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Preparation and Evaluation of Pimecrolimus Nanoemulsion for Topical Delivery

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Abstract

One of the innovative approaches in order to improve the dispersibility of drug and thus improving its release and permeation is nanoemulsion which was adapted in this study for the poorly soluble drug pimecrolimus. Pimecrolimus is a low water soluble drug used to treat psoriasis, eczema, atopic dermatitis and vitiligo. This work describes the high-energy ultrasonication technique used to prepare pimecrolimus nanoemulsion. A pseudo-ternary phase diagram was created in order to determine the ideal ratios of oil-surfactant/co-surfactant mixture for the creation of nanoemulsions. Based on pimecrolimus's solubility in various oils, surfactants, and co-surfactants, Tween 80 was chosen as a surfactant, ethanol as a co-surfactant, and benzyl alcohol as an oil phase .The work involved creating twelve NE formulas for optimization, and evaluated for their droplet size, visual transparency, drug content, zeta potential, physical stability, and in vitro release. Droplet size is 45.1 nm, drug content is 92.59%, PDI is 0.402, Zeta potential is -0.8 mV, were displayed by the ideal NE formula F8 which consist of 10% benzyl alcohol (oil phase). 40% Smix (tween80 and ethanol) and 60% water, which released 76.3% in 5h and 96.6% of pimecrolimus in 24h compared with the commercial cream which released 26.2% in 5h and 42.5% of the drug in 24h. This suggests that the prepared formula has promising potential as an effective nanocarrier to enhance drug solubility and permeability and, consequently, its therapeutic effectiveness topically through the skin.

Key words: Nanoemulsion, Pimecrolimus, Benzyl alcohol, Tween80, RP-HPLC.

Introduction

With the topical delivery of medications to the skin, the topical therapy for skin disorders has improved. Because topical dosage forms, like ointments and creams, have a lower risk of systemic side effects, they are used to treat skin conditions. Lately, lipid-based carriers have emerged as a crucial method for enhancing the safety, efficacy, and medication penetration of topical formulations. Nanoemulsion is a useful and easy-to-prepare that effectively delivers medication with only mild side effects[(1)].Since nanoemulsions have so many advantages, they are used in both cosmetics and personal care: The gravitational force is significantly lessened due to the small droplet size, and Brownian motion might be adequate to defy gravity.This suggests that neither creaming nor sedimentation occur while the product is being stored. The droplets' minuscule size also prevents flocculation, and the system remains dispersed. Because the tiny droplets are non-deformable and do not fluctuate on the surface, they also prevent their coalescence. Furthermore, the liquid film between the droplets cannot be disrupted due to the substantial surfactant film thickness (in relation to droplet radius).

Active substances can be effectively delivered via the skin using nano-emulsions. Actives can enter the emulsion system quickly because to its vast surface area. Nano-emulsions' small size allows them to pierce the "rough" skin surface, improving actives' penetration. Their skin feel and aesthetic appearance may be pleasing because of the system transparency, and fluidity (at the right oil concentrations). The homogenous deposit on substrates is made possible by the droplets' small size. Enhancement of spreading, penetrating, and wetting may also be facilitated by the system's low surface tension and the droplets' low interfacial tension [(2)].Nanoemulsion droplet sizes range from 20 to 200 nm on average, with narrow size distributions. These nanoemulsions are either oil-in-water or water-in-oil, because they are made of water, surfactant, co-surfactant, and oil. An oil-water nanoemulsion consists of a dispersed oil phase and a continuous water phase. NEs are produced by high-energy emulsification or dispersion processes [(3)].There are numerous approaches that have been proposed for creating nanoemulsion. which are phase inversion method, sonication method, and high pressure homogenizer[(4)].Pimecrolimus, a novel

selective inflammatory cytokine release inhibitor derived from ascomycin macrolactam, has been demonstrated to suppress disease activity in psoriasis, contact dermatitis, and atopic dermatitis[5]. The color of pimecrolimus is off-white to white in fine crystalline form. It is insoluble in water but soluble in methanol and ethanol and belong to BCS class II[6].The aim of this research is to prepare and optimize pimecrolimus nanoemulsion that may enhance drug release and permeation to be a potential candidate to deliver an effective concentration of the drug throughout the skin.

Materials and methods

Materials

Pimecrolimus was from bidepharm (China). Benzyl alcohol, anise oil, peppermint oil, cardamom oil, and clove oil sourced from CDH in India, PEG400 and PEG200 from BDH in England, along with tween 80 obtained from J.T Baker China, ethanol from J.T Baker in China, acetonitrile used of HPLC grade.

Methods

Analysis of pimecrolimus by reverse phase HPLC (RP- HPLC)

A previously reported and validated RP-HPLC analysis for pimecrolimus was adapted in this work [7]. The phosphate buffer solution (pH 3) containing acetonitrile in a 55:45 ratio made up the mobile phase. The mobile phase's flow rate has been modified to 0.8 mL/min. With a particle size of 5 micrometer and dimensions of 250 x 4.6 mm, an Inertsil ODS, C18 column was used for the separation process. The column was kept at a constant temperature of 30°C, and each injection consisted of a volume of 10 µL. The column was subjected to a pre-injection equilibration period of at least 30 minutes, during which the mobile phase was allowed to pass through the system. The eluents were observed at a wavelength of 258 nm.

