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Original article

RELEASE OF VITAMIN E FROM DIFFERENT TOPICAL COLLOIDAL DELIVERY SYSTEMS AND THEIR *IN VITRO-IN VIVO* EVALUATION

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Abstract

In this study, topically applicable o/w microemulsion (ME), o/w simple emulsion (SE) and solid lipid nanoparticle (SLN) cosmetic delivery systems were prepared containing vitamin E. Characterization of the systems was performed by various techniques, particle size and zeta potential measurement, X-ray diffraction, NMR, vitamin E quantification by validated HPLC method. Release properties of vitamin E from the formulations were determined in vitro using Franz diffusion cells with a synthetic membrane (0.22 µm). Antioxidant effects of the formulations prepared were investigated in vitro by 2,2-diphenyl-1-picrylhydrazyl (DPPH) test. Antioxidant efficacy was also tested in vivo by measuring net skin elasticity and skin roughness values. Vitamin E could be incorporated successfully into ME, SE and SLN delivery systems. The best long-term physical stability was obtained for the microemulsion system. As a result of skin efficacy tests, it can be concluded that SLN formulation containing vitamin E improves skin moisture while decreasing skin roughness ($p \leq 0.01$) indicating an antiaging efficacy. ME and SE formulations were also found to decrease skin roughness ($p \leq 0.001$).

Key words: *Vitamin E, Microemulsion, Emulsion, Solid lipid nanoparticle, In vivo evaluation on skin*

E Vitamininin Farklı Topik Kolloidal Taşıyıcı Sistemlerden Salımı ve *in vitro-in vivo* Değerlendirilmesi

Bu çalışmada, E vitamini yüklenmiş topik uygulanabilir y/s mikroemülsiyonu (ME), y/s basit emülsiyonu (SE) ve katı lipit nanopartikül (SLN) kozmetik taşıyıcı sistemleri hazırlanmıştır. Sistemlerin karakterizasyonu için partikül boyutu, zeta potansiyel ölçümü, X-ışını kırınımı, NMR gibi çeşitli yöntemler kullanılmış ve E vitamini miktar tayini valide edilmiş yüksek performanslı sıvı kromatografisi (YPSK) metodu kullanılarak yapılmıştır. E Vitamininin formülasyonlardan salımı sentetik membran (0.22 µm) ile Franz Difüzyon Hücreleri kullanılarak incelenmiştir. Formülasyonların antioksidan etkisi 2-2-difenil-1-pikrilhidrazil (DPPH) testi ile in vitro olarak belirlenmiştir. Antioksidan etki cilt esnekliği ve pürüzsüzlüğü değerlendirilerek in vivo olarak değerlendirilmiştir. Yapılan analizler sonucunda E vitamininin formülasyonlara başarı ile yüklendiği ve en iyi kararlılığın ME sisteminde olduğu gözlenmiştir. In vivo cilt etkinlik testlerine gore SLN formülasyonu 4 haftalık uygulama sonucunda cilt nemini arttırmakta ve antioksidan etkinin neticesi olarak pürüzlülüğü azaltmaktadır ($p \leq 0.01$). ME ve SE formülasyonlarının da cilt pürüzlülüğünü azalttığı gözlenmiştir ($p \leq 0.001$).

Anahtar kelimeler: *E vitamini, Mikroemülsiyon, Emülsiyon, Katı lipit nanopartikül, In vivo cilt değerlendirmesi*

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INTRODUCTION

Vitamin E is a chain-breaking antioxidant which prevents the propagation of free-radical reactions (1). It is a primary and reference antioxidant that interrupts autoxidation by reacting with lipid radicals as an electron donor and converts free radicals into more stable species (2). Vitamin E is known to have a skin antiaging effect due to its antioxidant property (3, 4). A well-known phenomenon in skin aging (photoaging) is the sun exposure (ultraviolet-UV and infrared-IR radiation) leading to the formation of free radicals (5).

The use of vitamin E in cosmetic products is limited due to its low stability. Aiming to solve the stability problem it was incorporated into ME, SE and SLN formulations in this study.

MEs are modern carrier systems which are single phase, transparent, optically isotropic and thermodinamically stable (6-9). When compared to SE, ME has a lower surface tension. They can easily penetrate into *Stratum corneum* with low or nearly zero surface tension (10, 11). They are attractive in cosmetic science because of their transparent character (12). They have a high solubilizing capacity and can increase the topical efficacy (13, 14).

Solid lipid nanoparticles (SLN), with a number of advantages for topical route of application can easily penetrate into *Stratum corneum* because of their small particles (15-20). Due to their solid matrix structure, sustained release and controlled drug delivery is possible (21). SLN systems can maintain the stability of labile compounds (22). Cosmetic benefits of empty SLNs include sunscreening and occlusive features leading to increased skin moisture (23-25).

A number of methods were described for the determination of vitamin E derivatives (26-28). In this study, a new validated HPLC method was developed for determining vitamin E.
The present study has a wide content of preparation, characterization, determining stability, *in vitro* antioxidant effect and *in vivo* skin evaluation of ME, SE and SLN systems containing vitamin E. The aim of this work is to develop topically applicable colloidal cosmetic formulations of vitamin E to maintain its stability.

EXPERIMENTAL

Materials

Glycerol behenate (Compritol ATO® 888) (Gattefosse, France), polyethylene glycol sorbitan monooleate (Tween® 80) (Sigma, USA), caprylic/capric triglyceride (Miglyol® 812) (Croda, Germany), polyethylene glycol (PEG 400) (Merck, Germany) and Tween® 80 (Sigma, USA) were used as received for preparing the formulations. 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Merck, Germany) was used for determining the antioxidant activity. Ethanol (E. Merck, Germany), methanol (E. Merck, Germany) and acetonitrile (E. Merck, Germany) were used in release and HPLC studies. α-Tocopherol (vitamin E) was supplied by Roche, Türkiye.

Methods

Preparation of ME and SE

ME was prepared proceeding calculations determined by triple-phase diagram obtained using titration technique (29-31). Co-surfactant/surfactant (CoS/S) ratio was kept constant while varying the amounts of oil and water. Several ME areas were obtained and the ME area was selected for the optimum ratio of co-surfactant/surfactant. Oil phase (Miglyol® 812), surfactant (Tween® 80), co-surfactant (PEG 400) and Vitamin E were mixed at 70°C and titrated by distilled water. The transparent and monophasic system was obtained spontaneously by gentle mixing. The stability of pre-formulations was the critical point selecting the optimum

For the preparation of simple emulsion, vitamin E was added to the oil phase at 70°C followed by the addition of the aqueous phase of the same temperature containing the surfactant (Tween® 80). Optimum formulations were given in Table 1.

