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Stability of cosmetic formulations containing esters of Vitamins E and A: Chemical and physical aspects

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Abstract

Cosmetic stability prediction relies on quantitative chemical determinations of active components after certain times and in different temperatures. However, physical stability, an important parameter in skin care products is not considered in these conditions. This study proposes the determination of cosmetic stability chemical and physical parameters validated by (HPLC) chromatography and rheological measurements, respectively, using a gel-cream containing retinyl palmitate and tocopheryl acetate as a model system. The predicted shelf life addresses both the physical and chemical aspects of the system. Results emphasize the importance of studying both parameters by showing the relation of components degradation and physical stability. Moreover, they contribute to an improved understanding of physical and chemical stability aspects of cosmetic formulations, mainly if they contain Vitamins A and E derivatives.

Keywords: Stability studies; Cosmetics; Vitamins; Rheology; Physical and chemical aspects

1. Introduction

Due to the dynamics of the cosmetic market, development of new products should be fast and accordingly, stability prediction is usually performed by accelerated storage conditions (Tadros et al., 2004). Temperature variation is the main parameter used to induce rapid chemical and physical alterations in formulations, which are usually detected by quantification of some components over time. To predict shelf life the kinetics of chemical degradation may be mathematically treated by using the Arrhenius equation (Magari et al., 2004), but physical stability is not considered in this case. Prediction based only on chemical parameters cannot be totally trusted especially for cosmetic formulations where physical stability is of utmost importance and other parameters should also be analyzed.

Combined analytical techniques, including HPLC methodology, are currently used to obtain chemical stability data about formulations over time (Austria et al., 1997; Frauen et al., 2002; Gaspar and Maia Campos, 2006).

Measurements of rheological behavior are important not only to evaluate physical stability (Spiclin et al., 2003; Tadros et al., 2004), but are also parameters indicating system quality, usefulness and purpose. Studies on these properties have become a crucial tool for analysis of cosmetic preparations, due to the possibility of producing correct profiles of physical and structural stability (Soriano et al., 2001). Thermal stress can alter parameters as viscosity, solubility, creaming facilitation, coalescence, melting of waxes or hydration of polymers (Lippacher et al., 2004), and consequently, it should be possible to predict instability processes by studying the rheological behavior of cosmetic formulations under these conditions.

In summary, accelerated stability studies using temperature stress, HPLC analysis and rheological determinations are useful to characterize formulations as a function of short time periods (Di Mambro et al., 2003; Tadros et al., 2004). The aim of this study was to validate chemical and physical methods for stability determination in cosmetic formulations, using a gel-cream containing retinyl palmitate and tocopheryl acetate as a model. The results should also contribute to a better understanding of physical and chemical stability aspects of cosmetic formulations, mainly if they contain derivatives of Vitamins A and E.

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2. Materials and methods

2.1. Materials

Vitamin A palmitate (1,000,000 UI/g), Vitamin E acetate and Vitamin K_1, with 97, 96 and 98% purity, respectively, were purchased from Sigma (St. Louis, MO, USA). HPLC-grade methanol and *iso*-propanol were obtained from Mallinkrodt (Paris, KY, USA).

Glycerin and propyleneglycol from Synth (Diadema, SP, Brazil) Sepigel™ 305 (polyacrilamide, C13-14, isoparaffin, Laureth-7) from Seppic (Paris, France), NET FS™ (silicon microemulsion) from Nikko Chemicals (Tokyo, Japan) and Phenova™ (phenoxyethanol and parabens) from Croda (Campinas, SP, Brazil) were used in the preparation of the gel-cream.

2.2. Formulations studied

Formulations were prepared in a Heidolph RZR 2021 (Schwabach, Germany) stirrer at 600 rpm. The gel-cream was obtained by adding to deionized water the following components to the final stated percentages (w/w): 5.0% Sepigel™ 305, 4.0% NET FS™, 2.0% glycerin, 0.8% Phenova™ dissolved in 3.0% propyleneglycol and 0.02% DL-α-tocopherol as antioxidant. The gel-cream was supplemented with retinyl palmitate and tocopheryl acetate to final concentrations of 1% (w/w) and 2% (w/w), respectively.

