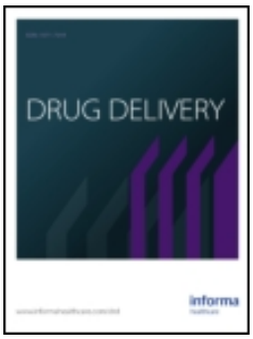

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Stability of Vitamins C and E in Topical Microemulsions for Combined Antioxidant Therapy

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An interesting strategy for protecting skin from excessive exposure to free radicals is to support the skin endogenous antioxidant system. As the balance between different skin antioxidants is very important, a combined therapy using at least two antioxidants is desirable. In the present work, o/w, w/o, and gel-like microemulsions (ME), all composed of the same ingredients, were selected as carrier systems for dermal delivery of vitamins C and E. Gel-like ME was found to offer the best protection for both vitamins, although other ME also significantly increased their stability compared with that solution. In the presence of vitamin C no decrease in vitamin E content occurred. To obtain ME appropriate for dermal use, their viscosity was increased by adding thickening agents. On the basis of visual examination of viscosity and physical stability of thickened systems, several thickeners were selected. The addition of thickener significantly increased the viscosity of ME and changed the behavior of systems from ideal Newtonian to thixotropic. Finally, the stability of both vitamins was examined as a function of thickening agent and of the location of vitamins in the ME. The addition of thickeners changed the stability of at least one vitamin, but the systems generally still protected vitamins better than solutions. It is likely that the changes in internal organization of ME resulting from the addition of thickener, confirmed by thermal analysis and changes in solubility of oxygen in the outer phase, were the most important factors that influenced the stability of vitamins in thickened systems.

Keywords Antioxidants, Microemulsion, Stability, Topical Application, Vitamins

Researchers generally agree that oxidative stress makes a major contribution to skin ageing, skin disorders, and skin diseases. Skin has developed several lines of defense against damaging reactive metabolites, the most important being enzymatic (e.g., SOD, catalase, peroxidase) and nonenzymatic (e.g., glutathione, α-tocopherol-vitamin E, ascorbate-vitamin C, β-carotene, ubiquinone) antioxidant systems. A promising strategy for enhancing skin protection from oxidative stress would be to support the endogenous skin antioxidant system. The most intensively studied antioxidants for preventing skin oxidative damage have been vitamin C, vitamin E, and β-carotene (Kohen 1999; Steenvorden and Beijersbergen van Henegoven 1997; Offord et al. 2002).

Vitamin C, unique in its high reactivity with all aggressive oxygen radicals, is a major—and the only essential—antioxidant in the aqueous cell compartment, whereas vitamin E is the most important chain-breaking radical scavenger in the liposoluble compartment, so constituting the major specific defense line against lipid peroxidation. There is increasing evidence that vitamin E and C, even though present in different compartments of the cell, act synergistically. Vitamin C potentiates the protective efficacy of vitamin E by regenerating its radical. Regeneration probably occurs at the interphase region (Figure 1) (Steenvorden and Beijersbergen van Henegoven 1997; Packer and Fuchs 1993).

As antioxidant defense mechanisms are linked, the balance between different skin antioxidants is important. If only one of the antioxidants is supported, another will soon become a limiting factor. Tocopheroxyl radical depletes other antioxidants by its own regeneration (Figure 1), so vitamin E monotherapy could result in an overall negative effect. As a consequence of the synergistic interplay of different antioxidants, a combined therapy of vitamin E with vitamin E recycling antioxidants, such as vitamin C, glutathione, ubiquinone, or carotenoids, is desirable (Kohen 1999; Packer and Fuchs 1993; Fuchs 1998; Turunen, Olson, and Dallner 2004).

Oral supplementation of ascorbate is not thought to increase its skin concentration sufficiently. In spite of its very low lipophilicity, mouse skin was found to be relatively highly permeable to vitamin C, indicating its possible topical use. Topical use of vitamin C also is recommended because of its depigmenting activity and its ability to promote the synthesis of collagen. As vitamin C in solution undergoes rapid oxidation, its use in pharmaceutical products is limited above all by its low stability.

Topical administration of vitamin E was found to be generally more effective in protecting skin against oxidative stress than a systemic treatment. Vitamin E is very slowly oxidized by atmospheric oxygen and generally is considered to be stable. However, it is possible that it could undergo photodegradation

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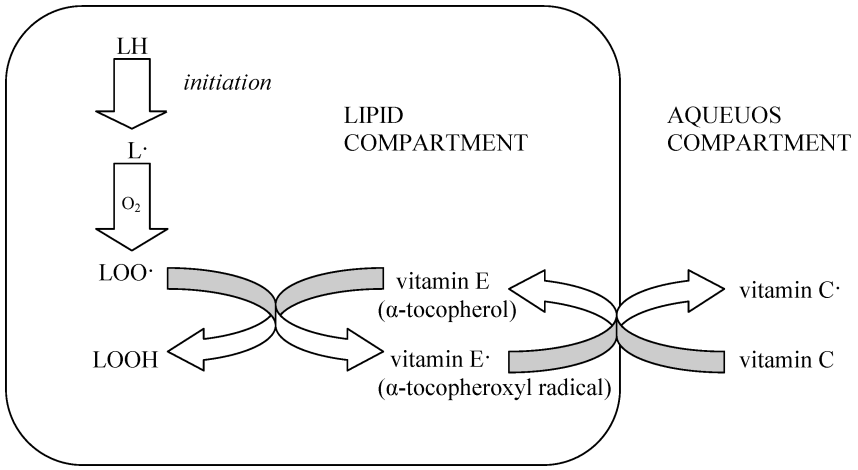

FIG. 1. Protective effects of vitamin E and vitamin C against lipid peroxidation. LH = lipid molecule, L^{-} = lipid radical, LOO^{-} = lipid peroxyl radical, LOOH = lipid peroxide.

when applied to skin that is exposed to ultraviolet (UV) radiation (Steenvorden and Beijersbergen van Henegoven 1997; Fuchs 1998).