Preparation of SLN

SLN formulation was prepared using hot homogenization technique with high pressure homogenizer APV Micron Lab 40 (APV GmbH, Denmark) at 500 bar applying three homogenization cycles (32, 33). Solid lipid was heated over 5-10°C of its melting point (73°C) and vitamin E was dissolved in the molten lipid (20, 32). The lipid phase containing vitamin E was dispersed in hot surfactant (78 °C) (Tween® 80) solution of distilled water using Ultra-Turrax T25 (Janke and Kunkel GmbH and Co KG, Germany) at 9500 rpm for 3 minutes; then cooled to room temperature. This pre-emulsion was then homogenized by high pressure homogenization applying three homogenization cycles at 500 bar. Optimum formulation was selected upon smaller particle size and higher zeta potential data. The composition of formulation was given in Table 1.

Characterization of ME and SE

Determination of type

Types of the ME and SE systems were determined by the aqueous dilution of the external phase. This procedure was repeated after 15 days and 1, 2, 3 and 6 months.

Globule size analyses and Conductivity measurements

Droplet sizes of ME and SE were determined at 25°C by photon correlation spectroscopy (PCS) (Malvern Instruments, England) (n=3).

Conductivity measurements

Conductivity of ME and SE were determined with *conductometer* (*Hanna* Instruments, *Germany*) by direct measurement.

Rheological analyses

Rheological characterization of the ME and SE formulations was performed using cone-plate rheometer (RVIII, Brookfield, USA). Tests were performed at 25±1°C. Continuous flow measurements were achieved by increasing/decreasing shear rates.

Characterization of SLNs

Particle size analyses and zeta potential measurements

Particle size and zeta potential measurements of SLN dispersions were performed by Zetasizer Nanoseries (Malvern Instruments, England). Measurements on each sample were repeated 3 times.

Rheological analyses

Rheological characterization of the SLN system was achieved as described for the emulsion systems.

X-ray diffraction analyses

X-ray diffraction analyses were performed for determining the crystalline nature of the lipid particles. Since dry particles are needed for analyses, samples were lyophilized at -52°C with the Lyovac-GT 2 laboratory freeze dryer (Leybold-Heraeus Lyovac GT–2, Germany) and X-ray diffraction analysis was performed on lyophilized samples using RIKAGU D/Max-3C (Japan). The X-ray diffraction analysis range was 2–40°C over 2θ with 2°C min-1 scanning rate, with 40kV voltage and current intensity level of 20mA.

Differential scanning calorimetric analyses

Differential scanning calorimetry (DSC) used was DSC-60 (Shimadzu, Japan). DSC analysis was performed on the lyophilized samples of the SLN system prepared. The heating rate of 10°C/min was employed in the temparature range of 30-100°C. Analysis was carried out under nitrogen with a scan rate of 5 K/min.

Fourier transform infrared spectroscopic analyses

Fourier transform infrared (FT-IR) spectroscopic analysis was performed on the lyophilized SLNs (34, 35). Spectra were obtained by FT-IR spectrophotometer (Perkin-Elmer, England) with KBr disk method between 400 cm^{-1} and 4000 cm^{-1} wavelength.

Nuclear magnetic resonance spectroscopic analyses

High resolution proton nuclear resonance (^1H NMR) spectra of SLNs were obtained on CP-MAS NMR spectrophotometer (Ultra Shield, Germany) at 25°C using lyophilized samples.

Quantification of vitamin E with HPLC

Vitamin E content of formulations was determined using reversed-phase HPLC method with HPLC apparatus consisting of a pump (LC 10-AD), a UV detector (SPD-20A) and data station (Shimadzu, Japan). C_{18} column (250 mm x 4.6 mm i.d., 5 µm particle size) was used. The mobile phase composed of acetonitrile:methanol (95:5) was degassed. The eluent flow rate was 1 mL/min. Injection volume was 20 µL at 30 °C and monitoring at 292 nm. During the validation procedures of the HPLC method, linearity was shown by coefficient of determination (r^2) value of the equation. Solutions were prepared at three different concentrations of vitamin E and analyzed 6 times a day for 3 consecutive days to assess the intra-assay precision, intermediate precision and accuracy of the HPLC method used.

Stability testing of formulations

To investigate the stability of ME, SE and SLN systems, formulations prepared were stored at 4°C, 25°C (room temperature), 40°C and 40°C+60 % relative humidity (RH) for 6 months. Characterization tests of each system were repeated every month.

Antioxidant activity of formulations

Antioxidant effects of formulations were determined by DPPH test (36, 37). During the determination of antioxidant activities of the formulations prepared control formulations were also tested because of the existence of excipients used in formulations. Ethanolic solution of vitamin E was used as a control. 5 mL of each formulation was diluted to 25 mL with water while the control solution was diluted with methanol. Various concentrations of formulations were mixed with methanolic solution containing DPPH radical. The reduction in DPPH radical was measured by an UV spectrophotometer (Shimadzu UV-Visible Recording Spectrophotometer UV-160 A, Japan) at 517 nm. Measurements were repeated three times.

In vitro release studies

Franz diffusion cells were used for *in vitro* release studies. The diffusion cells were thermoregulated with a water jacket at 32°C. Polypropylene membranes of 0.22 µm pore size were mounted on *Franz* diffusion cells after saturating them 20 minutes in the donor phase. The receptor chamber was filled with ethanol. 1000 µL of ME, SE and SLN formulations were then applied to the membrane as the donor phase. 0.5 mL aliquots were withdrawn from the receptor phase at the 5^{th}, 15^{th}, 30^{th} minutes and up to 240 minutes. Equivalent amount of ethanol was added to the receptor solution to replace the amount withdrawn. . Typically, in release studies the receptor phase is usually an isotonic solution or another water-based medium. In this study the use of such mediums was impossible due to the extremely low solubility of VE, therefore ethanol was used as a receptor phase for better evaluation of complete penetration of VE from

the formulations prepared (38). Vitamin E in the samples was analyzed by HPLC. Flux (J) and permeation coefficient (k_p) were determined from the slope of the steady-state portion of this profile the amount of drug permeated versus time was plotted.

