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ORIGINAL ARTICLE

Dual influence of colloidal silica on skin deposition of vitamins C and E simultaneously incorporated in topical microemulsions

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Abstract

Background: Colloidal silica is the thickener of interest for topical formulations and can therefore be used to optimize the viscosity of both hydrophilic and lipophilic microemulsions (MEs). To the best of our knowledge, no information is available about the effect of topically applied colloidal silica on skin penetration of drugs. So, our aim was to determine its influence on the effectiveness of ME in the simultaneous delivery of vitamins C and E to the skin. *Methods*: Two different aspects of silica possible function were investigated. Its effects on formulation characteristics were studied by determination of partition coefficient of the vitamins, their solubility and release profile. The direct impact of silica on the skin was further evaluated by transepidermal water loss measurements, scanning electron microscopy (SEM), and cell toxicity determination (MTT assay). *Results*: The addition of colloidal silica to ME was shown to increase significantly the vitamins' solubility and their partition to the phase in which they were less soluble. Its presence also increased the amount of both vitamins in epidermis, which was confirmed by release studies. Furthermore, we demonstrated that colloidal silica interacts with excised skin. It decreased transepidermal water loss, probably by retaining water in the *stratum corneum* because of its massive accumulation in the upper layers, as revealed by SEM. *Conclusion:* The results confirmed that addition of colloidal silica in ME simultaneously loaded with vitamins C and E enhanced vitamins' skin bioavailability by its dual influence on delivery characteristics of ME as well as on skin properties.

Key words: *Colloidal silica; microemulsions; skin delivery; solubilization capacity; vitamin C; vitamin E*

Introduction

Microemulsions (MEs) are optically isotropic and thermodynamically stable nanosized systems of water, oil, and surfactants. In contrast to classical course emulsions they are stable, transparent, and able to form spontaneously; these characteristics, coupled with the possibility of solubilizing both water- and oil-soluble drugs, make them a very interesting drug delivery system[1–3]. MEs have been demonstrated to improve (trans)dermal delivery of several drugs over the conventional topical preparations such as emulsions, gels, or aqueous solutions[3]. Because MEs are usually low-viscosity Newtonian fluids, their rheological properties can make effective skin application difficult. A possible solution to the low viscosity lies in ME gels—transparent systems consisting of lamellar phases that retain the advantages of ME but have higher viscosity. These systems, however, remain a challenging task for formulators because their microstructures are frequently destroyed on application of small shear stresses, by small changes in temperature, or even following incorporation of drug[4–6]. A more conventional way to optimize the viscosity of topical ME is the addition of thickener to liquid ME[5]. Selecting an optimal thickener can be demanding, because it should improve rheological behavior of ME without significantly modifying other characteristics such as stability and high water–oil

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interface area[7]. Moreover, thickeners appropriate for oil-continuous are rarely compatible with water-continuous ME. An example of a thickener forming a gel in oil-continuous as well as in water-continuous ME is hydrophilic colloidal silica[8].

Colloidal silica (fumed silica, colloidal silicon dioxide) is a fine, white, light, and amorphous powder with particle size usually less than 100 nm. Several grades that differ in particle size, surface area, degree of hydrophilicity, and density, produced by modifying the manufacturing process, are commercially available. Hydrophilic colloidal silica can convert nonpolar liquids such as olive oil, liquid paraffin, or isopropyl myristate (IPM) into transparent gels. It is also used as a thickening agent for polar liquids[9,10]. The increased viscosity of formulations thickened with silica is a consequence of weak interactions between silica particles that form agglomerates. As the size of the particles is not uniform, there are some spaces in the structure inside which the fluid phase is caught[11]. Silica gels are distinguished by high viscosity with little temperature dependence and by pronounced thixotropic behavior[9,10]. Colloidal silica is generally regarded as an essentially nontoxic and nonirritant excipient. It is GRAS listed and included in the FDA Inactive Ingredients Guide[10] as well as in Ph. Eur. (monograph 'Colloidal Anhydrous Silica') and in USP (monograph 'Colloidal Silicone Dioxide').

Skin is frequently and directly exposed to pro-oxidative environments, including ultraviolet radiation and air pollutants. To counteract the harmful effects of reactive oxygen species, skin is equipped with antioxidant systems that prevent oxidative stress. However, these systems can be depleted and dermal supplementation of skin endogenous antioxidants plays an important role in prophylaxis and treatment of oxidative stress[12]. Vitamins C (L-ascorbic acid) and E (α-tocopherol) are the skin's major water- and lipid-soluble antioxidants, respectively. They can, especially when delivered topically, inhibit acute ultraviolet damage like erythema and sunburn, as well as chronic photoaging and skin cancer[13–15]. Both are highly effective depigmenting agents. Topical vitamin C also increases collagen synthesis. Moreover, because vitamin C regenerates oxidized vitamin E, their combination is synergistic. It has been shown that a topical combination of L-ascorbic acid with α-tocopherol gives twofold greater protection against UV-induced erythema than either vitamin alone[13,16–18]. MEs are promising delivery systems for combined delivery of vitamins C and E to the skin because of their potential to incorporate hydrophilic and lipophilic molecules in the same system[8,19]. Moreover, they provide protection against (photo)oxidation of both vitamins[8], improve the solubilization of vitamin E, and hence enhance their bioavailability.