The specific structure of microemulsion (ME) allows the incorporation of lipophilic vitamin E and hydrophilic vitamin C in the same system. ME are clear, thermodynamically stable dispersions of water and oil, stabilized by an interfacial film of surfactant molecules. They are able to increase drug solubility, modify drug release, and protect labile drugs (Lawrence and Rees 2000). ME are often very low viscosity Newtonian fluids and cannot be applied directly to the skin. However, from phase diagrams constructed for the selected components, areas with lamellar structures and consequently increased viscosity can be formed (Podlogar et al. 2004). The more usual way to solve the viscosity problem is to add a suitable thickening agent that can modify the rheological behaviour without significant influence on other features of ME, such as stability, transparency and spontaneous formation (Osmann-Gardabbou et al. 2000, Spiclin et al. 2003).

The aim of our work was to develop ME that simultaneously contain the lipophilic (vitamin E) and the hydrophilic (vitamin C) antioxidants and are suitable for dermal use. Because the formulation can significantly affect drug stability, the potential of w/o and o/w ME to protect vitamins from oxidative degradation was evaluated. We investigated the extent to which the ME structure influences the stability of vitamin C and E and also to what extent vitamin C increases the stability of vitamin E. As vitamin E was found to be reasonably stable under normal storage conditions, its degradation will be accelerated by UVA radiation. The rheological behavior of o/w and w/o ME was modified to be appropriate for dermal application by adding various thickeners. The best thickening agents and their optimal concentrations were determined. Finally the influence of selected thickeners on the stability of both vitamins was assessed for both ME types.

MATERIALS AND METHODS

Isopropyl myristate (IPM) was obtained from Fluka Chemie, Switzerland, and used as the lipophilic phase. Tween 40—polyoxyethylene (20) sorbitan monopalmitate (Fluka Chemie)—was used as surfactant and Imwitor 308—glyceryl caprylate (Condea, Germany)—as cosurfactant. Twice distilled water was used as the hydrophilic phase. α-Tocopherol (vitamin E) and ascorbic acid (vitamin C) were from Fluka.

The following thickening agents were used: hydroxypropyl methyl cellulose—HPMC (Methocel K4M, Colorcon, UK), sodium alginate (Protanal LF 120M, FMC BioPolymer, USA), xanthan gum (Sigma Aldrich, Germany), locust bean gum (Sigma Aldrich), microcrystalline cellulose (Lek, Slovenia), colloidal silica (Aerosil 200, Degussa, Germany), magnesium stearate (Fluka, Germany), cetostearyl alcohol (Lanette O, Cognis, Germany), titanium dioxide (Ridel de Haen, Germany), zinc dioxide (Lex Slovenia), white wax (Pharmachem, Slovenia), carbomer (Carbopol 974 PNF, BF Goodrich, Belgium) and poloxamer (Lutrol F68, Basf, Germany).

Preparation of Microemulsions

The components of ME are acceptable for dermal use. Both types of nonthickened ME and gel-like ME differ only in quantitative composition (w/w percent), as shown in Table 1. All

TABLE 1
Composition of w/o, o/w, and gel-like ME (w/w %)

Component	ME W/O	ME O/W	Gel-like ME
Water	10	45	60
Isopropyl myristate	60	25	10
Tween 40	15	15	15
Imwitor 308	15	15	15

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ME were prepared in the same way. The surfactant and cosurfactant were blended in a 1:1 mass ratio to obtain the surfactant mixture. IPM and distilled water were then added. Components were mixed with a magnetic stirrer for 5 min at room temperature. Vitamin C (0,4% w/w concentration) and/or E (1% w/w concentration) were incorporated in ME by stirring with a magnetic stirrer for 30 min. No preservatives were added to avoid possible interactions with vitamins.

Viscosity Modification of ME

Thickening agents were added to ME containing vitamins and stirred with a magnetic stirrer for 30 min. When cetostearyl alcohol or white wax were used as thickeners, the system was blended at 55°C. In that case the vitamins were added to ME after thickening. The thickened ME were left covered for at least 24 hrs. Afterward they were checked visually for physical stability.

Rheological Measurements

A Rheolab MC 100 Paar Physica, equipped with a cone and plate sensor system MK 22, was used for rheological characterization. Rheological measurements were carried out under destructive and nondestructive conditions at 20 ± 1°C. Under nondestructive shear conditions of oscillatory shear, the linear viscoelastic regime was determined at a frequency of 0.5 Hz. The frequency dependence of dynamic moduli was measured under conditions of linear viscoelastic response at a constant strain amplitude 0,1. All measurements were made in triplicate. The absolute dynamic viscosity of nonthickened ME was determined using a Hoeppler viscometer at 25°C.

Differential Scanning Calorimetry Measurements

A Model Pyris 1 (Perkin Elmer) was used for differential scanning calorimetry (DSC) measurements. Approximately 15 mg of sample was weighed precisely into a small aluminium pan and sealed hermetically. The empty sealed pan was used as a reference. Samples were cooled from 20 to –40°C at a rate of 10 K/min.

Vitamin E (Photo) stability

The stability of vitamin E was determined in samples stored in closed glass flasks at room temperature (22+/−1°C) over a period of 2 months. Half the samples were stored in the dark and half in the light to study the influence of light. The amount of nondegraded vitamin E was determined quantitatively by HPLC immediately after the preparation of sample and subsequently on the 1st, 2nd, 3rd, 7th, 14th, 28th, and 56th days of storage.

For the studies of photostability 10 g of samples were irradiated in test tubes without stirring for 48 hr. A (UVA) lamp Spectroline ENF-260C/FE 0,17 ampere, $\lambda = 365$ nm was used. The distance of the test tube from the UVA lamp was 5 cm. Approximately 500 mg of sample was accurately weighed into a glass flask, diluted to 50 ml with methanol, and analyzed by HPLC. HPLC analysis was carried out with a Knauer HPLC pump 64, a sample injector Rhodyne 7125 with a 20 μL sample loop, and a Knauer variable wavelength detector.

Chromatographic conditions: the stationary phase was a 120 × 4 mm ID column packed with 5 μm Nucleosil C18; the mobile phase was methanol-acetonitrile 70:30. The flow rate was 1,5 ml/min and UV detection at 291 nm. The method was validated for the determination of vitamin E in ME.

Vitamin C Stability

The stability of vitamin C was determined in samples stored in well-closed glass flasks at room temperature (22+/− 1°C). The amount of nondegraded vitamin C was determined quantitatively by HPLC immediately after preparation of the sample and subsequently on days 1, 2, 3, 7, 14, and 28. For HPLC analysis, approximately 500 mg of sample was accurately weighed into a glass flask and diluted to 100 ml with methanol. All samples were prepared in triplicate.

