Expert Opinion

Main approaches for delivering antioxidant vitamins through the skin to prevent skin ageing

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Introduction: One of the major contributions to skin photoageing and diseases is oxidative stress, caused by UV radiation inducing reactive oxygen and nitrogen species. Successful prophylaxis and therapy would necessitate control of the oxidant/antioxidant balance at the affected site, which can be achieved through the external supply of endogenous antioxidants.

Areas covered: This review discusses possible strategies for dermal delivery of the antioxidant vitamins E and C, as oral supplementation has proved insufficient. These antioxidants have low skin bioavailability, owing to their poor solubility, inefficient skin permeability, or instability during storage. These drawbacks can be overcome by various approaches, such as chemical modification of the vitamins and the use of new colloidal drug delivery systems. New knowledge is included about the importance of: enhancing the endogenous skin antioxidant defense through external supply; the balance between various skin antioxidants; factors that can improve the skin bioavailability of antioxidants; and new delivery systems, such as microemulsions, used to deliver vitamins C and E into the skin simultaneously.

Expert opinion: A promising strategy for enhancing skin protection from oxidative stress is to support the endogenous antioxidant system, with antioxidants containing products that are normally present in the skin.

Keywords: antioxidants, colloidal delivery systems, skin delivery, vitamin C, vitamin E

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1. Introduction

It is generally agreed that oxidative stress makes a major contribution to skin ageing, diseases and disorders. According to the free radical theory, lipid peroxidation, DNA damage and inflammation, all caused by free radicals, play a key role in skin pathologies [1]. This discovery has led to a medical revolution that stresses the role of antioxidants and free radical scavengers as prophylactic and therapeutic agents [2,3]. Free radicals are ubiquitous in the body and are generated by normal physiological processes, including aerobic metabolism and inflammatory responses, where their production and detoxification are more or less balanced. However, in excess concentrations (i.e., during pathological processes) free radicals cause oxidative damage to molecules [4]. Skin is an organ subjected to a high degree of oxidative stress from both endogenous and exogenous sources (radiation, smoking, chemical air pollutants, wind, etc.), the most important being UV radiation. The induction of oxidative damage by UV radiation, which induces reactive oxygen (ROS) and nitrogen species such as superoxide anion, hydroxyl radical, hydrogen peroxide and nitric oxide, has been demonstrated to occur in lipids, proteins and DNA [5-7]. Adverse reactions include sunburn in short-term exposure, Langerhans cell depletion and local immunosuppression caused by longer UV exposure and long-term effects such as cutaneous photoageing and skin cancer [6,8-9].

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Article highlights.

- This review focuses on photoageing in view of dermal delivery of antioxidants for enhancing skin protection from oxidative stress, a major contributor to skin ageing.
- Vitamins E and C are two of the most studied antioxidants for preventing skin oxidative damage; moreover, they act synergistically.
- Major limitations for successful dermal delivery of vitamins C and E are inadequate stability, permeability and solubility.
- There are two strategies for delivery of antioxidant vitamins through the skin, namely, chemical modifications of antioxidant molecules and/or new drug delivery systems.
- Among delivery systems, microemulsions are capable of delivering vitamins E and C simultaneously.

This box summarizes key points contained in the article.

As free radicals can inflict cellular damage, organisms have evolved several lines of defense to protect cells from free radicals and to repair DNA damage [6]. In skin, the most important lines of defense are enzymatic (e.g., superoxide dismutase, catalase, peroxidase) and non-enzymatic (e.g., glutathione, α-tocopherol or vitamin E, ascorbate or vitamin C, β-carotene and ubiquinone) antioxidant systems – see **Figure 1**. As the capacity of these systems is limited and they can be overwhelmed by excessive exposure to ROS, a promising strategy for enhancing skin protection from oxidative stress would therefore be to support the endogenous skin antioxidant system.

Photoageing is accelerated ageing of the skin resulting from environmental damage. The process is mainly due to UV irradiation and it can be prevented. It accounts for most of the ageing symptoms on sun-exposed skin. Knowledge of the mechanisms involved in photoageing provides the key approach for prevention and treatment of aged skin. The most important measure is continuous use of sunscreens, followed by topical or systemic antioxidant supplementation and use of compounds capable of repairing DNA and stimulating collagen synthesis [10]. A new, recent review of photoageing with an emphasis on prevention and topical treatment is given in [11]. Topical retinoids, particularly tretinoin, isotretinoin and tazarotene, are medical therapies with proven benefits for the treatment of photodamaged skin derived from randomized clinical evidence. However, antioxidants have also been shown to have beneficial effects on reversing damage to photoaged skin. Antioxidants are a very heterogeneous group of compounds varying from those similar to physiological species to numerous substances derived from nutritive plants, particularly polyphenols, catechins, flavonoids and acyclic carotenoids, which also demonstrate strong antioxidant activity. Some substances of natural origin, such as curcumin, capsaicin and gingerol, have also been shown to reduce photocancerogenesis, not only because of their antioxidant activity but also because of their capacity to prevent inflammation, gene mutation and immunosuppression. As plant-derived antioxidants are beyond the scope of this article, they are not discussed further.

This review focuses on antioxidant vitamins E and C and especially on possible strategies for delivering them into the skin. Oral supplementation of ascorbate is not thought to increase its skin concentration sufficiently. Thus, topical delivery is an attractive alternative. Furthermore, topical use of vitamin C is also recommended because of its depigmenting activity and its ability to promote collagen synthesis. Third, there is increasing evidence that vitamins E and C, even though present in different compartments of the cell, that is, vitamin E is in lipophilic membranes whereas vitamin C is in the aqueous cell compartment, act synergistically. Emphasis is therefore placed on those delivery systems that make possible simultaneous delivery of both vitamins or their derivatives.

2. Antioxidant vitamins E and C in human skin

Numerous antioxidants have been tested for their ability to reduce free radicals to less reactive molecules, thus reducing oxidative damage to critical cellular constituents [11]. They were also confirmed as supplements and to augment the photoprotection provided by sunscreens [12]. The antioxidants for preventing skin oxidative damage that have been studied most intensively are vitamins E and C, coenzyme Q_{10} and lipoic acid, referred to as 'network antioxidants', which work synergistically to regenerate one another (**Figure 2**). Although glutathione, coenzyme Q_{10} and lipoic acid can be synthesized by humans, levels of vitamins C and E depend on their oral intake or topical delivery [13].