A clean, dry 50 mL volumetric flask was filled precisely with 10 mg of pimecrolimus to produce a stock solution with a concentration of 200µg/mL. Subsequently, 30 milliliters of the diluent (a 50:50 blend of acetonitrile and water) were introduced into the flask. The volume was then increased to 50 mL followed by sonicating it for 30 minutes. In a volumetric flask, one milliliter was extracted from the stock solution and diluted to ten milliliters to be analyzed using HPLC.

Construction of calibration curve for pimecrolimus by RP-HPLC.

Several aliquots were taken from the stock solution (200µg/mL =200ppm) and diluted to prepare samples with concentrations of 5, 10, 15, 20 and 25ppm and analyzed by RP-HPLC with UV detector at 258nm. Peak area versus concentration was plotted to create a calibration curve.

Pimecrolimus solubility determination

Pimecrolimus's solubility in different types of oil was determined (clove oil, cardamom oil, peppermint oil, anise and benzyl alcohol) and emulsifiers (triton x 100, capryol 90, cremophor RH and tween 80 as surfactant, transcutol P, PEG400, PEG200, and ethanol as co-surfactant). 5 mL of each liquid medium, an access amount of the drug, were combined and placed in a sealed glass vial. After that, a magnetic stirrer was used to continuously stir the mixture at 500 rpm for 48 h at (25°C). Then a syringe filter (0.45µm) was used to filter each equilibrated sample, then appropriate volume of mobile phase was added[8]. The drug's solubility at a wavelength of 258 nm was measured using HPLC.

Pseudo-ternary phase diagrams construction

By using the aqueous titration method, the pseudo-ternary phase diagrams were created. The best emulsifiers and solubilizing oil were selected; the co-surfactant was ethanol, the surfactant was tween 80, and the oil phase was benzyl alcohol. The triangle coordinate diagrams were developed for the purpose of assessing various ratios of surfactant/cosurfactant mixtures (Smix). For the purpose of creating oil-in-water (o/w) emulsions, various Smix ratios, such as 1:1, 1:2, 1:3, and 2:1, were used in a hope to achieve the desired hydrophilic-lipophilic balance (HLB) value range of 12–18. For the determination of the limits of the emulsion area, from 1:9 to 9:1, oil to Smix ratios were prepared and underwent water titrations. Each time, 0.5 ml of water at room temperature was added little by little while being gently stirred at a speed of about 500 rpm. The water quantity at which the shift takes place, specifically from a state of transparency to the point of turbidity, was determined based on the volume measurements [9].

When turbidity followed by phase separation, the sample was eliminated from the phase diagram. Once the solutions have been stirred and exhibited transparency and clarity, the monophasic samples must be positioned inside the phase diagram. "Emulsion area" refers to the area of space that includes points that show a uniform solution. The constructed diagrams were drawn with ProSim Ternary Diagram software (Stratage; France).The emulsion composition with a bigger region of emulsion in the phase diagram, indicating a better hydration capacity, was determined to be the optimal choice[10] .

Pimecrolimus nanoemulsion preparation

Based on the pseudo ternary phase diagram results, pimecrolimus o/w formulas were created. Where the oil and Smix mixture was used to dissolve the drug. Next, drop by drop, water was added at room temperature while the magnetic stirrer was spinning at about 500 rpm to create a clear formula. Each formula has a 10 mL volume containing 100 mg pimecrolimus. After that, the formulas were

subjected to ultrasonic force for 10 minutes at 20 KHz and 90 W using a probe sonicator (Qsonica sonicator; USA). This was done to reduce size, but sonication caused heat generation, which was avoided by placing the formula in ice water[11]. The composition of the prepared formulations is displayed in Table 1.

Table 1. The components of pimecrolimus NE formulas.

Formula cod	Drug in (mg)	Benzyl alcohol %	Smix (surfactant: cosurfactant)	Smix ratio	Smix %	Water to reach 10 mL	Ultrasonication time (min)
F1	100	5	Tween80: ethanol	1:2	30	Qs	10
F2	100	5	Tween80: ethanol	1:2	40	Qs	10
F3	100	5	Tween80: ethanol	1:2	50	Qs	10
F4	100	5	Tween80: ethanol	1:2	60	Qs	10
F5	100	5	Tween80: ethanol	1:2	70	Qs	10
F6	100	10	Tween80: ethanol	1:2	30	Qs	10
F7	100	10	Tween80: ethanol	1:2	40	Qs	10
F8	100	10	Tween80: ethanol	1:2	50	Qs	10
F9	100	10	Tween80: ethanol	1:2	60	Qs	10
F10	100	10	Tween80: ethanol	1:2	70	Qs	10
F11	100	10	Tween80: ethanol	1:2	60	Qs	20
F12	100	10	Tween80: ethanol	1:2	60	Qs	30

Determination of the nanoemulsion's mean droplet size, polydispersity index, and zeta potential

The mean droplet size of the pimecrolimus nanoemulsion was identified by the dynamic light scattering method. The instrument used was a Zetasizer Nano Series (model ZEN 3600 Nano ZS, Malvern, UK). Every experiment was conducted in triplicate, and the temperature was recorded at 25 ºC [13].

Drug content determination

The purpose of this test was to find how much drug in each NE formula. HPLC was used to ascertain the pimecrolimus content in the NE formulation. One milliliter of each formula was centrifuged for twenty minutes at room temperature and 3500 rpm[14]. The supernatant was analyzed using HPLC/ UV detector (SYKAM; Germany) at 258 nm wavelength.

