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Research Article

Formulation and evaluation of retinyl palmitate and vitamin E nanoemulsion for skin care

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Abstract

Background: Improving and maintaining the skin integrity and health are the most essential targets in long-term care. The aim of this study is to formulate a serum of retinyl palmitate (RP) and vitamin E (VE) as nanoemulsion (NE) for achieving healthy skin.

Methods: The solubility of RP and VE was studied in different oils. The NEs were prepared using oil, water, and different surfactant-co-surfactant mixtures, and then the medicated nanoemulsion was prepared by addition of 0.5% RP and VE to the oil phase, and vitamin C as an antioxidant to the aqueous phase with different preservatives. The prepared NE was characterized in terms of particle size, charge, rheology, diffusion, and irritation to the skin.

Results and conclusion: The data showed that the highest solubility of both RP and VE was in safflower oil. Tween 20, Ceteareth 20 with ethanol, PEG 200, and cremophor RH40. This combination was able to produce NE with good integrity and acceptable particle size. The prepared formulations gave particle size of 60–70 nm and showed Newtonian flow. Irritation tests on rats showed that the formula was safe to be applied on the skin with no signs of irritation up to 72 hours. A preliminary stability study at room temperature showed good stability up to 6 weeks. In conclusion, RP and VE could be formulated successfully as NE with good stability and physical characteristics.

Keywords

Co-surfactant, Nanoemulsion, Retinol, Skin care, Surfactant, Tocopherol

Introduction

Our skin reflects our age and health status. Additionally, skin surface features such as tone, color, uniformity, and pigments are indicators of the wellness of human skin. Improving and maintaining the skin integrity and health are the most essential targets in long-term and acute skin care (Nakrem et al. 2009). Typical skin care routine is the washing of the skin followed by the administration of topical treatments to preserve and enhance the function and integrity of the skin (Lichterfeld et al 2015). Skin care products) pharmaceutical and cosmetic (are important in wellness and health care (Cowdell and Steventon 2015; Lichterfeld-Kottner et al. 2020).

Vitamin A is crucial for the health of the skin, both natural and synthetic types of vitamin A have been used pharmaceutically to treat a wide range of skin problems,

such as acne vulgaris, psoriasis, as well as photodamage (Booij and Van De Kerkhof 2011). Vitamin A serves as a necessary micronutrient that the body cannot produce on its own and must be obtained primarily through diet (Harrison 2012). The active forms of vitamin A are retinal, retinol (main type), and retinoic acid, which contain an end group of aldehyde, alcohol, and carboxylic acid respectively (Polcz and Barbul 2019). The structure of retinol is shown in Fig. 1.

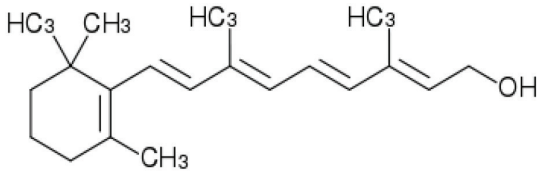

Figure 1. The structure of retinol (Butnariu 2016).

Tocopherol, often known as vitamin E, is a fat-soluble vitamin that serves as an antioxidant defending the cell membrane. Vitamin E cannot be produced by the body, and usually produced only by plants throughout their photosynthetic activities. Vitamin E needs to be ingested in limited amounts from diet or supplements (Colombo 2010). Vitamin E serves as a free radical (FR) scavenger, and it prevents oxidative stress of biological components (Colombo 2010). The most prevalent type of vitamin E found in skin care products seems to be the antioxidant alpha-tocopherol-acetate (Thiele and Ekanayake-Mudiyanselage 2007). Vitamin E is present as alpha-tocopherol or as tocopherol acetate in quantities varying between 1.0–5.0% (Zingg 2018). The combination of vitamins C and E is recommended to boost defense against erythema as well as sunburn, offering potential protection versus skin aging and skin cancer. Applying vitamin E to the skin prior to exposure to the sun can prevent the production of UVB-induced cyclobutane pyrimidine dimer (Butt et al. 2017). The structure of vitamin E is shown in Fig. 2.

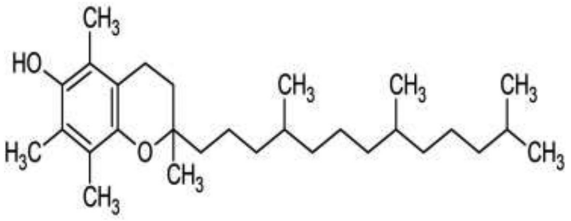

Figure 2. The structure of vitamin E (Colombo 2010).

