STABILIZATION OF PURE VITAMIN C IN AQUEOUS COSMETIC PREPARATIONS

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Summary
The cosmetic industry associated L-ascorbic acid (LAA) or not with its salts and esters, to be employed for fighting the cutaneous aging process. In large part, in the segment of cosmetics, the salts and esters of the LAA alone are employed more frequently than the pure LAA, since the former are chemically more stable, but result in less effective products. The present work refers to a process for stabilizing LAA in an aqueous medium, which includes the step of placed the LAA in contact with Polyvinylalcohol (PVA) through weak force like Van der Waals interaction. The PVA provides stability for LAA aqueous solution, which is stable for 106 days with a LAA content decrease only of 10% w/v.

Introduction
The levogyre ascorbic acid (LAA) is a compound known since long ago for its uses, in medicine and more recently in the cosmetic industry. Its application aims at the most varied benefits, from the treatment of scurvy, regeneration of skin aging. (The expression "skin signs" as used herein means the signs of skin aging, such as wrinkles resulting from the action of time and from external factors such as climatic actions as sunshine, wind and temperature) [1].
The aging manifests itself on the skin by the loss of elasticity, loss of turgency, formation of wrinkles and the appearance of distribution in skin pigmentation. Among the physiological alterations, which actively contribute to this process, we can mention decrease in immunological functions, decline of basal metabolism, alterations in the structure of the conjunctive tissue, decrease in the capability of renewing the cutaneous lipids and in the hygroscopic components. Leading to a lesser power of hydric retention and, consequently, to dehydration of the skin. All of these alterations are the reflex of a series of internal and external factors, which jointly contribute to the aging process [2].
The technologies for treatment of the skin have tried to treat not only the visible signs of aging, but also to act more and more deeply on the causes thereof, by preventing potential risks which might contribute to the aging process. On this basis, the active principles are developed for act at specific sites, directly where they are required, that is to say, in deeper layers of the skin and directly on the aspects, which guarantee the cutaneous maintenance [1].
Biochemically, the LAA is essential in the biosynthesis of collagen. LAA acts as an important cofactor of its fundamental enzymes: prolylhydroxylase and lysylhydroxylase. Those enzyme act converting proline into hydroxyproline (an aminoacid that is fundamental to the stability of the triple helix of procollagen). Also, converting lysin into hydroxylysin (another aminoacid that is fundamental to the stabilization of the structure of collagen, participating in the formation of intermolecular links and imparting mechanical stability to the fiber) [3-5].
The LAA is also one of the main biological antioxidants, by virtue of its reducing properties, being capable of neutralizing these highly reactive species, important players in the aging process. It can also act an antioxidant in the peroxidative processes of metabolization of fats and formation of free radicals, besides contributing to maintain vitamin E in the organism, also an important antioxidant, in its reduced form, assisting in the protection against lipoperoxidation and, consequently, avoiding injury to the cellular membranes. It should be stressed that qualitatively, the antiradical action of the LAA is superior to that of Vitamin E and Gluthation, even if associated [5].
Due to this instability, although it shows greater efficiency when in its original form, in its molecular formula, the LAA is used in the form of its salts or esters. The resulting compositions have greater stability for long periods of time, thus preventing degradation due to contact with the oxygen dissolved in water and absorbed from atmospheric air. However, many works have been carried out for the main purpose of achieving stability of the LAA in its molecular form, in order to enable its use in medicaments and cosmetic compositions [6-8].
Therefore, it is an objective of the present work to provide an effective process for stabilizing LAA in an aqueous medium, which will enable the use of LAA in its molecular form, Particularly in cosmetic and/or pharmaceutical formulations.

Materials and Methods.

Materials: Levogyre ascorbic acid (LAA) was from F. Hoffmann-La Roche & Co. Polyvinylalcohol was from Clariant GmbH. All the others compound was from cosmetic grade.

Measurement of stability LAA solutions on UV-radiation: Five solutions were prepared as shown in Table I. After appropriate dilution, UV-spectra were obtained with determined period, without removing the cell of sampling compartment in order to receive UV radiation constantly, to promote the degradation of the LAA. After UV-radiation exposure, non-degraded LAA concentration was determined.

TABLE I. Solution composition content LAA, S1 is a new stable LAA aqueous solution; S2 is a market product; S3 is same S1 with citric acid; S4 is a simulation laboratory of market product and S5 is a simple LAA aqueous solution.