Dye solubilization test

Each prepared pimecrolimus NE was mixed with 2 mg/mL of methyl orange dye which is a water soluble dye to identify the type of continuous phase. After that, the color was assessed visually to see if the dye had spread uniformly throughout the external continuous phase (w/o nanoemulsion) (o/w nanoemulsion) [15].

pH measurement

Digital pH meter was used to determine the pH of the prepared NE formulas (F1 -F12). After dissolving 1ml of each formula in 100 mL of distilled water, the pH was determined. This test was repeated three times[16].

Dilution test (dispersity test)

This test was conducted to confirm the NE's physical stability. One millilitre of each formulation was continuously stirred to 50 millilitres, 100 millilitres, and 500 millilitres at 37°C using a magnetic stirrer (Dragon Lab; USA) set at 50 rpm. The formulas' turbidity, clarity, and phase separation were visually assessed[17].

Characterization of pimecrolimus nanoemulsion

Visual transparency

Using a good light source and transparent glass vials containing the formulas, and the prepared NE (F1–F12)'s visual transparency was assessed [12].

Viscosity

Viscometer (Visco, ATAGO's viscosity metre VISCOTM; Japan) was used to measure the prepared NE viscosity (F1-F12). For formulas with low viscosity (less than 50 mPa.s) UL was used, while spindle A1 was used for formulas with moderate to high viscosity[11].

Studies of nanoemulsion's physical stability

Centrifugation test

The formulas (F1–F12) were all centrifuged by a Fanem, 206-R Centrifuge from Brazil for 30 minutes at 3500 rpm. The formulas that passed this test, which required the formulas to maintain homogeneity, were deemed stable and moved on to the tests below[18].

Test of the heating/cooling cycle

The aim of this experiment was to investigate the ability of nanoemulsion to tolerate abrupt temperature changes. Six cycles total, each lasting 48 hours: four at 4°C in refrigerator and four at 45°C in oven. For two days, each formula was heated and cooled by first being kept at 4°C in a refrigerator and then being placed at 45°C in an oven. This cycle was carried out six times in a row [19].

Freeze/ thaw cycle

After a day at -20°C in the deep freezer, the formulas were removed and stored at 25°C. Within 2–3 minutes, the stable NE should take on its initial shape. Every formula experienced two cycles[20].

In-vitro release study

Using the dialysis-bag method (Molecular cut off 12000 Dalton "Da"), the release of

pimecrolimus from formulas with high %EE was assessed. The dialysis bags were completely wet. Each dialysis bag had both sealed ends, and 5 mL of the NE formulation was added. After that, the dialysis bags were immersed in a dissolving medium made of 100 mL of phosphate buffer pH 7.4 containing 30 mL of acetonitrile. In a USP dissolution apparatus type II (paddle method) rotating at 100 rpm, the experiment was conducted at 37±10ºC. At the specified times (1, 2, 3, 4, 5, 6, 12 and 24 hours). 5mL sample was taken and examined by HPLC and replaced with new dissolving media[8].

Selection of optimum NE formula

When selecting the optimal formula, factors such as the ideal droplet size, zeta potential, PDI, pH, viscosity, and in- vitro release were taken into account.

Field emission scanning electron microscopy (FE SEM)

Analysis to determine the nanoemulsion's morphology. 1-2 µL sample of the drug was placed on a silicon strip and dried by using oven , then the powder sample placed on the field emission scanning electron microscopy sample site (INSPECT F 50 FE SEM, FEI, Netherlands) to observe the morphology of the optimized emulsion[21].

Statistical analysis

The statistical analysis of the formulations was performed with (ANOVA) one-way analysis of variance, is used in SPSS version 26. When the p-value is less than 0.05, the observed discrepancy is deemed statistically significant.

Results and discussion

RP-HPLC analysis for pimecrolimus

Figure (1) shows the spectrum for pimecrolimus where the retention time was 3min for pimecrolimus. Depending on the area under the peak that obtained from HPLC, calibration curve was plotted as shown in Figure[2].

Pimecrolimus solubility study

The oil which has the larger capacity for solubilization of the drug will be chosen as an oil phase[22] .In this study the drug gave a solubility of 150.87mg/mL in benzyl alcohol which was higher than other oils, therefore it was chosen as an oil phase and it was reported to be used as an oil phase for different drugs such as ketoprofen NE [23] . The best surfactant was tween80 (although it gave lower solubility than triton x100) but it gave better miscibility and clear emulsion. The non-ionic surfactant Tween80 has an HLB value of 15, which is necessary to create an o/w emulsion [24]. The best solubility of pimecrolimus in a co-surfactant (ethanol)which is used to enhance the solubility of different drugs and used as a co- surfactant in the formulation of emulsion of different drugs like ondansetron nanoemulsion [9]. Table 2 shows the results of solubility of pimecrolimus in different media.

