	35	15	50	**F-20**	1:4	30	10	60
F-10	4:1	35	5	60	**F-21**	1:4	30	15	55
F-11	4:1	35	10	55					

Thermodynamic stability study

Results demonstrate stability of nineteen APX-NE and discarding two (F-6 and F-21).The inherited high stability could be attributed to steric stabilization of nonionic surfactant triton-X-100[25] which confers long shelf life to nanoemulsions as compared to ordinary emulsions[26].

Characterization of APX-NE

Droplet size and Polydispersity Index (PDI)

Average droplet size measured for all formulations passed thermodynamic stability tests successfully. Among tested formulations, nine APX-NE demonstrate ultrafine droplet size of less than 50 nm[27] including F-2, 7, 8, 9, 10, 11, 12, 13 and 16 with lower size obtained of 12.32 nm for F-12. According to the results, as presented in Table 2, a decrease in droplet size 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. This could be attributed to 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[28].

PDI values (Table 2) of ≤ 0.3 indicates homogenous monodispersed nanoemulsion formation with good stability and uniformity in droplet size distribution upon dilution[29].

Table 2. Results of PSD, PDI, pH, electrical conductivity, T%, APX content%, for the prepared APX-NE formulations, (mean ± SD, n = 3).

F-code	PSD (nm)	PDI	pH	Electrical conductivity (µs/cm)	Transmittance %	% APX content
F-1	672.48	0.430	5.25 ± 0.02	194.58 ± 1.24	99.56 ± 0.02	97.05 ± 0.11
F-2	38.63	0.420	5.12 ± 0.05	181.11 ± 0.84	99.79 ± 0.01	98.23 ± 0.21
F-3	281.70	0.296	5.07 ± 0.01	167.08 ± 0.18	97.34 ± 0.03	99.01 ± 0.13
F-4	106.01	0.432	5.15 ± 0.02	191.64 ± 1.29	99.81 ± 0.01	96.94 ± 0.26
F-5	283.76	0.567	5.07 ± 0.05	182.79 ± 0.91	98.86 ± 0.01	98.84 ± 0.17
F-7	26.41	0.302	5.38 ± 0.05	185.84 ± 1.14	97.13 ± 0.01	99.63 ± 0.14
F-8	23.06	0.279	5.16 ± 0.01	172.63 ± 0.82	97.46 ± 0.02	99.37 ± 0.2
F-9	15.49	0.235	4.91 ± 0.04	164.34 ± 0.66	99.95 ± 0.01	99.65 ± 0.18
F-10	37.17	0.321	5.35 ± 0.03	188.57 ± 0.61	99.94 ± 0.01	99.56 ± 0.11
F-11	35.83	0.329	5.27 ± 0.04	177.46 ± 0.55	97.72 ± 0.06	99.73 ± 0.26
F-12	12.32	0.234	5.16 ± 0.01	168.18 ± 1.18	98.05 ± 0.03	99.98 ± 0.18
F-13	16.34	0.308	5.07 ± 0.01	190.89 ± 1.33	97.81 ± 0.02	99.67 ± 0.13
F-14	114.59	0.434	4.98 ± 0.03	181.47 ± 0.89	98.49 ± 0.07	96.88 ± 0.18
F-15	150.16	0.252	4.91 ± 0.03	173.34 ± 0.54	99.91 ± 0.04	97.04 ± 0.19

Table 2. Continued results of PSD, PDI, pH, electrical conductivity, T%, APX content%, for the prepared APX-NE formulations, (mean ± SD, n = 3).

