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STABILITY OF VITAMIN E AND VITAMIN E ACETATE CONTAINING COSMETIC PREPARATIONS

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Abstract: Tocopherol (T) and tocopherol acetate (TA) are widely used ingredients in cosmetics. The present study was carried out to evaluate the content and stability of T/ TA contained in marketed and experimental cosmetic formulations. T/TA in four marketed products (A-D) and two experimental formulations (F1 and F2), stored under different temperatures, were investigated by HPLC. The results indicated variable degree of stability according to the storage temperature and product type. The stability progressively decreased upon storage at 37 °C > 25 °C > 2-8 °C. TA containing formulations showed higher stability compared to T. Further studies are in progress to optimize such formulations for improving vitamin E stability and transdermal permeation to eventually achieve the expected therapeutic and cosmetic outcomes.

Keywords: Vitamin E, Vitamin E acetate, Commercial cosmetics, Experimental preparations, Stability.

INTRODCUCTION

Inclusion of botanical extracts, vitamins, anti-microbials, etc. to cosmetics has become an important marketing advantage, mostly without scientific proof. Claims based on these additives must be carefully phrased to maintain the product(s) in the cosmetic area. In addition, such claims are rarely based on rigorous scientific evidence of performance. A case in point is the use of different components of green tea for antioxidant purposes on the skin surface. Although these substances exhibit systemic effects upon ingestion, evidence for skin benefits from topical application has not been established [1]. Topical over-the-counter products alleged to benefit ageing skin are immensely popular among cosmetics consumers. Patients often seek over-the-counter products due to market availability, comparably cheaper prices, and the lack of the physician bottleneck. Vitamin E (α -tocopherol, T) is one of the widely used ingredients in OTC products for protection against skin ageing. Vitamin E is a lipid-soluble antioxidant which plays key roles in protecting cell membranes from lipid

peroxidation by free radicals [2,3]. Furthermore, Gensler and Magdaleno, 1991 [4] concluded that, in terms of parameters of tumor incidence and tumor burden, α -tocopherol significantly reduced photocarcinogenesis. The term vitamin E embraces all tocopherols and tocotrienols showing the biological activity of the isomer RRR- α -tocopherol [2]. Vitamin E is normally distributed in skin, with the highest levels in the deepest layers [5]. It has been documented that α -tocopherol is the major antioxidant in the human epidermis, and that its depletion is an early and sensitive marker of environmental oxidative damage [6]. Vitamin E topical applications have been demonstrated to increase stratum corneum hydration and enhance water binding capacity [3]. However, the stratum corneum represents the major barrier against drug delivery and considered the limiting factor to permeation of drugs across the skin [7]. Ricciarelli et al., 1999 [8] pointed out that α -tocopherol reduced the age-dependent increase of collagenase expression by inhibiting protein kinase C

activity. The protective effects of vitamin E against photoaging have been demonstrated in various animal and *in vitro* skin models [9-11]. Proposed mechanisms for antiaging effects on skin range from antioxidant properties to improved collagen synthesis or protection from collagen breakdown. Despite the media attention and consumer popularity that these ingredients have generated, there have been few scientific studies to support these claims [12]. Vitamin E is available as the free alcohol or its esters in commercial cosmetic formulations. However, successful delivery of topically administered ingredients and liberation of the active form (α -tocopherol) is of crucial value for the efficacy of such preparations. This fact was demonstrated in a human study, in which the acetate ester of tocopherol showed no evidence of conversion to the biologically active form, α -tocopherol, despite adequate absorption into the skin [13]. Furthermore, a recent study on the metabolic conversion of α -tocopherol acetate (TA) into α -tocopherol in skin demonstrated that permeation and metabolism of α -tocopherol acetate was highly dependent on the delivery system, re-emphasizing the importance of formulation in cosmetic preparations [14]. The importance of actives vs. inactive forms, appropriate concentration, consistent delivery and product stability remain hurdles which most of the published literature has yet to cross. Although cosmetics and cosmeceuticals are tested for safety, testing to determine whether beneficial ingredients actually live up to a manufacturer's claims is not mandatory [15]. Accordingly, academia and state agencies should contribute in reviewing and verifying the claims made by manufacturers to protect the consumers and ensure valid and scientifically founded claims for cosmetic products. Furthermore, the fast oxidizable T and TA in the marketed cosmetic products present a scientific challenge on the optimal storage conditions. Thus, the objectives of the