The oil in water (O/W) NEs help improving the solubility of the lipophilic medications in the oil phase as well as the continuous phase (Klang et al. 2010; Kong et al. 2011; Zhou et al. 2009). Droplet sizes of nanoemulsions (NE) are approximately 100 nm. Water, oil, as well as an emulsifier are the common components of NEs (Mason et al. 2006; Abu Hijleh et al. 2021). NEs are created by the emulsification technique using low and high-energy approaches (Wang et al. 2008; Abu Hijleh et al. 2020). In comparison to typical topical medicines like ointments, gels, as well as creams, utilizing NEs as carriers might improve the transport of pharmaceuticals throughout the skin (El Maghraby et al. 2014; Mostafa Moslehi et al. 2014). Small droplets inside an NE make it possible to deliver active ingredients to the skin efficiently, and thus enhancing medication penetration (El Maghraby et al. 2014; Mostafa et al. 2014). The aim of this study is to produce a topical serum of retinol and vitamin E in the form of NE and the evaluation of this serum in terms of Active Pharmaceutical Ingredients (APIs) content, physical stability, diffusion, rheology and comparing the results with a marketed product.

Abbreviations: NE: nanoemulsions; RE: retinyl palmitate; VE: vitamin E; Vit C: vitamin C; FR: free radical; S_{mix}: Mixture of surfactant and co surfactant; PDI: Polydispersity index; PB: phosphate buffer, DDISS: Draize dermal irritation scoring system; APIs: Active Pharmaceutical Ingredients; RT: Retention Time; O/W: Oil in Water; SQRT: Square Root of Time; J: Flux.

Material and methods

Materials

Retinyl palmitate and alpha tocopherol was a purchased from Ambeed company (USA). Vitamin C (Ascorbic acid) was obtained from Omega-touch labs (USA). Safflower oil, Coconut oil, Jojoba oil, and Sesame oil were purchased from Now Foods (USA). Liquid paraffin was obtained from Gold Cross (Australia). Tween 20, Tween 60, Tween 80, and Ceteareth 20 were purchased from Janssen (USA). Cremophor RH40 from BBC chemical for lab (Germany). All other organic solvents or materials (Chloroform, methanol, and acetonitrile) were of HPLC, analytical or pharmaceutical grades.

Animals

Three healthy male albino Wistar rats weighing around (250±15g) were housed and acclimatized at the Laboratory Animal Research Unit at the Applied Science University. Each rat was individually housed in a cage and maintained under controlled conditions of temperature (20±3 °C), humidity (50±15%), and photoperiod cycles (12 light/12 h dark) with a conventional laboratory diet and unrestricted supply of drinking water. The Ethical approval was obtained from the Al-Ahliyya Amman University ethical committee (Decision no. AUP: AAU 1/2/2022-2023) which gained the approval of the Applied Science University.

Methods

Analytical methods

Chromatographic separation and quantitative analysis of RP and VE was performed on a Thermo Finnigan Surveyor HPLC system (Germany). The column used was a Hypersil Thermo Electron Corporation, C18 250 × 4.6, mm, 5µm (Thermo Fisher Scientific/Germany). The mobile phase was composed of Acetonitrile: Methanol

(70:30 v/v). The mobile phase was filtered through 0.45 µm nylon filter and degassed by an ultrasonic water bath (Model UCB 100, Spectralab). The flow rate was 1.0 mL/min and the column was maintained at ambient temperature. The injection volume of 10 µL was used, and detection wavelength was 220 nm.

Retinyl palmitate (RP) and vitamin E (VE) standard stock solutions were prepared by accurately weighing 10 mg of RP and 25 mg of VE and transferring them into 100 ml volumetric flasks. Then 70ml of mobile phase was added and sonicated for 15 minutes to solubilize RP and VE. The solutions were diluted with the mobile phase to give a final concentration of 0.1 mg/mL and 0.25 mg/mL for RP and VE respectively. Standard solutions were prepared by diluting stock solutions and injecting them into the HPLC system. The experiments were performed in triplicates and the average was calculated.

Solubility of retinyl palmitate and vitamin E

The solubility of RP and VE was measured in different types of oils to ensure the therapeutic concentration, and to choose the proper oily phase in the formulated emulsion. The solubility was tested in safflower oil, jojoba oil, coconut oil, olive oil, sesame oil, and liquid paraffin. Two mL of oil was added in a screw cap test tube and an excess of RP was added in each tube, shaken for few minutes on the vortex, then left to equilibrate in a water bath with a shaker at 25 rpm for 48 hours. Then, the samples were centrifuged at 3000 rpm and 0.5 ml of the supernatant was suitably diluted by the mobile phase and measured by the HPLC. The same procedure was repeated for VE. Measurements were taken in triplicate and the results were expressed as solubility in mg/g oil at ambient temperature.