In vivo skin efficacy studies

Prior to the study, 10 female volunteers, aged 24-44 were investigated for their facial skin uniformity. Volunteers signed a Volunteer Protocol prepared previously. As mentioned before, empty SLNs have a moisturizing effect due to their occlusive effect. To test the cosmetic benefit of the formulations prepared, skin moisturizing efficacy of all the formulations were tested besides the skin antiaging tests, measurement of skin elasticity and skin roughness. Formulations were applied twice a day to ten women volunteers (cheeks and crow's feet area) for a period of one month and skin measurements were repeated once a week (39). Skin moisture was measured using the Corneometer® (Courage&Khazaka, Germany) and skin elasticity values were obtained by the Cutometer® (Courage&Khazaka, Germany). Skin replicas were each time taken and the condition of skin surfaces was evaluated with a camera and the skin roughness values were measured by Skin Visiometer® and VisioScan® (Courage&Khazaka, Germany) (40). Statistical evaluation of the skin measurements was performed on the computer using SPSS (41). The in vivo study protocol was approved by the Scientific Ethical Committee of Eskişehir Osmangazi University, Türkiye (Protocol No: 06-08-22-1).

RESULTS AND DISCUSSION

Characterization

Compositions of formulations were given in Table 1. ME areas versus co-surfactant/surfactant ratios obtained are given in Figure 1 and the phase diagram of MEs is given in Figure 2. Pseudoternary phase diagrams of the prepared microemulsions with different Km (surfactant/cosurfactant) were generated to determine the optimal Km according to the size of the microemulsion area. According to Figure 1 the largest microemulsion area was the 1:1 (co-surfactant:surfactant) ratio and the optimum ME area was given in Figure 2. The ME areas were calculated with three-phase diagram. The combinations of water-co-surfactant:surfactant-oil at the center of gravity of this optimum ME area was used in *in vivo* studies considering the higher stability during the storage period (29, 30). In Figure 2 it should be noted that not every combination of components produce microemulsons over the whole range of possible compositions (42). The limited area show the o/w ME formation. Heat and sonication is often used particularly in ME systems , to speed up the process (30, 42).

Table 1. Optimum formulations of ME, SE and SLN

Ingredients (in % w/w)	ME	SE	SLN
Vitamin E	8	8	0.8
Caprylic/capric triglyceride	16.56	32	-
Polyethylene glycol sorbitan monooleate	33.81	16	6
Polyethylene glycol	33.81	-	-
Distilled water	7.82	44	84
Glycerol behenate	-	-	9.2

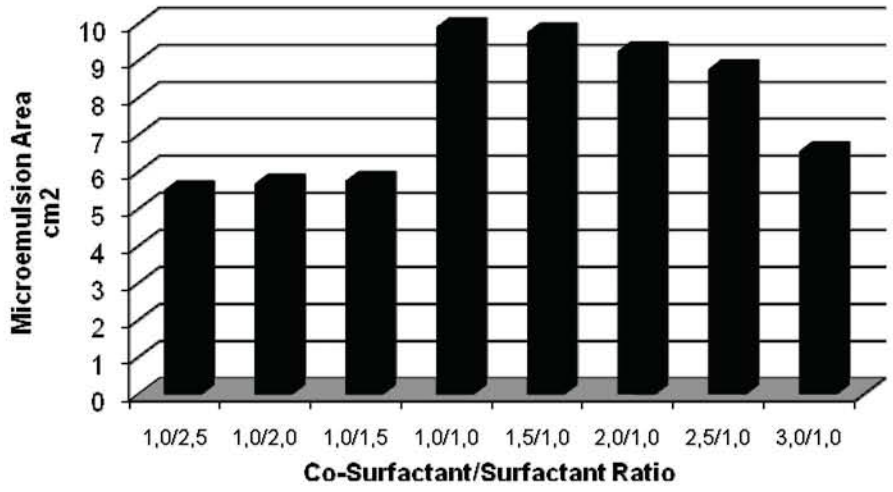

Figure 1. ME areas versus co-surfactant/surfactant ratio

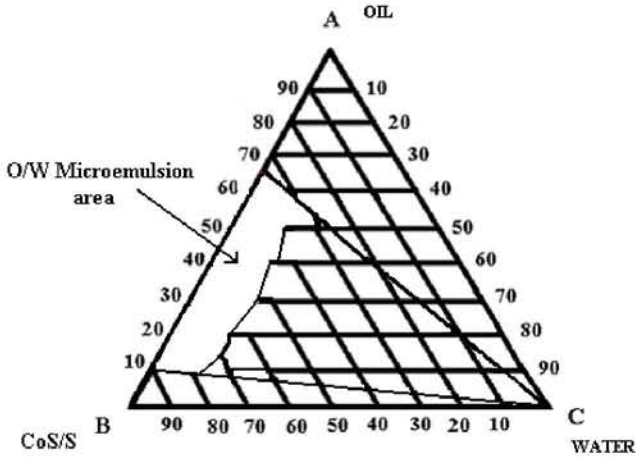

Figure 2. Phase diagram of optimum ME formulation [co-surfactant:surfactant ratio=1:1]

The spontaneously formed formulation of ME was isotropic, slightly viscose and transparent. SE obtained was milky in appearance and homogeneous.

Upon determination of the types of ME and SE systems, the external phase was verified to be aqueous, *ie* the ME and SE prepared were of o/w type.

Globule sizes of ME and SE systems are important when discussing the characterization and stability (43, 44). Mean globule sizes of the freshly prepared ME and SE systems were found to be 0.183±0.000 μm and 2.105±0.000 μm, respectively (Table 2).

Table 2. Mean globule sizes of ME and SE systems

Globule Size (μm) ± se				
Time	**ME System**			
	Storage Conditions			
	25°C	**4°C**	**40°C**	**40°C+60 % RH**
Fresh	0.183±0.000	-	-	-
15th Day	0.189±0.000	0.210±0.000	0.202±0.000	0.186±0.000
1st Month	0.197±0.000	0.213±0.000	0.219±0.000	0.209±0.000
2nd Month	0.219±0.000	0.226±0.000	0.225±0.000	0.206±0.000
3rd Month	0.223±0.000	0.247±0.000	0.269±0.000	0.242±0.000
6th Month	0.221±0.000	0.258±0.000	0.272±0.000	0.240±0.000
Time	**SE System**			
	25°C	**4°C**	**40°C**	**40°C+60 % RH**
Fresh	2.105±0.000	-	-	-
15th Day	2.149±0.000	2.584±0.000	2.482±0.000	2.455±0.000
1st Month	2.447±0.000	2.687±0.000	2.688±0.000	2.581±0.000
2nd Month	2.561±0.000	2.780±0.000	2.754±0.000	2.667±0.000
3rd Month	2.545±0.000	2.784±0.000	2.782±0.000	2.687±0.000
6th Month	2.544±0.000	3.113±0.000	2.789±0.000	2.680±0.000

se: Standard Error; n=3

Conductivities of the freshly prepared ME and SE systems were 3.00±0.00 $\mu S \cdot cm^{-1}$ and 80.00±0.33 $\mu S \cdot cm^{-1}$, respectively (Table 3).