It has been demonstrated that colloidal silica drastically improves epidermal concentration of a hydrophilic vitamin C and a lipophilic vitamin E in reconstructed human epidermis[20]. To the best of our knowledge, no other published study has been done to investigate the effect of colloidal silica in topical preparations on skin penetration of drugs. Therefore, our focus in this work was to elucidate the influence of colloidal silica on isolated pig ear skin, as the most relevant in vitro animal model for human skin, by studying skin deposition of the same two vitamins. Two aspects of the function of colloidal silica in the ME were evaluated: first, its ability to affect formulation characteristics (partition coefficient of the vitamins, solubility, and release), and secondly, its direct impact on skin by transepidermal water loss (TEWL) measurements, scanning electron microscopy, and cell toxicity by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) determination.

Materials and methods

Preparation of formulations

Colloidal silica (Aerosil 200) was obtained from Degussa, Düsseldorf, Germany. IPM was from Fluka Chemie, Buchs, Switzerland, and used as the lipophilic phase of ME. Tween 40—polyoxyethylene (20) sorbitan monopalmitate (Fluka Chemie)—was used as surfactant and Imwitor 308—glyceryl caprylate (Condea, Witten, Germany)—as cosurfactant. Purified water was used as hydrophilic phase. α-Tocopherol (vitamin E) and ascorbic acid (vitamin C) were from Fluka. The composition of tested formulations is given in Table 1.

ME components (oil and hydrophilic phases, surfactants) were mixed with a magnetic stirrer for 5 minutes at room temperature. Vitamins C and E were incorporated into the ME by stirring with a magnetic stirrer for 30 minutes. Colloidal silica was added to formulations containing vitamin(s) and stirred with a plastic spoon. The formulations were left covered for at least 24 hours before use.

Vitamins' isopropyl myristate/water partition coefficient determination

The partition coefficients of the vitamins were determined in the presence or absence of colloidal silica (5% (w/w)) for IPM—water system containing 10% (w/w) vitamins. The system was vortexed in a centrifuge tube and then shaken overnight. Next day the system was centrifuged at $7168 \times g$ for 10 minutes to separate the phases. Concentrations of the vitamins in each phase were determined by high-performance liquid chromatography (HPLC). Experiments were repeated in triplicate at 25 ± 1°C.

Table 1. Composition of formulations.

Component (w/w %)	o/w ME	o/w ME colloidal silica	w/o ME	w/o ME colloidal silica	Aqueous solution	Aqueous solution colloidal silica
Tween 40	14.79	13.29	14.79	13.29	—	—
Imwitor 308	14.79	13.29	14.79	13.29	—	—
Isopropyl myristate	24.65	22.15	9.86	53.16	—	—
Purified water	44.37	39.87	59.16	8.86	99.60	89.60
Vitamin E	1.00	1.00	1.00	1.00	/	/
Vitamin C	0.40	0.40	0.40	0.40	0.40	0.40
Colloidal silica	—	10.00	—	10.00	/	10.00

Solubilization capacity of formulations

An excess amount of vitamin C (0.5 g) or vitamin E (3 g) was added to 4 g of ME. Systems were allowed to reach equilibrium by stirring at 25 ± 1°C. After 24 hours samples were centrifuged at 56,096 × *g* for 20 minutes at 5 ± 1°C to separate excess vitamin from formulation. After dilution and filtration, samples were analyzed by HPLC.

Release studies

Release rates of vitamins C and E from formulations were measured through 0.45-μm cellulose acetate membrane (Sartorius, Goettingen, Germany) soaked in receptor solution 24 hours before experiment. Franz diffusion cells with a diffusion area of 0.785 cm^2 and 8 mL receptor volume were used. IPM with 0.5% (w/w) Tween 40 and 0.5% (w/w) Imwitor 308 was used as receptor fluid for vitamin E to obtain sink conditions and miscibility of receptor fluid with ME. About 0.9% NaCl was used as receptor solution for vitamin C and 500 mg of ME was dosed in the donor compartment. The diffusion membrane was kept at 32°C in a water bath and the receptor fluid was stirred continuously. At predetermined time (i.e., at 15-minute intervals for 1.5 hours, then at 30-minute intervals for the next 1.5 hours, and finally each hour for 3 hours), 0.3-mL samples were taken and replaced by the same volume of fresh preheated receptor solution. Vitamin concentrations were determined by HPLC. Each experiment was done in quadruplicate.