F-code	PSD (nm)	PDI	pH	Electrical conductivity (µs/cm)	Transmittance %	% APX content
F-16	13.12	0.300	4.86 ± 0.07	196.96 ± 1.36	97.48 ± 0.01	99.89 ± 0.11
F-17	371.53	0.414	4.73 ± 0.01	187.66 ± 0.81	99.99 ± 0.01	97.72 ± 0.34
F-18	495.17	0.411	4.67 ± 0.02	180.74 ± 0.65	99.63 ± 0.04	98.04 ± 0.21
F-19	321.68	0.415	4.95 ± 0.01	195.77 ± 1.08	99.57 ± 0.05	97.62 ± 0.11
F-20	353.85	0.386	4.82 ± 0.04	185.39 ± 1.36	97.84 ± 0.01	98.02 ± 0.19

Transmittance percent and electrical conductivity

All tested APX-NE exhibit high light transmittance (Table 2) with close approximation to 100% indicating optically clear, transparent and nanosized droplets(30). Electroconductivity reveal the formation of o/w nanoemulsions with a high degree of electrical conductivity as water represents the external phase and can conduct electrical current[31].

pH and APX Content Determination of APX-NE

pH readings summarized in Table 2 and reveal comparable values with skin pH, which ranges from 4.5 to 6.5, indicating its suitable application for transdermal use without skin irritation or sensitization[32].

APX content presented in Table 2 and reveal acceptable values that set within official range (85 % - 115 %) according to the United States Pharmacopeia (USP) indicating successful APX loading without any precipitation or degradation of the drug.

APX-NE gel preparation

APX-NE (F-12) formulation was selected for gel preparation, as F-12 demonstrate desirable properties of droplet size, PDI, pH and APX content. The prepared APX-NE gel demonstrate consistent and viscous gel that is appropriate for transdermal use.

Compatibility studies

Fourier Transform Infrared (FTIR)

The FTIR spectrum of pure APX backbone structure (figure 1) display bands at 3479 and 3313 cm^{-1} assign for N-H stretching asymmetric and symmetric of primary NH, while 2937 and 2837 cm^{-1} *bands refer to* C-H stretching asymmetric and symmetric of CH_3. Bands at 1512-1400 cm^{-1} assign for C=C stretching of benzene ring, 1184 and 1595 cm^{-1} refer to N-C and -C=O stretching vibration for amide. Same characteristic bands of pure APX appeared after drug formulation into NE and NE-gel with small shifting, as shown in figure 2, indicating compatible APX-excipient mixing without chemical interaction[33].

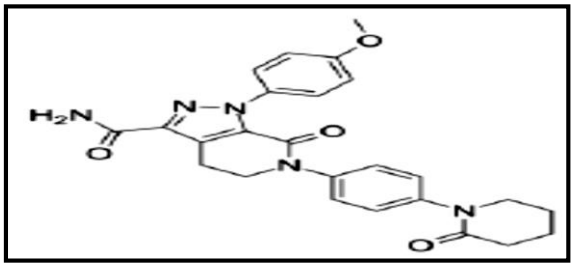
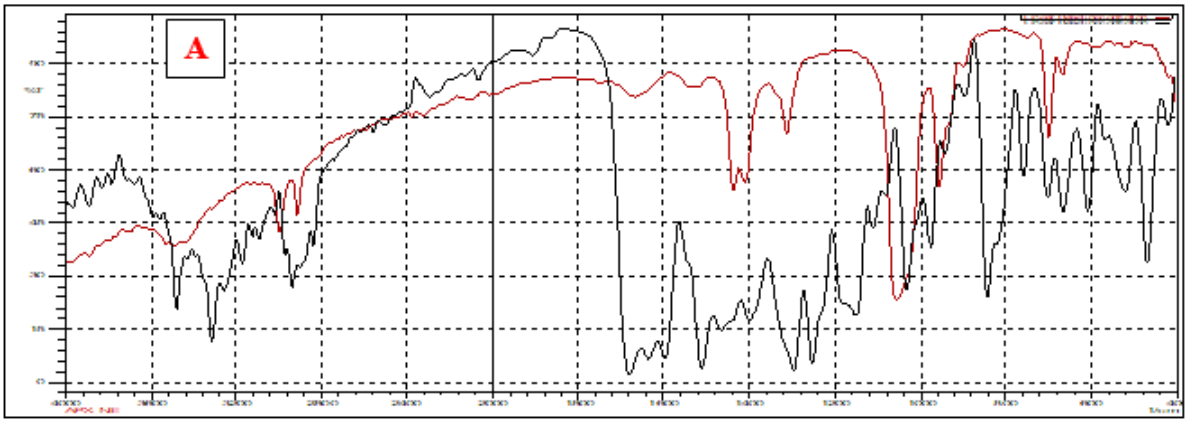


Figure 1. Chemical structure of apixaban (APX)[6].