HPLC analysis was carried out with a Knauer HPLC pump 64, a sample injector Rhodyne 7125 with a 20 μL sample loop, and a Knauer variable wavelength detector. Chromatographic conditions: the stationary phase was 250 × 4 mm ID column packed with 5 μm Nucleosil C18-NH_2, and the mobile phase methanol—acetonitrile—0.02 M phosphate buffer pH 3.5 (20:30:50). The flow rate was 1 ml/min and UV detection at 243 nm. The method was validated for the determination of vitamin C in ME. Limit of quantification (LOQ) for chromatographic determination of vitamin C was determined from the calibration curve and was 22.7 μM; limit of detection (LOD) was 7.5 μM.

Statistical Analysis

Fractions of nondegraded vitamin are reported as arithmetic means ± standard deviation. Statistical analysis was performed using a two-tailed Student's test with the software package SPSS®. Significance was tested at the 0.05 level of probability.

RESULTS AND DISCUSSION

Viscosity Modification of ME

According to the broad definition of ME by Danielsson and Lindmann, they are optically clear and thermodynamically stable systems. This definition does not mention the microstructure of ME. However, it is well known that an optically clear system can have a wide range of microstructures, the most frequent being spherical droplets, rod-like micelles, lamellar and hexagonal phases (Lawrence and Rees 2000). The nonthickened ME used in our experiments consisted of fine droplets of inner phase dispersed in continuous medium, as characterized by Podlogar and coworkers (2004; Podlogar, Bester Rogac, and Gasperlin 2005). They are stable, transparent liquids with limited use for dermal application because of their low viscosity.

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TABLE 2
Thickening agents tested for w/o and o/w ME

Microemulsion type	Thickening agent	Concentration (w/w %)	Stability after 24 hr	Viscosity after 24 hr
O/W	Hydroxypropyl methyl cellulose	1	Stable system	Too low
	HPMC	3	Sedimentation	/
	Microcrystalline cellulose	3	Phase separation	/
	Xanthan (xan)	2	Stable opalescent system	Too low
	Xanthan	3	Inhomogeneous system	Sufficient
	Locust bean gum (LBG)	1	Stable opalescent system	Too low
	Locust bean gum	3	Phase separation	/
	Sodium alginate (alg)	1	Stable opalescent system	Too low
	Sodium alginate	3	Sedimentation	/
	Mixture of xan and lbg	3 (1:1)	Inhomogeneous system	Sufficient
	Mixture of xan and alg	3 (1:1)	Stable opalescent system	Sufficient
	Mixture of xan and alg	5 (1:1)	Inhomogeneous system	/
	Poloxamer 168	5	Stable opalescent system	Too low
	Carbomer	2,5	Stable opalescent system	Sufficient
	Colloidal silica	10	Stable system	Sufficient
W/O	White wax	10	Stable milky system	Sufficient
	Magnesium stearate	2	Sedimentation	/
	Cetostearyl alcohol	5	Macroemulsion	/
	Titanium dioxide	5	Sedimentation	/
	Zinc oxide	5	Sedimentation	/
	Colloidal silica	10	Stable system	Sufficient

The rheological properties therefore were modified by addition of various thickeners. A wide range of thickeners was tested (Table 2).

The physical stability and viscosity of thickened systems were checked visually after 24 hr. On that basis colloidal silica, a 1:1 w/w mixture of xanthan and alginate (xan-alg mixture) and carbomer were selected as thickeners for o/w ME. For w/o ME colloidal silica and white wax were the only ones found to be appropriate. The physical stability of thickened ME was checked every week over 2 months and no instability was observed. The flow characteristics of nonthickened ME and of selected thickened systems were characterized by rheological measurements under destructive (Figures 2 and 3) and nondestructive conditions (Figure 4).

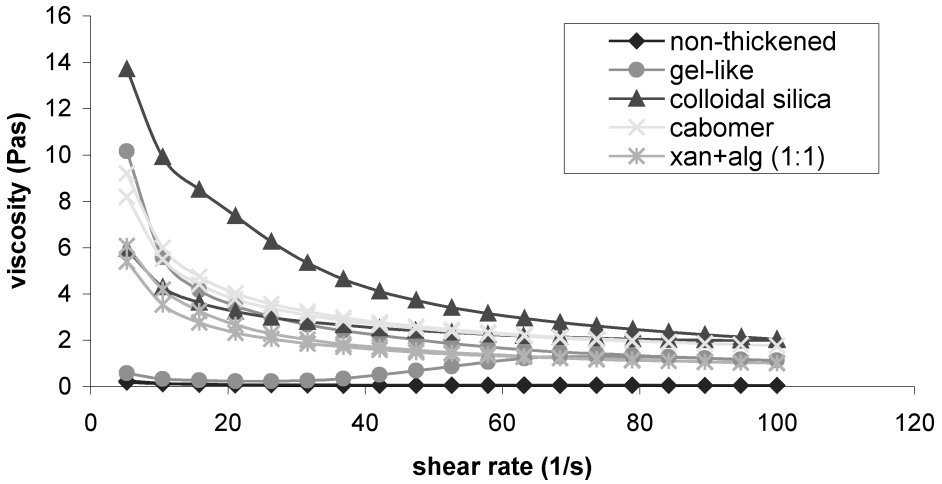

FIG. 2. Flow curves of nonthickened, thickened, and gel-like o/w ME.

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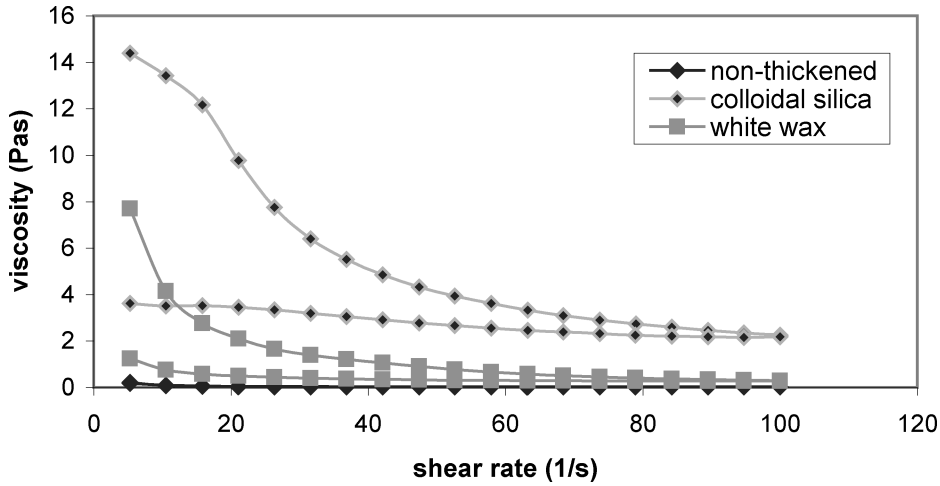

FIG. 3. Flow curves of nonthickened and thickened w/o ME.