Formulation of the Nanoemulsion (NE)

Nanoemulsion (NE) was prepared using the suitable oily phase from the solubility study. Several formulations of nanoemulsion contain different ratios of oily phase, aqueous phase, and different ratios of the surfactant (Tween 20, Tween 60, Tween 80, Ceteareth 20, and cremophor RH40) and cosurfactant (ethanol and PEG 200) were prepared as shown in Table 1. The selection criteria of the best formula depended on the physical appearance (translucent), particle size (preferably less than 100 nm), and stability at room temperature.

Table 1. The composition and ratios of prepared (Smix)s.

Smix code	Type of surfactant	Type of co-surfactant	Ratio surfactant : Co-surfactant (w:w) in grams
Smix 1	Tween 20	Ethanol	1:1
Smix 2	Tween 20	Ethanol	1:2
Smix 3	Tween 60	Ethanol	1:1
Smix 4	Tween 80	Ethanol	1:1
Smix 5	Tween 80	Ethanol	1:2
Smix 6	Tween 80	Ethanol	1:3
Smix 7	Tween 80	PEG 200	1:1
Smix 8	Ceteareth 20	Ethanol	1:3
Smix 9	Ceteareth 20	PEG 200	1:1
Smix 10	Cremophor RH40	Ethanol	1:1
Smix 11	Cremophor RH40	PEG 200	1:1
Smix 12	Cremophor RH40	–	–

Preparation of surfactant and co surfactant mixture (Smix)

The mixture of surfactant and co-surfactant (Smix) s was prepared by mixing them in a beaker at 300 rpm for 15–20 min. When the surfactant was very viscous like tween 80 or waxy like ceteareth 20, it was heated to 40 °C until suitable viscosity achieved. Then the cosurfactant was added while the surfactant cooled down. Twelve samples of (Smix)s were prepared as shown in Table 1.

Preparation of the nanoemulsion (NE)

The NE was prepared by high energy method (sonication). The ratio of the oily phase, aqueous phase, and the (Smix)s was decided based on preliminary experiments. All prepared (Smix)s were tried in the formulation of NE that contained fixed portions of oil and aqueous phases (15–30% of oil and 40–65% of water). Ten formulae were prepared as shown in Table 2. The oil phase, Smix, and the aqueous phase were accurately weighed, then Smix was mixed with the oily phase, and the aqueous phase was added gradually with continuous stirring using a vortex mixer for a few minutes. The probe sonicator was inserted in the primary emulsion and turned on at a frequency of 3.0 per second. When the mixture turned translucent, the emulsion was formed. The prepared emulsions were evaluated visually and then left aside for a few hours before choosing the proper formula. Five grams from each formulation were prepared.

Table 2. The composition and ratios of the blank NE.

Formula Code	% Oil phase	% & Type of Smix	% Aqueous phase
F1	30	30 - Smix1	40
F2	25	25 - Smix4	50
F3	20	27 - Smix 5	53
F4	15	27 - Smix 7	58
F5	25	10 - Smix 8	65
F6	30	20 - Smix 9	50
F7	25	25 - Smix 10	50
F8	25	10 - Smix10	65
F9	25	10 - Smix11	65
F10	30	5- Smix 12	65

Preparation of the final formulation

Two selected formulae (FF1 & FF2) of the NE were prepared as shown in Table 3. The final formula contained Vitamin C (Vit C) as an antioxidant, methylparaben, propylparaben, and Germall plus as preservatives in addition to RP and VE. Twenty grams of each formula were prepared by dissolving RP, VE, and propylparaben (if added) in the oily phase. Then Smix was added to the oily phase and mixed thoroughly. Vit C, methylparaben, and Germall plus were dissolved in the aqueous phase. Then NE was prepared as described above.

Table 3. Composition of the final formulation of the Nanoemulsion (NE) serum.

Ingredients	% by weight in FF1 % (w/w)	% by weight in FF2 % (w/w)
Aqueous phase	57.45	57.5
Oily phase	30	30
Smix	10	10
Vit C	1	1
RP	0.5	0.5
VE	0.5	0.5
Germall plus	–	0.5
Methyl paraben	0.4	–
Propyl paraben	0.15	–
Total	100	100

Characterization of the prepared NE

The prepared NEs were characterized in terms of droplet size and charge, content of RP and VE, rheological behavior, and diffusion study.