Table 3. Conductivity values of ME and SE systems

Conductivity (μS·cm-1) ± se				
Time	**ME System**			
	Storage Conditions			
	25°C	**4°C**	**40°C**	**40°C+60 % RH**
Fresh	3.00±0.00	-	-	-
15th Day	3.00±0.00	3.00±0.00	3.00±0.00	3.00±0.00
1st Month	3.00±0.00	3.00±0.00	3.00±0.00	3.00±0.00
2nd Month	2.00±0.00	2.00±0.00	2.00±0.00	2.00±0.00
3rd Month	2.00±0.00	2.00±0.00	2.00±0.00	2.00±0.00
6th Month	2.00±0.000	1.98±0.00	2.00±0.00	2.00±0.00
Time	**SE System**			
	25°C	**4°C**	**40°C**	**40°C+60 % RH**
Fresh	80.00±0.00	-	-	-
15th Day	80.00±0.00	81.67±0.33	78.66±1.86	80.00±0.00
1st Month	79.33±0.33	82.00±0.58	76.33±0.33	80.67±0.67
2nd Month	81.00±0.00	83.33±0.33	76.67±0.33	80.33±0.33
3rd Month	80.33±0.33	80.67±0.33	76.67±0.33	80.33±0.33
6th Month	80.33±0.33	80.10±0.33	76.67±0.33	80.12±0.33

se: Standard Error; n=3

Newtonian flow model was detected for ME systems prepared, as expected (Figure 3). After 6 months storage period, rheological behaviour of ME was not changed and showed Newtonian flow. SE system prepared was determined as non-Newtonian flow model. After 6 months storage period, rheological behaviour of SE was changed and this indicates the instability of SE formulation (44). Rheological examination indicates the characterization and performance of the formulations during the storage period of 6 months (45, 46).

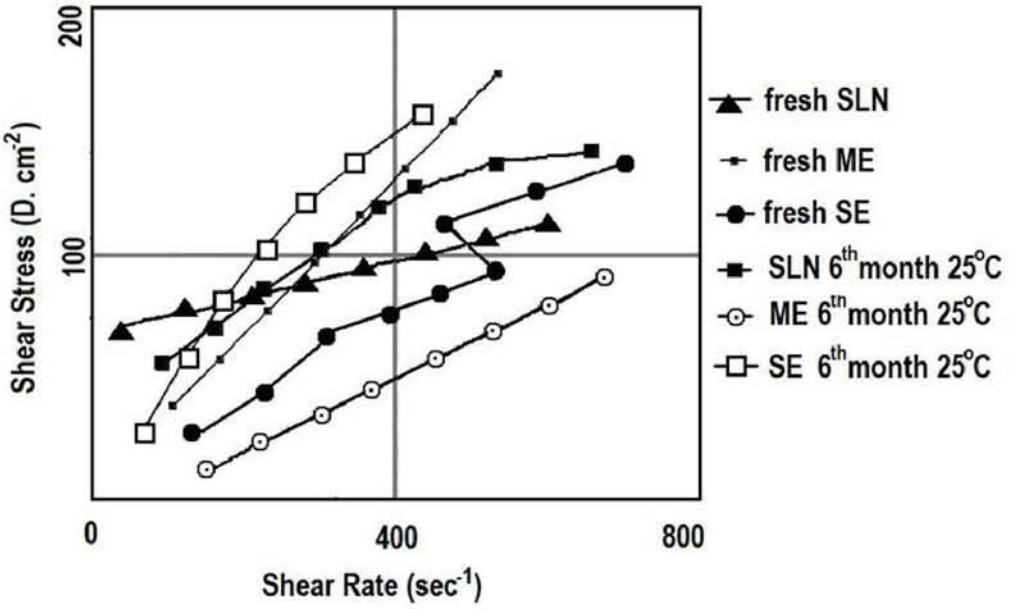

Figure 3. Shear stress of SLN and ME as a function of shear rate at 25° C.

Table 4. Mean particle sizes and zeta potentials of SLN system

Time	Particle Size (μm) ± SE			
	Storage Conditions			
	25°C	**4°C**	**40°C**	**40°C+60 % RH**
Fresh	0.183±0.000	-	-	-
15th Day	0.183±0.000	0.354±0.000	0.313±0.000	0.320±0.000
1st Month	0.327±0.000	0.391±0.000	0.335±0.000	0.305±0.000
2nd Month	0.275±0.000	0.325±0.000	0.928±0.000	0.311±0.000
3rd Month	0.388±0.000	0.350±0.000	1.480±0.000	0.968±0.000
6th Month	0.480±0.000	0.378±0.000	1.450±0.000	1.330±0.000
Time	**Zeta Potential (mV) ± SE**			
	Storage Conditions			
	25°C	**4°C**	**40°C**	**40°C+60 % RH**
Fresh	-11.3±0.0	-	-	-
15th Day	-11.6±0.0	-12.7±0.0	-14.8±0.0	-14.7±0.0
1st Month	-18.6±0.0	-13.9±0.0	-18.9±0.0	-12.4±0.0
2nd Month	-28.3±0.0	-13.6±0.0	-13.2±0.0	-11.3±0.0
3rd Month	-21.8±0.0	-13.3±0.0	-12.4±0.0	-10.6±0.0
6th Month	-21.8±0.0	-13.3±0.0	-12.4±0.0	-10.6±0.0

SE: Standard Error; n=3

Mean particle size and zeta potential of the freshly prepared SLN formulation was determined to be 0.183±0.000 μm and -11.3±0.0 mV, respectively (Table 4).

Results of the other methods of SLN characterization are given under the heading of *Stability*.