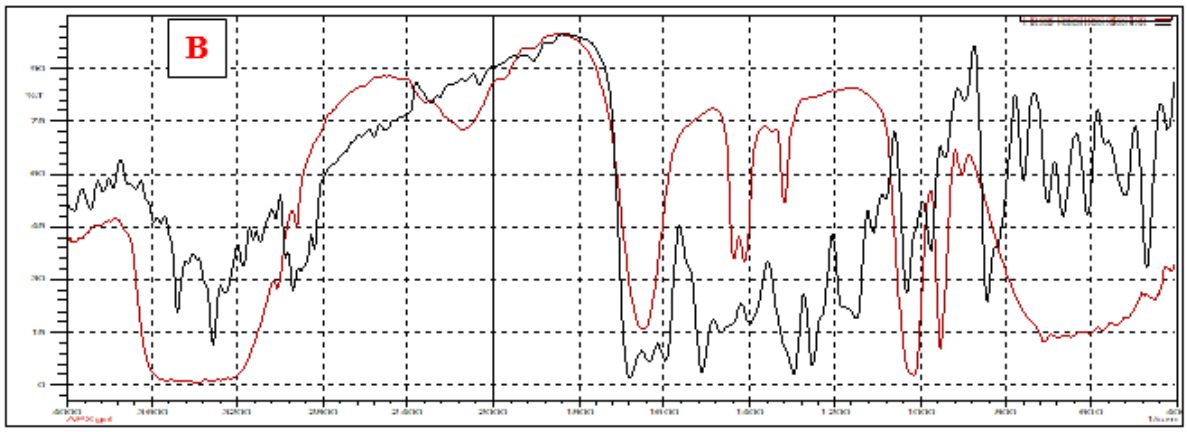


Figure 2. Comparative FTIR spectra of pure APX in black color with the red spectra of (A) APX-NE and (B) APX-NE based gel.

X-ray Diffraction (XRD)

The XRD diffractograms of tested samples presented in figure 3. XRD diffractogram of pure APX revealed highly crystalline structure, as it displayed sharp intense narrow diffraction peaks noted at 2θ angles 17.0362, 22.2967 and 32.7404 degrees. Same peaks also displayed in APX-NE gel diffractogram indicating APX/NE-gel components compatibility without any chemical modification of APX[34]. Additionally, APX-NE gel displayed no sharp peaks with significant ($p \leq 0.05$) lower intensities indicating amorphous structure of APX within NE-gel[35].