Nonthickened ME of both types are Newtonian fluids with viscosities less than 0.5 Pas (Figures 2 and 3, Table 3). The third investigated structure was gel-like ME. This term was chosen only to distinguish this system from liquid o/w ME. The gel-like ME has a higher content of water, which allows strong hydration of the hydrophilic chains of nonionic surfactant. Hydrated chains are connected by hydrogen bonds, resulting in strong interactions between droplets (Podlogar et al. 2004), which is expressed by slightly increased dynamic viscosity (Table 3) and also can be seen from Figure 2 as increased viscosity at higher shear rates and by the rheopexic behavior. The viscosity of gel-like ME was characterized as appropriate for dermal use.

The addition of thickening agent significantly increased the viscosity of o/w and w/o ME and changed the ideal Newtonian to thixotropic behaviour. As clearly seen from Figure 2, the o/w ME thickened with colloidal silica had a much higher viscosity at lower shear rates and greater hysteresis than the systems thickened with polymers. The increased viscosity of ME thickened with silica is a consequence of the weak forces between the particles that form agglomerates. As the size of particles is not uniform, there are some empty spaces in the structure they form and the fluid phase is caught inside. When the shear force increases, the structure is destroyed and the fluid is released, the result being lower viscosity (Olhero and Ferreira 2004). Forward and reverse rate sweep tests show a distinct hysteresis loop that indicates the pronounced thixotropic character of the system.

TABLE 3
Absolute dynamic viscosities of nonthickened and gel-like ME determined with a Hoeppler viscometer

	Absolute dynamic viscosity (mPas)
W/O ME	25,8
O/W ME	27,2
Gel-like ME	79,6

Silica also was selected as a thickener for w/o ME. It increased the viscosity, which was comparable to that of the opposite type of ME. The viscosity of the system thickened with white wax was comparable to that of o/w ME thickened with carbomer (Figures 2 and 3).

More information on the network structure of thickened ME can be obtained from oscillatory measurements. Typical curves of the frequency dependence of storage and loss modulus for thickened ME are shown in Figure 4. ME thickened with silica and carbomer form a weak gel that, in the latter case, is the consequence of physical entanglement of polymeric chains. The addition of xan-alg mixture to o/w ME resulted in a highly viscous polymeric solution. Because of the inhomogeneous parts in the systems with higher concentration of polymers it was not possible to prepare weak gels with mixed xan-alg. The exposure of thickened and non-thickened ME to UV light for 48 hr did not change their rheological behavior.

Thermal Analysis

The microstructure of ME can be examined by thermal analysis. The state of water in a microemulsion system, and consequently its microstructure, can be determined by controlled cooling of the sample (Podlogar et al. 2004, 2005). Figure 5 shows the cooling curves of o/w ME and water dispersion of a xan–alg mixture. Two peaks are seen on the thermograms of ME. The broad peak at $\sim -5^\circ$C represents the solidification of IPM. The second peak between -10°C and -20°C represents the freezing of bulk water (Podlogar et al. 2004; Podlogar et al. 2005). The freezing peaks of water in nonthickened and gel-like o/w ME appear, as expected, at approximately the same temperature and have very similar shapes, indicating the similar structure of the two samples.

The addition of colloidal silica broadened the water peak slightly, indicating interactions between silica particles and water. Thickening of ME with polymers resulted in change of water

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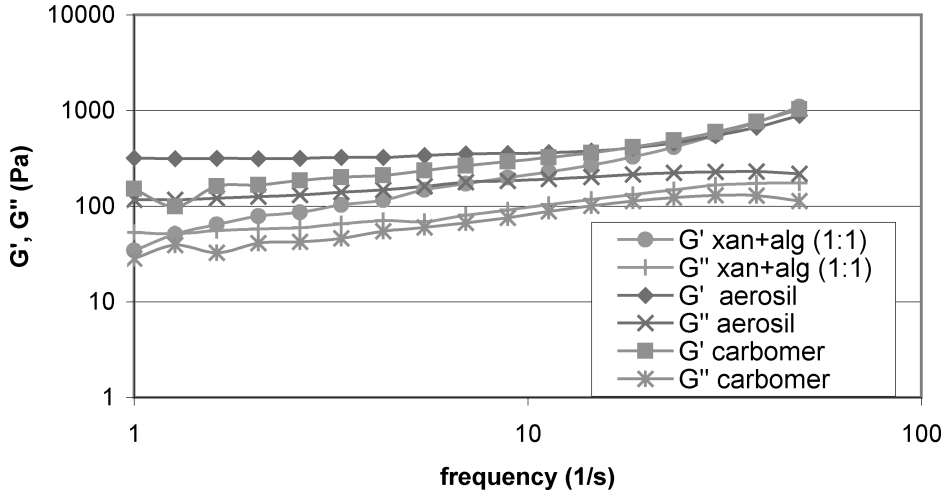

FIG. 4. Frequency dependence of G′ and G″ for selected thickened o/w ME at constant strain amplitude.

behavior. A shift of freezing point toward higher temperatures was observed, meaning that the bulk water was less tightly bound than in nonthickened ME. When the system was thickened with carbomer the peak remained sharp, which is characteristic of polymer dispersion with well defined structure. In contrast, the freezing peak of water became much broader in the presence of the xan-alg mixture, indicating a less homogenous mixture of natural polymers. The cooling curves also were less reproducible. The control run with water dispersion of xan-alg led to the conclusion that the water is mostly involved in hydration of the polymer chains (Figure 5).