The cumulative amount of vitamin released was plotted against square root of time according to

$$Q(t) = k \times t^{(1/2)}, \tag{1}$$

where $Q(t)$ is the cumulative percentage of vitamin released in time t (<60%), k is the rate constant, and t is time (minutes).

Permeation studies

Pig ears were obtained from a local slaughterhouse. The skin was kept frozen until use. Before use, skin was briefly washed under tap water, hairs were removed, and skin was sliced (thickness <1 mm). Skin slices were mounted on Franz diffusion cells and filled with receptor fluid to equilibrate overnight in a temperature-controlled water bath, resulting in a membrane surface temperature of 32°C. Immediately before the experiment the whole receptor compartment was emptied and refilled with fresh preheated medium. About 8 mL of 0.9% NaCl containing 3% chicken egg albumin to increase vitamin E partition into receptor fluid (Sigma Aldrich, Steinheim, Germany) was used as the receptor fluid. The area available for diffusion was 0.785 cm^2. At predetermined time intervals (30, 60, 120, 180, 240, 360, and minutes), 1 mL of sample was taken from the receptor compartment and replaced by fresh preheated medium. Vitamins were extracted from collected samples with methanol and analyzed by HPLC. After 6 hours, the formulation was removed and the skin surface was cleaned. Epidermis was separated from dermis by heat treatment. Both were cut into small pieces and vitamins extracted with MeOH. Samples were analyzed by HPLC. The experiments were conducted in quadruplicate.

The steady-state flux of vitamin, J, was estimated from the slope of the straight line portion of the cumulative amount of drug permeated per surface area plotted against time.

HPLC analysis

HPLC analysis was carried out with an Agilent 1200 series HPLC system

Chromatographic conditions for vitamin E: the stationary phase was a 120 × 4 mm i.d. 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 limit of quantification (LOQ) for chromatographic determination of vitamin E was determined from the calibration curve to be 1.25 μM; the limit of detection was 0.412 μM.

Chromatographic conditions for vitamin C: the stationary phase was a 250 × 4 mm i.d. column packed with 5 μm Nucleosil C18-NH_2, and the mobile phase was

methanol—acetonitrile—0.02 M phosphate buffer of pH 3.5 (20:30:50). The flow rate was 1 mL/min and UV detection at 243 nm. LOQ for chromatographic determination of vitamin C was 22.7 μM and limit of detection was 7.5 μM.

Scanning electron microscope imaging of stratum corneum

Pig ear skin was treated as in the permeation experiments. After 6 hours of contact formulations were removed and the surface was cleaned with 5 × 300 μL of MeOH and dried with a cotton swab. Before observation, tape stripping was done with double-sided conductive tape (diameter 12 mm, Oxon, Oxford Instruments, Abingdon, UK) that was afterward fixed onto metallic studs. A Supra 35 VP (Zeiss, Oberkochen, Germany) scanning electron microscope (SEM) was used with an acceleration voltage of 1.00 kV and a secondary detector.

Transepidermal water loss measurements

The European Centre for the Validation of the Alternative Method-recommended procedure was followed[21] with slight modifications. Pig ears were washed under tap water and skin samples were prepared by removing the whole skin carefully from the underlying cartilage. The skin was mounted on Franz cells containing 8 mL of 0.9% aqueous solution of NaCl. During the experiments the temperature was kept at 25 ± 1°C. The Franz cells were placed in a water bath overnight and the next morning TEWL was measured with an MPA 5 Tewameter® (Courage Khazaka, Köln, Germany). Each formulation (50 mg) tested was placed on the surface of the skin. After 4 hours, the formulations were removed and the surface was cleaned with 2 × 300 μL of phosphate-buffered saline (pH 7.4) and dried with a cotton swab. The TEWL was measured again 4 hours after the removal of the formulation. Measurements were made in triplicate.

Cell viability by MTT assay

Episkin® kits containing 12 cell cultures with 1.07 cm^2 surface area on the nutritive gelatin gel were a gift from Episkin (Lyon, France). Upon receipt of the kit, cell cultures were removed from the nutritive gel and transferred under aseptic conditions into a sterile 12-well culture dish containing 2 mL of the maintenance medium per well. Maintenance medium was provided by Episkin. The cultures were incubated at 37°C, 5% CO_2, and saturated humidity until their use.