present study were to assess the content and effect of storage condition on vitamin content of such cosmetic preparations for determining the optimum storage conditions. This investigation aims at determination of vitamin E/acetate in 4 commercially available cosmetic products in Kuwaiti Market (A, B, C, and D) and two experimental cosmetic formulations (F1 and F2), as well as evaluation of vitamin stability in the above products.

MATERIALS AND METHODS

Materials

Methanol, acetonitrile, hexane and ethanol used in the study were of HPLC grade (Merck, Darmstadt, Germany). Vitamin E Acetate was obtained from BASF Ludwigshafen, Germany. Vitamin E, soybean oil and corn oil were procured from Sigma Aldrich Chemie GmbH, Steinheim, Germany. Propylene glycol (Generico Medical Practice, AB Almere, Holland), stearic acid, white soft paraffin, potassium hydroxide (Loba CHEMIE-India), lanoline, glycerol, sorbitol (Gainland Chemical Company, UK), Captex SBE and Acconon S-35 (ABITEC Corporation, Janesville, USA). All other chemicals used were of analytical grade.

Methods

Commercial cosmetic products

Four commercial products (A, B, C and D) were obtained from retail pharmacies in Kuwait and they contained only TA without declaration of its quantity. The study was conducted before reaching the expiry dates. No products containing T were found in the market and, therefore, the experimental lab formulations were prepared to study the stability of T as well as to serve for evaluation of extraction method for analysis.

Preparation of Vitamin E-containing laboratory products

Two cream-emulsion cosmetic formulations (Table 1), each containing about 0.5%w/w of T/TA, were prepared in

the laboratory to simulate the complex composition of commercial cosmetic preparations. The cream was prepared by melting stearic acid in a porcelain dish over water bath (75-80 °C), then the semisolid ingredients (lanoline, white soft paraffin and Captex SBE) were added until all the mixture is melted, and finally soybean oil and corn oil were added (oily phase). Potassium hydroxide was dissolved in water followed by Acconon S-35, then glycerin and sorbitol were added and the mixture was heated to 75-80°C (aqueous phase). The aqueous phase was added to the oily phase with trituration and the mixture was then removed from water bath and mixing was continued until a homogenous creamy liquid was obtained. Accurately weighed amounts of T and TA were added to the mixture, avoiding addition of the vitamin to the cream while hot to avoid possible degradation; resulting in nominal concentration of 0.532 and 0.539% w/w, respectively. The final cream was filled in a well-closed plastic jars.

Method of Extraction of Vitamin E/ Acetate from Cosmetic Products

A 500 mg sample of each formulation was extracted with methanol, centrifuged, and the supernatant of extract was filtered through 0.45 µm cellulose filter and injected into the HPLC for estimating T and TA [16].

HPLC Method for the Estimation of T and TA

The method involved Waters 2690 HPLC (Waters 2690 Separations Module Milford, MA, USA) with variable wavelength PDA detector, a disposable guard column C-18 and RP Waters Symmetry C-18 column (4.6 X 150mm, 5 µm particle size). The column temperature was maintained at 25° C. Samples (50 µl) were injected and the flow rate of the mobile phase (3% v/v water/methanol) was

adjusted at 1.5 ml/ min. The eluents were monitored at 290 nm and 283 nm for T and TA, respectively. The peak areas for T and TA were recorded, and analyzed using the Millennium Software Empower from Waters. The peak areas of T and TA were subjected to regression analysis against their concentrations. The details of the procedure and validation of the method was reported previously [16]. The reported HPLC analytical method was precise (inter- and intraday variation was less than 3.5%) and accurate (> 98% of recovery after adding known quantity of T and TA to cosmetic products).