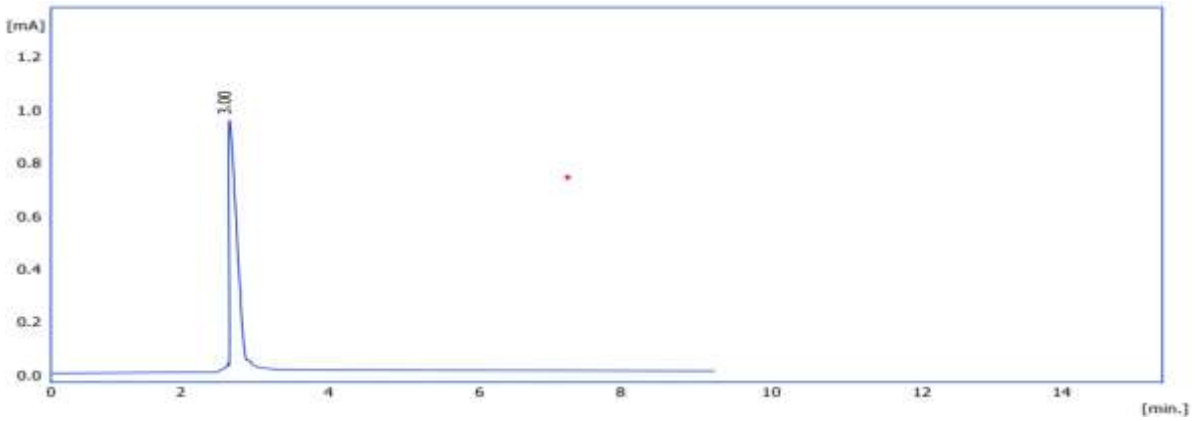


Figure 1.HPLC chromatogram for pimecrolimus .

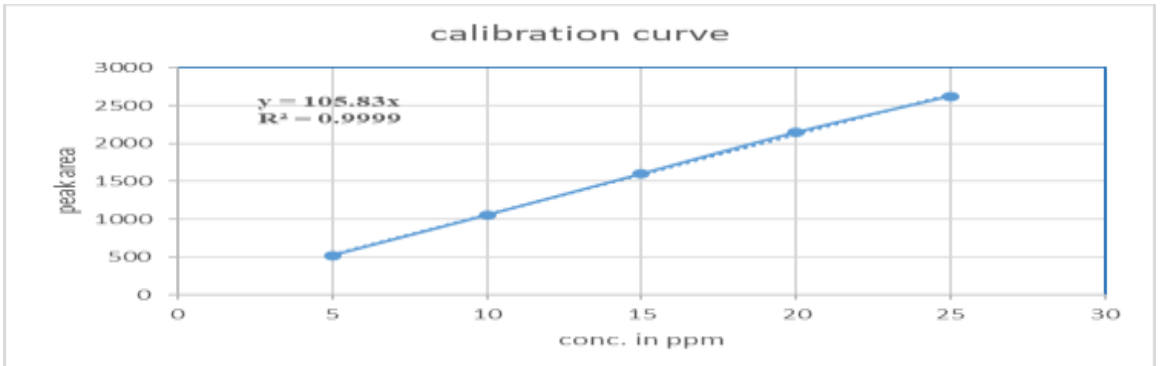


Figure 2. The RP-HPLC pimecrolimus calibration curve using acetonitrile: phosphate buffer (pH 3) 45:55 ratio as the mobile phase.

Table 2. The solubility of pimecrolimus in various oils, co-surfactants, and surfactants

oil Type	Solubility (mg/mL)	Surfactant type	Solubility (mg/mL)	Co-surfactant type	Solubility (mg/mL)
Clove oil	9.073	Triton x100	2.273	Transcutol p	2.237
Cardamom oil	2.424	Capryol 90	1.986	PEG400	1.257
Peppermint oil	2.552	Cremophor RH	2.012	PEG200	1.325
Anise oil	4.323	Tween80	1.879	ethanol	6.543
Benzyl alcohol	150.87				

Construction of pseudo-ternary phase diagrams

Pimecrolimus is a very lipophilic drug and has a very low water solubility so o\w emulsion was prepared to enhance its solubility. Different ratios of Smix (2:1, 1:1, 1:2, and 1:3) were used for creating the pseudo-ternary phase diagram, with benzyl alcohol serving as the oil phase. Figure 4 illustrates the optimal outcome (largest area of monophasic, NE) was with the Smix ratio 1:2. Tween80 is a non-ionic surfactant with a hydrophilic-lipophilic balance (HLB) value of 15. Both hydrophilic and lipophilic components of its chemical structure can be concentrated and adsorbed onto the water-oil interface (benzyl alcohol). In an oil-in-water emulsion, the adsorption process forms a barrier that encircles the dispersed droplets. Reducing interfacial tension stabilizes the emulsion by causing the droplets to make less physical contact with one another and to be less likely to coalesce [(25)] .

Co-surfactants are typically composed of tiny molecules, such as short glycols and alcohols (like ethanol) with low molecular weight (C2-C10 long). By being absorbed into the interfacial cover of nanoemulsion droplets, co-surfactant molecules' small size enables them to permeate the surfactant film, decreasing fluidity while giving the film enough flexibility to accommodate the curvature required for the creation of fine transparent films [(26)] .

Characterization of pimecrolimus nanoemulsion

Visual transparency

Each prepared NE formulation had a clear optical appearance. This transparency could be caused by the small size of NEs droplets, which gave weak light scatters and demonstrated the effectiveness of the used technique[(27)].

Determination of the zeta potential, polydispersity index, and droplet size of nanoemulsion

Table 2 displays the formulated NE's zeta potential, PDI, and droplet size average. The quantity of Smix and the concentration of the dispersed phase in addition to the process variables such as the kind of energy input and emulsification duration, all have an impact on the NEs' droplet size[(28)]. The prepared NE formulas had an average droplet size of between 45.1 and 191.1 nm. The findings indicated that the formation of a film around dispersed globules was significantly influenced by the amount of Smix (using a 1:2 ratio of Tween 80: ethanol and maintaining a 5% oil phase). As the concentration of Smix increased, the droplet sizes (F1-F4) and (F6-F8) decreased non-significantly ($p>0.05$). Where in F1 in which the Smix percentage was 30% (droplet size 178.3nm), F2 with percentage 40% (droplet size 174.6nm) and F3 with Smix percentage 50% (droplet size 169.2nm) , while F4 with Smix percentage 60% (droplet size 166.7nm) .