MTT assay was performed according to the method of Mosmann[22] with modifications proposed by Fentem et al.[21]. Cell cultures were incubated overnight in the maintenance medium at 37°C, 5% CO_2, and saturated humidity. The next day 50 mg of aqueous dispersion of 10% colloidal silica or distilled water (negative control) was applied to the cell culture and cells were incubated for 4 hours in 2 mL of assay medium. The surface of the culture was rinsed several times with a gentle stream of phosphate-buffered saline. Two milliliters of MTT dissolved in the assay medium (2 mg/mL) was put in each plate and cells were incubated at 37°C and 5% CO_2 during 3 hours. MTT was extracted from the cells by placing the culture into 2.6 mL of isopropanol overnight at room temperature. The next day optical densities were read on an UV/visible spectrophotometer Shimadru Brukard Instrumente AG, Basel, Switzerland, at 540 nm. All tests were done in triplicate. The direct reduction of MTT by test product was checked as follows: 25 mg of 10% colloidal silica aqueous dispersion was added to 1 mL of MTT solution (1 mg/1 mL of medium). The solution was incubated in the dark for 60 minutes at 37°C. As the solution remained yellow, it was assumed that the formulation cannot reduce the MTT.

Data analysis

Two-tailed Student's *t*-test was used to compare the partition coefficients determined with and without colloidal silica. The influence of the formulation on the vitamins' solubility in formulation and on their retention in skin layers was evaluated by one-way analysis of variance. Bonferroni's test was used for post hoc comparisons. Significance was tested at the 0.05 level of probability.

Results and discussion

The influence of colloidal silica on delivery characteristics and skin permeation ability of ME simultaneously loaded with vitamins C and E

Modification of vitamins isopropyl myristate/water partitioning in the presence of colloidal silica

Colloidal silica influenced the partitioning of both vitamins in IPM/water. To test the influence of colloidal silica on vitamin partition coefficient, it was added to IPM/water system in 5% (w/w) because in 10% (w/w), which was used in ME, pickering emulsion gel was formed, making phase separation difficult. Vitamins C and E were added together to an IPM/water system at 10% (w/w) to ensure that the concentrations of both vitamins in all phases were always above LOQ (Table 2). On the addition of colloidal silica to the ME, the partitioning of hydrophobic vitamin E into water increased 230-fold and that of the hydrophilic vitamin C into IPM 1.2 times (Table 2). Although the change of vitamin C partition was less pronounced than that of vitamin E, it was still statistically significant ($P = 0.0011$).

Table 2. Partition coefficients for vitamins C and E in isopropyl myristate/water at 25 ± 1°C.

	(log [IPM/H_2O])	
System	Vitamin C	Vitamin E
Isopropyl myristate/water	−5.22 ± 0.00	4.82 ± 0.02
Isopropyl myristate + colloidal silica/water + colloidal silica	−5.15 ± 0.02*	2.46 ± 0.01*

*$P < 0.05$ for comparison with the isopropyl myristate/water system in the absence of colloidal silica.

Addition of colloidal silica increases the solubilization of vitamins in ME

For all experiments two types of nonthickened ME—oil continuous (w/o) and water continuous (o/w) ME, all composed of the same components (Table 1)—were used. To increase viscosity they were thickened with colloidal silica. The addition of colloidal silica to both types of ME increased solubilization of both vitamins C and E (Table 3). This effect was more pronounced when vitamins were incorporated in the inner phase of ME (vitamin C in w/o ME; vitamin E in o/w ME) and can be explained by increased affinity of vitamins for the continuous phase as a consequence of silica particles with high surface area (200 m^2/g)[10] as already indicated by partition coefficient results. Interestingly, as for partition coefficient, the improvement in solubility was greater for lipophilic vitamin E although colloidal silica used in our study was hydrophilic. Vitamin E, even though being lipophilic, has an ether group and a phenol group that can each participate in interactions with a hydrophilic surface[23]. Moreover, it has been observed that poorly water-soluble drugs can also be adsorbed to the surface of hydrophilic colloidal silica[24,25].

The influence of colloidal silica on the release of vitamins C and E from ME

The release of vitamins C and E was slower and less complete from ME containing colloidal silica than from nonthickened ME (Figure 1a and b, Table 4). The main reason is the increased viscosity of formulation that was approximately the same for both types of thickened ME[20]. The release of vitamin C from nonthickened ME was slower from w/o than from o/w ME, but the differences were small. The same was also observed for ME containing colloidal silica. However, the release of vitamin E from o/w and w/o ME containing colloidal silica was the same despite the pronounced difference in its release kinetics from nonthickened o/w and w/o ME. As expected, in nonthickened ME, vitamin E release rate was higher from w/o ME where it was mainly located in the continuous phase. The unexpectedly high release

