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Stability of vitamin C derivatives in topical formulations containing lipoic acid, vitamins A and E

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Synopsis

The stability of ascorbyl palmitate, sodium ascorbyl phosphate and magnesium ascorbyl phosphate in topical formulations was investigated by direct reverse phase high performance liquid chromatography after sample dilution with a suitable buffer – organic solvent mixture. Ascorbyl palmitate, sodium ascorbyl phosphate and magnesium ascorbyl phosphate are derivatives of ascorbic acid which differ in hydrolipophilic properties. They are widely used in cosmetic and pharmaceutical preparations. According to the results, ascorbyl esters showed significant differences: sodium ascorbyl phosphate and magnesium ascorbyl phosphate are more stable derivatives of vitamin C than ascorbyl palmitate and may be easily used in cosmetic products.

Résumé

On recherche la stabilité du palmitate d' ascorbyl et phosphate de magnésium dans des formulations topiques par RP-HPLC directe après dilution de l'échantillon avec une mélange approprié tampon-dissolvant organique. Les propriétés hydro-lipophiles du palmitate d'ascorbyl, du phosphate d'ascorbyl et sodium et du phosphate d'ascorbyl et magnésium, dérivés du acide ascorbique, sont différentes. Ils sont utilisés vastement dans des préparations cosmétiques et pharmaceutiques. Selon les résultats, ils montrent différences significatives: le phosphate d'ascorbyl et sodium et le phosphate d'ascorbyl et magnésium, sont les dérivés plus stables de la vitamine C, et peuvent être utilisés facilement dans des produits cosmétiques.

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Introduction

Vitamin C (L-ascorbic acid) (Fig. 1a) has been used in cosmetic and dermatological products because it has many favourable effects on the skin. As a reducing agent, vitamin C can scavenge and destroy aggressive oxidizing agents and radicals. Because of its capability to suppress pigmentation of the skin and decomposition of melanin, it can be used to whiten the skin. Vitamin C also improves the formation of collagen [1]. Furthermore, there is considerable evidence that vitamin C plays an important role in the prevention of a large number of chronic diseases, such as cancer, cerebral apoplexy, diabetes, dermatitis, myocardial infarction and acquired immunodeficiency syndrome. These biological activities of vitamin C result from its enediol structure, which manifests a strong electron-donating ability [2]. However, its low stability is a serious limitation. It is easily oxidized, especially under aerobic conditions and light exposure, being degraded first in a reversible step to dehydroascorbic acid and second to oxalic acid in a irreversible fashion [3].

Chemical modification of ascorbic acid has led to more stable derivatives such as ascorbyl esters

with C_6 to C_{18} fatty acids or ascorbyl phosphate salts. Among the lipophilic derivatives, ascorbyl palmitate (Fig. 1b) is often used in topical preparations against oxidative changes of biological components of the skin, and as an anti-oxidant to protect lipophilic ingredients in formulations [4]. Because of its lipophilic character, ascorbyl palmitate penetrates more easily, whereas ascorbyl phosphate salts being prodrugs must be converted by an enzymatic hydrolytic process before penetrating into the skin [1]. The phosphate esters of vitamin C serve as hydrophilic anti-oxidants and increase the stability towards alkali, oxidation and prolonged storage. In particular, magnesium ascorbyl phosphate (Fig. 1c) has been widely used as a bleaching ingredient for cosmetics [2]. The sodium ascorbyl phosphate (Fig. 1d) is one of the most effective free radical quenchers and has the greatest potential for slowing down the detrimental effects resulting from photodamage. It protects the cells against free radicals, promotes collagen formation and acts on the melanin formation process [5].

The stability of these derivatives has been studied in different formulations: ascorbyl palmitate [1, 3, 4], sodium ascorbyl phosphate [1, 5, 6] and magnesium ascorbyl phosphate [3, 7]. The simultaneous high performance liquid chromatography (HPLC) determination and quantitation of such ingredients has not yet been reported in the literature. Only a few methods have been reported in the literature for the determination of ascorbyl palmitate [1, 3, 4, 8], sodium ascorbyl phosphate [1, 5, 6] and magnesium ascorbyl phosphate [3, 7–9].