Quantification of vitamin E with HPLC

The relative retention time of VE upon HPLC analysis was found to be 13.8 min. The linear equation for vitamin E was determined to be y = 9061.608x + 8675.301. The regression equations relating peak area (y) to injected amounts (x, μg) of VE. According to the HPLC chromatogram of VE (Figure 4) and of the receptor phase (Figure 5), selectivity of the HPLC method used was validated.

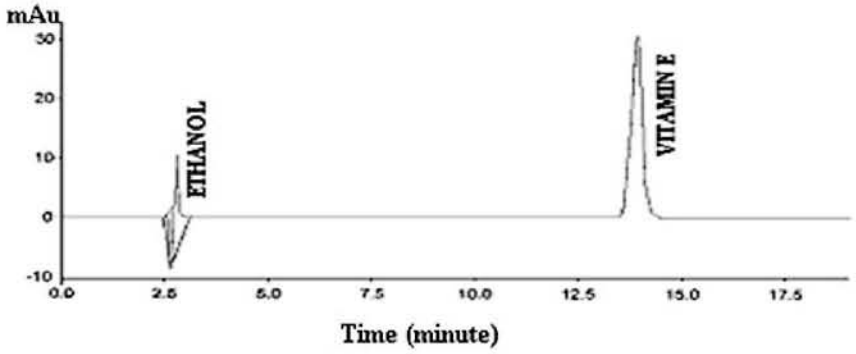

Figure 4. Chromatogram of vitamin E (50 μg/mL)

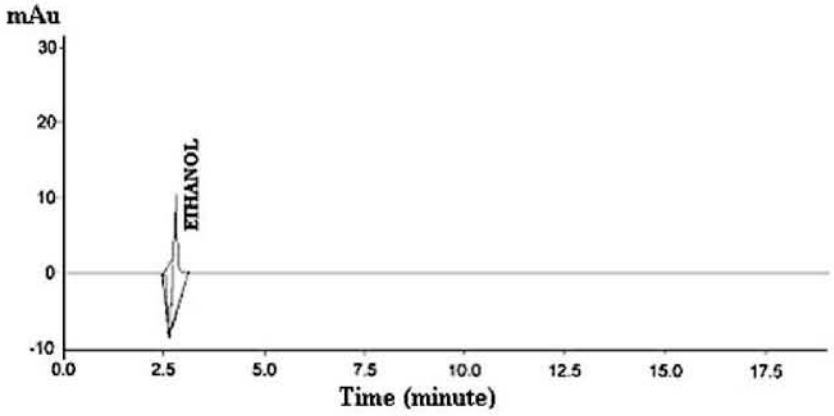

Figure 5. Chromatogram of receptor phase

The Limit of Quantification (LOQ) value which is defined as the lowest concentration of vitamin E which can be detected with acceptable precision was determined to be 0.025 μg/mL while the Limit of Detection (LOD) value defined as the lowest detection limit was 0.008 μg/mL.

Stability

There were no visual changes in the ME and SE systems during the storage period of 6 months. However, SE formulation stored at 40ºC and 40ºC+60 % RH showed phase separation at the end of 6 months.

There were no significant changes in globule sizes of ME and SE systems during the storage period ($p>0.05$) (Table 2).

The aqueous phase becomes a continuous phase and high conductance is detected (47) if phase inversion occurs. When conductivities of the ME and SE systems were tested to investigate any phase inversion and stability, it was found that there were no significant changes in conductivity measurements ($p>0.05$) (Table III). This indicates that no phase inversion happened in the emulsion systems (48, 49).

Rheology is an important aspect of pharmaceutical and cosmetic formulations. It has a great effect on application of emulsion systems. Rheological behavior is expected to change during storage (50). Viscosities of formulations stored at different conditions were investigated to understand the effect of temperature on viscosity (51). ME system was determined to show Newtonian flow model and this characteristic lasted for 6 months during the storage at different conditions. SE and SLN systems showed non-Newtonian flow when prepared but after 6 months they show different rheological types. At first Bingham model was seen in SLN system but after 6 months it was changed to Casson model (44). This change indicates the instability of formulation (42). The rheograms of formulations are shown in Figure 3.

After a 6-month storage time, SLNs stored at 4ºC and 25ºC demonstrated no physical changes. The formulations stored at 40ºC and 40ºC+60 % relative humidity (RH) were converted into viscose cream formulations most probably due to the high temperatures (52).

Particle size is an important parameter in SLN stability (53). The particle size data of the SLN system kept at different storage conditions is given in Table IV. The changes seen in the particle sizes of SLNs which were stored at 40ºC and 40ºC+60 % (RH) were not significant ($p>0.05$). Zeta potential values of SLNs during the 6-month storage period are also shown in Table IV. It was determined that the zeta potentials of the SLN formulation remained at negative values and there were no significant changes occurring ($p>0.05$). The use of Tween® 80 may be an important factor stabilizing zeta potentials (54).

Regarding the rheological behavior of the SLN system, lipid used in the SLN formulation was reported to be important for the end viscosity of the system (55). Viscosity was found to change with temperature for the SLN formulations prepared. Similar to emulsions, SLNs showed viscoelastic character (56).

Internal structure of lipid nanoparticles was investigated by X-ray diffraction (35). Diffraction patterns of the freshly prepared SLN and the sample stored at 25ºC for 6 months are given in Figure 6. Degree of crystallinity was found to be lower for the SLN system stored for 6

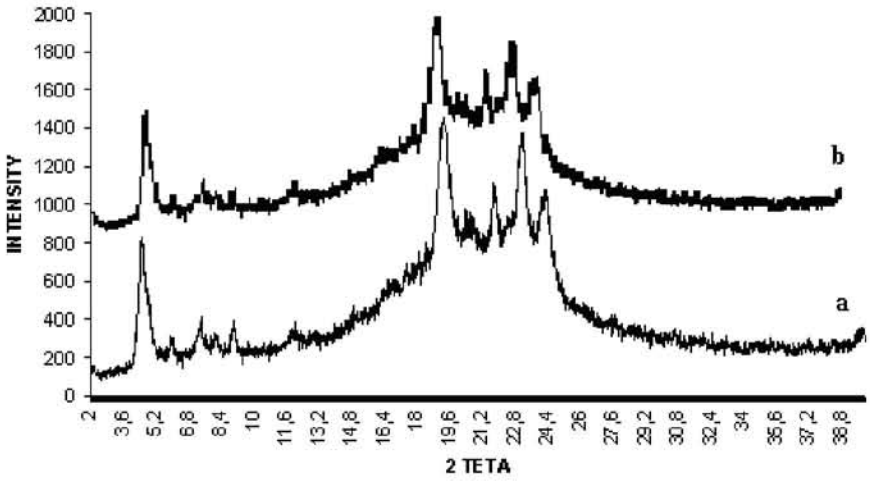

Figure 6. X-ray diffraction patterns of : (a) SLN stored at 25º C ± 1º C for 6 months; (b) Fresh SLN

months when compared to the freshly prepared sample as indicated by the weaker peaks following storage for 6 months. X-ray analysis is not adequate for deciding on the crystallinity and stability of lipids. It is useful to evaluate data of DSC and X-ray together.