2.1 Vitamin E

Vitamin E (practically insoluble in water, logP = 10) is the most important chain-breaking radical scavenger in the liposoluble cell compartment, thus constituting the principal specific defense line against lipid peroxidation. The initial oxidation product of tocopherol is a metastable tocopheroxyl radical, which can be reduced to tocopherol or can react with another lipid peroxyl radical, yielding tocopherol quinine. As shown in **Figure 3**, the term vitamin E refers collectively to eight naturally occurring molecules that show vitamin E activity [14].

α-Tocopherol is the predominant vitamin E homologue in human skin. Higher levels have been found in epidermis than in dermis [15]. Irradiation of the skin with low UV doses (below the minimal erythema dose) depletes vitamin E in the stratum corneum by almost 50%, indicating that its depletion from the uppermost skin layer is a very early and sensitive event of photo-oxidative damage. The high susceptibility of stratum corneum vitamin E to UV depletion might be due, at least in part, to a lack of co-antioxidants; for example ascorbate, which is present only at very low levels

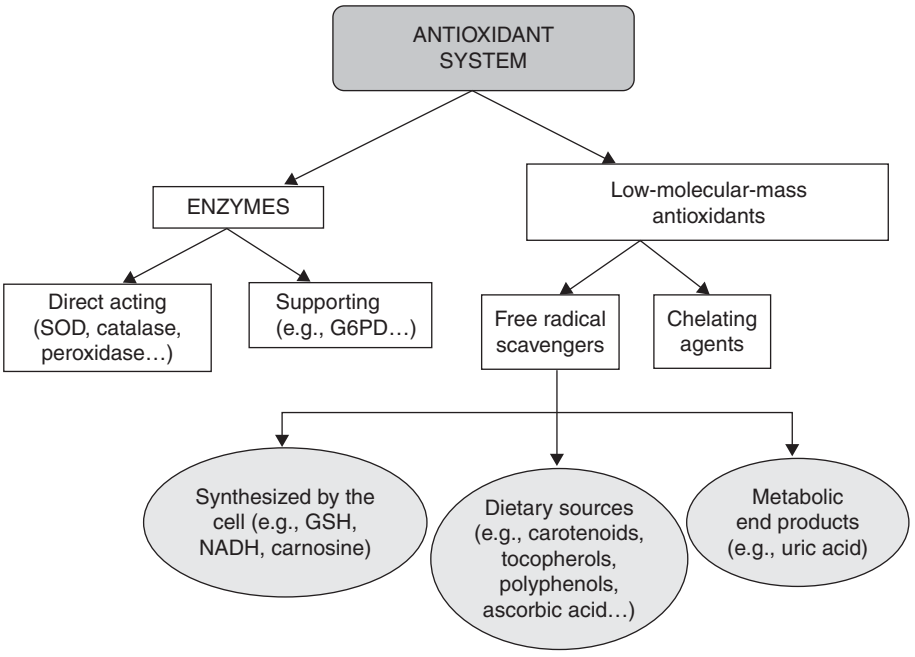

Figure 1. The antioxidant defense system of the living cell.

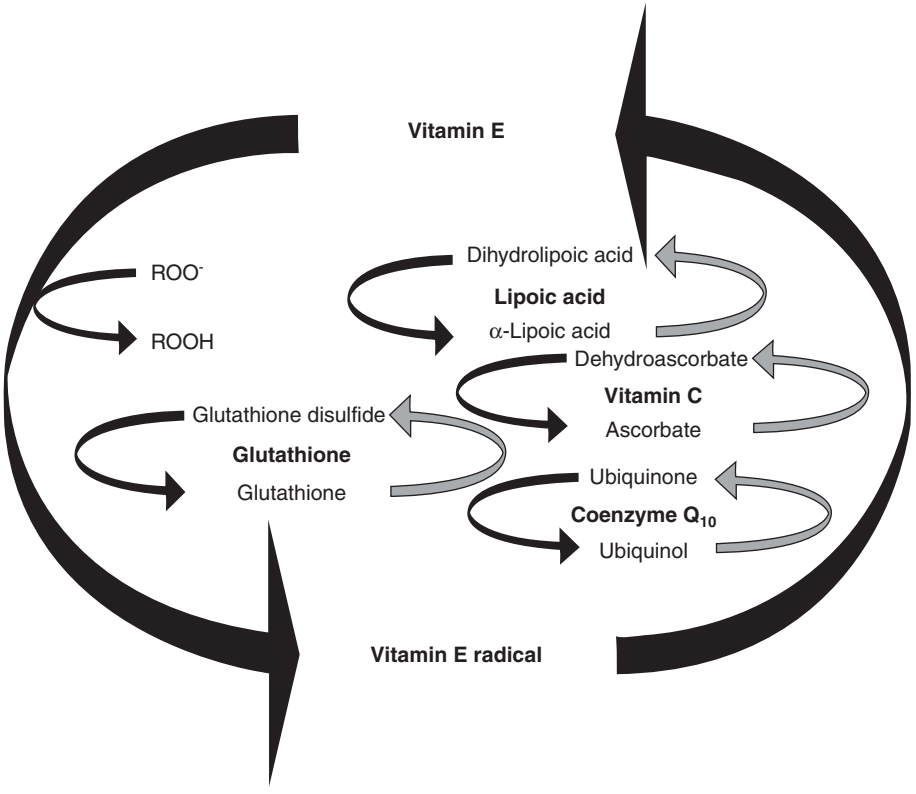

Figure 2. Regeneration pathways of 'network antioxidants'.

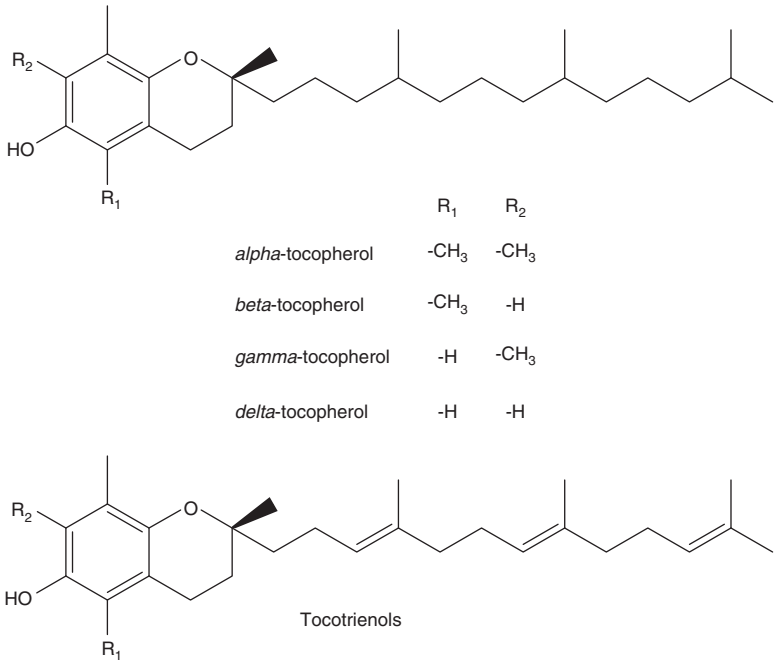

Figure 3. Chemical structures of tocopherols and tocotrienols.

in the stratum corneum compared with dermal and epidermal levels [16].