We have included these vitamin C derivatives to enhance vitamin A stability. Previous studies have shown that lipoic acid was not very stable in these formulations, but the presence of vitamin A favours its chemical stability [10]. The aim of this work was to compare the stability of ascorbyl palmitate, sodium ascorbyl phosphate and magnesium ascorbyl phosphate in o/w emulsions for cosmetic application. The chemical analyses were carried out by HPLC using the method described by Spiclin [1].

Materials and methods

Materials and reagents

Ascorbyl palmitate was provided by Hoffmann La Roche (Basel, Switzerland), sodium ascorbyl phosphate by BASF (Ludwigshafen, Germany), magnesium ascorbyl phosphate by Merck (Darmstadt, Germany), butylhydroxytoluene by Eastman Chemical Company (Kingsport, TN, U.S.A.) and Vitamin C by Kromberg (Buenos Aires, Argentina). Vitamin A (as palmitate) and vitamin E (as acetate) were provided by Merck and lipoic acid by Labochim (Laboratorio Chimico Internazionale, Milan, Italy).

The emulsions consisted of silicone fluid (Dow Corning, Sao Paulo, Brazil); mineral oil, vaseline (R.A.A.M., Buenos Aires, Argentina) and acetylated lanolin, Acelan L (Fabriquímica, Buenos Aires, Argentina) as oil phase; polyoxyethylenated fatty alcohol, Ceral PW (Fabriquímica, Argentina) as surfactant; methyl *p*-hydroxybenzoate and propyl *p*-hydroxybenzoate (Clariant, Leeds, U.K.) as

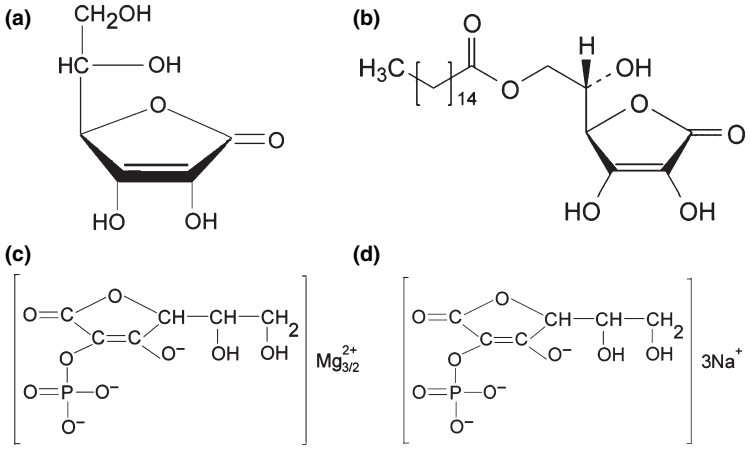

Figure 1 (a) Vitamin C, (b) ascorbyl palmitate, (c) magnesium ascorbyl phosphate, (d) sodium ascorbyl phosphate.

preservatives; propylene glycol (Dow Chemical, Midland, MI, U.S.A.) and demineralized water as hydrophilic phase. All chemicals used were of analytical grade. Methanol, acetonitrile and water were of HPLC grade. Solvents were filtered through a 0.45-μm membrane and degassed.

Preparation of the emulsions

The non-ionic emulsifier was melted into a stainless steel container; then acetylated lanolin, silicone fluid, propyl *p*-hydroxybenzoate and mineral oil were added. It was mixed by slow agitation avoiding the incorporation of air and keeping the temperature between 72 and 74°C. Lipoic acid and butylhydroxytoluene were then added. It was stirred at the maintained temperature until a full dispersion was obtained. Demineralized water, propylene glycol and methyl *p*-hydroxybenzoate were mixed into another stainless steel container. This mixture was heated up to 75°C. Both phases were filtered by gravity filtration. The mixture 1 was incorporated into mixture 2 and stirred at 63 ***g***, for 5 min. Then, cooling was started and stirring was slowed down. Vitamins A, E, ascorbyl palmitate, sodium ascorbyl phosphate, magnesium ascorbyl phosphate and vitamin C diluted in water were incorporated at 45°C. The emulsions were stored for 30 months for system 1 and 18 months for system 2 at room temperature, and were analysed under the same conditions in all cases. The quantitative compositions of the formulations are shown in Table I and II.