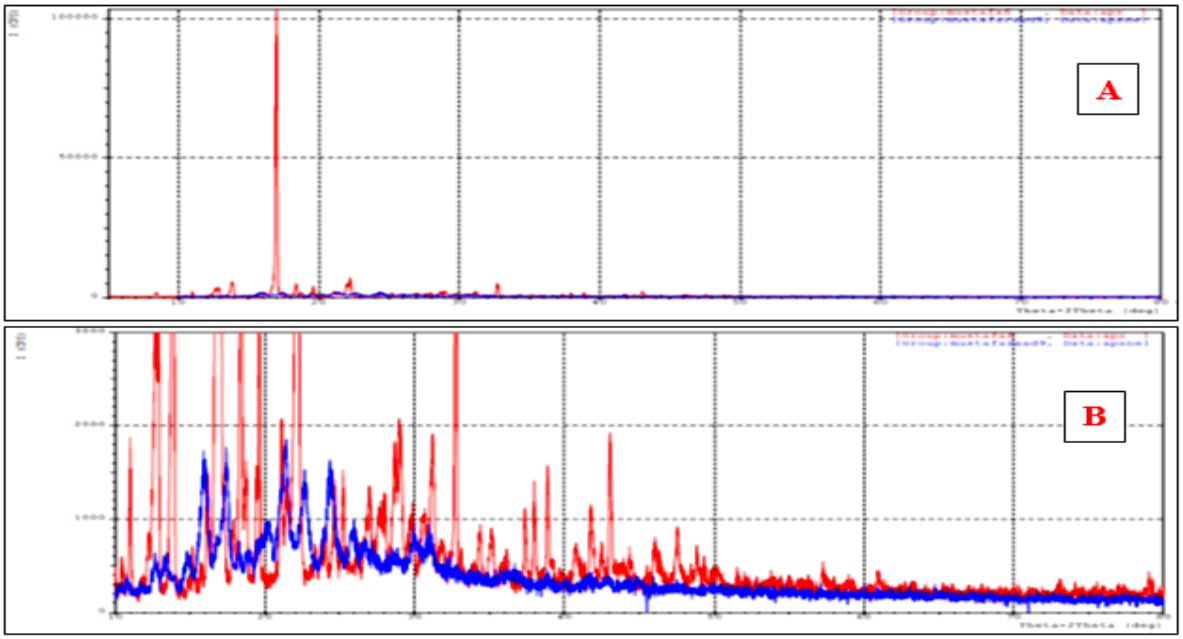


Figure 3. Comparative XRD of APX (blue) and APX-NE gel (red) at intensity (A) 100000 and (B) 3000.

Microscopic morphology studies

Transmission Electron Microscopy (TEM)

Photomicrographs of APX-NE and APX-NE gel shown in figure 4 and reveal discrete, dark spherical shaped droplets with almost uniform particle size distribution of less than 50 nm. These results confirm ultrafine (< 50 nm) APX-NE gel formation and fit with droplet size analysis of APX-NE F-12 (12.32 nm) using dynamic light scattering (DLS) technique. Furthermore, no signs of droplet coalescence observed and thus, indicating physical stability of formulation[36].

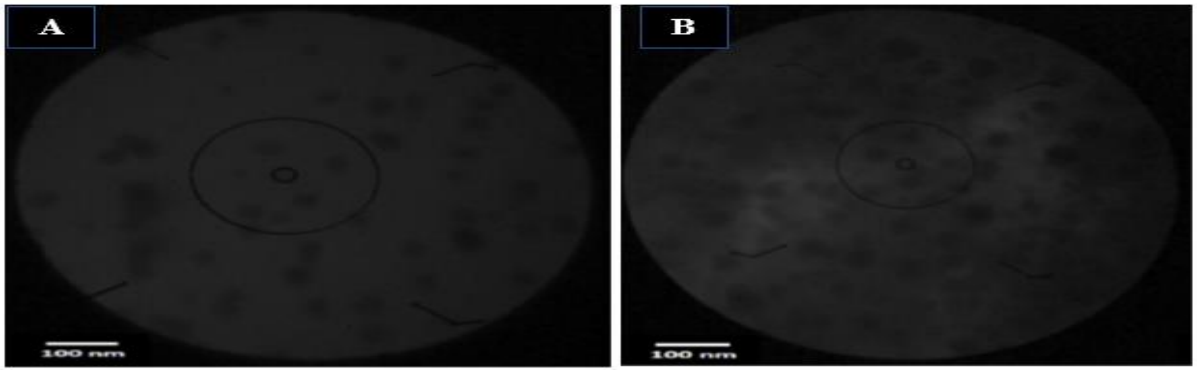


Figure 4. TEM of (A) APX-NE and (B) APX-NE gel.