The same results obtained in dutasteride nanoemulsion [(29)] .Upon further increase in Smix as in F5(70%) the droplet size increased (191.1nm), due to the additional free surfactant accumulated in the external phase [(30)] . The same results observed upon increasing Smix percentage (keeping oil phase at 10%) where the droplet size decreased in (F6-F10) similar to that observed for oil phase 5%. The same finding was observed with lidocaine NE, where variations in the amount of oil and surfactant influenced the surfactant's penetration into its hydrophobic zone, which in turn affected the surface curvature and droplet size[(31)].Droplet size decreased from 178 nm to 114.9 nm, respectively, when the oil phase (benzyl alcohol) increased from 5% in F1 to 10% in F6. This decrease was significant statistically ($p<0.05$). As the oil concentration was raised from 5% to 10%, the specific surface area increased and the droplet size decreased because the cavitation threshold was lowered along with the cavitating medium's viscosity, which further reduced the dispersed phase droplets[(32)] .

The same was observed in ketoprofen nanoemulsion[(23)].Increasing the time of sonication more than 10 min led to significant ($p<0.05$) in droplet size, as in formula F11prepared with sonication time of 20 min had droplet size of 147.8nm and F12 prepared with sonication time of 30min had droplet size of 147.4nm in comparison with F9 (contain the same amount of the oil phase and the Smix) prepared with sonication time of 10 min had a droplet size of 58.9nm. This is because every time a droplet forms from the breakage of an original droplet, then a new interface could be created. Due to the shorter collision timescale than adsorption timescale, As a result, some of the newly formed droplets receives insufficient surfactant covering, which causes coalescence[(33)].The PDI of the prepared formulas ranged between (0.386-0.601) which is in the acceptable range (less than 0.7) indicating that the nanoemulsion droplets have uniform distribution,

homogeneity and high quality[34]. Similar to the results observed with ondansetron NE[9].

The zeta potential for the formulas (F1-F12) ranged between (-0.113 to -1.78). Theoretically, surface charge development shouldn't occur when non-ionic surfactants like Spans and Tweens are used to make nanoemulsions. However, a small negative charge is frequently noticed, which may be related to ionic pollutants. Furthermore, contribution to stability of nanoemulsions, the presence of more hydrogen bonds formed between the polar polyol groups' proton acceptors and the hydrogen atoms on the aqueous medium's surface. Furthermore, the stability of the nanoemulsion containing a non-ionic surfactant was improved by a steric effect brought on by the hydrophobic interactions between the hydrocarbon tails and the oil phase[35]. Figure 4 illustrate peak charts for (A) particle size and (B) zeta potential.

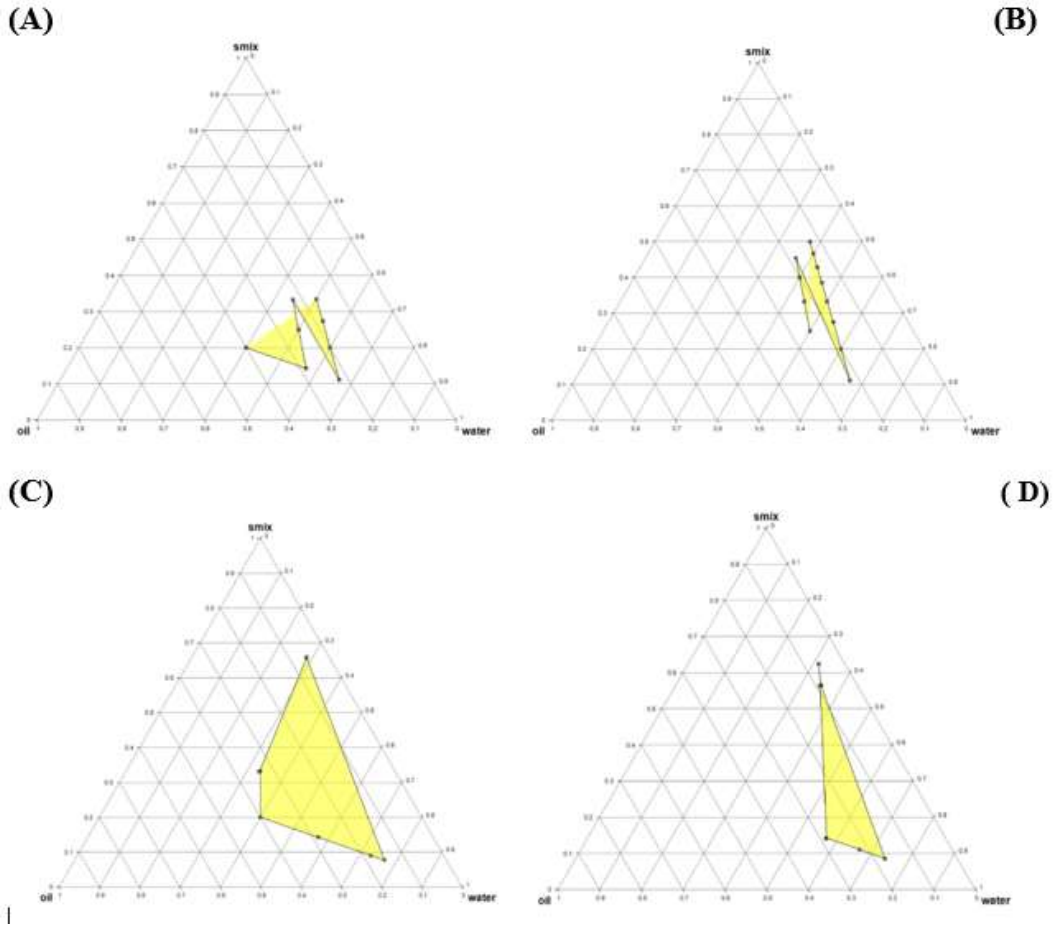
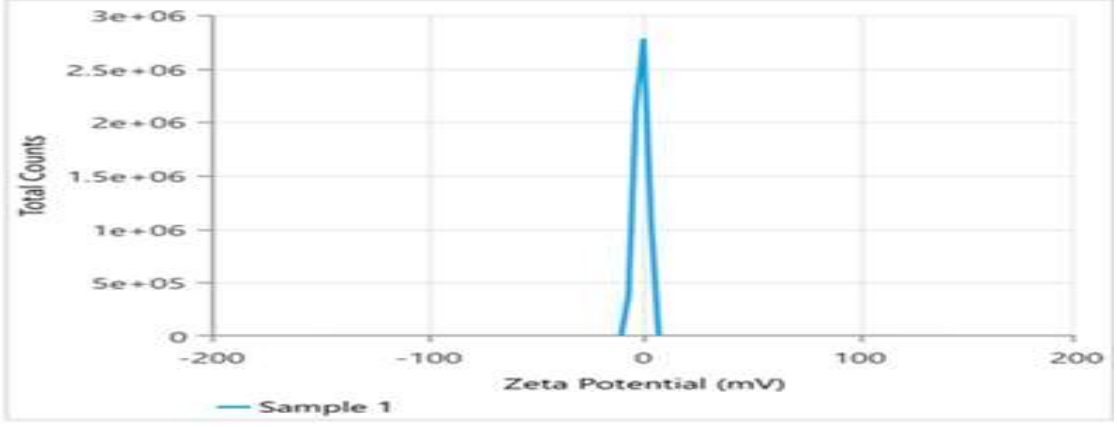