Measurement of droplet size and charge

Droplet size and charge were measured by zeta sizer (Malvern / UK). 100 µL of the sample was diluted with distilled deionized water to one mL and placed in the instrument. All formulae that gave translucent NE were subjected to this test. Average droplet size and polydispersity index (PDI) were recorded. Three measurements were made and the average ±SD was calculated. Zeta potential was also measured and recorded as average ±SD.

Assay of RP and VE in the selected formulae (FF1 and FF2)

An assay of the prepared formula was made using the developed HPLC method. One gram of each formula was taken, and centrifuged at 3000 rpm to break the emulsion. 100 µL were taken from the oil phase, appropriately diluted with the mobile phase and measured for RP and VE concentration.

Rheological study

The viscosity and rheological behavior of the prepared serum were determined using a cone and plate viscometer (Anton Paar, Rheometer Germany GmbH, Model MCR 302). All measurements were carried out at a temperature of 25±1 °C, using spindle Cp 50. The formula was loaded between the concentric cylinders in a volume of 5–10 ml to ensure accurate results. To assure accuracy, the rheometer was calibrated and programmed via a computer controlled RheoCompass software (Anton Paar). The flow curve is constructed by plotting shear rate (1/s) versus shear stress (in Pascals) as well as the viscosity (in mPascas) versus temperature (in Celsius) curve. Ebanel/ Vitamin C serum (USA) was used as reference for the rheological properties. The same procedure was repeated for the reference serum.

Diffusion study

The diffusion study was performed using the Franz Diffusion Cell model (SES GmbH-Analyse systeme/Fridhofstr 7-9D 55234 Bechenheim/Germany). The membrane used was dialysis tubing (Medical International Ltd/ M wt. 12–14000 daltons), with a volume of donner compartment = 12 mL, diffusion media was phosphate buffer (PB) (potassium dihydrogen phosphate buffer pH 7.0), and cross-sectional area of diffusion = 1.7 cm^2. To ensure sink conditions, 100 mg of each of RP and VE were added to 10 mL phosphate buffer pH 7.0 with 5% Tween 80, then placed in a shaking water bath for 24 hours at 26 °C. The next day the samples were centrifuged, and the aqueous phase was analyzed for both compounds. Samples were applied to the membrane and readings were taken at 0.5, 1, 2, 3, 4, 5, 6, 7, 8, and 24 hours and the volume was replaced by fresh media to keep the same sink conditions. The test was performed on the final formula (FF2) whereby 200 mg sample which theoretically contained 1 mg of each RP and VE was put in each of the 6 cells and average readings were calculated. Due to the sensitivity of RP to the light, the whole instrument was covered with foil and turning off all lights and shutting down the curtains of the Lab.

Skin irritation/corrosive potential test

Three healthy male albino Wistar rats (250±15g) were used for assessment of skin irritation/corrosive potential and the reversibility of dermal effects of a topical preparation of RP and VE (FF2). The rats numbered as follows: 1 a negative control with no treatment, 2 a positive control given a blank formula without RP and VE, and 3 received the formula FF2. The presented test was conducted according to the OECD Guidelines for Testing of Chemicals, adopting Guideline 404 for Acute Dermal Irritation/ Corrosion (OECD 2002). Before the experiment, fur was removed by closely clipping the dorsal area of the trunk of the rats using an electric clipper while having the rat restrained humanely, then the application of the formulation was performed as described by the guidelines. Table 4 shows the Draiz system guidelines of evaluation.

Table 4. Draize dermal irritation scoring system (DDISS).

Erythema and Eschar Formation	Value	Edema Formation	Value
No erythema	0	No edema	0
Very slight erythema (barely perceptible)	1	Very slight edema (barely perceptible)	1
Well-defined erythema	2	Slight edema (edges of area well defined by definite raising)	2
Moderate to severe erythema	3	Moderate edema (raised approximately 1 mm)	3

Preliminary stability study at room temperature

A preliminary stability study was conducted on formula FF2 to determine its physical stability and concentration of RP and VE at room temperature. FF2 was prepared and put in amber test tubes and wrapped with aluminum foil and left on

the bench in the Lab where the temperature was around 24–26 °C during the day. Samples were taken at time zero and examined after 1, 2, 3 and 4 weeks, for physical appearance of the NE as well as estimation of RP and VE content. Particle size and zeta potential were also measured after 4 weeks.