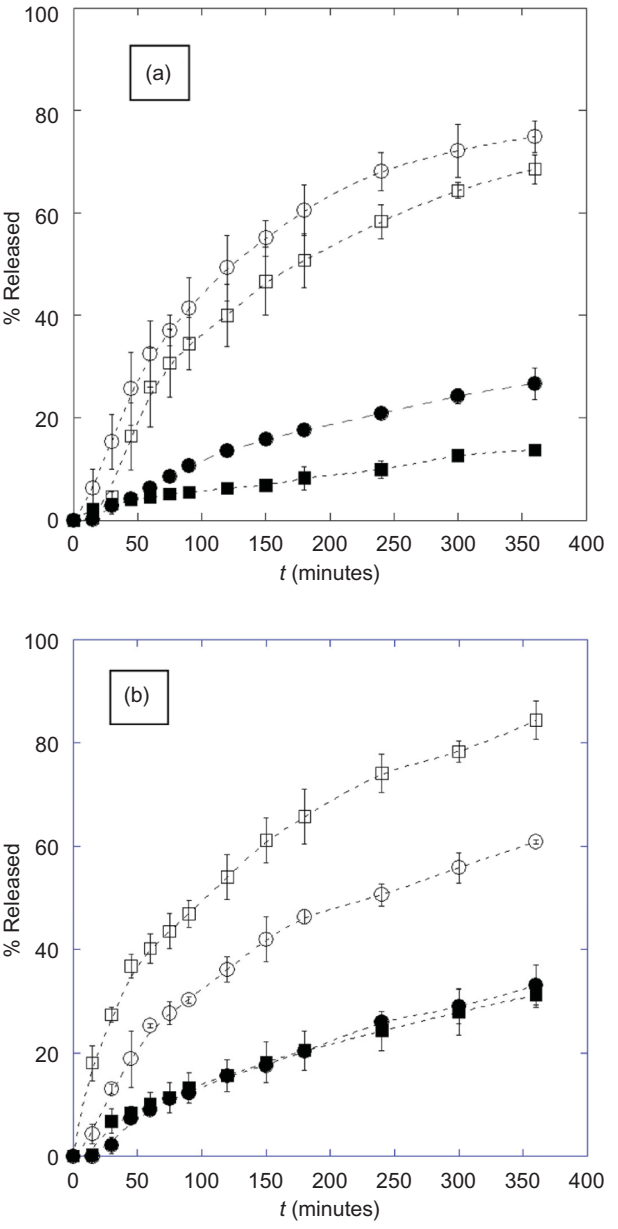

Figure 1. (a) Vitamin C release from o/w ME (○), w/o ME (□), thickened o/w ME (●), and thickened w/o ME (■). Experiments were performed in quadruplicate. (b) Vitamin E release from o/w ME (○), w/o ME (□), thickened o/w ME (●), and thickened w/o ME (■). Experiments were performed in quadruplicate.

Table 3. Solubility of vitamins C and E in o/w and w/o ME at 25 ± 1°C.

	Solubility (mg/g)	
Formulation	Vitamin C	Vitamin E
o/w ME	85.5 ± 2.6	134.4 ± 4.9
o/w ME colloidal silica	92.3 ± 5.6*	342.5 ± 6.4*
w/o ME	21.8 ± 0.6	355.4 ± 3.3
w/o ME colloidal silica	33.5 ± 0.5**	509.4 ± 2.0**

*$P < 0.05$ compared to o/w ME in the absence of colloidal silica.
**$P < 0.05$ compared to w/o ME in the absence of colloidal silica.

Table 4. Rate constant (k) for vitamin release from ME with and without colloidal silica.

	k ($min^{-1/2}$)		Pearson's coefficient[a]	
Formulation	Vitamin C	Vitamin E	Vitamin C	Vitamin E
o/w ME	5.7	3.8	0.995	0.997
o/w ME colloidal silica	1.8	2.1	0.998	0.998
w/o ME	5.1	5.0	0.988	0.992
w/o ME colloidal silica	0.7	1.8	0.985	0.999

[a]Coefficient indicating the extent of linear relationship between $Q(t)$ and $t^{(1/2)}$ (Equation 1).

rate of vitamin E from thickened o/w ME with respect to thickened w/o ME can be attributed to increased vitamin partition to the aqueous continuous phase of o/w ME in the presence of colloidal silica.

To allow comparison with permeation experiments, the vitamins release rate, characterized by k, the rate constant that was calculated from the slope of the linear portion of the plots of cumulative vitamin released ($Q(t)$) against $t^{1/2}$ (Equation 1), was evaluated (Table 4). Pearson's coefficients, proving good fitting of experimental data to assumed kinetics, are also listed.

In vitro skin delivery of vitamins C and E

To prove the efficacy of ME simultaneously loaded with vitamins C and E, we performed skin absorption studies. The amounts of vitamins accumulated in epidermis and dermis are shown in Table 5. The results show relatively high concentrations of both vitamins delivered to the tested skin layers. ME thickened with colloidal silica significantly increased the amount of both vitamins in epidermis. Overall molar concentrations of vitamin C in the dermis were negligible compared to those recovered in epidermis and receptor fluid (Figure 2, Table 5).