Lyophilized SLN samples are needed to perform DSC analysis on lipid stability of SLNs and therefore the SLN system prepared was lyophilized before the measurement (53, 57). DSC measurements show the melting and crystallization behavior of lipids by the changes in endothermic and exothermic peaks (53, 58). Thermograms (Figure 7) of the SLN formulations stored at 4ºC and 25ºC showed no physical changes at the end of 6 months while the thermograms of the samples stored at 40ºC and 40ºC+60 % (RH) showed changes in the melting points most probably due to the high temperature (59).

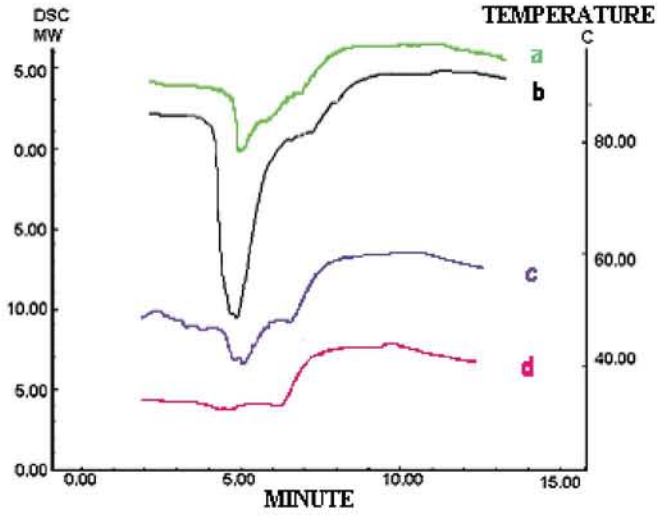

Figure 7. DSC thermograms of: (a) SLN stored at 25º C after 6 months; (b) 4º C; (c), 40º C; (d) 40º C+60% RH

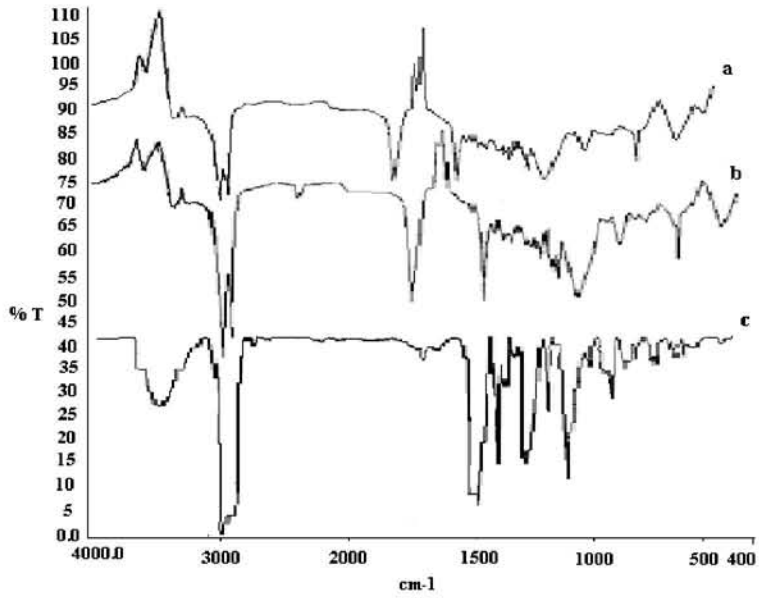

Figure 8. FT-IR spectra ranging from 4000-400 cm^{-1}: (a) control SLN; (b) SLN; (c) Compritol

In the FT-IR spectrum (Figure 8) of SLNs, characteristic 1600-1700 cm-1 bands for C=C formation and 900-1200 cm-1 of C-O formation occurred due to the presence of α-tocopherol. Since no peaks of α-tocopherol were detected in SLNs prepared without the active agent, it can be concluded that vitamin E was succesfully incorporated into the SLN system. Peaks which characterize Compritol® were seen in both SLNs.

NMR is another method to investigate the stability of SLNs (34, 56, 58). Investigations were performed on lyophilized freshly prepared samples and after 6 months. There were no changes occurring in signals (Figure 9) indicating the stability of SLNs stored at 25ºC.