Statistical analysis

All statistical analysis results were performed using Microsoft Excel. The results were presented as mean ± SD.

Results and discussion

Method of analysis of Retinyl palmitate (RP) and vitamin E (VE)

HPLC analysis has been used to determine the amount of RP and VE in the formulation. Fig. 3 shows one chromatogram of RP and VE and the clear separation of the two APIs. It also shows very clear sharp peaks obtained of RP and VE at RT = 7.8 min and 5.5 min respectively.

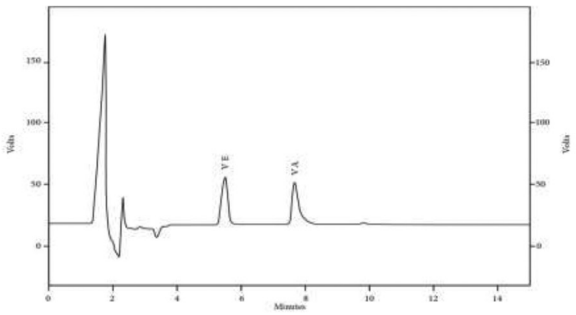

Figure 3. A Chromatogram showing the separated peaks of RP and VE and their retention times from the linearity test (conc. = 4 µg/ml for RP and 20 µg/ml for VE).

Solubility study of retinol palmitate (RP) and vitamin E (VE)

The solubility of RP and VE is a crucial step in the choice of the oil phase of the emulsion. Both APIs are hydrophobic materials, intended to be solubilized in the oil phase of the NE. The target percentage of the proposed formulation is 0.5% w/w for both. Results illustrated in Fig. 4 show that the highest solubility of RP was achieved in Safflower oil (190.83 mg/g) while the lowest was in sesame oil (30.66 mg/g). For VE, results revealed that the highest solubility was obtained in safflower oil (298.00 mg/g and the lowest of 79.48 mg/g in coconut oil). Therefore, it was decided that safflower oil is the oil phase of the proposed NE where the solubility of both RP and VE covers the required concentration that is 0.5% w/w for both.

Formulation of the Nanoemulsion (NE)

The NE was successfully prepared, and it was described as "translucent". The milky color emulsion was considered as "failed preparation" because its particle size would be large in microns to give the white color. Table 5 shows that the ratio of oil in the final preparation was 25–30% and the aqueous phase 40–60%. Tween 80 with co-surfactant (Smix1), Ceteareth 20 with co-surfactant PEG200 (Smix 9), Cremophor RH40 with co-surfactant ethanol or PEG (Smix 10 and 11) and alone (5%) were all successful in preparation of the NE in terms of visual evaluation as reported in some studies in the preparation of NE (Gurpret and Singh 2018). In this formula, no successful preparation was achieved using Tween 60 as well as Tween 80.

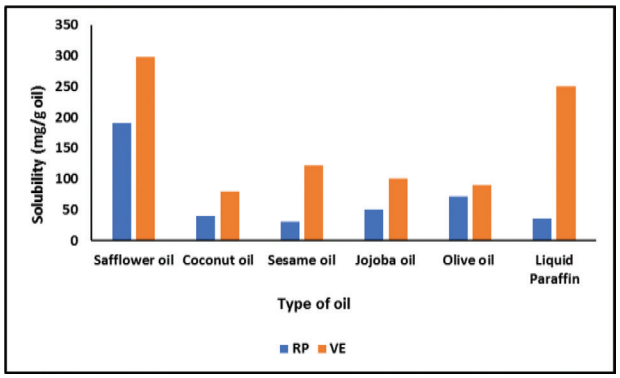

Figure 4. Histogram showing results of solubility study of RP and VE (in mg/g) in different oil phases (Safflower, coconut, sesame, jojoba oil, olive oil and liquid paraffin).

Table 5. Result of NE preparation of the ten proposed formulas.

Formula Code	Percent of oil phase	Percent and type of Smix	Percent of aqueous phase	Result (visual)
F1	30	30- Smix1	40	Translucent
F2	25	25- Smix4	50	White milky
F3	20	27- Smix 5	53	White milky
F4	15	27- Smix 7	58	White milky
F5	25	10- Smix 8	65	White milky
F6	30	20- Smix 9	50	Translucent
F7	25	25- Smix 10	50	Translucent
F8	25	5- Smix10	70	Translucent
F9	30	5- Smix11	65	Translucent
F10	30	5- Cremophor	65	Translucent

Characterization of NE

Particle size measurement and Zeta potential

The particle size of O/W NE is a function of the oil phase to water phase ratio, type, and concentration of surfactant, type, and concentration of co-surfactant (if added), and the preparation method (Uchechi et al. 2014).