Figure 3. Triangular coordinate oil-water emulsion diagram with different Smix (tween80: ethanol) ratios (A) 2:1, (B) 1:1, (C) 1:2, (D) 1:3 using an oil phase (benzyl alcohol).



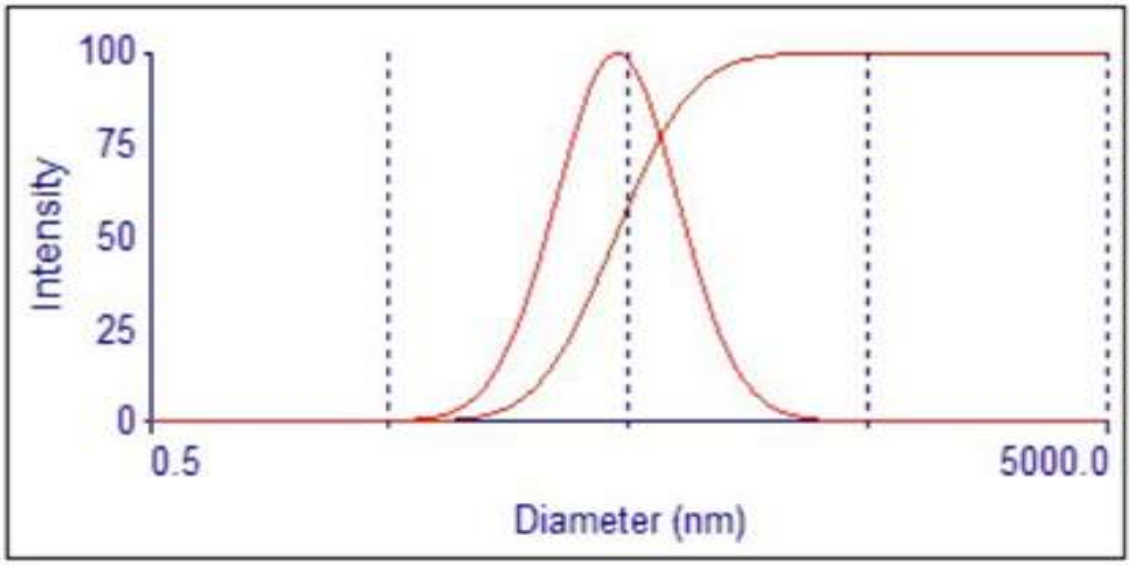


Figure 4. peak charts of (A) particle size and (B) zeta potential.

Drug content in the preparation of NE formulas

The drug content results are displayed in Table 5. A high value (96.7% or above) was seen in all of the NE formulations that were created, demonstrating that the components used, their concentrations and ratios, and the method used were all suitable[14].

Dye solubilization test

Water serves as the external phase, and since every NE formulation yielded an orange colour, the NE is of the o/w type[36].

pH measurement

The nanoemulsion formulations that prepared were found to have pH values ranging from 6 to 6.7, which is acceptable for skin preparations (Table 6). This means that the formulations were suitable for topical application and did not irritate the skin due to their pH value[37].

Dispersity test (dilution test)

The findings demonstrated that all 12 formulations exhibited clear NE without any precipitation or cracking in less than a minute following the addition of water (continuous phase), implying the stability of the prepared NE formulations [38].