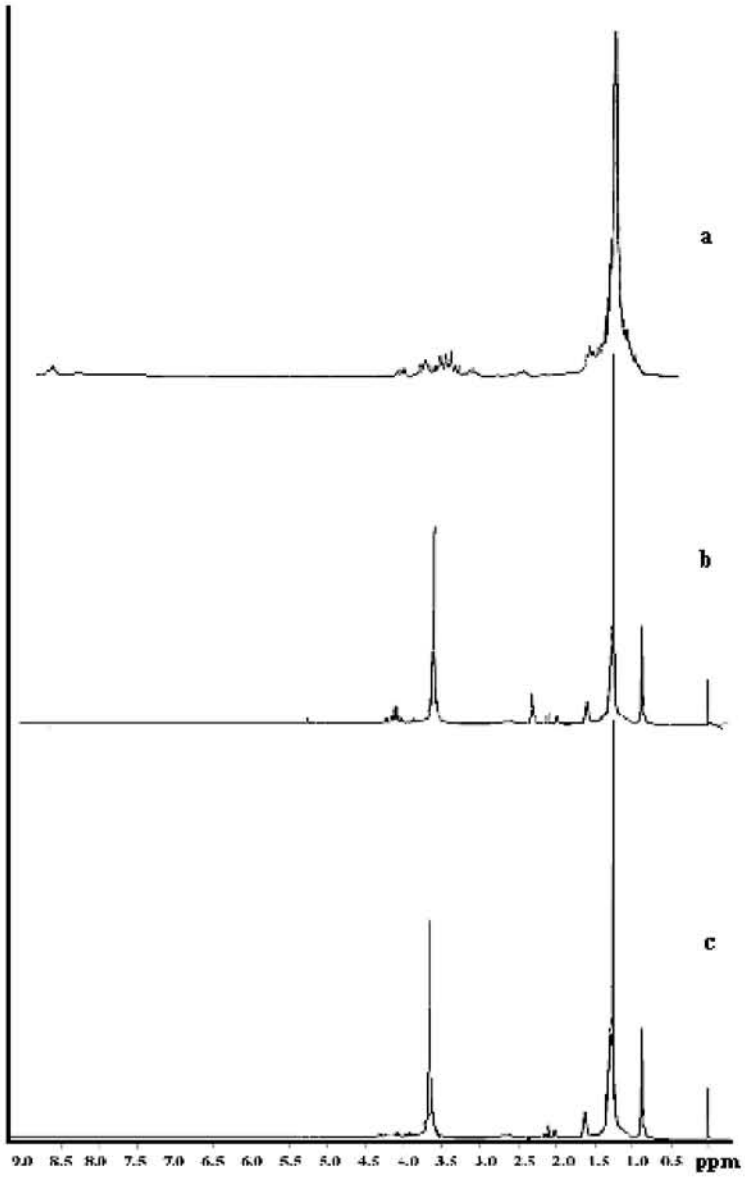

Figure 9. [1]H-NMR spectra of: (a) control SLN; (b) freshly prepared SLN; (c) SLN stored at 25° C for 6 months

For better evaluation of the production temperature (for ME 70°C and for SLN 78°C) effect on the stability of VE, pure VE was heated up to 80 °C and cooled down to room temperature. HPLC analysis and IR analysis were carried out on heated VE (H-VE). There is no change

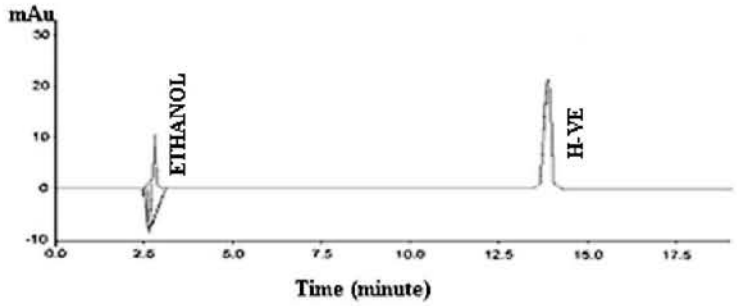

Figure 10. Chromatogram of H-VE (40µg/mL)

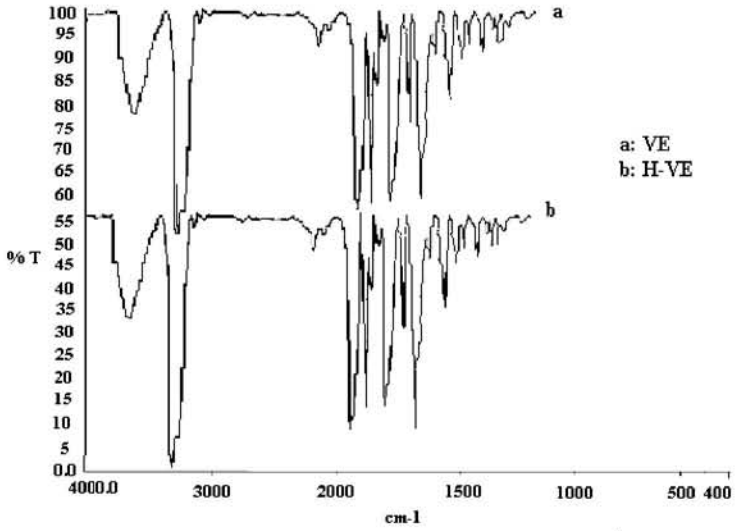

Figure 11. FT-IR spectra ranging from 4000-400 cm^{-1}: (a) VE; (b) H-VE

occurred in peak areas (Figure 4 and Figure 10) and recovery time of chromatogram and same peaks were evaluated in IR spectrum (Figure 11).

Antioxidant activity of formulations

The DPPH assay is widely used for the measurement of free radical scavenging capacity in phytotechnology, food technology, and pharmacology/toxicology (36). The DPPH is a free radical that easily accepts an electron or hydrogen radical to become a stable diamagnetic molecule (37). All formulations containing vitamin E were found to show antioxidant activities according to the data obtained (Table 5). According to identify the antioxidant effect of VE, formulations without VE and pure VE were also investigated with DPPH method.

Due to the results, all of the formulations have more antioxidant activity after adding VE to the systems; but there is no difference determined between formulations antioxidant activity statistically ($p>0.05$).

Table 5. Scavenging activity of formulations according to DPPH test

Scavenging % DPPH ± SE							
Samples	**Pure Vitamin E**	**Control ME**	**ME**	**Control SE**	**SE**	**Control SLN**	**SLN**
250µL. 3 mL^{-1}	94.05±0.00	7.14±0.00	20.26±0.00	8.89±0.00	23.40±0.00	4.14±0.00	17.63±0.00
100µL. 3 mL^{-1}	93.89±0.00	5.23±0.00	14.66±0.00	6.34±0.00	16.82±0.00	2.26±0.00	11.56±0.00
50µL. 3 mL^{-1}	93.73±0.00	2.52±0.00	10.35±0.00	4.91±0.00	12.50±0.00	1.12±0.00	5.38±0.00

SE: standart error; n=3

In vitro release from formulations

Figure 12 shows the released amounts of vitamin E from formulations. Vitamin E was released 99.94 % from the ME system at the end of 150 minutes, 99.96 % from the SE system at the end of 120 minutes and 99.99 % from the SLN system at the end of 240 minutes. This showed that encapsulating vitamin E in the SLN system prolongs the release of the active agent. (20, 23, 24).

The permeability coefficients (k_p) of Vitamin E from the ME, SE and SLN formulations were calculated to be $2.75x10^{-4} \pm 0.001$ cm^2. h^{-1}, $7.5x10^{-4} \pm 0.001$ cm^2.h^{-1} and $3x10^{-4} \pm 0.001$ cm^2.h^{-1}, respectively.

There is no doubt that the release of a drug from a topical formulation can be effectively influenced by the vehicle in which it is applied. In this study three different topical delivery systems were used and evaluated. The results indicated that SE formulation exhibited significantly higher drug release than other vehicles due to the permeability coefficient. The observed higher drug release rate may be due to lipophilic character of SE (60). The release amount and profile of the systems will be change according to use modified human skin in Franz diffusion cell experiments (61, 62).