Topical delivery of vitamin E protects the skin against UV-induced cutaneous damage, carcinogenic and mutagenic activity of ionizing radiation and chemical agents [14,17]. The most notable evidence for the beneficial role of topical vitamin E exists in photoprotection when applied before UV exposure. Topically applied vitamin E protects the skin against UV-induced erythema [18], mouse skin against UV-induced lipid peroxidation [19], UV-induced photoageing changes [20,21] and UV-induced photocarcinogenesis [22,23]. There have also been reports about protection against the formation of DNA photoproducts in mouse skin, which strengthens the arguments for its use as topical protection against photoageing [24,25]. In addition, areas yet to be explored include tocotrienols, also referred to as the vitamin E of the twenty-first century. Their role against cancer and other chronic diseases is being studied; however, there is evidence that topical α-tocotrienol supplementation inhibits lipid peroxidation after benzyl peroxide treatment of human skin [26].

Vitamin E is widely used in cosmetics because it has been shown to decrease the fine lines and wrinkles induced by photoageing. It has been touted as an excellent moisturizer, resulting in increased softness and smoothness of the skin [13,27]. In dermocosmetic products it is used in concentrations ranging from 1 to 5% [10].

For commercial purposes vitamin E esters, mainly succinate and acetate, are often used because of their better stability. However, the skin has only a limited capacity to cleave the inactive esterified forms to active free tocopherol, so the antioxidant activity of esters in the skin is minimal [28].

Although vitamin E is widely used in many topical products, reports of side effects such as allergic or irritant skin reactions are rare [14]. The question of pro-oxidant effects of the tocopherols remains a mystery. High levels of tocopherols *in vitro* induce lipid peroxidation, but *in vivo* evidence is still lacking [15].

2.2 Vitamin C

Vitamin C (ascorbic acid; freely soluble in water, logP = -0.5, pK_{a1} = 4.2, pK_{a2} = 11.6; **Figure 4**), unique in its high reactivity with all aggressive oxygen radicals, is a major – and the only essential – antioxidant in the aqueous cell compartment. Most animals synthesize their own vitamin C; only humans and other primates lack the enzyme α-glucono-γ-lactone oxidase, essential for vitamin C synthesis.

As active transport of vitamin C from the gastrointestinal tract is limited, even massive oral doses do not increase its skin concentration to optimal levels [28]. The skin contains relatively low amounts of ascorbic acid, ~ 41 ng/mg (dry weight) for the entire skin, with the stratum corneum containing only 7 ng/mg (dry weight) [15]. Its level in the skin, especially in the epidermis, also decreases with age [29,30].

As in the case of vitamin E, skin levels of vitamin C can also be severely depleted by UV radiation and environmental pollutants. Even minimal UV exposure (a 1.6 minimal erythema dose) decreases the level of vitamin C to 70% of the normal level and exposure to 10 p.p.m. of ozone in city pollution

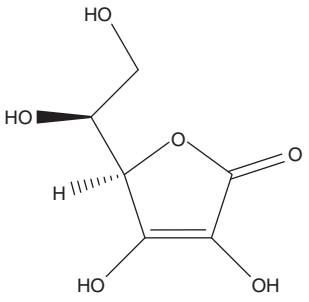

Figure 4. Structural formula of vitamin C.

decreases its epidermal level by 55% [28]. Topical application has been shown to increase significantly its cutaneous levels.

Vitamin C is important in treating skin pathologies ranging from mild inflammation to skin cancer. Topical vitamin C treatment can significantly retard UV-A-mediated damage to the skin. It plays a vital role in the metabolism of collagen, where it is necessary for the hydroxylation of lysine and proline in procollagen [13]. It has been further proven as an anti-inflammatory agent because it decreases the activation of the transcription factor nuclear factor kappa beta, which is responsible for many pro-inflammatory cytokines such as TNF-α and interleukins (IL-1, IL-6, IL-8). It is also an excellent depigmenting agent owing to inhibition of the enzyme tyrosinase and consequent reduction of melanin production. All of these actions contribute to reversal of photoageing: the synthesis of collagen and inhibition of metalloproteinase I have been confirmed to reduce wrinkles and inhibition of tyrosinase and anti-inflammatory activity result in depigmenting solar lentigines. Another important activity of vitamin C is that it increases the synthesis of several specific lipids on the skin surface [28,31-33].

Photoprotective properties of ascorbic acid have been studied on porcine skin; the authors demonstrated protection against UV-A and UV-B phototoxic injuries measured by erythema and sunburn cell formation [34,35]. In the last few years several studies on human subjects have confirmed some anti-ageing potential of vitamin C after topical application. In a randomized study with 19 patients, the results demonstrated significant clinical, subjective and photographic improvements in wrinkles, roughness, course rhytids, laxity and sallow skin after application of 10% ascorbic acid on half of the face compared with an unloaded vehicle on the other half [36]. Another double-blind study with 10 patients demonstrated a decrease in photoageing scores of the cheek and perioral area after a 3-month application of 10% vitamin C formulation [37]. Furthermore, the potential of 5% ascorbic acid to improve the clinical appearance of photoaged skin and to reduce facial wrinkles after a 6-month period was shown in a study that involved 20 subjects [38]. Although the results of human studies are optimistic, greater scientific confidence demands studies with more subjects as well as histological proof of skin ageing reversal.

Despite interesting scientific research, topical vitamin C preparations have often been disappointing for several reasons. Most preparations are very unstable on exposure to light and air. In aerobic conditions ascorbic acid is reversibly oxidized to L-dehydroascorbic acid, which can be irreversibly degraded to oxalic acid [39]. Oxidation occurs very rapidly, and once oxidized these preparations are useless. Even when products are stable, many of them do not penetrate the stratum corneum [28].

At high concentrations vitamin C can act as a pro-oxidant, but the pro-oxidant activity is dependent on the availability of free metal ions, which are usually kept at a low level in the cells. As a result, the antioxidant activity will dominate under normal circumstances [40].