Stability of Commercial and Experimental Products

Three sets of each of the commercial and developed products were stored at different temperatures; namely 2- 8°C (refrigerator), 22-25°C (shelf), and 37°C. The aged preparations were monitored visually for physical change, if any, such as color, odor, and homogeneity and package integrity. For evaluation of chemical stability, samples were taken at zero time, and after appropriate time intervals, followed by extraction as described above, and analyzed for vitamin E / vitamin E acetate content as described earlier [16]. To study the effect of repeated exposure of the products to outside environment on vitamin stability (simulating real use conditions), samples were kept in individual vials, each vial opened and used once for analysis at each storage time interval, and results were compared with those obtained from original bulk containers. Furthermore, samples were removed from the top surface of the bulk container and compared with samples withdrawn from the interior of the same container to assess the difference in vitamin oxidation due to repeated exposure of the product to surrounding environment (simulating the real condition of use by consumers).

RESULTS AND DISCUSSION

Evaluation of Vitamin E Content in Commercial Products

Market survey in Kuwait regarding vitamin E/ester-containing cosmetic preparations revealed that no one product carry a quantitative claim of the vitamin content and all products contain only TA. Four commercial products containing TA were chosen for the purpose of evaluation of content and stability upon storage at three different temperature levels. Analysis of the commercial products revealed that the initial concentration of the vitamin ester (TA) ranged between 0.12 and 0.68%. The mean concentrations were 0.12, 0.68, 0.53, and 0.49% for products A, B, C and D, respectively. Previous study on use of vitamin E and its derivatives in topical preparations marketed in USA and Europe revealed that concentrations of the vitamin ranged between 0.0001% and more than 20% [6]. Notably, there is a striking lack of published data on dose-

response studies defining the optimal dosage of the vitamin E. This could be certainly due to limited-efficiency control requirements for non-pharmaceuticals, such as vitamin E. Furthermore, it may be attributed to ill-defined study end points and to the difficulty of measuring oxidative stress *in vitro*. The authors remarked that if the product claim is to improve antioxidant protection of skin barrier, topical formulations with vitamin E at concentrations ranging from 0.1 to 1% are likely to be effective. Accordingly, the same authors suggested using vitamin E in combination with co-antioxidants such as vitamin C to help enhancing antioxidant effects and stability of vitamin E [6]. Although the tested commercial products in the present study pointed out a concentration range similar to that suggested by Thiele et al., 2005 [6], nevertheless these products contain the acetate ester prodrug rather than the active free vitamin E.

Table 1: General formula of laboratory formulations containing 0.5% (w/w) of vitamin E or vitamin E acetate.

Ingredient	Percent w/w
Stearic acid	6
White soft paraffin	4
Lanoline	4
Soybean oil	8.4
Corn oil	8.3
Captex SBE (Caprylic/ Capric/Stearic Triglyceride)	4
Glycerol	11
Sorbitol	15
Acconon S-35 (PEG-35 SoyGlycerides)	5
Potassium hydroxide	0.28
Water	34.02

Stability of Vitamin E/Acetate in Experimental and Commercial Products

The tested products were subjected to stability study upon storage at three different temperature levels; refrigerator (2-8 °C), room temperature (22-25 °C) and elevated temperature at 37 °C.

Physical Stability

In product A, the consistency and the color of the cream stored at 37 °C was changed. The cream became thinner and the color changed from white color to off-white. Regarding product C, the tube showed leakage at the sealed end after 5 months at 37 °C. A loss of the cream through leakage of the cream and separation of waxy

material was also observed. The loss of aqueous phase due to water evaporation might explain the reported unexpected increase in the vitamin concentration in the last two months of storage. For product *D*, the samples stored at 37 °C were no longer homogenous in appearance after 5 months. On the other hand, experimental cosmetic preparations maintained the initial consistency, homogeneity, color and appearance.

Chemical Stability

Storage of the commercial and experimental formulations at various storage conditions revealed different stability profiles after different storage periods. The results of the initial and remaining concentrations after different time intervals reported for the experimental and commercial formulations are presented in Figs. 1 & 2 and Figs. 3-6, respectively.