In contrast, considerable amounts of vitamin E were found in dermis. Although the presence of colloidal silica enhanced vitamin E deposition in dermis, the differences

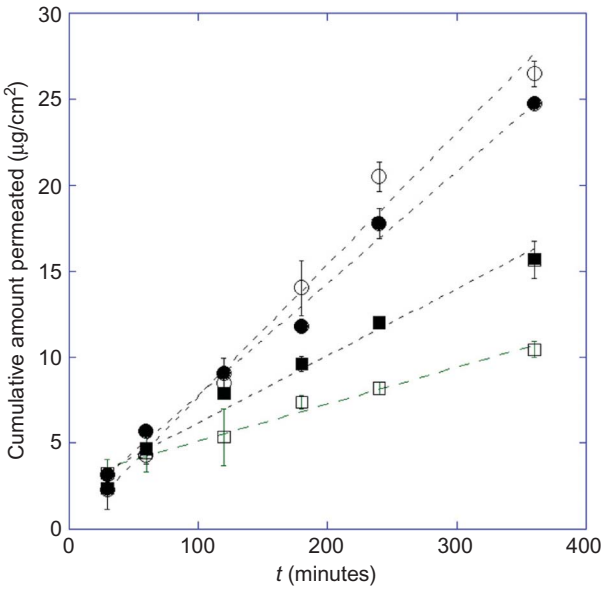

Figure 2. Cumulative amounts of vitamin C permeated through the skin following topical application of o/w ME (○), w/o ME (□), thickened o/w ME (●), and thickened w/o ME (■). Experiments were performed in quadruplicate.

between ME with or without colloidal silica were not statistically significant. No vitamin E was found in receptor fluid. It probably bounds to skin tissue where it formed a very strong reservoir[26] and consequently its partitioning from skin to receptor fluid was not favored.

Cumulative amounts of vitamin C permeated through the skin following topical application of different MEs are shown in Figure 2. In contrast to vitamin E, vitamin C permeated the skin and was found in receptor fluid after only 30 minutes. Results with ME thickened with colloidal silica are again as in case of vitamin E outstanding; more vitamin C was found in receptor fluid than expected from the release profiles (Figures 1a and b and 2). The permeation rates (steady-state flux) of vitamin C from ME are shown in Table 6. Both release and permeation rates of vitamin C from o/w ME and

Table 5. Skin absorption of vitamins C and E from ME with and without colloidal silica.

	Amount in epidermis (μg)		Amount in dermis (μg)	
Formulation	Vitamin C	Vitamin E	Vitamin C	Vitamin E
o/w ME	2.65 ± 0.41**	16.1 ± 4.0	0.436 ± 0.117	43.2 ± 6.6
o/w ME colloidal silica	4.62 ± 0.65*,**	26.0 ± 4.3*	0.189 ± 0.015	64.8 ± 6.1
w/o ME	0.057 ± 0.004*	21.8 ± 4.2	0.158 ± 0.027	58.0 ± 6.8
w/o ME colloidal silica	2.12 ± 0.66**	34.6 ± 5.2	0.128 ± 0.061	70.9 ± 5.7*
Aqueous solution	0.006 ± 0.000*	/	0.000 ± 0.000	/
Aqueous solution colloidal silica	0.188 ± 0.039*	/	0.113 ± 0.020	/

*$P < 0.05$ compared to o/w ME in the absence of colloidal silica.
**$P < 0.05$ compared to w/o ME in the absence of colloidal silica.

Table 6. Steady-state flux of vitamin C through pig ear skin from ME with and without colloidal silica.

Formulation	Steady-state flux (nmol/cm^2*h)	Pearson's coefficient[a]
o/w ME	26.2 ± 3.1	0.993
o/w ME colloidal silica	22.2 ± 0.89	0.996
w/o ME	7.31 ± 1.17	0.992
w/o ME colloidal silica	13.3 ± 0.97	0.988

[a]Pearson's coefficient indicates the degree of a linear relationship between cumulative amounts of vitamin C permeated and time.

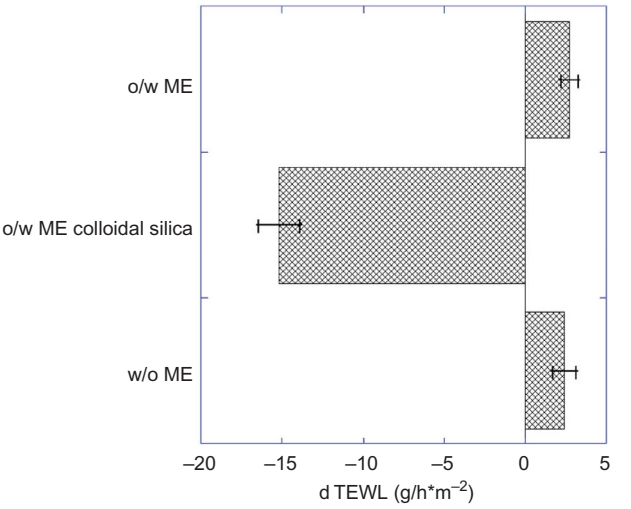

Figure 3. Effect of addition of colloidal silica to ME on transepidermal water loss (TEWL). Measurements were done in triplicate.