Table I System 1

Materials	(g/100 g)		
Polyoxyethylenated fatty alcohols	7.000	7.000	7.000
Silicone fluid	0.500	0.500	0.500
Mineral oil	5.000	5.000	5.000
Acetylated lanolin	1.000	1.000	1.000
Methyl *p*-hydroxybenzoate	0.200	0.200	0.200
Propyl *p*-hydroxybenzoate	0.100	0.100	0.100
Propylene glycol	9.000	9.000	9.000
Vitamin A palmitate	0.120	0.120	0.120
Vitamin E acetate	0.400	0.400	0.400
Lipoic acid	0.500	0.500	0.500
Butylhydroxytoluene	0.015	0.015	0.015
Ascorbyl palmitate	0.500	–	–
Magnesium ascorbyl phosphate	–	0.500	–
Sodium ascorbyl phosphate	–	–	0.500
Demineralized water to volume	100.000	100.000	100.000

Table II System 2

Materials	(g/100 g)	
Polyoxyethylenated fatty alcohols	7.000	7.000
Silicone fluid	0.500	0.500
Mineral oil	5.000	5.000
Acetylated lanolin	1.000	1.000
Methyl *p*-hydroxybenzoate	0.200	0.200
Propyl *p*-hydroxybenzoate	0.100	0.100
Propylene glycol	9.000	9.000
Vitamin A palmitate	0.120	0.120
Vitamin E acetate	0.400	0.400
Lipoic acid	0.500	0.500
Vitamin C	0.500	0.500
Magnesium ascorbyl phosphate	0.500	–
Sodium ascorbyl phosphate	–	0.500
Demineralized water to volume	100.000	100.000

Equipment

The HPLC system consisted of a dual piston reciprocating Spectra Physics pump (Model ISO Chrom LC pump: Spectra Physics, Irvine, CA, USA), a UV–Vis Hewlett Packard detector (Model 1050), a Hewlett Packard integrator (Series 3395: Hewlett Packard, Avondale, PA, USA) and a Rheodyne injector (Model 7125).

Chromatographic conditions

1 For ascorbyl palmitate, the stationary phase was 125 mm × 4 mm column packed with 5 μm LiChrospher RP-18 and the mobile phase was methanol : acetonitrile : 0.02 M phosphate buffer pH 2.5 (75 : 10 : 15). The flow rate was set at 1.5 mL/min and the determination by ultraviolet (UV) detection at 254 nm.
2 For sodium ascorbyl phosphate and magnesium ascorbyl phosphate, the stationary phase was a 250 mm × 4 mm column packed with 100 μm Microsorb NH_2 and the mobile phase was acetonitrile : 0.3 M phosphate buffer pH 4.0 (40 : 60). The flow rate was set at 0.8 mL/min and the determination by UV detection at 258 nm.

The determinations were performed at room temperature.

Preparation of standard solutions

A standard stock solution of ascorbyl palmitate (0.4 mg/mL) was prepared by dissolving appropriate

amount in methanol. The standard solution was obtained by diluting the standard stock solution with methanol to yield a solution containing 0.04 mg/mL.

A standard stock solution of the phosphate esters of vitamin C (0.4 mg/mL) was prepared by dissolving appropriate amount in tetrahydrofuran : 0.3 M phosphate buffer pH 4.0 (1 : 100,v/v) (diluting solution). The standard solution was obtained by diluting the standard stock solution with diluting solution to yield a solution containing 0.04 mg/mL.