	S1	**S2**	**S3**	**S4**	**S5**
LAA	2.5 g	(*)	2.5 g	2.5 g	2.5 g
Water	20 mL	(**)	20 mL	20 mL	97.5 mL
Propylenoglycol	5.0 mL	(**)	5.0 mL	5.0 mL	---------
Hydroxyethylcellulose	---------	(**)	---------	0,25 g	---------
Polyvinylalcohol	0.25 g	---------	0.25 g	---------	---------
Citric acid	---------	(**)	1,52 g	1,52 g	---------

(*) - LAA concentration estimated in 10 % w/v.
(**) – Unknown.

Measurement of stability of LAA solution in storage conditions: The S1 solution (Table I) was prepared and placed in four storage conditions (room temperature, 37°C, 45°C and Sun exposure), in amber glasses with normal atmosphere headspace. For each condition, three samples were prepared. UV-spectra were obtained, in triplicate, at determined period (days), after appropriate dilution. LAA concentration was determined spectrometrically. The S5 solution was prepared in same condition and exposure only at room temperature.

Other methods: LAA was measured by UV-spectrophotometer at room temperature through band intensity at 260 nm.

Results are shown as means ± Sd. Statistical comparisons were made using ANOVA and Student T-test.

Equipment

A Cary-3G spectrophotometer (Varian Analytical Instrument).

Results and Discussion

Measurement of stability LAA solutions on UV-radiation: To determine the LAA concentrations after exposure UV-radiation, it was necessary to build standard curve. The figure 1 shows the remaining LAA (% w/v) in function UV-radiation exposure time (minutes). After 20 minutes, S5 solution shows a LAA degradation of 30%. This is the expected result for LAA instability when in aqueous solution and it on UV-radiation. The S1 solution shows a smaller degradation index, about 3 %, and the results show a better performance than S2 solution (statistically significant). The S2 solution is a market product with claims "Ascorbic Acid Pure".
The S3 and S4 solutions are laboratory preparations for to compare between our solution and literature/market product solution with addition of citric acid. This addition gives a high LAA degradation index those S1 and S2 solutions, respectively.

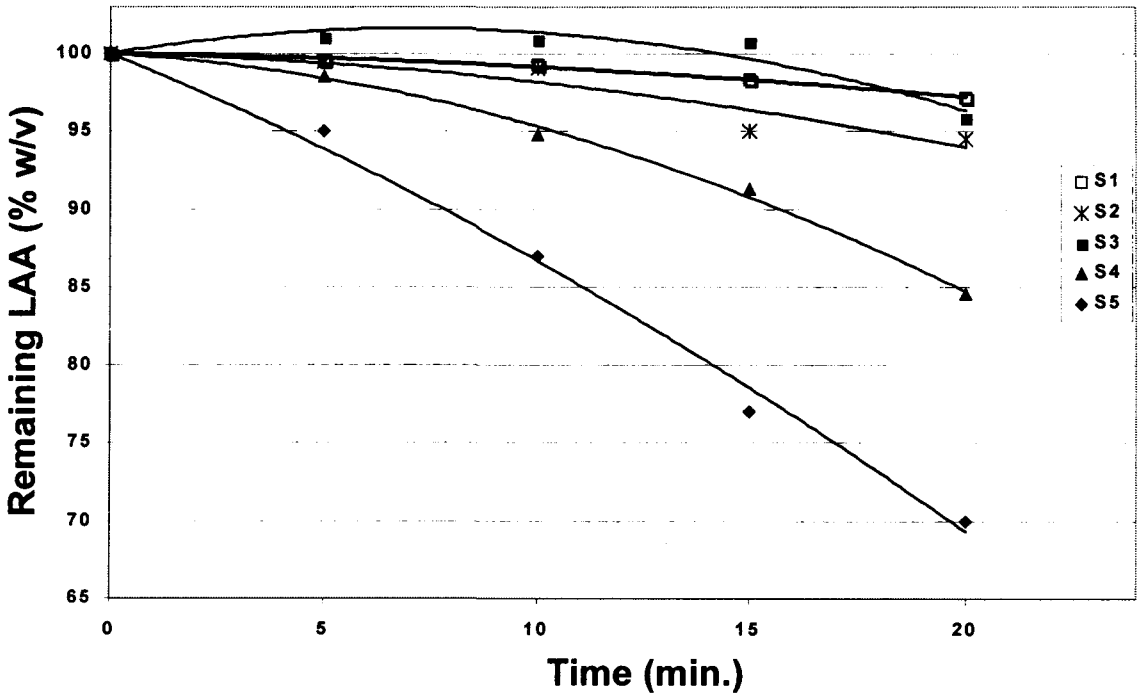
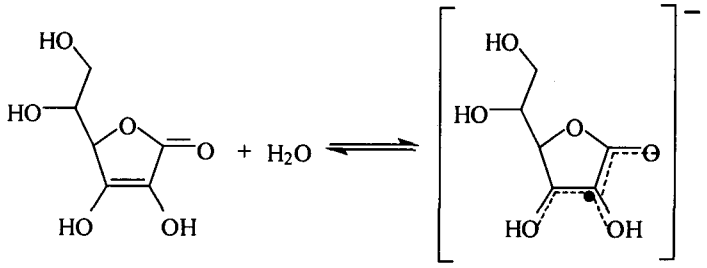