The results of measuring particle size are illustrated in Table 6. It was investigated that the major factors affecting the droplet size are composition of the emulsion (ratio of the oil phase to aqueous phase), the type and concentration of the emulsifying agent. Since the preparation was intended for skin care, and the active materials (VA and VE) are needed to be absorbed, that's why small droplets were needed but not less than 20 nm. The evaluation of

Table 6. Particle size measurement of the prepared NE.

Formula Code	Percent oil phase	Percent and type of Smix	Percent Aqueous phase	Particle size±SD) (nm)	PDI
F1	30	30- Smix1	40	90.2±5	0.68
F6	30	20- Smix 9	50	130.5±7	0.8
F7	25	25- Smix 10	50	180±5	0.63
F8	25	10- Smix10	55	65.6±19	0.4
F9	25	10- Smix11	65	76.3±5	0.52
F10	30	5- Cremophor RH40	65	70.5±2.5	0.41

the best combination was made based on the smallest droplet size obtained and the smallest PDI, which reflect homogeneity of the NE. Bases on the solubility study, the volume of oil phase 25–30%, was enough to load the required dose of both VA and VE. Since all formulas were prepared using the safflower oil, the ratio of oil phase, type of surfactant and co-surfactant were the major factors affecting the particle size. Oil phase less than 25% was not enough to stabilize the emulsion and increasing the ratio of co-surfactant had no effect in decreasing the particle size. Cremophor RH40 without co-surfactant was also able to form NE. Based on the results of a particle size measurement, F8 which contained 25% safflower oil, 65% aqueous phase and 10% cremophor RH40-ethanol (1:1) was chosen to formulate the medicated NE.

Two medicated formulations were prepared from F8 named "FF1" which contained the paraben as a preservative and "FF2" that contained Germall plus as preservative in addition to the RP, VE and vitamin C were prepared as shown in Table 3. Germall plus is natural broad spectrum preservative that is compatible with most cosmetic products. Parabens have the advantage of mixing the hydrophobic ones with the oil phase and the hydrophilic with the aqueous phase. It was decided to give option according to the availability and cost of the final preparation for industrial purposes.

Particle size was measured in both formulas, FF1 gave a particle size of 50.3±5 nm with PDI of 0.32 and FF2 gave particle size of 52±3.6 nm with PDI of 0.34. These particle size values are not significant from F8 (before addition of APIs ($p>0.05$) .Small particle size would help in the good absorption and penetration of the APIs to the skin. Arianto & Cindy prepared sunscreen NE composed of sunflower oil as an oily phase and Tween 80 as a surfactant. The evaluation showed that the prepared formulation had an average particle size of 124.47 nm with yellowish color, a clear, transparent appearance (Arianto, and Cindy 2019). Uchechi et al. reported that nanoparticles ranging 50–500 nm are suitable for dermal and transdermal delivery of APIs. Very small of 1–20 nm might be cleared quickly and particles > 1 micron are retained on the surface of the skin (Uchechi et al. 2014). FF1 and FF2 have droplet size slightly larger than 50 nm which is suitable for topical absorption to the underlying skin layer. Zeta potential of FF1 and FF2 were +0.917 mV and +0.995 mV respectively. These were small values that might create stability problems. The small zeta potential is expected since the NE was prepared using a non-ionic surfactant. It could be increased by using a buffer with higher ionic strength as an aqueous phase.

Assay of RP and VE in FF1 and FF2

Estimation of RP and VE in the final preparation showed that percent RP content was 97.3± 0.5% and 96.6±10% for FF1 and that of FF2 was 97.9±0.8 and 98.8±0.5 for RP and VE respectively.

Rheological study

The rheological study aimed to investigate the rheological properties of the formulation. This gives an idea about filling the formula in suitable containers, pourability, spreadability on the skin, and the behavior during storage. Both FF1 and FF2 in addition to the reference product (Ebanel Vitamin C serum, USA) produced a linear relationship between shear rate and shear stress, which means that they follow the Newtonian system and the reference product showed higher shear stress needed at the same shear rate values used for FF1 and FF2. The viscosity of FF1and FF2 decreased with increased temperature logarithmically. The viscosity of FF1 at 32 °C was 3.66 mPas which seems to be suitable as the final dosage form is like a