2.3 Interactions of vitamins E and C

There is increasing evidence that vitamins E and C, even though they occupy different compartments of the cell, act synergistically. Vitamin C, mostly located in cytoplasm, reinforces the protective efficacy of vitamin E, located in cell membranes, probably by regenerating its radical. *In vitro* lipid oxidation studies showed that by combining vitamins C and E the free radical activity of each vitamin is enhanced [41-43]. Ascorbate and α-tocopherol have been proved to protect human fibroblasts by scavenging free radicals, therefore a constant supply of both vitamins can favor cell protection against oxidative stress [44].

The *in vivo* photoprotective effect of a topical combination of vitamins C and E and melatonin was investigated in a randomized, double-blind study. After exposure to UV radiation, vitamin C or vitamin E alone produced only small effects on erythema, whereas the combination of both vitamins enhanced the photoprotective response. Even better protection was obtained by using a combination of melatonin with both vitamins [45]. Topical formulation of both antioxidant vitamins was also found to give fourfold protection against UV-induced erythema, compared with twofold protection by either vitamin alone [46]. A clinical study also confirmed that oral intake of vitamins C and E can reduce solar-simulated irradiation-induced skin inflammation, in contrast to either vitamin alone, which failed to protect the skin [47]. Several double-blind, placebo-controlled clinical studies have shown that systemic intake of vitamins C and E in combination acts synergistically to reduce sunburn reaction and increase the minimal erythema dose, as reviewed by Bialy *et al.* [48].

3. Approaches to delivering antioxidant vitamins into the skin

At present, there are many antioxidant products on the market that have been formulated into conventional dosage forms, with vitamins leading the group. These conventional forms are easy to formulate and are relatively less expensive. However, antioxidants that have been used in diet or

formulated into classical drug delivery systems have some common problems in their efficacy related to their physicochemical and biopharmaceutical properties, such as low solubility, poor permeability, high instability and/or biotransformation before they reach the site of action [2]. Addressing these problems with the help of conventional dosage forms is difficult, and the help of advanced delivery systems is a must for maximizing the potential roles of antioxidants in prophylaxis and therapy. Various types of strategy have been used by different groups to achieve successful antioxidant delivery. The major approaches used are chemical modifications of drug molecules and/or new drug delivery systems, which offer better targeting to the upper skin layer, faster onset and lower concentrations.

3.1 Chemical modifications of the drug molecule

Numerous modifications of antioxidants have been attempted in order to obtain more stable derivatives, increase their solubility, prolong their half-life *in vivo*, protect them from degradation/inactivation in the gastrointestinal tract (GIT), improve skin penetration or attain targeting to tissues or cells. Some of the major chemical modifications of vitamins C and E are discussed briefly in this section.

3.1.1 Ascorbic acid derivatives

Chemical modification of ascorbic acid has led to more stable derivatives such as ascorbyl esters with fatty acids or ascorbyl phosphate salts [49].

3.1.1.1 Alkyl esters of ascorbic acid

One of the most studied lipophilic esters is ascorbyl palmitate (AP; practically insoluble in water, logP = 7.19; **Figure 5A**), which is often used in cosmetic and food preparations as an alternative source of ascorbic acid. It can penetrate into the skin more easily when compared with the acidic form owing to its amphiphilic character. Although it is often acknowledged as more stable, its stability is still not adequate. The main problem is oxidation mediated by transition metal ions present in traces. Studies have indicated that AP's chemical stability depends on the structural properties of the topical preparation used, which therefore offer further opportunities to overcome the stability problem [50].

Among the formulations, colloidal lipid carriers such as nanoemulsions, microemulsions, nanosuspensions, liposomes, solid lipid nanoparticles (SLNs) and oil-loaded SLNs (also described as nanostructured lipid carriers [NLCs]) have been studied extensively (**Table 1**). Almost all authors indicated the importance of carrier type and structure for AP protection against degradation. Instability of AP in NLCs was further overcome by adding antioxidants during the production step, flushing the preparation with nitrogen gas and storage at low temperatures (4°C) [51,52]. Subsequent investigation by Ahlin Grabnar *et al.* focused on AP stability in nanoemulsions, SLNs and NLCs with a focus on the type of components. The SLN and NLC dispersions investigated showed no greater AP protection from the aqueous environment than nanoemulsions. Importantly, the study confirmed that AP stability in NLCs is enhanced by the higher total lipid concentration and the type of phospholipid; hydrogenated phospholipids in dispersions offer better protection [53]. Another attempt to enhance AP stability was the preparation of nanosuspensions using high-pressure homogenization, which were subsequently transformed to dry powders by lyophilization [54].

A comprehensive study by Kristl *et al.* evaluated the influence of specific domains and composition of nanostructured systems, that is liposomes, SLNs and microemulsions, because the protective ability depends on the location of AP hydrophilic moiety sensitive to oxidation. Consequently, AP was found to be most stable in SLNs and non-hydrogenated phospholipid liposomes [55]. Microemulsions also do not offer sufficient long-term AP stability. However, it was found that high concentrations of AP reduced the extent of its degradation, whereas light accelerated it. The location of AP in microemulsions and oxygen dissolved in the system collectively influence the stability of the compound [56]. The results presented clearly demonstrate that formulation alone makes a limited contribution to drug stability. The addition of a co-oxidant of the oxime type into microemulsions seems to be an innovative approach that resulted in enhanced AP stability in hydrophilic microemulsions [57].

In addition to enhancing stability, the effectiveness of AP against free radical formation in porcine skin has been reported. Ascorbyl palmitate incorporated in both types of micoemulsion, oil-in-water (o/w) and water-in-oil (w/o), decreased the level of free radical formation determined with electron paramagnetic resonance (EPR) spectroscopy using a spin trapping technique. Its effectiveness was found to be significantly dependent on the type of microemulsion and its concentration: o/w type delivered AP to skin more efficiently [58].

Gopinath *et al.* investigated the possibility of converting AP into bilayer vesicles with a view to exploiting them as carriers for drug delivery. These vesicles were termed aspasomes [59]. In comparison with ascorbic acid, AP was found to be more stable and the antioxidant potential of aspasome was enhanced.