Figure 1. LAA remaining after UV-radiation time, □ S1 solution; ∩ S2 solution (market product); ■ S3 solution is same S1 with citric acid; √ S4 solution literature with citric acid and | S5 solution is LAA ascorbic acid solution.

In the ionization of the LAA in water there is formation of ascorbate ion ($LAA^{\ominus}$), which is a metastable structure in water due to the electronic resonance between the carbons 1, 2, and 3 of the LAA, and the release of proton ions (eq.1). The first degradation step is the formation dihydroxyascorbic acid. In this step the reaction is reversible through pH decrease. The next steps are irreversible degradation reactions.



Eq. 1

In order for the minimum amount of ascorbate to be formed as a product of ionization and, therefore, for the maximum of LAA to remain stable, it was believed that the pH of the solution should be as low as possible. For this purpose, the common practice to various earlier solutions was to add any acid whatever as a citric acid.

The S3 and S4 solutions, which include citric acid with the function of lowering the respective pH values and thereby maintaining the LAA stability, were analyzed. These solutions show that the inclusion of the acid did not fulfil its expected role, there having been a drop in the remaining amount of LAA or loss of stability, whereas S2 solution presents an acceptable stability under UV radiation.

***Measurement of stability of LAA solution in storage conditions*:** The results that assay are very important (Figure 2). S1 solution has LAA degradation index larger that S1 solution in T= 45°C

storage condition, it evidence Polyvinylalcohol (PVA) addition is an auxiliary compound in LAA aqueous stabilization.
The LAA degradation index increase with increase temperature, which shows LAA degradation, is temperature function for S1 solution. After 98 days, S1 solutions in room temperature and sun exposure storage conditions show about 95% w/v of remaining LAA. All are spontaneous reactions and are first-order reactions.

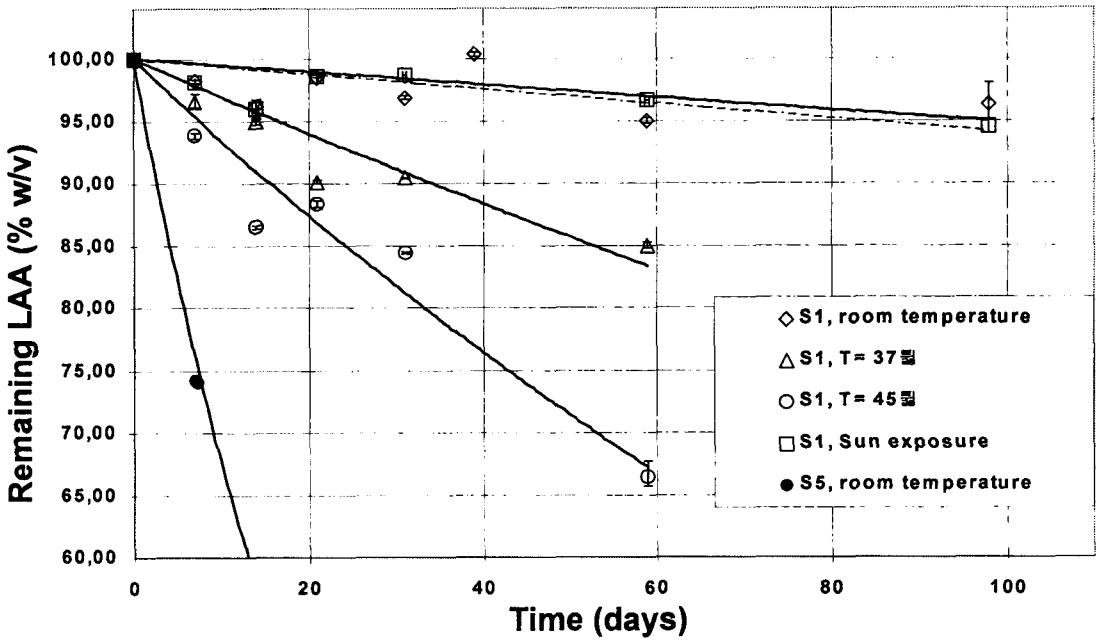


Figure 2. Experimental values plot of LAA thermal degradation S1 and S5 solutions.