Experimental Products

The developed two experimental emulsion cream formulations containing 0.57 and 0.58% w/w of T or TA, designated as *F1* and *F2* respectively, served to compare the stability of T relative to TA. These experimental products were also used to compare the stability of TA with that of TA in commercial products, as well as to evaluate the extraction efficiency of the tested methods for extraction of T/TA. The results of stability study are graphically represented in Figs. 1 and 2. It appears from the previous results of the experimental formulations that the free tocopherol is more susceptible to degradation, in comparison with the acetate ester. The free -OH in tocopherol is more easily oxidized by atmospheric oxygen, which is protected in case of the ester form. *F1* cream containing T lost about 80% of the initial concentration after storage for only 20 weeks at 37 °C. On the other hand, TA containing formulation (*F2*) lost only about 10% of the initial concentration after the same storage period

at 37 °C. This may be explained by the higher sensitivity of T and absence of any added antioxidant to these formulations. Avoiding addition of antioxidant was intended to objectively assess the effect of formulation on unprotected T/TA in such preparations upon storage. The degradation was quite appreciable and proportional with the increase of the storage temperature in the order of 37 °C > 25 °C > 2-8 °C (Figs. 1 and 2).

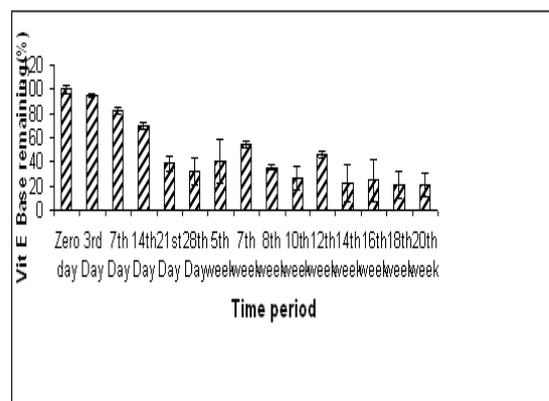


Fig.1a: Stability of Experimental cream F1 at 2-8°C.

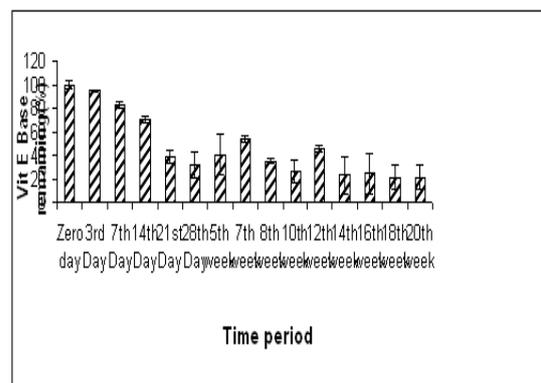


Fig.1b: Stability of Experimental cream F1 at 25°C.

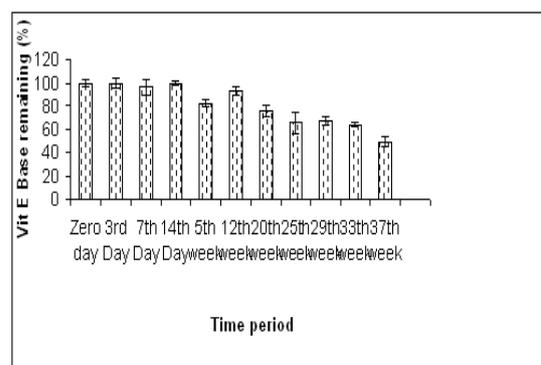


Fig. 1c: Stability of Experimental cream F1 at 37 °C.

Commercial Products

Since none of the investigated commercial products declares the actual content of TA in a quantitative manner, the values determined on starting the stability study were considered as the initial (zero point) concentration for each product. It is worthy mentioning at this point that although product A label shows that it contains only tocopherol acetate, the results of analysis revealed the presence of tocopherol also. In contrast, manufacturer of product B claims the presence of both vitamin E and vitamin E acetate under the ingredients, yet the results of analysis indicated the presence of the ester form only. Inclusion of T in this formulation may be intended as a protective antioxidant to TA, the last is the main ingredient.