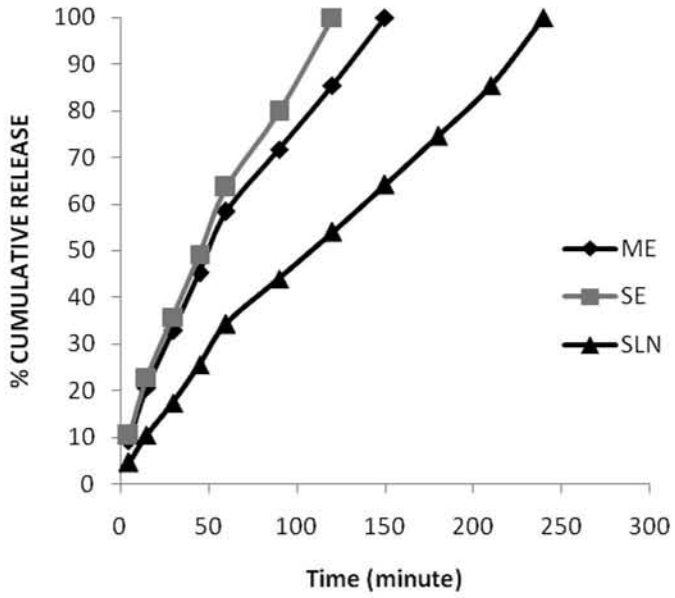

Figure 12. Release profiles of vitamin E from formulations

In vivo skin efficacy studies

According to in vivo efficacy tests, skin moisture was increased (Figure 13) by the 4-week application of the SE ($p \leq 0.001$) and SLN formulations ($p \leq 0.01$) (24, 15). ME formulation also improved skin moisture but this change was not significant statistically ($p > 0.05$).

Determination of the skin elasticity is of significance in identifying the skin age. Since the young skin is well supplied with blood, it is very elastic. Various parts of the body have different degrees of elasticity. According to the skin elasticity values obtained with the Cutometer®, no significant changes could be determined for all formulations (Figure 12) ($p > 0.05$) in a 4-week application period (39). Other biomechanical devices or an older patient panel are needed to visualize these effects (24).

After the 4-week application of ME and SE, skin roughness was decreased (Figure 13) ($p \leq 0.001$) and there was no statistical difference between the two emulsion formulations ($p > 0.05$). SLN formulation was also found to decrease skin roughness significantly ($p \leq 0.01$) (17, 24).
Due to the data obtained, there is a significant in vivo difference between the ME and SLN formulations in favor of SLN ($p < 0.05$).

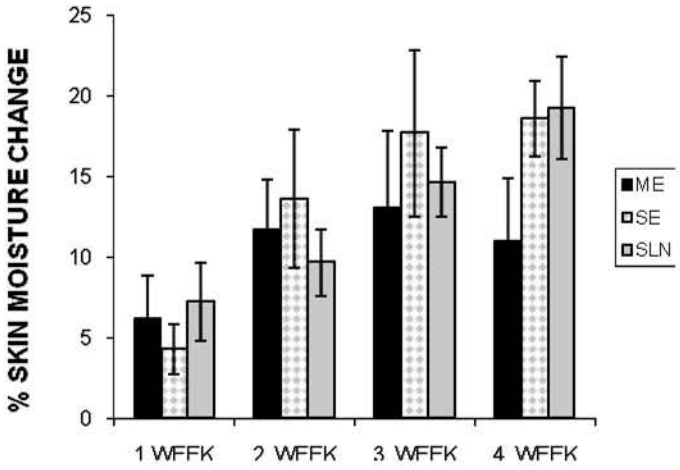

Figure 13. Changes in skin moisture values during 4-week application of formulations

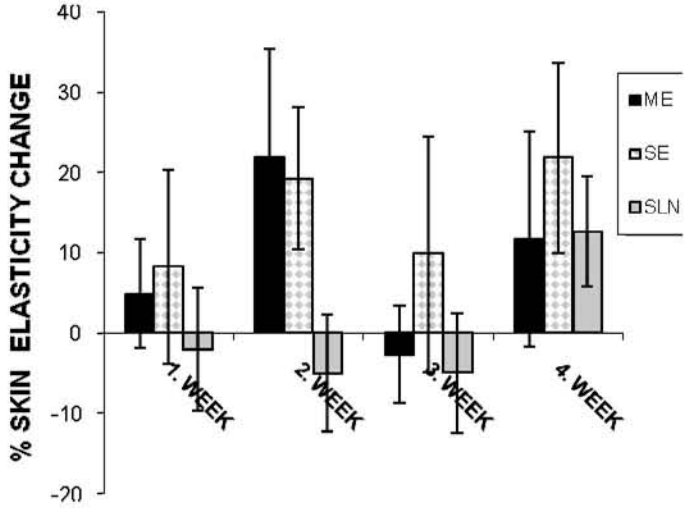

Figure 14. Changes in skin elasticity values during 4-week application of formulations

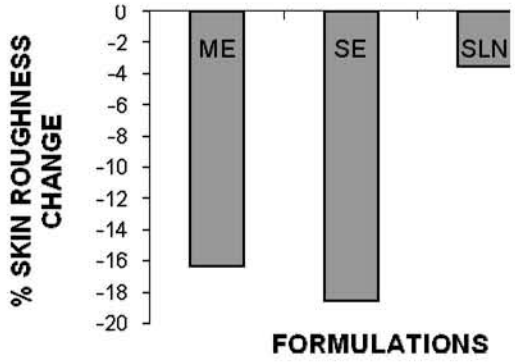

Figure 15. Changes in skin roughness values at the end of 4-week application

CONCLUSION

The results obtained demonstrated that vitamin E could be incorporated into stable microemulsion and solid lipid nanoparticle delivery systems successfully. SE formulation is instable during 6 months period. Physical stability of the ME system was found to be better than the SE and SLN formulations at the elevated temperatures. All of the formulations showed in vitro antioxidant effect. There is no difference occurred statistically with antioxidant activity. In vitro release of vitamin E from the formulations were sustained in the order of SLN > ME > SE which was expected. As a result of skin efficacy tests, it can be concluded that SLN and SE formulations containing vitamin E improves skin moisture while decreasing skin roughness indicating an antiaging efficacy. ME and SE formulations were also found to decrease skin roughness.

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