The cooling curves of nonthickened and thickened w/o ME are shown in Figure 6. Due to the large amount of IPM, and consequently lower water content, the latter is present in the inner phase. It freezes at ∼ –35°C. The addition of colloidal silica broadens both peaks slightly, whereas thickening with white wax almost eliminates the peak of water, and the broadening of the IPM peak is even more pronounced. The observed behavior indicates that white wax is solubilized in IPM and the surfactant mixture, which influences their lipophilicity and probably perturbs the packing characteristics of the surfactant, causes changes in the internal organization of ME.

Stability of Vitamins E and C in Nonthickened ME

We first examined the stability of vitamin E, alone and in combination with vitamin C, under normal storage conditions (Figure 7). Vitamin E dissolved in IPM and incorporated in both types of ME also was found to be stable. Neither carrier nor light had any significant influence on its stability. The addition of vitamin C also did not affect the stability of vitamin E.

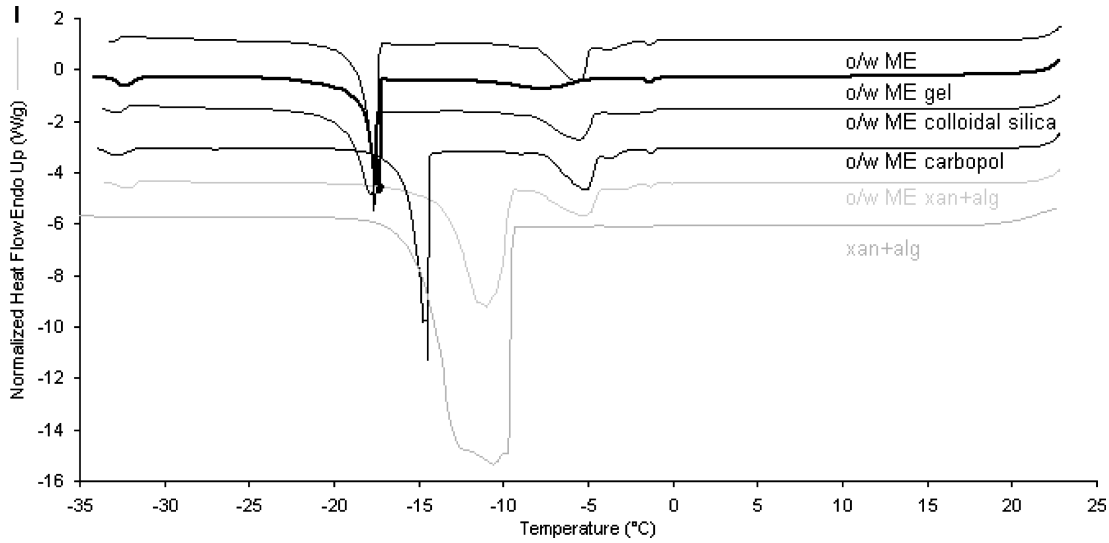

FIG. 5. DSC cooling curves of the nonthickened, gel-like, and thickened o/w ME, and water dispersion of xan-alg mixture.

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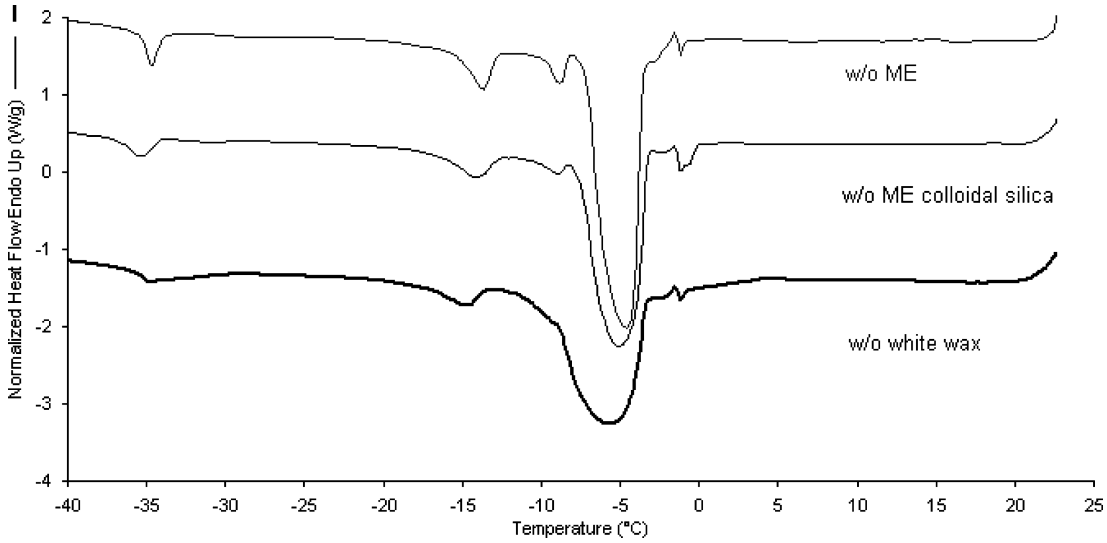

FIG. 6. DSC cooling curves of the nonthickened and thickened w/o ME.

Vitamin E may undergo photo-oxidation on exposure to UV light (Steenvorden and Beijersbergen van Henegoven 1997; Gallarate et al. 2004). After application of vitamin E to skin exposed to sun its photoinstability leads to increased levels of tocopheroxyl radical that can induce lipid peroxidation or deplete other antioxidants (Kohen 1999; Gallarate et al. 2004). Therefore incorporation into a formulation that would enhance its photostability is desirable. To find out to what extent different types of ME influence the photostability of vitamin E, samples were UVA irradiated for 48 hr (Figure 8). Long exposure time was the consequence of weaker light emission of lamp used in our experiments (3.5×10^{-4} W/cm^2) than the standard solar irradiation (1.1×10^{-3} W/cm^2). UVA light constitutes 90–99% of the UV radiation that reaches the surface of the earth and is particularly important in causing oxidative stress in the skin by inducing free radicals (Halliday 2005).

All types of nonthickened ME afford better protection from UVA light than oily solution. Gel-like ME was found to be the best protective system, followed by o/w and w/o ME. When vitamin E was incorporated into water continuous ME, it became dispersed in the fine droplets of the oily phase, where it was more protected because water and the interface area can act as a barrier for UV light and oxygen. Nevertheless, even w/o ME protected vitamin E from UV light better than the oily phase alone. Because of the large amount of surfactants in ME, they also can be present in the continuous phase (Podlogar et al. 2004; Podlogar et al. 2005). Thus, in w/o ME, vitamin E can be dissolved in the outer phase as well as being incorporated into the surfactant micelles.