Sample Preparation

Approximately 0.4 g of cream was exactly weighed, placed into a 50 mL volumetric flask, taken to volume with methanol and shaken for about 5 min for ascorbyl palmitate analysis. Approximately 0.4 g of cream was exactly weighed, placed into a 25 mL volumetric flask, taken to volume with diluting solution and shaken for about 5 min for the analysis of phosphate esters of vitamin C. The solutions were passed through a 0.45-μm membrane filter before injection.

Stability studies

All samples were stored in well-closed 250 g polyethylene flasks. During storage, samples were kept at room temperature (22 ± 1°C) in the dark. The amount of non-degraded active ingredients was determined quantitatively by HPLC.

Results and discussion

Chromatographic analysis of sodium ascorbyl phosphate and magnesium ascorbyl phosphate was performed using an amino column as a stationary phase, whereas ascorbyl palmitate was performed in an RP-18 column. Specification data of sodium ascorbyl phosphate and magnesium ascorbyl phosphate provided by its producers show that neutral or basic solutions guarantee the highest stability, whereas in acid solutions, these ascorbic acid derivatives are extremely unstable and may be easily hydrolysed to ascorbic acid and inorganic phosphate. Medium acid pHs rather than basic solutions are more suitable for the formulation of topical products because this pH is typical to that of the skin [11].

Despite the stability studies found in the literature, we have studied these formulations for 18 and 30 months. We examined the behaviour versus time of ascorbyl palmitate, sodium ascorbyl phosphate and magnesium ascorbyl phosphate in the same type of formulation. Samples were periodically directly analysed by HPLC after dilution with the proper solvent mixture. No interference peaks were detected in the chromatographic patterns and the applicability of both methods was verified.

The definitive differences in behaviour in system 1 (Fig. 2) between the derivatives was evident: sodium ascorbyl phosphate and magnesium ascorbyl phosphate kept its stability to nearly 60–70% even after 365 days of storage in the dark at ambient temperature, whereas ascorbyl palmitate already showed great instability (none detected) after the same time.

In a second test, in system 2 (Fig. 3), we introduced vitamin C instead of butylhydroxytoluene to evaluate the stability of sodium ascorbyl phosphate and magnesium ascorbyl phosphate. In this way, we presumed that the stability of these molecules with vitamin C in the formulation would increase. Both emulsions stored at ambient temperature in

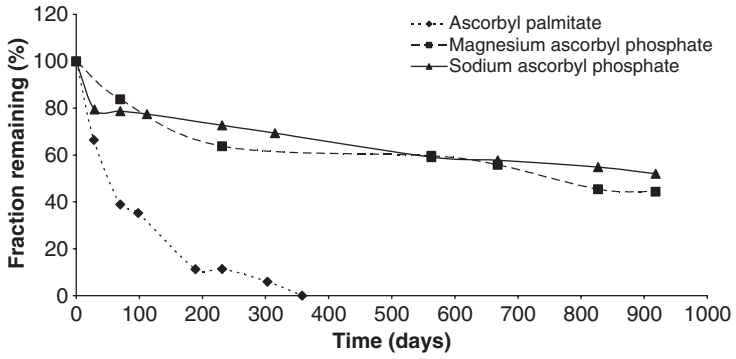

Figure 2 Content of ascorbyl palmitate, magnesium ascorbyl phosphate and sodium ascorbyl phosphate in system 1 after 30 months storage at room temperature.

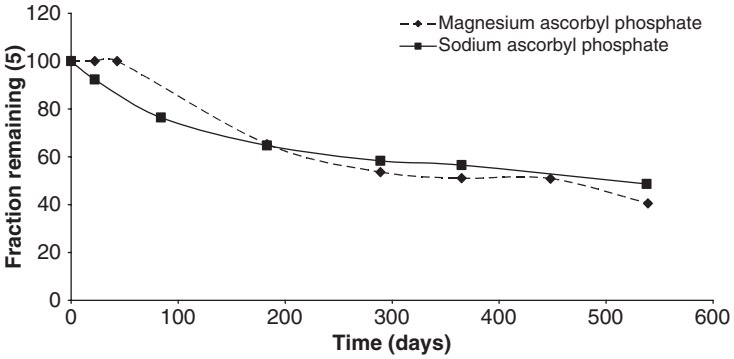

Figure 3 Content of magnesium ascorbyl phosphate and sodium ascorbyl phosphate in system 1 after 18 months storage at room temperature.