thickened o/w ME (Tables 4 and 6) were higher for the former. However, the differences in its release rates from o/w ME and o/w ME thickened with colloidal silica were pronounced, whereas the differences in the permeation rates were minimal. Furthermore, for oil-continuous ME the release rate of vitamin C was higher from w/o ME, whereas the permeation rate was higher from w/o ME containing colloidal silica, showing that no simple correlation exists between vitamin release and permeation.

To clarify the influence of colloidal silica on skin deposition of vitamin C, the experiment was repeated with (a) its aqueous solution and (b) its aqueous solution thickened with 10% colloidal silica. Aqueous solution thickened with colloidal silica delivered 33-fold higher amounts of vitamin C into epidermis than in the absence of silica (Table 5). Furthermore, the amount of vitamin C delivered to epidermis by an aqueous dispersion of colloidal silica was three times higher than that delivered in epidermis by nonthickened w/o ME (Table 5). Neither solution enabled permeation of vitamin C through the skin, but the one containing colloidal silica allowed its penetration into the dermis (Table 5). These results lead to conclusion that colloidal silica alone acts as an enhancing agent for the delivery of hydrophilic vitamin C into epidermis.

The impact of colloidal silica on skin

The permeation experiments have shown that the addition of colloidal silica to ME increased epidermal concentration of both vitamins and also delivered, relative to release studies, disproportionately large amount of vitamins C and E to receptor fluid and dermis, respectively. The significant impact of colloidal silica on the skin delivery of both vitamins is therefore confirmed. Moreover, the pronounced enhancement of skin deposition of vitamins from formulations containing colloidal silica implies that apart from its influence on vitamin–vehicle interactions demonstrated by partition coefficient and solubility studies, colloidal silica could also affect skin properties. Therefore, two other techniques, TEWL measurement and SEM imaging, were used to clarify its role in the skin permeation of vitamins. Finally, the influence of topically applied colloidal silica gel on the viability of keratinocytes has been tested using reconstructed human epidermis as the European Centre for the Validation of the Alternative Methods recommended model for irritancy and corrosion testing of topical products[21,27,28].

Transepidermal water loss

TEWL is the outward diffusion of water through the skin and is a commonly used technique for assessing skin barrier function, because its increase reflects an impairment of the water barrier[29]. Interestingly, TEWL was considerably lower after exposure of pig skin to ME containing colloidal silica (Figure 3). Because the skin surface was thoroughly cleaned and colloidal silica was the only component that differed from the other two MEs tested (which both increased TEWL), the reduction of TEWL suggests the deposition of colloidal silica particles in the stratum corneum. Hydrophilic silica particles that accumulated in stratum corneum could absorb water and consequently decrease its flux across the skin. This hypothesis explains the massive accumulation of vitamins in epidermis from formulations containing colloidal silica.

SEM images of pig ear skin treated with ME containing colloidal silica

The accumulation of colloidal silica particles inside the skin was investigated by SEM. The massive accumulation of colloidal silica was observed in the upper layers of stratum corneum from ME containing colloidal silica (Figure 4b), which is in accordance with the TEWL results. With increasing tape strip number the amount