Another factor that influences the stability of vitamin E in ME is its partition coefficient between the oily and surfactant phases. The molecule of vitamin E has two distinct domains; a

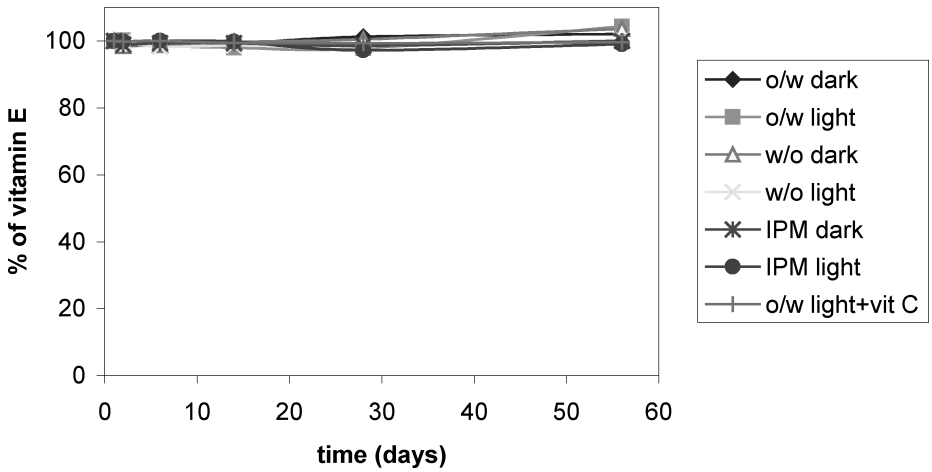

FIG. 7. The influence of different vehicles and storage conditions on the stability of vitamin E.

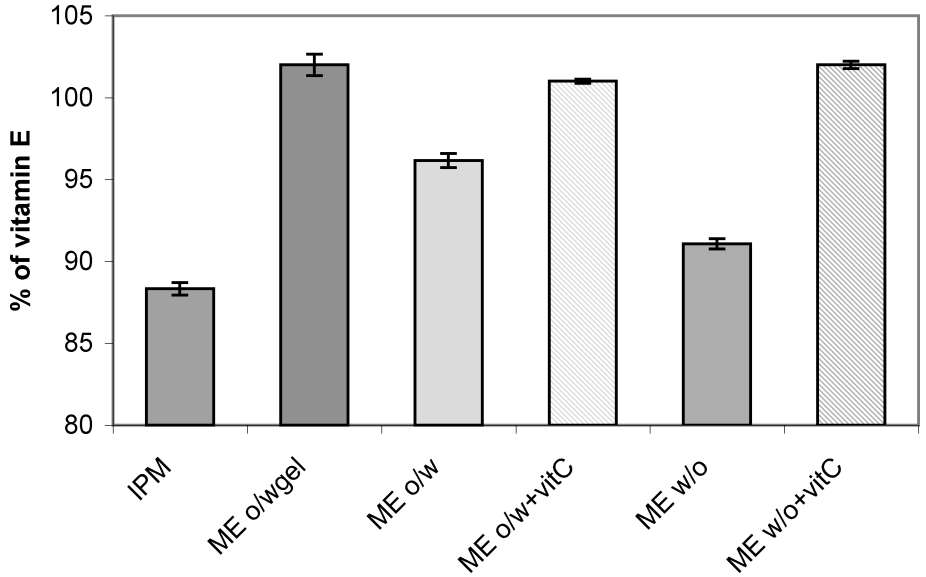

FIG. 8. Fraction of nondegraded vitamin E in ME and oily solution after 48 hr of exposure to UVA light.

chromanol nucleus terminated by a polar OH group and a long hydrophobic phytyl side chain (Figure 9) (Bongiorno et al. 2006; Avellone et al. 2002). We confirmed that vitamin E is freely soluble in the oily phase as well as in the surfactant mixture of ME, so we assume that, in the ME, it partitions between the oily phase and the surfactant mixture. Avellone et al. (2002) showed that the chromanol ring of vitamin E competes with water molecules for binding sites on hydrophilic regions of surfactant. When the content of water is increased, the concentration of vitamin E in the surfactant mixture decreases. In o/w ME, strong hydration of the surfactant mixture is thus desirable, because it hinders the incorporation of vitamin E into surfactant mixture, leaving a higher fraction dispersed in the inner phase, in which it is more protected. This explains why the gel-like ME are found to be the best protective vehicles. In ME of opposite type the incorporation of vitamin E into the surfactant film is desirable as the chromanol ring is oriented toward the inner phase and so less sensitive to photodegradation than when present in the outer phase. This results in vitamin E being more stable than in oily solution.

The addition of vitamin C to ME in the same molar concentration as vitamin E significantly protected the latter from UV degradation. Vitamin C is mainly present in the aqueous phase of ME, but its good solubility in the surfactant mixture and hydration of the surfactant film indicates that it is also present in the interfacial area, contributing to the regeneration of vitamin E.

We further evaluated the stability of vitamin C incorporated into nonthickened ME. Aerobic degradation of vitamin C was studied in ME stored in the dark to assess whether the partition influences its resistance to oxidation (Figure 10). After 14 days, w/o ME was found to protect vitamin C significantly better than o/w ME. This was expected for at least two reasons:

1. The presence of continuous oily phase, in which vitamin C is insoluble, provides protection, despite the fact that oxygen solubility in oils is roughly one order of magnitude higher than in water (Gallarate et al. 1999). The interface may act as a barrier for oxygen diffusion into the inner aqueous phase.
2. The degradation of vitamin C is highly concentration-dependent. Although both ME contained the same total amount of vitamin C, the latter is soluble only in the aqueous phase and in the surfactant mixture, which means that its local concentration in the hydrophilic compartments of ME was higher in the case of w/o ME. The higher the concentration of vitamin C, the more stable it is.