the dark showed a significant increase in its stability during the first month. Sodium ascorbyl phosphate and magnesium ascorbyl phosphate kept its stability to nearly 51–57% after 365 days of storage. On the basis of experimental results, we concluded that butylhydroxytoluene favours the long-term chemical stability of vitamin C derivatives. Between vitamin C derivatives, it seems that sodium ascorbyl phosphate is more stable than magnesium ascorbyl phosphate in the long-term studies.

Anti-oxidants work by consuming oxygen at a faster rate than the drug substance reacts with oxygen, and in such cases they will protect the drug substance until they are completely used up. Because of their chemical structure, the molecules of ascorbyl palmitate are orientated with the palmitic residue in the lipophilic phase and the cyclic ring in the aqueous phase. Only the cyclic ring is sensitive to oxidation. In o/w emulsions, the cyclic ring is in the external aqueous phase. The instability of ascorbyl palmitate is highly dependent on the oxygen dissolved in the aqueous phase. In o/w emulsions, the dissolution of oxygen from the outside compensates the oxygen consumed in the degradation reactions.

The second-order reaction is described by the general rate equation: $-dA/dt = k_2[A][B]$. In this case B is oxygen. The first-order reaction is: $-dA/dt = k_1[A]$. Degradation profiles of ascorbyl palmitate, sodium ascorbyl phosphate and magnesium ascorbyl phosphate were evaluated by fitting the experimental data to different order kinetics. The data were transformed for linear regression analysis for zero, first and simple second-order reactions. The calculated Pearson values are listed in Tables III and IV, bold print indicating the best fits. For ascorbyl palmitate, we found first-order kinetics. In this case, as oxygen is in excess and its cyclic ring is sensitive to oxidation, a reduction of second order to pseudo-first order fits better.

Table III Pearson coefficients for the case of zero-, first- and second-order degradation kinetics of ascorbyl derivatives in system 1

Anti-oxidant	Zero order	First order	Second order
Ascorbyl palmitate	0.8916	**0.9882**	0.9608
Sodium ascorbyl phosphate	0.9148	0.9508	**0.9744**
Magnesium ascorbyl phosphate	0.9356	0.9599	**0.9686**

Table IV Pearson coefficients for the case of zero-, first- and second-order degradation kinetics of Ascorbyl derivatives in system 2

Anti-oxidant	Zero order	First order	Second order
Sodium ascorbyl phosphate	0.9268	0.9573	**0.9804**
Magnesium ascorbyl phosphate	0.9563	0.9746	**0.9828**

Sodium ascorbyl phosphate and magnesium ascorbyl phosphate are water-soluble agents. The introduction of phosphoric group in second position protects the molecule from break-up of the enediol system of the molecule against oxidation. Ascorbyl phosphate salts cannot act as anti-oxidant agents to stabilize formulations. In the case of ascorbyl phosphate salts, the reaction follows a second-order kinetic model. These results confirmed the capability of the phosphoric group to protect enediol system from hydrolysis, even when it is included in cosmetic emulsions, whereas

the lipophilic ester in sixth position does not protect vitamin from degradation.

In conclusion, the results reported demonstrate that phosphate ester of vitamin C formulations are more stable than ascorbyl palmitate formulations. In particular, esterification with palmitic acid in sixth position reduces the hydrolysis of ascorbic acid but does not guarantee satisfactory stability levels in the finished products. Instead, the introduction of phosphoric group in second position protects the molecule from break-up of the enediol system, thus confirming phosphate ester of vitamin C as stable derivatives of vitamin C that may be easily used in cosmetic products.

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