The initial concentration of the vitamin E acetate (TA) in the commercial products ranged between 0.12 and 0.68%. The mean concentrations were 0.12, 0.68, 0.53, and 0.49% for product A, B, C, and D respectively. The percent of TA remaining after different times and storage conditions are graphically represented in Figs. 3-6. The dramatic degradation upon storage of experimental preparation F1 at 37 °C (Fig. 1) containing T was not observed with any of the tested commercial preparations containing TA. Products A, B and C retained 88-92% of the initial TA concentration after storage at 37 °C for 7 months. Product D showed the maximum loss after storage under the same conditions, wherein the remaining concentration was found to be 76%. These findings explain why the manufacturers of commercial products use basically the more stable acetate ester TA instead of the free tocopherol, yet the last is the active form.

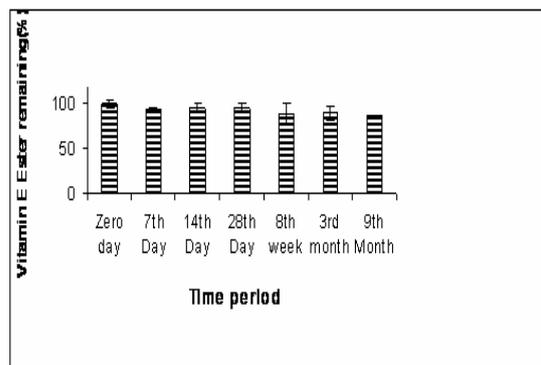


Fig. 2a: Stability of Experimental Cream F2 at 2-8°C.

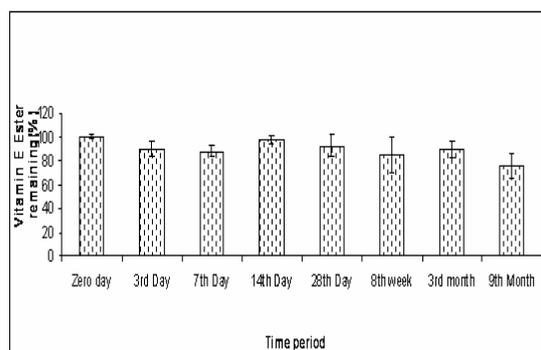


Fig. 2b: Stability of Experimental Cream F2 at 25°C.

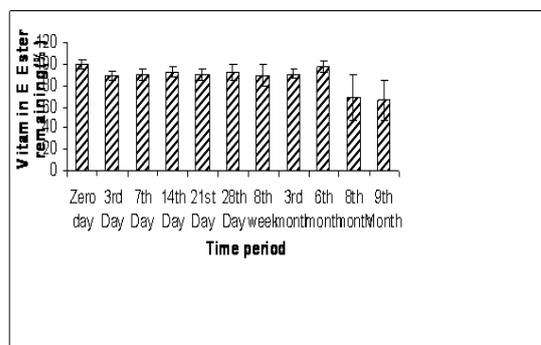


Fig. 2c: Stability of Experimental Cream F2 at 37°C.

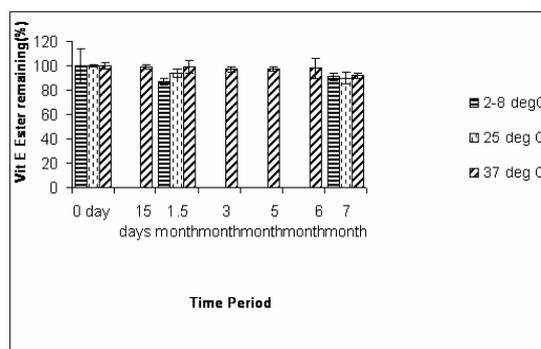


Fig. 3: Stability of commercial product (A).