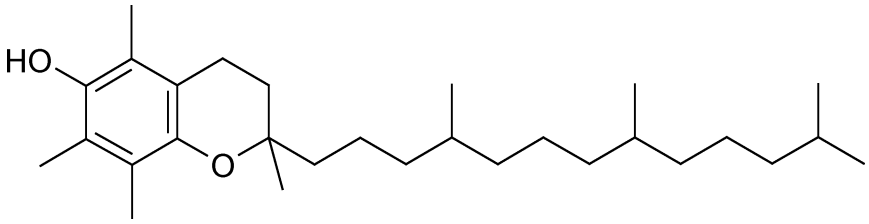

FIG. 9. The structure of vitamin E.

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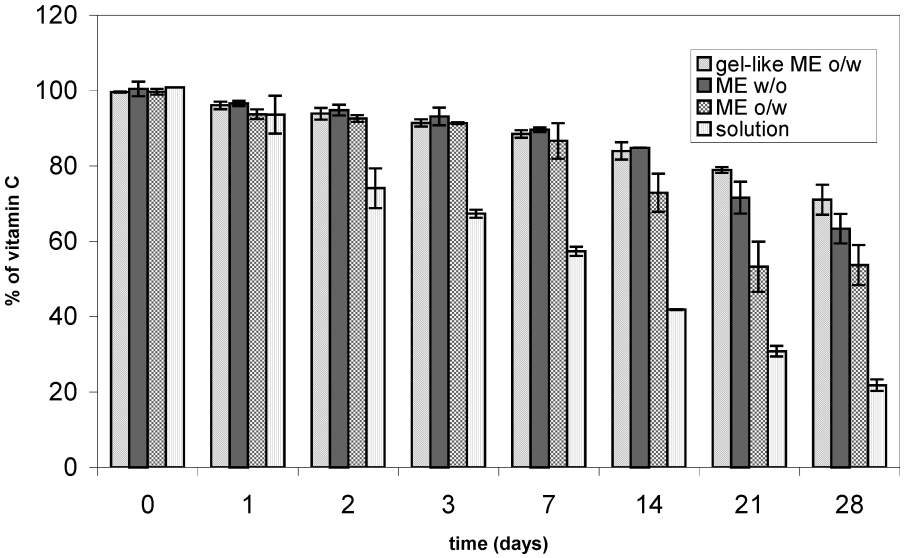

FIG. 10. Fractions of nondegraded vitamin C in nonthickened ME and in aqueous solution during 1 month storage.

According to the above explanation, one would expect that vitamin C would be least stable when incorporated into gel-like ME that has water as continuous phase and the highest percent of hydrophilic components, but the opposite was in fact the case. In the gel-like ME the surfactants are highly hydrated, so less water is available for dissolving vitamin C. As a consequence, vitamin C is located to a higher degree in the surfactant film. The increased viscosity also reduces the rate of diffusion of oxygen.

Stability of Vitamins E and C in Thickened ME

The majority of thickened ME did not offer the same level of protection for vitamins as nonthickened ME although, compared with solutions, the stability of vitamins was in most cases improved (Figures 11, 12, and 13). When the xan-alg mixture was used as thickening agent, the stability of both vitamins decreased significantly. After 28 days no vitamin C could be detected (Figure 12). Its instability is most probably the consequence of the pro-oxidative effect of alginate (Trommer and Neubert 2005). Thickening of o/w ME with carbomer had no significant influence on the stability of vitamin C, but it significantly decreased that of vitamin E. Vitamin E was significantly less stable in o/w ME thickened with polymers (carbomer, xan-alg mixture) than in IPM. As seen from the thermograms of ME thickened with polymers (Figure 5), the water was used predominantly for hydrating the polymer, so less free water was available for

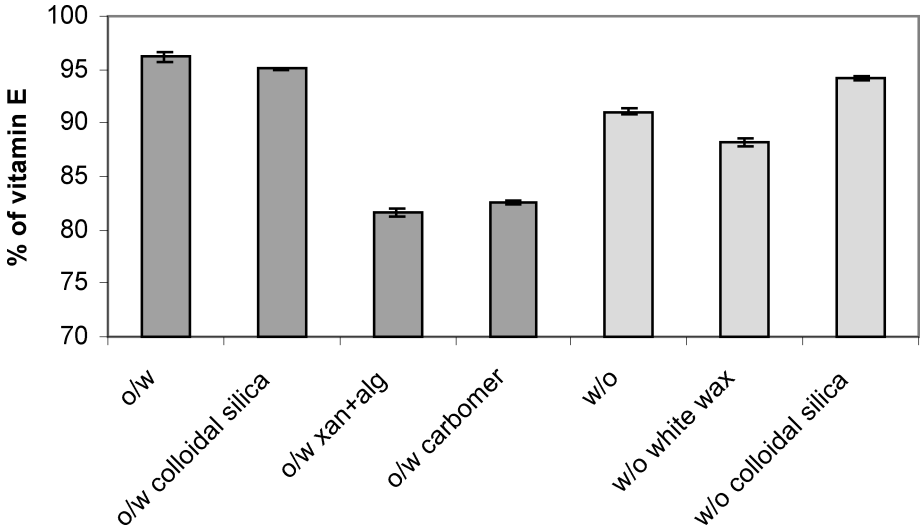

FIG. 11. Fraction of nondegraded vitamin E in different ME after 48 hr of exposure to UVA light.

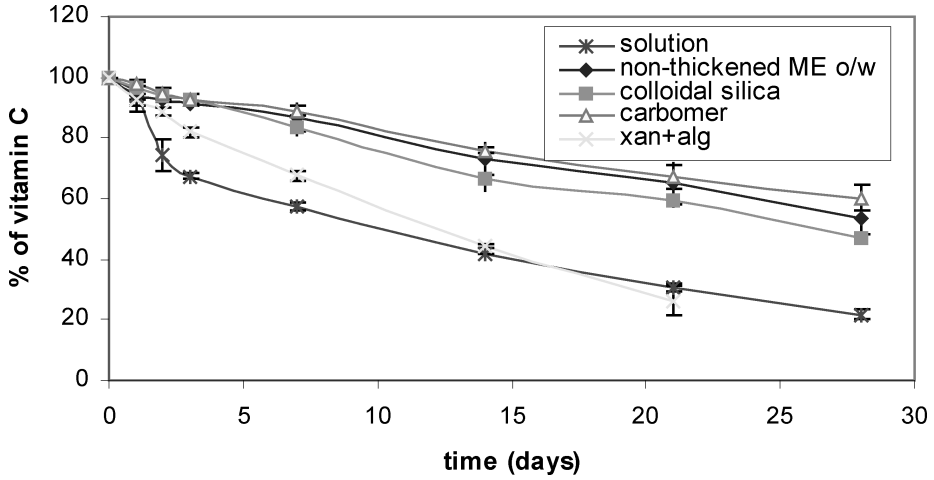

FIG. 12. Fractions of nondegraded vitamin C in thickened and nonthickened o/w ME and in solution stored in the dark for 1 month.