The effect of temperature on reaction rate is given by the well-known van't Hoff equation (eq.2) [9].

$$\ln k(T_2) = \ln k(T_1) - \frac{\Delta H^o}{R}\left(\frac{1}{T_2} - \frac{1}{T_1}\right) \qquad \text{(Eq.2)}$$

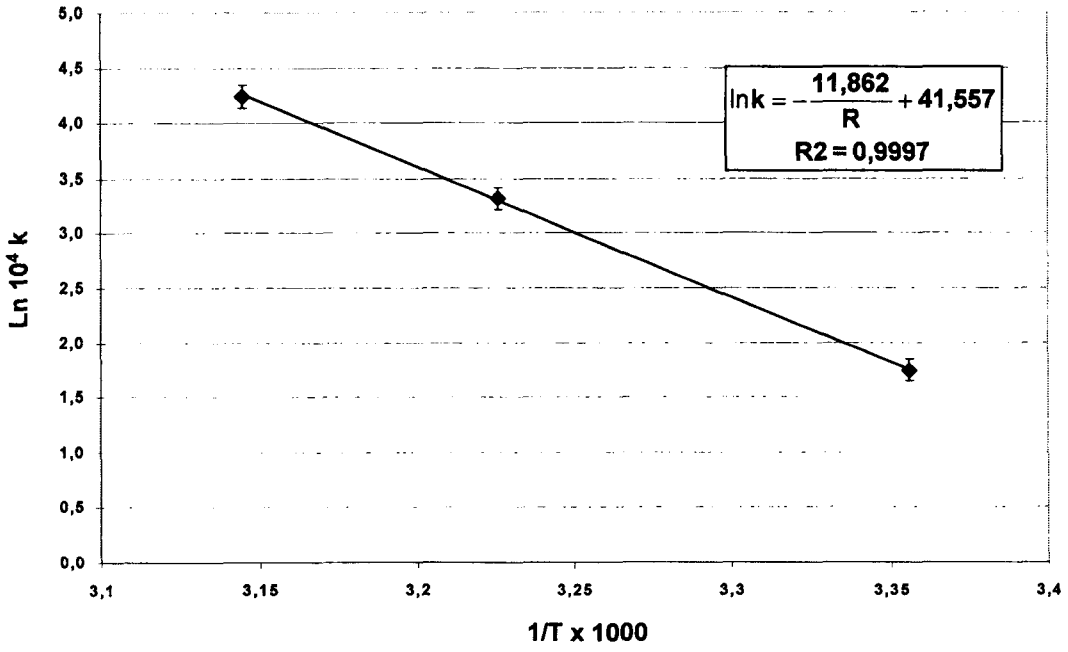


Figure 3. Van't Hoff plot of first order rate constant of S1 solution.

Where k is the rate constant at temperature T, ΔH° is the enthalpy, R is the gas universal constant (8,31431 $J{\cdot}K^{-1}.mol^{-1}$) and T is the absolute temperature. Figure 3 shows plot in accordance with equation 2. The curve is linear and decrease, which shows an endothermic process (ΔH° = 98,5 KJ).