Ex Vivo APX-NE gel permeation study

Human abdominal skin of adult female utilized for APX-NE gel permeability assessment to get best fit with *in vivo* conditions using Franz cell apparatus. Figure 5 present cumulative amount of APX permeated through human stratum corneum from APX-NE gel and pure APX gel as comparative diagram. Results reveal complete APX permeation from APX-NE gel after 7 h, whereas 6.89 % drug permeate from pure APX gel after same time, indicating significantly ($p \leq 0.05$) higher cumulative amount of drug permeated from NE-gel than that of pure APX gel with 14.5-fold increment. The reasons for this superior performance include the ultrafine (< 50 nm) sized globules of APX-NE within hydrophilic Carbopol 940 gel structure, and penetration enhancing properties of triton-x-100, carbitol and triacetin oil components of APX-NE, which disrupt stratum corneum lipid bilayer thereby affecting its integrity leading to pore formation and increased APX amount fluxed across skin[37]. The non-ionic surfactant triton-x-100 can solubilize lipids of stratum corneum and hence enhance APX penetration and absorption(38). Carbitol binds keratin filaments resulting in corneocytes disruption and enhance drug entrance. Additionally, APX present in dispersible form within NE-gel structure, resulting in either increased drug uptake directly or hold within NE as a vehicle for drug transport[39].

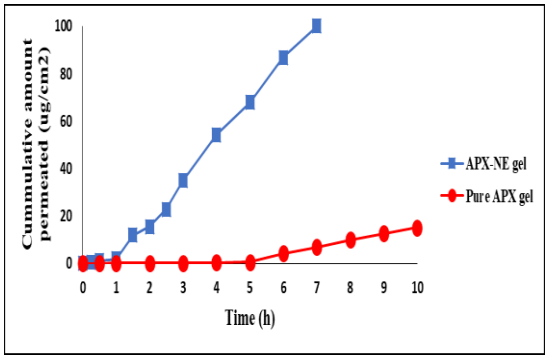


Figure 5. Comparative *ex vivo* permeability through human stratum corneum of pure APX gel (red) and APX-NE gel (blue)

Ex vivo permeation data analysis

Permeation parameters of APX-NE gel through human stratum corneum illustrated in Table 3 and indicate significant ($p \leq 0.05$) faster permeation than pure APX gel with higher Jss, PC and Er values, and shorter T_{lag}.

Table 3. Permeation parameters results of APX-NE gel.

Parameter	Through human stratum corneum	
	Pure APX gel	APX-NE gel
Flux (µg/cm ^{2}.h)	2.8914	16.741
PC (cm/h) × 10 $^{-3}$	1.15656	6.696
T_{lag} (h)	5	1
Er	1	5.79

Conclusion

APX-NE and APX-NE gel were prepared successfully with ultrafine droplets (< 50 nm) as indicated by DLS and TEM, in addition to the significant ($p \leq 0.05$) increase in *ex vivo* permeation of APX-NE gel through human stratum corneum in comparison with pure APX gel indicating penetration enhancing properties of formulation it-self without using chemical or physical enhancing methods.

References

1. 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.
2. 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.
3. 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.
4. 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.