3.1.1.2 Ascorbyl phosphate salts

Ascorbyl phosphate salts (sodium, magnesium) are hydrophilic derivatives of ascorbic acid. According to literature data and producer specification they are among the most stable ascorbic acid derivatives. This high stability is a result of their chemical structure; sodium ascorbyl phosphate (SAP) is represented in **Figure 5B**. Introduction of the phosphate group in the second position of the cyclic ring protects the enediol system of the molecule against oxidation, and so ascorbyl phosphate salts cannot act as antioxidant agents to stabilize formulations [50]. To achieve antioxidant action

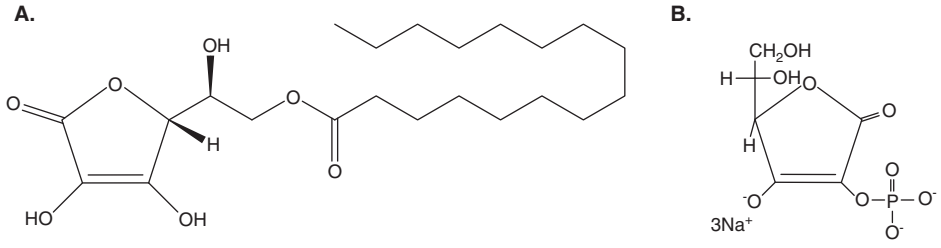

Figure 5. Structural formula of (A) ascorbyl palmitate and **(B)** sodium ascorbyl phosphate.

Table 1. Enhanced features of individual vitamins C and E, their derivatives and combinations with other antioxidants (Nos 13 – 16) when incorporated in various delivery systems.

No.	Antioxidant	Delivery system	Observed effect	Ref.
1	Ascorbic acid	w/o/w multiple emulsions	Slower degradation rate	[100]
2	Ascorbic acid	o/w/o multiple emulsions	Increased stability and controlled release	[102]
3	Ascorbic acid	Fullerene	Suppressed erythema, ROS and apoptosis index	[105]
4	Ascorbyl palmitate	ME	Decreased level of free radical formation after irradiation; improved skin delivery (o/w ME)	[58]
5	Ascorbyl palmitate	Aspasomes	Better antioxidant activity	[59]
6	Ascorbyl palmitate	NLC	Enhanced stability	[53]
7	Sodium ascorbyl palmitate	Liposomes	Enhanced penetration through stratum corneum and deeper skin layers	[62]
8	α-Tocopherol	Liposomes and o/w ME	Increased photostability; increased skin deposition (ME)	[89]
9	α-Tocopherol	Chitosan microspheres	Enhanced skin moisture and elasticity, decreased skin wrinkle volume	[107]
10	α-Tocopherol	Deformable liposomes	Improved photostability and skin deposition	[92]
11	α-Tocopherol and α-tocopherol acetate	Liposomes	Increased deposition in rat skin	[88]
12	α-Tocopherol and α-tocopherol acetate	SLN	Improved chemical stability	[96,97]
13	Ascorbic acid and α-tocopherol	ME	Increased stability, enhanced absorption in reconstructed human epidermis, increased pig ear skin bioavailability	[82-84]
14	β-Carotene and α-tocopherol	SLN	Improved photostability	[98]
15	Ascorbic acid and wheat protein	w/o/w multiple emulsions	Increased skin moisture	[103]
16	All-*trans* retinoic acid and α-tocopherol	Nanofibers	Gradual monotonous increase in the cumulative release	[108]

ME: Microemulsions; NLC: Nanostructured lipid carriers; SLN: Solid lipid nanoparticle.

within the skin, it must be converted into free ascorbic acid by enzymatic degradation in the skin.

A preliminary study on SAP stability in microemulsions endorsed its use in cosmetic and pharmaceutical preparations because > 95% of non-degraded compound remained after 2 months. SAP was stable in both types: o/w and w/o microemulsion, composed of the same ingredients, with no significant influence of its location in the carrier system. When incorporated in the internal aqueous phase, sustained release profiles were also observed [60]. Its effectiveness when incorporated in microemulsions against free radical formation was confirmed and was found to be concentration dependent [61]. In comparison with AP, incorporated in the same systems, the application time also had a significant influence on SAP effectiveness [58].

Furthermore, SAP-loaded liposomes as carriers for cutaneous photoprotection were prepared from non-hydrogenated or hydrogenated soy lecithin and cholesterol using the thin film method. Size and zeta potential of SAP-loaded liposomes were found to be significantly greater than for empty liposomes. It was also shown that liposomes enhance antioxidant penetration through the stratum corneum in deeper layers of the skin when compared with an aqueous solution [62].

3.1.1.3 Other ascorbic acid derivatives

Retinyl ascorbate, an ester co-drug of vitamins C and A, is proposed as a topical antioxidant for reducing UV-induced generation of free radicals and disrupted dermal cell growth. Abdulmajed *et al.* hypothesized that this co-drug

could enhance dermal uptake properties relative to both parent moieties, being more lipophilic as well as more stable owing to an ester bond between vitamins C and A. Free radical scavenging activity, ability to penetrate full-thickness human skin and pig ear skin, dissolution in lipids and binding to keratin in significant amounts were confirmed [63].

The new amphiphilic vitamin C derivative, disodium isostearyl ascorbyl phosphate, with C_{18} alkyl chain attached to the stable sodium ascorbyl phosphate, was synthesized and evaluated against reactive oxygen species on normal human dermal fibroblasts. The derivative is characterized by its high stability in various aqueous solutions and its non-reducibility. In addition, antioxidant and antiageing effects were confirmed. Namely, the pretreatment of fibroblasts with disodium isostearyl ascorbyl phosphate resulted in significant protection against cell damage induced by oxidative stress as well as in a suppressive effect against collagen decrease and metalloproteinase excess production induced by UV-A irradiation [64].

3.1.2 *Vitamin E derivatives*

In the case of vitamin E, the acetate and acid succinate esters are commonly used for their high stability. Despite their ability to penetrate the epidermis when applied topically, these esters rely on endogenous esterases to produce α-tocopherol activity. Whether human skin can hydrolyze tocopherol esters in a timely and quantitative fashion has not been decided, but it has been proven for murine and rat skin [65]. The hydrochloride salt of D-α-tocopheryl *N,N*-dimethylaminoacetate is another prodrug of vitamin E that has high solubility and stability [66].

A great deal of effort is being put into designing and synthesizing new derivatives of vitamin E. One of them, δ-tocopherol glucoside, has demonstrated improved stability and skin capability to metabolize the prodrug to active form (i.e., δ-tocepherol) when compared with the acetate form. Its diffusion rate was lower with a reservoir effect, but together with the ability to be metabolized this agent is an excellent candidate for continuous reinforcement of the antioxidant network in the skin [67].