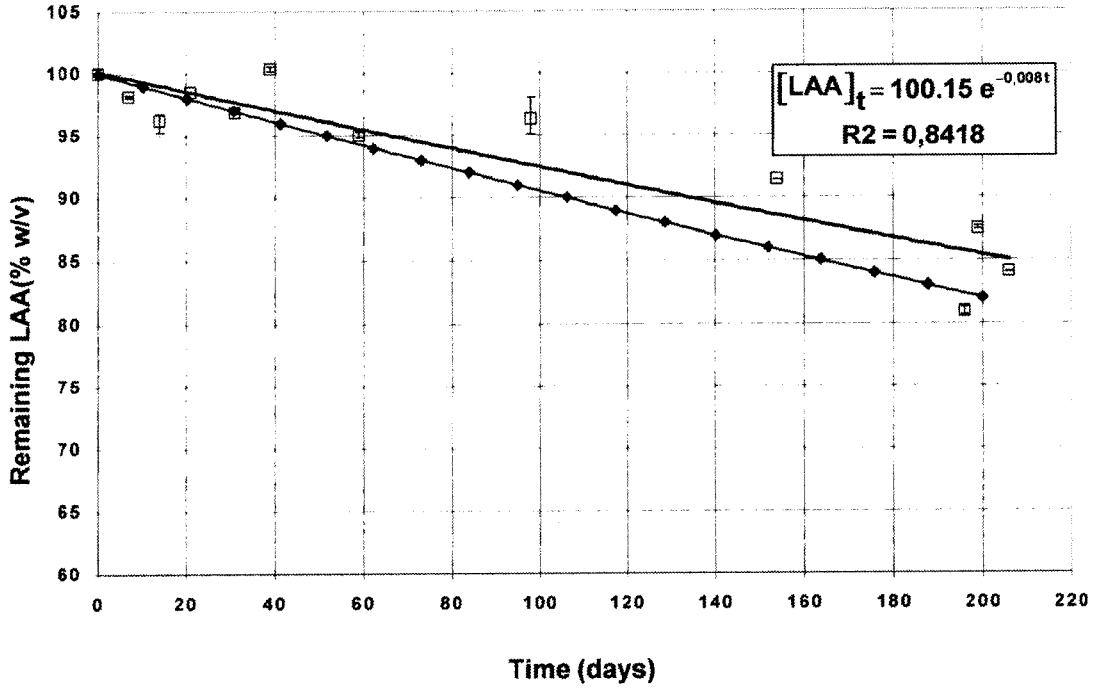
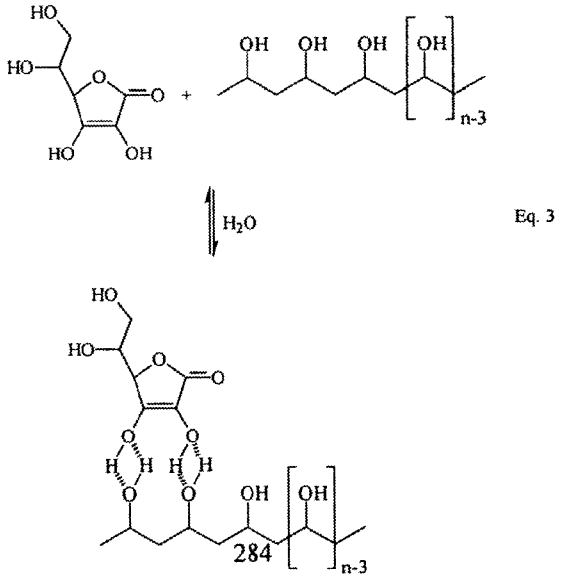


Figure 4. Comparative LAA degradation plot between ( ) S1 solution at room temperature and ( ) predictive curve.

Cosmetics are expected to maintain their quality and compositional integrity until they are used. Appropriate stability test and predictive methods must therefore, determine the shelf life for a product. Every since chemical reaction kinetics has been employed for stability studies many methods for the prediction of degradation has been reported [10].

Figure 4 shows the predict curve for shelf life product and experimental values of the LAA for S1 solution at room temperature. The temperature use in prediction calculus was 25°C. it's hope for expiration date for a product 90% of initial concentration. In the predict curve this time was 106 days and for experimental values was 134 days (more that 4 months) above the prediction, which is a good result for LAA aqueous solution at room temperature. The shift must, probability, that room temperature is above 25°C in our laboratory.



Eq. 3

The PVA addition into LAA aqueous solution is the stabilizer agent through Van der Walls forces (eq.3), which is a phenomena parallel and competes with ionization of LAA. This competition results in a delay in LAA degradation.

Conclusions

The LAA has been one of the most researched elements for this purpose, the main focus being the obtainment of its stabilization.

The LAA plays a vital role in the growth and repair of connective tissue. Many studies have proven its action in the process of cellular regeneration and skin protection, by a series of mechanisms. The LAA is directly involved in the stimulation of the biosynthesis of collagen, a macromolecule that is fundamental for maintaining the tonus of the skin. As an antioxidant substance, it fights directly the free radicals, which are elements connected with the cutaneous aging (an action proved by studies "in vitro"). Besides, the LAA is capable of protecting the skin against UV radiation and its subsequent damages. [11]

Continuous measurement of solutions, through UV-spectrophotometer, is an important tool for screening of LAA solutions.

The LAA degradation is an endothermic reaction in the experimental conditions and the enthalpy is minor when to compare whit LAA in water only. Therefore, the LAA is stabilized in studied solution by PVA, which form Van der Waals interactions maintaining all biological LAA proprieties.

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