Effect of Exposure/Container on Vitamin E stability

Before starting the stability study, samples from the surface and bulk of commercial cream packed in open-mouth container, exemplified by product *B*, were analyzed. The results revealed insignificant difference in TA concentration; namely 0.651 (RSD=2.3%) and 0.678% (RSD=0.065%) for the surface and bulk of containers respectively. Similarly, to assess the effect of oxidative exposure of the experimental products containing T/TA to the surrounding environment, samples from *F1* and *F2*, filled in frequently opened bulk (simulating customer use) or single-use containers stored at 25 °C, were analyzed and their contents were significantly indifferent as verified by Kruskal-Wallis test. After 9 months storage of *F1* at 25 °C, the remaining concentration of T in the individual and bulk containers was 57.69 and 49.12% respectively. The corresponding values for *F2* containing TA were 80.5 and 75.75%, respectively, after the same storage period. Although no significant difference was observed between the two container systems, however, the rate and extent of degradation of T was by far higher than TA. The results indicate that the commercial product *B* is exhibiting the same stability profile, whereas the experimental formulations showed reduced stability when stored in bulk containers. This might be due to increased oxidation of T and TA on exposure to larger surface area. The absence of such a phenomenon in product *B* might be due to the possible presence of antioxidants.

CONCLUSIONS

Stability of vitamin E and its acetate ester is questionable during use of cosmetic products by consumers. Free vitamin E is much more sensitive to oxidative degradation, compared to the acetate derivative. This finding explains why

commercial products include often the acetate ester, which is inactive prodrug, instead of the active free vitamin.

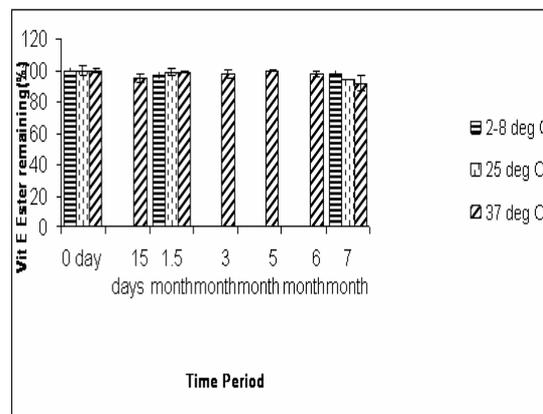


Fig. 4: Stability commercial product (B).

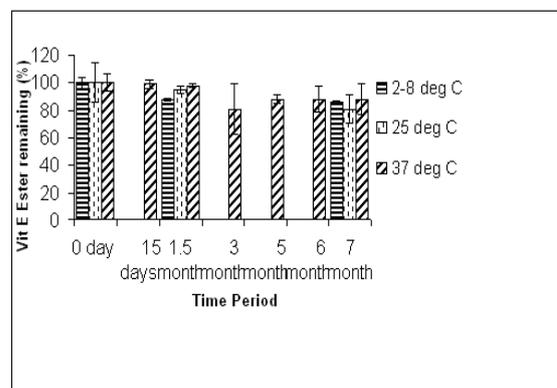


Fig. 5: Stability of commercial product (C).

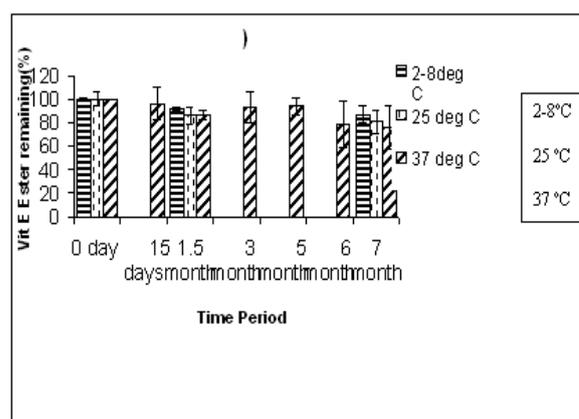


Fig. 6: Stability of commercial product (D).

Storage of vitamin E products at 37°C for 1-2 months results in appreciable loss of activity. Therefore, care must be experienced to store such preparation in controlled room temperature before and after reaching the end-users.

Drug control authorities should take care about packaging storage conditions while auditing cosmetics' manufacturers or distributors. In addition, the cosmetic manufacturers should recommend storage of such products at low temperatures, preferably in a refrigerator.

Further studies to optimize such formulations are required to improve vitamin E stability and transdermal

permeation to eventually achieve the expected therapeutic and cosmetic outcomes.

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