Esters of α-tocopherol with amino acids were synthesized and evaluated by Ostacolo *et al.* They offer several advantages, such as higher amounts accumulated in rabbit skin when compared with the acetate form and easier incorporation into conventional dermal formulations such as gels and creams owing to their hydrophilic character. Most importantly, enzymatic metabolism of these new derivatives generates not only vitamin E but also amino acids, components of the natural moisturizing factor for a synergistic effect [68].

Several other α-tocopherol derivatives have been shown to be effective prodrugs against lipid peroxidation in the liver; for example, α-tocopherol-hydroquinone, α-tocopherol-quinone and α-tocopherol-succinate [66].

3.2 New drug delivery systems

For useful topical application of antioxidants several obstacles must be overcome. Delivery systems help antioxidants to reach the site of action, rather than altering their chemical nature or biological activity. New drug delivery systems can be applied to improve the solubility, permeability and stability of antioxidants. The use of conventional delivery systems in several cases showed little or no improvement because antioxidant molecules are inherently unstable (ascorbic acid) and are susceptible to photodegradation (vitamin E) and/or presence of oxygen. Instability makes them difficult to formulate in an acceptable formulation. In addition, many antioxidants are deeply colored. A recent review by Kaur *et al.* emphasizes the role of new delivery system for topical delivery of antioxidants [69].

In **Table 1**, delivery systems for antioxidant vitamins C and E as well as their derivatives and combinations with other antioxidant agents are summarized in view of reported improvements. Chemical modifications of vitamins C and E that present one of the approaches to achieve successful antioxidant delivery have already been discussed in previous sections, therefore the main focus in the following sections is on delivery systems for vitamins C and E. Special attention is given to microemulsions because they make simultaneous skin delivery of both vitamins possible. In addition, possible pathways for hydrophilic/lipophilic molecules and new carrier systems across the startum corneum are represented in **Figure 6**. To obtain more precise information concerning skin penetration pathways for new carrier systems, refer to [70-73].

3.2.1 *Microemulsions*

Microemulsions are clear, thermodynamically stable dispersions composed of water, oil and surfactants. These spontaneously formed systems possess specific physicochemical properties, such as transparency, optical isotropy and low viscosity. An essential requirement for their formation and stability is the attainment of a very low interfacial tension between oil and water, which is achieved by the proper choice of surfactants as well as their responding concentration [74,75]. Topical microemulsions are the subject of active development, as reflected by numerous publications and patents being granted on these systems [76]. Their specific structure gives them considerable potential to act as drug delivery vehicles by incorporating lipophilic, hydrophilic and/or amphiphilic drugs. They have been shown to increase drug solubility, increase rate and extent of absorption, modify drug release, protect labile drugs, reduce patient variability, mask unpleasant odor and significantly increase bioavailability compared with classical dosage forms such as emulsions, gels and solutions [74,75,77-80]. The formation of microemulsions as well as most of their advantages arises from the relatively high surfactant concentration required. As surfactants are possible irritants, it is crucial to select mild, biocompatible, non-irritant compounds or their mixtures

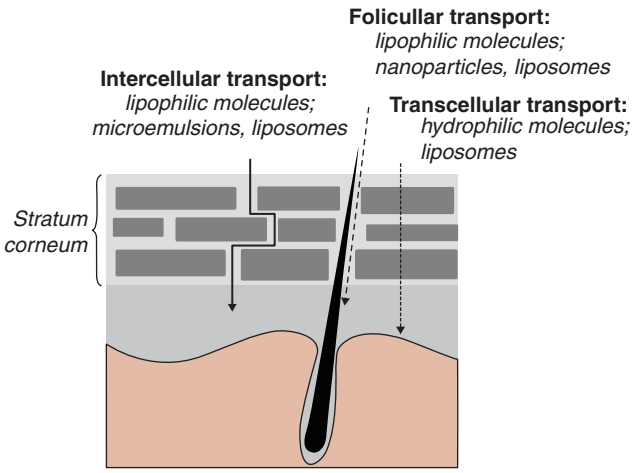

Figure 6. Schematic representation of possible pathways for hydrophilic/lipophilic molecules and new carrier systems across the stratum corneum.

and to keep their concentration as low as possible to enable microemulsion formation.

The specific structure of microemulsions allows the incorporation of lipophilic vitamin E and hydrophilic vitamin C in the same system [81]. This is advantageous owing to balance between various skin antioxidants, thus a combined therapy using at least two antioxidants is desirable.

For topical application the viscosity of liquid systems such as microemulsions plays a decisive role. The most common way to enhance viscosity is the addition of an appropriate thickener; for microemulsions simultaneously loaded with vitamins C and E, carbomer, colloidal silica, xanthan/alginate mixture, white wax and colloidal silica have been found suitable. The changes in internal organization of microemulsions resulting from the addition of thickener, as well as changes in the solubility of oxygen in the outer phase, were the most important factors that influenced the stability of vitamins in thickened systems [81]. However, it is worth mentioning another strategy that is an innovative approach to adjusting viscosity; namely, a transparent isotropic microemulsion gel formed by the addition of specific amounts of water to liquid o/w microemulsion. This was found to offer the best protection for both vitamins, although other microemulsions also significantly increased their stability compared with solution. The temperature-driven changes in the microstructure of gel-like microemulsions together with their influence on skin deposition of vitamins C and E were confirmed [82].

By varying the internal structure of non-thickened (o/w, w/o and gel-like) and colloidal silica-thickened microemulsions, absorption of vitamins C and E in reconstructed human epidermis can be significantly modulated, but it was generally enhanced in the epidermal and collagen layer by microemulsions when compared with solutions. The location of the antioxidants in the microemulsions and affinity for the vehicle appeared to be crucial in the case of non-thickened microemulsions [83].

Colloidal silica is a thickener of interest because it can be used for both types of microemulsion. Adding it increases pig ear skin bioavailability of both vitamins as compared with non-thickened microemulsion. The dual impact of colloidal silica on skin delivery was confirmed: it affected formulation characteristics and had a direct impact on the skin [84].

3.2.2 *Liposomes and other 'somes'*

Liposomes and niosomes are defined as structures consisting of one or more concentric spheres of lipid bilayers separated by water. Liposomes are predominantly formed by phospholipids, whereas niosomes are prepared from suitable nonionic surfactants. Their advantages are based on the similarity of vesicle bilayer structure to that of biological membranes, resulting in enhanced penetration in the stratum corneum, localized depot within the deep skin layers and consequently sustained release of dermally active compounds with a reduction of the amount of drug absorbed into systemic circulation [85-87].