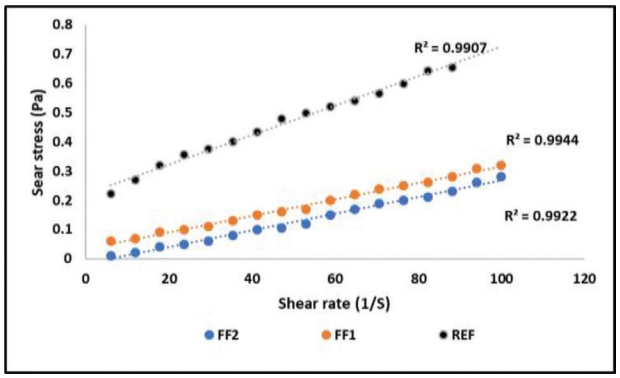

Figure 5. Shear rate – shear stress plot of FF1, FF2 and reference product (Vit C serum from Ebanel/USA) showed linearity of Newtonian flow.

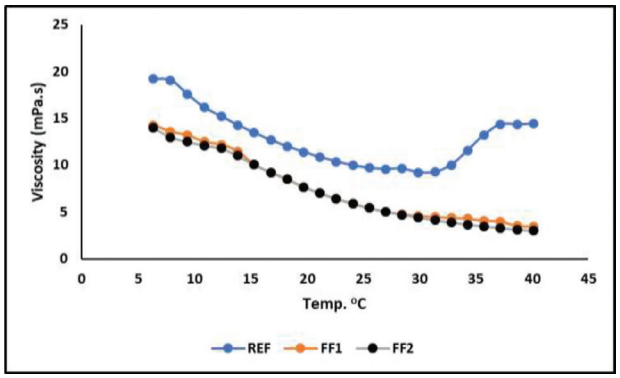

Figure 6. Viscosity – temperature plot of FF1, FF2 and reference product (Ebanel Vit C serum).

thin serum, and spreads on the face easily. FF2 gave a very close value of 4.1 mPas since it has almost the same composition except for small difference that did not affect the rheological behavior. The reference product showed also Newtonian flow and measured viscosity of 10.0 mPa.s at 32 °C, which was higher than the prepared FF1 and FF2. Viscosity decreased with temperatures until 30 °C then started to elevate slightly at higher temperature possibly due to the inclusion of other thickeners and components. Results are shown in Figs 5, 6.

Diffusion study

The aim of our formulation is to offer a good absorption of both RP and VE into the human skin, and a diffusion study helps in the prediction of successful preparation. Results showed that in the presence of Tween 80, the solubility of RP was equal to 1 mg/mL of RP and 1.265 mg/mL for VE. A solubility of approximately 250 µg/mL for RP and VE could be enough to ensure the sink condition. In this test, 4 times the required solubility was achieved for RP and about 5 times for VE than the required limit which ensured sink condition.

According to the guidelines, the highest concentration of API should not exceed 10–30% of its maximum solubility. The diffusion study used phosphate buffer pH 7.0 with 5% Tween 80 as a solubilizer. Following analysis, the amount of drug released was calculated at each time point. The flux (J) was calculated by dividing the amount released in micrograms by the surface area of the membrane (1.7 cm^2). Then, Flux was plotted versus the square root of time (SQRT); see Fig. 7. The samples were taken up to 24 hours, but results were presented up to 6 hours as per the guidelines.

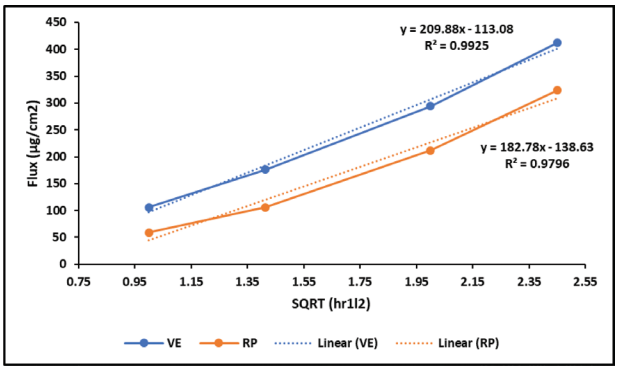

Figure 7. Flux versus square root of time plot of VE and RP from the FF1 (time = 6 hrs).

The flux versus time in hours plot for 6 hours showed linearity (0.99) for both RP and VE, the slope represents the "rate of diffusion" in (µg/cm^2/hr). The rate of diffusion of RP from the formula was equal to 51.3 µg/cm^2/hr and that of VE was equal to 62.5 µg/cm^2/hr. This difference might be attributed to the higher solubility of VE in the receiving media. The total amount of RP released in 24 hours was 66.6% of the total dose and that of VE 80% of the total dose during 24 hr and 36.6% RP and 40% VE during the first 6 hours.