Table 5. The mean droplet size, zeta potential, PDI, and drug content of the prepared pimecrolimus NE

Formulas code	Droplet size ±SD	PDI ±SD	Zeta potential ±SD	Drug content% ±SD
F1	178.3±10.0	0.537±0.049	-1.78±0.14	97.23±6.3
F2	174.6±11.2	0.477±0.042	-1.358±0.11	98.16±7.5
F3	169.2±8.7	0.544±0.05	-1.309±0.09	97.52±8.4
F4	166.7±9.3	0.502±0.048	-0.7879±0.072	98.19±6.5
F5	191.1±12.4	0.568±0.051	-0.6±0.04	98.24±8.0
F6	114.9±7.5	0.02±0.001	-0.5735±0.04	96.49±7.3
F7	106.2±6.3	0.601±0.056	-0.7662±0.06	98.21±6.4
F8	45.1±2.2	0.402±0.034	-0.8002±0.065	99.45 ±8.7
F9	58.9±3.1	0.44±0.040	-0.6242±0.054	99.17±7.5
F10	99.5±4.6	0.385±0.029	-0.113±0.0097	98.27±7.9
F11	147.8±6.0	0.485±0.042	-0.7268±0.068	98.14±6.8
F12	147.4±3.2	0.462±0.037	-0.8246±0.072	98.36±5.0

Viscosity

Table 3 showed that formulas F1–F5 (with a 5% oil phase) viewed a statistically significant ($p<0.05$) increase in viscosity when the amount of water was decreased because the relative dynamic viscosity of the emulsion depends on the concentration of water in the water phase[39]. When the oil phase percentage is 10%, as in formulas F6–F10, the viscosity increase was non-significant ($p>0.05$). The formulas containing 10% oil phase showed lower viscosity than those containing 5% oil because of the low viscosity of benzyl alcohol (oil phase) which was reported to be 5.555 mPa.s.[40].

Table 6. pH value and viscosity for the prepared pimecrolimus NE

Formula code	pH value ±SD	Viscosity mPa.s. ±SD
F1	6.05±0.5	19.8±1.7
F2	6.23±0.60	19.5±1.8
F3	6.03±0.54	31.5±2.4
F4	6.15±0.49	30.79±2.8
F5	6±0.51	32.7±3.0
F6	6.12±0.53	18.3±1.4
F7	6.3±0.59	26.17±2.3
F8	6.3±0.52	26.3±2.1
F9	6.4±0.60	24.02±1.9
F10	6.7±0.57	23.9±2.0
F11	6.31±0.61	21.4±1.7
F12	6.28±0.58	22.79±2.17

Physical stability studies of nanoemulsion

Physical stability study revealed that none of the NE formulas was separated or creamed during the various stress tests, which include centrifugation, heating/cooling, and freeze/thaw cycles[16].

In-vitro release study

The purpose of the in-vitro release study was to check the drug release from the prepared formulas (F1–F12) to that of the commercial product, pimecrolimus cream (elidel)®, over a 24-hour period (figure 4).Concerning the Smix percent, it was observed that, F1 containing Smix 30% and(oil phase of 5%) had a % release of 94.1% within 24 h while F5 containing Smix of 70% and (oil phase of 5%) showed slight increase in %release (96.3%) , the same was observed with F6 with Smix % of 30% and(oil phase 10%) gave a release of 97.9% while F10 with Smix of 70% and(oil phase of 10%) had slight increase in drug release (98.4%) within 24 h , because the amount of co-surfactant increased as the Smix increase and the co-surfactant reduced interfacial tension and increased interface fluidity, which increased drug release because the droplets had a high surface to volume ratio and could solubilize the drug effectively. A similar discovery was made regarding the self-emulsifying artemether drug delivery system[41].

The formulas F6–F10, which contain 10% oil phase, had a slightly higher release than the formulas F1–F5, which contain 5% oil phase. This may be explained by the fact that (F6–F10) had smaller droplet sizes, which increased surface area and facilitated the drug dissolution and more extensive diffusion through the dialysis membrane [42], where for example F8 which contain 10% oil phase (size 45.1 nm) had a %release of 76.3% in 5 h and 96.6% within 24 h while F1containing 5% oil phase (size 178.3nm) had % release of 88.7 within 24h . Similar results observed with eugenol NE[43]. Formulas F9, F11, F12 containing the same Smix % and oil phase % with sonication time 10min, 20min and 30min respectively, where F9 had a drug release% of 98.8%within 24 h slightly higher than F11 and F12 which had a release percent of 92.02% and 83.2%. This was due to the larger droplet size of F11 and F12 as compared to F9.

The commercial marketed pimecrolimus cream showed significantly ($p<0.05$) lower drug release of 26.2% in5 h and 42.5% in 24 h than all the prepared NEs , indicating the efficiency of NE in enhancing the solubility of the water insoluble drug (pimecrolimus) , as well as the fact that their tiny globule size eventually results in a larger surface area, allowing for a faster rate of drug release and better drug penetration[22].