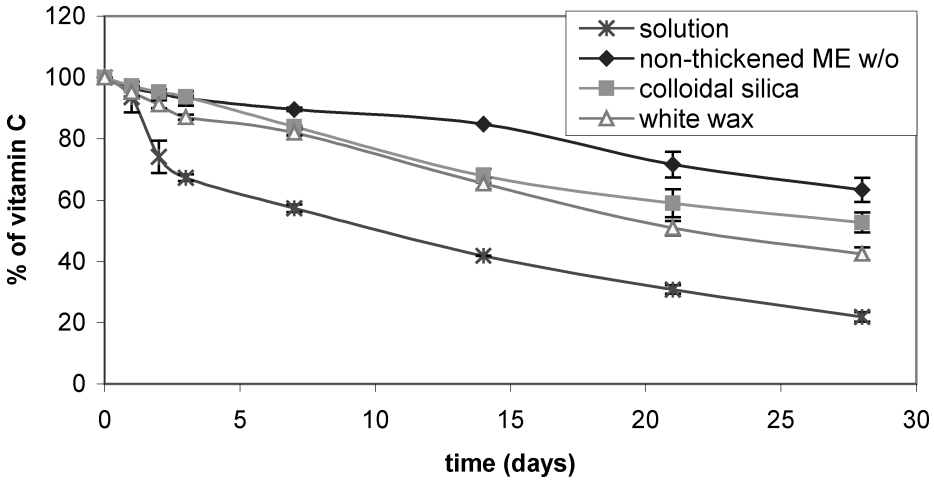

FIG. 13. Fractions of nondegraded vitamin C in thickened and nonthickened w/o ME and in solution stored in the dark for 1 month.

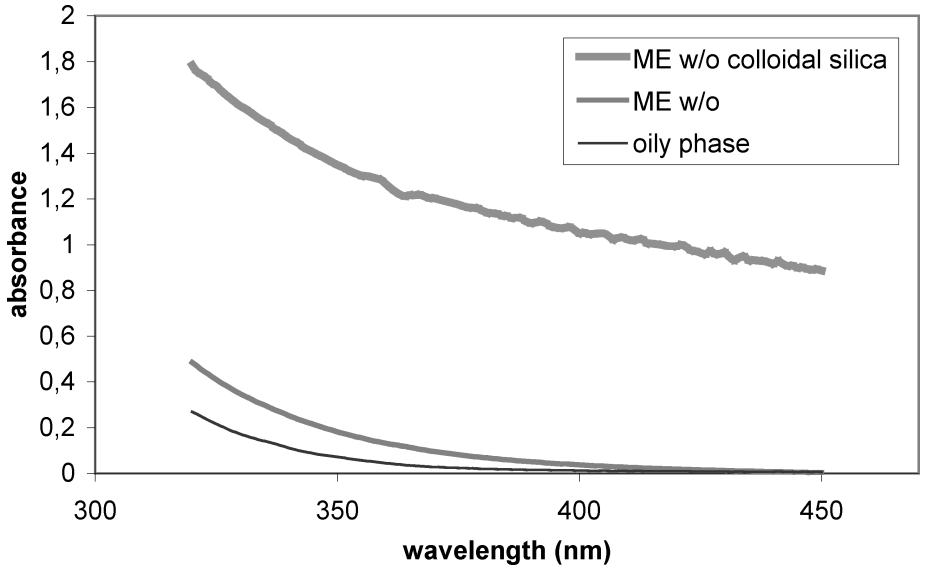

FIG. 14. Absorbance as a function of wavelength for w/o ME thickened with colloidal silica, nonthickened ME, and oily phase.

hydration of surfactants. The dehydrated surfactant film could not shield vitamin E from UV light so efficiently and, at the same time, incorporated more vitamin E, where it was more exposed to light.

O/w ME thickened with colloidal silica lowered the stability of both vitamins, but not significantly (Figures 11 and 12). In w/o ME it significantly enhanced the stability of vitamin E and accelerated the degradation of vitamin C (Figures 11 and 13). The better protection of vitamin E could be explained by the ability of silica dispersion to absorb UV light (Figure 14). As the samples with vitamin C were stored in the dark, UV absorption was not important. Another factor that could influence vitamin stability is the increased oxygen transport in gels formed with colloidal silica (http://freepatentsonline.com/5091275.html). As colloidal silica is soluble in all three phases of ME, the reduced stability of vitamin C was probably due to increased oxygen transport in the sample.

Both vitamins in w/o ME thickened with white wax are less stable than in nonthickened ME. The oxygen solubility in lipophilic vehicles depends on the polarity of the vehicle (Akoh, Min, and Akoh 2002). The more polar the vehicle, the lower the oxygen solubility. When white wax is added to the oily phase of ME, the oxygen solubility increases and accelerates the degradation of vitamins. As white wax produced by bleaching was used, it is also possible that it contained traces of impurities that were oxidants. Further, the addition of white wax also influences the structure of ME; this is probably more important for the stability of vitamin C, which is incorporated into the inner phase (Figure 6).

CONCLUSION

The incorporation of vitamins E and C into nonthickened o/w and w/o ME, and into gel-like o/w ME, enhanced their stability. Gel-like o/w ME was found to be the best protective system for both vitamins. The presence of vitamin C at the same time improved the UVA stability of vitamin E. Addition of a selected thickener (carbomer, xan-alg mixture, colloidal silica, and white wax), that suitably modified the rheological behavior of ME, changed either oxygen solubility in the outer phase or structural organization of ME and consequently influenced the stability of vitamins. As nonthickened and thickened ME, with the exception of those thickened with xan-alg mixture, increased the stability of vitamin C and E compared with that in water and oily solution, respectively, we can confirm the tested ME to be suitable carrier systems for combined antioxidant topical therapy.

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