A liposome-based antiageing topical preparation was launched on the cosmetics market in 1986 by L'Oréal and Dior (Capture™). At present, several liposome-based antiageing creams and skin moisturizers are available on the market. However, it is still a challenging task for formulators to enhance stability in the final preparation because liposomes are faced with numerous chemical and physical destabilization factors, such as surfactants, temperature, pH and lipid peroxidation [73].

The results of systematic statistical study of liposome formulations for topical delivery of α-tocopherol acetate confirmed their great potential owing to increased drug deposition in rat skin as compared with control formulations. Moreover, liposomal dispersion and liposomal gel were found to be stable for 3 months at 30°C and 65% humidity [88]. α-Tocopherol-loaded liposome dispersions, gel-emulsions and o/w microemulsions, consisting of skin-compatible emulsifiers, were studied in terms of drug photostability against UV-B light as well as skin deposition within intact pig ear skin. The best protective effect was noted in o/w microemulsions. Liposome dispersions also significantly increased α-tocopherol photostability, but with no effect on skin deposition [89].

From another point of view, a liposome system could be used as an alternative model system simulating biomembranes. A liposome model made of anionic and cationic surfactants and methyl linoleate was used to investigate the antioxidant activity of vitamins E and C [90].

Recent approaches in modulating drug delivery through the skin have resulted in the design of a new vesicular carrier. Cevc introduced the first generation of elastic vesicles referred to as transfersomes [91]. They have been defined as specially designed vesicular particles consisting of at least one inner aqueous compartment surrounded by a highly deformable

membrane. A major benefit when compared with liposomes is their ability to penetrate pores much smaller than their own size. Gallarate *et al.* formulated α-tocopherol-loaded deformable liposomes. When applied non-occlusively, they significantly improve skin deposition of α-tocopherol and its photostability. In comparison with non-deformable vehicles, skin deposition of α-tocopherol was found to be almost four times higher [92]. Also, ethosomal vitamin E preparations have been prepared. They were shown to enhance vitamin permeation into dermal fibroblast 3.5 times compared with liposomes [93].

3.2.3 Solid lipid nanoparticles

Lipid nanoparticles are colloidal systems consisting of spherical solid lipid particles in the nanometer range of 20 – 1000 nm. Solid lipid nanoparticles and nanostructured lipid carriers are based on solid lipids; however, they can be distinguished by their inner structure. SLNs consist of solid lipids, whereas NLCs, despite being solid at body temperature, contain a certain percentage of extra liquid lipid, providing more space for drug loading [94]. The enhanced chemical stability of agents, the occlusion effect in correlation with enhanced skin bioavailability and increased skin hydration as well as physical stability in topical formulations – a major advantage when compared with liposomes – are the principal benefits of SLNs in dermal delivery [95].

Protection of labile antioxidants has been reported for retinol and vitamin E, as well as for vitamin E acetate, all of which have been successfully incorporated into SLNs [96,97]. In a study by Trombino *et al.*, stearyl ferulate-based SLNs were formulated to improve the photochemical stability of incorporated β-carotene and α-tocopherol. After treatment with a pro-oxidant and/or exposure to sunlight, the antioxidants entrapped in SLNs were extremely stable, to such an extent that a dermatological formulation to prevent skin damage was suggested [98].

3.2.4 Multiple emulsions

Multiple emulsions are defined as emulsions in which both types of emulsion exist simultaneously. There are two types: w/o/w and o/w/o. Multiple emulsions offer many benefits, such as incorporating incompatible agents in the same formulation, their protection from environmental conditions and, most importantly, providing slow or controlled release [99].

Preparation of a stable multiple emulsion is a must but still a challenge. Gallarate *et al.* performed a study in which ascorbic acid was added to various emulsified systems; specifically, o/w microemulsion, w/o emulsion and w/o/w emulsion. Its stability against oxidation was studied at 45°C in aerobic conditions and compared with that in aqueous solutions. In all emulsified systems the degradation rate of ascorbic acid was slower than in solutions. The highest protection was obtained when it was dissolved in the inner aqueous phase of w/o/w multiple emulsion [100]. In addition, a recent study confirmed that w/o/w multiple emulsions containing ascorbic acid and using paraffin oil could be formulated. However, they were stable only at lower temperatures, thus exposing a need for further stabilization [101]. Oil/w/o multiple emulsions that showed an occlusive effect and differed in surfactant types and ratios were loaded with ascorbic acid. Most stable systems were prepared from non-ionic siliconized surfactants, sorbitan derivatives and co-surfactants such as polyglyceryl derivatives. Increased stability of vitamin C and, moreover, its zero-order controlled release, were confirmed [102].

In a study by Akhtar and Yazan, a multiple emulsion prepared using natural macadamia nut oil was tested as delivery system for two antiageing agents (vitamin C and wheat protein) presuming a synergistic effect. Unloaded and loaded multiple emulsions were applied to the cheeks, and skin moisture, skin sebum and skin elasticity were monitored. It was found that both formulations increased the skin moisture; however, there were no significant variations among other parameters because both behaved similarly from the dermatological point of view [103].

3.2.5 Miscellaneous

Fullerenes are compounds composed solely of an even number of carbon atoms, the most common one being C60 (also known as the 'buckyball'). Fullerene and its derivatives, especially water-soluble ones, are known to be potent antioxidants and their role in preventing oxidative damage has been established [104]. A study by Ito *et al.* evaluated the co-application effects of fullerene and ascorbic acid on UV-B-irradiated mouse skin. Fullerene itself had no phototoxic effect, whereas erythema, the ROS index and the apoptosis index decreased with the application of fullerene. The co-application with ascorbic acid significantly suppressed erythema and both the ROS and apoptosis index. The binding in fact impairs the Fenton reaction between ascorbic acid and Fe-protein that generates reactive oxygen species [105].

For lipophilic antioxidative molecules encapsulation might constitute an appropriate means to preserve their properties during storage and enhance their physiological potencies. Encapsulation for skin deposition requires vitamin protection against various stresses (light, oxygen, etc.) and particle penetration through the skin [106]. Vitamin E was incorporated in chitosan microspheres. According to *in vitro* release studies, microspheres showed burst release after the fifth min after application; however, release lasted for 6 h. *In vivo* studies showed that the system enhanced the skin moisture and elasticity while decreasing the skin wrinkle volume, therefore being a promising candidate as an antiageing product [107].