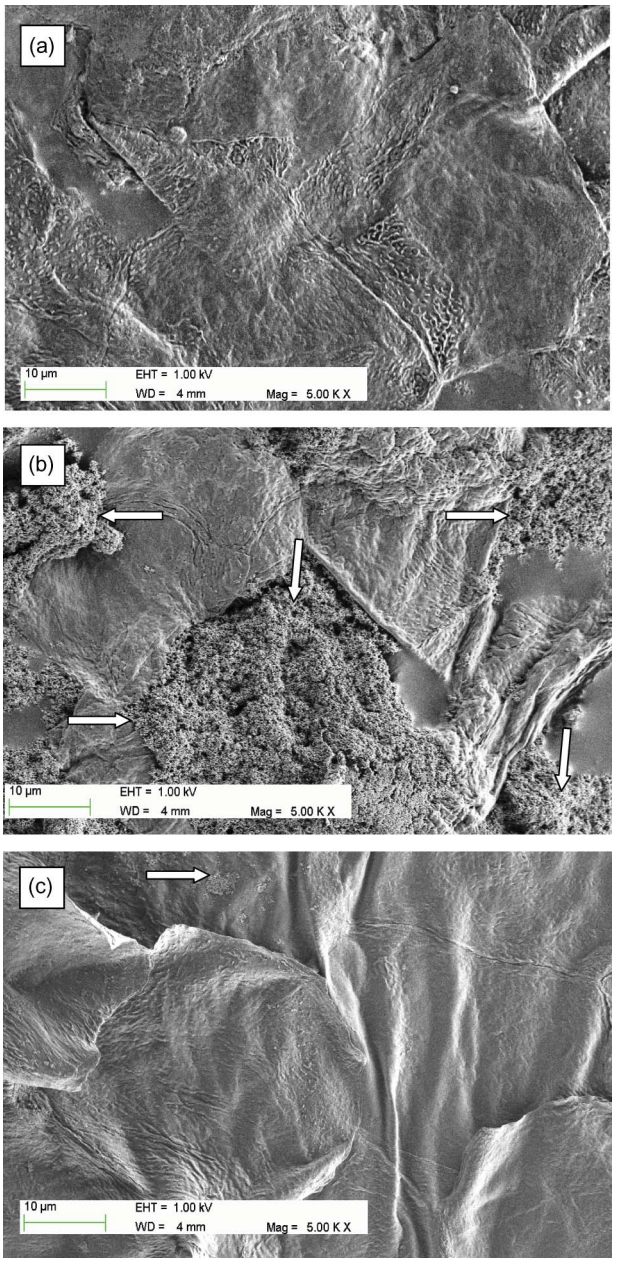

Figure 4. SEM pictures of Stratum corneum: (a) untreated skin second tape strip; (b) skin treated with w/o ME colloidal silica second tape strip; (c) skin treated with w/o ME colloidal silica eighth tape strip.

of colloidal silica decreased considerably (Figure 4c) and was on some tapes even absent.

Cytotoxicity of formulations containing colloidal silica

The cell viability of reconstructed human epidermis (Episkin® large model) after exposure to three different vehicles, aqueous dispersion of colloidal silica, o/w ME, and o/w ME thickened with colloidal silica, is shown in Figure 5. Cell viability was determined by the reduction of

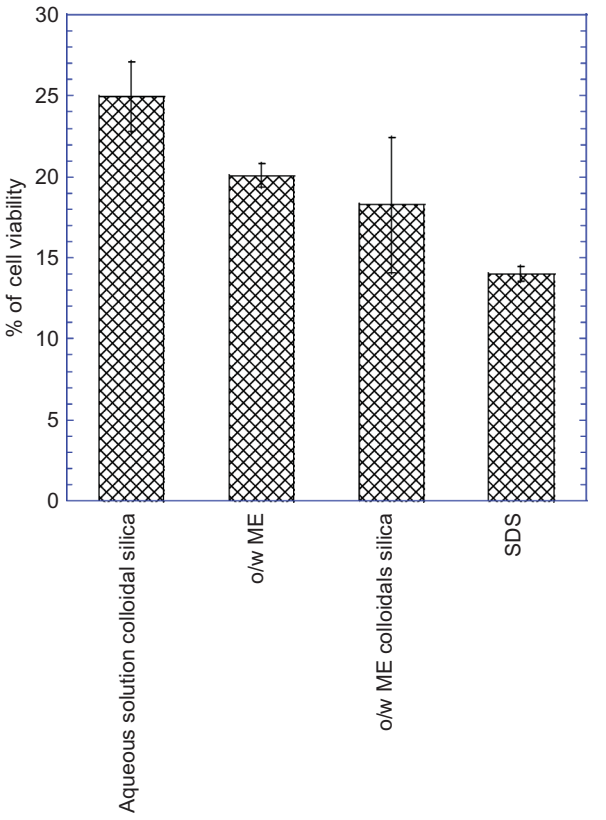

Figure 5. Cell viability measured by the MTT reduction test following topical application of various formulations. All tests were done in triplicate.

mitochondrial dehydrogenase activity measured by formazan production from MTT. Purified water and SDS solution (4 mg/mL) were used as negative (nontoxic) and positive controls. The unchanged viability of cell cultures treated with water (98 ± 2%) confirmed that cell viability decreased only because of exposure of cell cultures to the test formulation. All formulations performed slightly better than SDS solution, a standard irritant, yet the differences were insignificant. The viability of cell culture treated with aqueous solution of colloidal silica was, however, unexpectedly low, taking into account the fact that colloidal silica has always been recognized as a safe excipient and is a widely used thickener[10]. o/w ME alone performed similarly to colloidal silica dispersed in water. The addition of colloidal silica to o/w ME lowered cell viability, but less than it would have been estimated from the performance of aqueous dispersion of colloidal silica and nonthickened o/w ME alone.

Conclusion

In conclusion, the results presented in this article confirmed that the inclusion of colloidal silica in ME

simultaneously loaded with vitamins C and E enhanced vitamins' skin bioavailability. Dual influence of colloidal silica was observed: it changed not only delivery characteristics of ME but also skin properties.

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Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this paper.

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