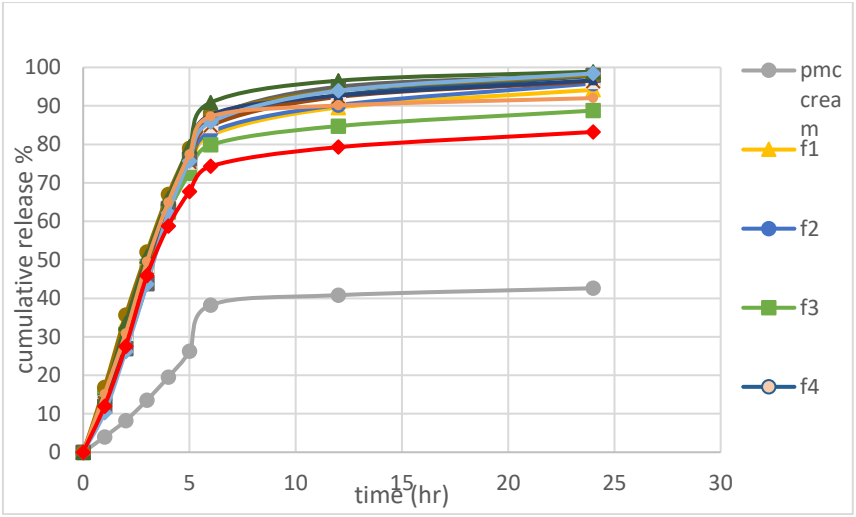


Figure 4. Drug release profile of pimecrolimus from the prepared nanoemulsion formulas (F1-F12) in comparison to pimecrolimus commercial pmc cream (elidel®) using phosphate buffer pH 7.4 as a dissolution medium.

Selection of optimum NE formula

The results indicated that the optimal pimecrolimus nanoemulsion formula was F8. As its % drug content of 99.45%, appropriate pH of 6.3, small droplet size of 45.1 nm, and PDI of 0.402. A high in- vitro release profile (96.9% release in 24 hours) was also demonstrated.

Field emission scanning electron microscopy (FE SEM)

The best nanoemulsion formula's morphology F8 was characterized using field emission transmission electron microscopy. The images show globules, spherical in shape with small droplet size. As shown in figure 5.

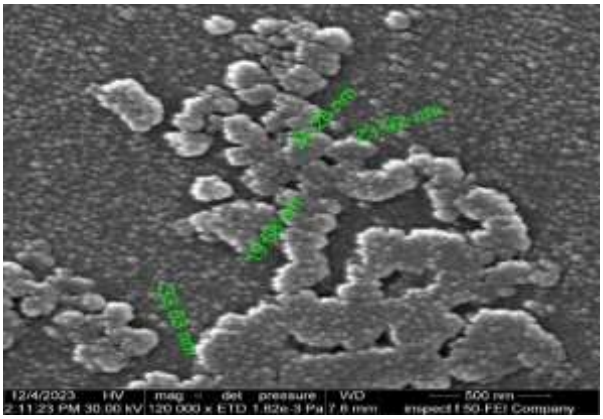


Figure 5. FE SEM for formula 8

Conclusion

Pimecrolimus loaded o/w nanoemulsion was successfully developed by high energy emulsification method with droplet size (45.1nm) and suiTable physical properties. Because of its small droplet size and low viscosity, it released drug approximately 2.5 times faster than commercial cream. Consequently, the generated NE shows characteristics of a nanocarrier that may aid in improving solubility and permeability, which may enhance its therapeutic efficacy when applied topically to treat dermatitis and eczema.

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Conflict of Interest

In regards to this work, the author would like to clarify that there are no conflicts of interest.

Author Contributions

Sara A. Challoob: contributed to data gathering, analysis, practical (follow the procedure) and written parts of the study. Nidhal K. Maraie gave final approval and agreement for all aspects of the study, supervision, revision, and rearrangement.

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تحضير و تقييم مستحلب البميكروليمس النانوي كعلاج موضعي

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الخلاصه

احد الاساليب المبتكرة لتوصيل الادوية موضعيا هي تقنية المستحلب النانوي و ذلك لسهولة تصنيعها و استقرارها على مدى فترة طويلة . بميكروليمس هو دواء قليل الذوبانية في الماء يستخدم لعلاج الصدفية ,الاكزيما , التهاب الجلد و البهاق . خلال هذا العمل يتم تحضير مستحلب البميكروليمس النانوي بواسطة تقنية عالية الطاقة (جهاز الموجات فوق الصوتية) .انشاء مخطط الطور الثلاثي الزائف و ذلك لمعرفة النسب المثلى للزيت و مساعدات الاستحلاب المستخدمة لتحضير المستحلب النانوي . تم استخدام الكحول البنزيلي كطور زيتي و توين٨٠ و الكحول الاثيلي كمساعدات الاستحلاب اعتمادا على ذوبانية الدواء في مختلف الزيوت و مساعدات الاستحلاب . تضمن العمل تحضير اثني عشر تركيبة مستحلب نانوي و اختبارها من ناحية الشفافية البصرية , حجم القطيرات ,مؤشر التشتت المتعدد , جهد زيتا , التخفيف,الاستقرارية , و تحرر الدواء من التركيبة. اظهرت تركيبة المستحلب النانوي المثلى حجم القطيرة, جهد زيتا ,كفاءة الانحباس ٤٥,١ نانومتر , ٠,٤٠٥ , ٠,٨ ملي فولت , ٩٢,٥٩٪ على التوالي و حررت افضل صيغة ٧٦,٣٪خلال ٥ ساعات و ٩٦,٦٪ من دواء البميكروليمس خلا٢٤ساعة مقارنة بالكريم التجاري الذي يحرر٢٦,٢٪ خلال ٥ ساعات و ٤٢,٥ % خلال ٢٤ ساعة مما يدل على الامكانات الواعدة للتركيبة المعدة كناقل نانوي فعال يعزز قابلية ذوبان الدواء و نفاذيته و بالتالي فعاليتة العلاجية .

الكلمات المفتاحية : مستحلب نانوي، بميكروليمس ، الكحول البنزيلي، توين ٨٠، الطور المعكوس لكروماتوغرافيا السائل ذو الضغط العالي .