Nanofibers have attracted much attention in the last decade and, among other applications, have also been developed as carriers for drug delivery. These ultrafine fibers with average diameters in the submicrometer down to nanometer range have appealing characteristics, such as a high ratio of surface area to mass or volume, porous structure and theoretically unlimited length, plus superior mechanical performance and flexibility compared with any other form of the same material.

Mats of electrospin cellulose acetate nanofibers, used as carriers for vitamins A and E, showed a gradual monotonic increase in the cumulative release of the vitamins over test periods, whereas the corresponding as-cast films showed a burst release [108].

4. Conclusion

The skin is subjected to a high degree of oxidative stress from both endogenous sources (metabolism, inflammation) and exogenous sources (UV irradiation, air pollution, smoking, etc.). Oxidative stress especially contributes to skin (photo) ageing and skin diseases. A promising strategy for enhancing skin protection against oxidative damage could therefore be supporting the endogenous skin antioxidant system with antioxidant-containing products. Among these, of special importance are those that are normally present in the skin, with vitamins E and C leading the group. To improve the solubility, permeability and stability of antioxidant vitamins, two approaches are mostly used: chemical modifications of the vitamin molecules (alkyl esters of vitamin C, ascorbyl phosphate salts, acetate and succinate esters of vitamin E, etc.) as well as new colloidal delivery systems. These delivery systems help to deliver antioxidants efficiently, rather than altering their chemical nature or biological activity. Of special interest are carriers capable of simultaneous skin delivery of hydrophilic vitamin C and lipophilic vitamin E for combined antioxidant therapy, such as microemulsions.

5. Expert opinion

In recent years great attention has been paid to antioxidants as pharmaceutical as well as cosmetically active agents. This is supported by the free radical theory, which proposes that lipid peroxidation, DNA and protein damage are caused by free radicals. Compared with other organs, skin is especially at risk because the most important source of free radical production is exogenous; namely, UV radiation. Today everyone is aware of the risk associated with excess sunbathing and skin cancer as its most serious consequence, thanks to dermatological prevention campaigns all over the world. Dermatologists agree that there is no such thing as a safe tan, and the authors fully concur. As the sun cannot be totally avoided, protective measures include avoiding sun exposure during the hours when UV irradiation is strongest, using good sunscreen cosmetics and physical protection of the eyes and head.

Whereas skin cancer is the most serious complication of excessive UV irradiation, photoageing is undoubtedly the most frequent one. Consequently, 'antiageing' has become a key marketing focus for skincare in recent years and antioxidants are used as active molecules to improve the skin's appearance by slowing down, stopping or even reversing environmental and age-related damage that leads to skin wrinkling, discoloration and loss of suppleness.

'Antioxidant' is a much misinterpreted word. Almost any chemical can exert an antioxidant effect *in vitro* under appropriate conditions. Halliwel and Gutteridge defined antioxidants as any substance that when present at low concentrations compared with those of an oxidizable substrate significantly delays or prevents its oxidation [109]. Among physiological antioxidants in the skin, vitamins E and C, coenzyme Q_{10} and lipoic acid have been studied intensively. Considering antioxidant delivery, skin is again an exception because oral supplementation is insufficient, especially in the case of increased needs. This is obviously true for vitamin C. Topical use of vitamin C is also recommended because of its depigmenting activity and its ability to promote collagen synthesis. Another fact that should not be ignored is that endogenous antioxidant defense mechanisms are linked, and therefore the balance between various skin antioxidants is very important. If only one of them is supported, another will soon become a limiting factor. The tocopheroxyl radical depletes other antioxidants by its own regeneration, and so vitamin E monotherapy could result in an overall negative effect. As a consequence of the synergistic interplay of various antioxidants, combined therapy of vitamin E with vitamin E recycling antioxidants, such as vitamin C, glutathione, ubiquinone or carotenoids, is desirable.

The physiological role and effects of antioxidant vitamins E and C are well documented as well as their interactions. Thus, *in vivo* studies have confirmed protection of topically applied vitamin E against UV-induced erythema, lipid peroxidation, photoageing changes and photocarcinogenesis. Protection against the formation of DNA photoproducts has also been reported in mouse skin. Tocotrienols, also referred to as the vitamin E of the twenty-first century, appear to be promising compounds. Although their role in fighting cancer and other chronic diseases is the primarily aspect being studied, inhibition of lipid peroxidation in human skin after topical delivery has also been confirmed.

Vitamin C's photoprotective properties were studied on porcine skin as well as in humans, and antiageing potential after topical application (improvement of wrinkles, roughness, course rhytids, laxity and sallow skin) was proved. Despite promising results for both vitamins, further clinical studies are still needed, especially in order to obtain full scientific confidence in them as therapeutic agents. Clinical studies have also confirmed that vitamins E and C, although present in different cell compartments (vitamin C in the hydrophilic compartment and vitamin E in the lipophilic compartment), act synergistically. Therefore, it is reasonable to deliver them simultaneously.

The use of vitamin C in cosmetic and pharmaceutical products is limited owing to its low stability. To solve the stability problem, derivatives of vitamin C were synthesized. According to published data lipophilic derivatives such as alkyl esters (ascorbyl palmitate) do not show enhanced stability in comparison with pure ascorbic acid. However, these derivatives possess higher lipophilicity and thus penetrate skin more easily.

Incorporation into colloidal delivery systems also does not sufficiently enhance the long-term stability of either ascorbyl palmitate or ascorbic acid. Thus, almost always extra measures such as flushing with argon or nitrogen gas and storage at a low temperature are needed. A promising approach seems to be formulation of lyophilized nanosuspensions or incorporation of extra antioxidant for ascorbic derivative protection. On the other hand, ascorbyl phosphate salts are chemically stable derivatives, but the problem is their hydrophilicity, which hinders their deposition into deeper skin layers.

Vitamin E is oxidized very slowly by atmospheric oxygen and is generally considered to be stable. However, it can undergo photodegradation when applied to skin that is exposed to UV radiation, which might be successfully overcome by the protection offered by appropriate delivery systems. In the authors' opinion the best choice is the simultaneous delivery of both vitamins with an appropriate delivery system that enables not only protection but also modification of the release kinetics by incorporation into various compartments of the systems.

To summarize, oxidative stress resulting from UV radiation and its damage to the skin is a fact that cannot be avoided. However, detailed and greater knowledge about protective mechanisms in skin as well as skin-protecting agents has led to the development of biomimetic formulations containing antioxidants as an active care principle. Formulations based on these objectives are a powerful weapon for combating photoageing. Thus, optimized topical delivery of antioxidant agents seems to remain a challenge for the future.

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