5. 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.
6. 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.
7. Byon W, Garonzik S, Boyd RA, Frost CE. Apixaban: A Clinical Pharmacokinetic and Pharmacodynamic Review. Clinical pharmacokinetics. 2019;58(10):1265-79.
8. Abdulbaqi MR, Rajab N. Apixaban Ultrafine O/W Nano Emulsion Transdermal Drug Delivery System: Formulation, In Vitro and Ex Vivo Characterization. Systematic Reviews in Pharmacy. 2020;11(2):82-94.
9. 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.
10. 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.
11. 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.
12. 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.
13. Ghareeb MM. Design and in vitro characterization of a topical nanoemulsion-enriched hydrogel of econazole nitrate. Journal of Applied Pharmaceutical Science. 2019;9(1):051-7.
14. 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.
15. Liu Z, Mendiratta S, Chen X, Zhang J, Li Y. Investigation of inverse emulsion assisted controlled release of polyacrylamides for enhanced oil recovery. RSC Advances. 2019;9(21):11968-77.
16. Parveen R, Akhtar N, Farooq MA, Ghayas S, Bushra R, Khan DH, et al. Preparation of microemulsion containing Lycopersicon esculentum extract: In vitro characterization and stability studies. Pakistan journal of pharmaceutical sciences. 2019;32(4 (Supplementary)):1821-7.
17. Lai W-C, Hong L-t, Hsueh C-Y. Size-tunable silica nanowires prepared using reverse microemulsion-gel systems. Journal of the Taiwan Institute of Chemical Engineers. 2019;99(2019):207-14.
18. Zsikó S, Cutcher K, Kovács A, Budai-Szűcs M, Gácsi A, Baki G, et al. Nanostructured Lipid Carrier Gel for the Dermal Application of Lidocaine: Comparison of Skin Penetration Testing Methods. Pharmaceutics. 2019;11(7):1-11.
19. Abd E, Benson HA, Mohammed YH, Roberts MS, Grice JE. Permeation Mechanism of Caffeine and Naproxen through in vitro Human Epidermis: Effect of Vehicles and Penetration Enhancers. Skin pharmacology and physiology. 2019;32(3):132-41.
20. Pradal J, Vallet CM, Frappin G, Bariguian F, Lombardi MS. Importance of the formulation in the skin delivery of topical diclofenac: not all topical diclofenac formulations are the same. Journal of pain research. 2019;12(2019):1149-54.
21. Kaewbanjong J, Amnuaikit T, Souto EB, Boonme P. Antidermatophytic Activity and Skin Retention of Clotrimazole Microemulsion and Microemulsion-Based Gel in Comparison to Conventional Cream. Skin pharmacology and physiology. 2018;31(6):292-7.
22. Parhi R, Reddy SS, Swain S. Transdermal delivery of ondansetron HCl from thermoreversible gel containing nanocomposite. Current Nanomaterials. 2019;4(2):137-47.
23. Rahman MA, Mujahid M. Development of self-nanoemulsifying tablet (SNET) for bioavailability enhancement of sertraline. Brazilian Journal of Pharmaceutical Sciences. 2018;54(1):1-14.
24. 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.
25. 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.

26. 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.
27. 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.
28. 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.
29. 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.
30. 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.
31. 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.
32. 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.
33. Gambhire M. S. , Gujar K. N., A. VA. Formulation, Optimization and Evaluation of Immediate Release Tablet of Apixaban. International Journal of ChemTech Research. 2017;10(10):424-35.
34. Gade S, Patel KK, Gupta C, Anjum MM, Deepika D, Agrawal AK, et al. An ex vivo Evaluation of Moxifloxacin Nanostructured Lipid Carrier enriched in situ gel for Transcorneal permeation on Goat Cornea. Journal of pharmaceutical sciences. 2019;108(9):2905-16.
35. Jangdey MS, Gupta A, Saraf S. Fabrication, in-vitro characterization, and enhanced in-vivo evaluation of carbopol-based nanoemulsion gel of apigenin for UV-induced skin carcinoma. Drug delivery. 2017;24(1):1026-36.
36. 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.
37. Shukla T, Upmanyu N, Agrawal M, Saraf S, Saraf S, Alexander A. Biomedical applications of microemulsion through dermal and transdermal route. Biomedicine & Pharmacotherapy. 2018;108(2018):1477-94.
38. Jurek I, Góral I, Mierzyńska Z, Moniuszko-Szajwaj B, Wojciechowski K. Effect of synthetic surfactants and soapwort (Saponaria officinalis L.) extract on skin-mimetic model lipid monolayers. Biochimica et Biophysica Acta (BBA)-Biomembranes. 2019 ;1861 (3) :556 - 64.
39. Abd E, Benson HA, Roberts MS, Grice JE. Minoxidil skin delivery from nanoemulsion formulations containing eucalyptol or oleic acid: Enhanced diffusivity and follicular targeting. Pharmaceutics. 2018;10(1):1-14.

Baghdad Iraqi Journal Pharmaceutical Sciences by bijps is licensed under a Creative Commons Attribution 4.0 International License. Copyrights© 2015 College of Pharmacy - University of Baghdad.