Irritation study

Interpretation of skin irritation/corrosive potential relied on Draize's Dermal Irritation Scoring model (Hemmati et al. 2016) as described in Table 7. This response was observed throughout all dermal testing.

Table 7. Dermal responses observed in individual rats.

Erythema					
Wistar Rat (1) control, Rat (2) test	**Evaluation after removal of test substance**				
	0 minutes	**60 minutes**	**24 hours**	**48 hours**	**72 hours**
(1) Control	0	0	0	0	0
(2)Test	0	0	0	0	0
Edema					
Wistar Rat (1) control. Rat (2) test	**Evaluation after removal of test substance**				
	0 minutes	**60 minutes**	**24 hours**	**48 hours**	**72 hours**
(1) Control	0	0	0	0	0
(2) Test	0	0	0	0	0

Results showed no signs of irritation, sensitivity, erythema or redness. Fig. 8 shows the status of rats in the test. The application of the formula on the rats' skin as a model for human skin showed no signs of irritation in the tested rat used in the experiment for 72 hours. The positive control which contains the formula without RP and VE gave the same results which indicated that the NE with its composition and preservative system is also safe on the skin. This indicates that the prepared formula FF1 could be safely applied on the skin regarding irritation risk.

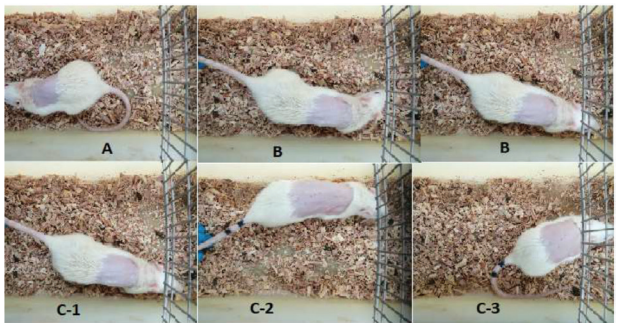

Figure 8. Results of irritation test. (**A**) Negative control with no treatment. (**B**) Blank formula without RP and VE and (C-1) Formula immediately after application, (C-2) after 1 hr, and (C-3) after 24 hours.

Preliminary stability test at room temperature

For 45 days, FF2 was stored at room temperature. Four readings were taken for the assay, particle size and zeta potential and physical appearance. Results are shown in Table 8, which shows that the prepared NE is stable for the tested time and conditions.

Table 8. Results of preliminary stability study at room temperature of FF2.

Time	Assay RP (%)	Assay VE (%)	Particle size (nm)	Zeta potential (mV)	Physical appearance
Zero	98.3±1.5	99.5 ±0.9	50 ± 8	0.991	Translucent, clear, plasma color
1 week	99.3 ±0.98	98.6 ±1.6	–	–	No change
Two weeks	98.1± 0.5	99.5 ±0.6	–	–	No change
Four weeks	97.5 ±1.6	97.6 ±1.2	–	–	No change
Six weeks	97.6 ± 2.1	97.3 ± 0.7	53 ± 5	0.971	No change

Conclusions

Retinol palmitate and Vitamin E are essential for improving and maintaining the skin health and integrity. The NE was prepared using oil and aqueous phase with different surfactant co-surfactant mixtures, followed by the addition of both RP and VE to the oily phase. Vitamin C as an antioxidant is added to the aqueous phase with different preservatives. Results showed that the highest solubility of both RP and VE was in safflower oil which was used in the preparation of the NE in a ratio of 25–30%. Tween 20, Ceteareth 20 with ethanol and PEG 200, and cremophor RH40 were able to produce NEs with good integrity and acceptable particle size. The prepared formulations gave particle size of 60–70 nm and showed Newtonian flow. Irritation tests on rats showed that the formula was safe to be applied on the skin with no signs of irritation up to 72 hours. Diffusion study showed that the total amount of RP released in 24 hours was 66.6% of total dose and that of VE 80% of total dose during 24 hr and 36.6% RP and 40% VE during the first 6 hours. It can be concluded that retinol palmitate and vitamin E could be formulated successfully in a nanoemulsion with good stability and physical characteristics.

Author contributions

All authors contributed to the study conception and design. All authors contributed to the material preparation and data collection and writing the manuscript. All authors read and approved the final manuscript.

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