2.3. Stability studies

Formulations containing vitamins A and E, were stored in PVC pots (37 mm in diameter × 29 mm deep), at 45, 37 and 25 °C and 75% relative humidity for up to 120 days. Vitamin quantifications and rheological measurements evaluated samples collected at 7-day intervals during the first 28 days and 60 and 120 days later. Quantification of the vitamins in the formulation samples was by a validated isocratic HPLC method. Its results and calculations of shelf-life through the Arrhenius equation characterized chemical stability (Garret, 1956a,b). Considering that an increase in storage temperature can decrease the chemical stability of a formulation (Magari et al., 2004), vitamin quantifications also represented degradation kinetics. Thus, chemical stability was calculated as the period of time in which formulations maintained at room temperature (25 °C), showed a 15% loss in the concentration of their main components.

2.4. Chromatography

The HPLC system consisted of a model LC-10AD Shimadzu Liquid Chromatograph (Kyoto, Japan) fitted with a variable wavelength UV detector (SPD-10A), connected to a personal computer. The integrator program consisted of a LC-10, Shimadzu.

Chromatographic separations were performed on a LiChrocart (125, 4 mm) Merck column, filled with 5 μm Lichrospher 100 RP-18 particles as the stationary phase. Degassed methanol was the mobile phase at a flow rate of 1.5 mL min^{-1}. The injection volume was 20 μL and analyses were performed in two wavelength channels: 326 nm for Vitamin A and 254 nm for Vitamin E. Vitamin K_1 was the internal standard analyzed at 254 nm.

For vitamin extractions, formulation samples (25 mg) were added to tubes containing *iso*-propanol and the internal standard (15 μL mL^{-1}) and vortexed for 3 min. After centrifugation of the suspension the filtered supernatants were used for HPLC injection. To quantify retinyl palmitate and tocopheryl acetate in the formulations, standard solutions were prepared daily and analyzed by HPLC in parallel to samples. Peak-area ratios were used for calculations following the internal standard method.

For precision assays, samples in four different concentrations were analyzed five times and the intra-assay relative standard deviation (R.S.D.) calculated. Inter-assay R.S.D. was determined analyzing the samples in seven different days. The intra- and inter-assay accuracy was also evaluated for each concentration, by assessing the agreement between the measured and nominal concentrations of the analytes. In addition, the efficiency of the extraction method ($n = 5$) was estimated from formulations containing different concentrations of Vitamins A and E.

2.5. Rheological measurements

Physical stability was assessed through rheological determinations performed in a model DV-III Brookfield rotational rheometer (Stoughton, MA, USA) with a cone-plate configuration, connected to a Brookfield software program, RHEOCALC Version V 1.01. Rheological parameters were determined at 25 °C, using a CP 52 spindle and 0.5 g of each sample, 24 h after preparation and after different storage times. Rheogram curves constructed with ascendant and descendant segments were obtained with rotation speeds increasing progressively (1–4 rpm) and gradually decreasing (4–1 rpm). With the results obtained, values for consistency index (related to the system viscosity) and flow index (related to the system pseudoplasticity) were mathematically calculated by the Ostwald law:

$$\tau = \kappa\gamma^{\eta}$$

where τ is the shear stress, κ the consistency index, γ the shear rate, η is the flow index. Data were statistically analyzed using Kruskal–Wallis, a non-parametric test.

3. Results and discussion

A representative HPLC chromatogram of vitamin extracts from gel-cream formulations is depicted in Fig. 1. It shows well resolved Vitamins E, K_1 and A peaks with retention times of about 4, 6 and 10.5 min, respectively, in a single and short run easily reproduced.The absolute recovery, intra- and inter-days precision and accuracy values for retinyl palmitate and tocopheryl acetate are presented in Tables 1 and 2, respectively. HPLC with UV detection is the most common technique used in retinoids and tocopherols determinations (Julianto et al., 1999; Alvarez and Mazancourt, 2001; Gimeno et al., 2001; Hartmann et al., 2001). Although determination of these substances at

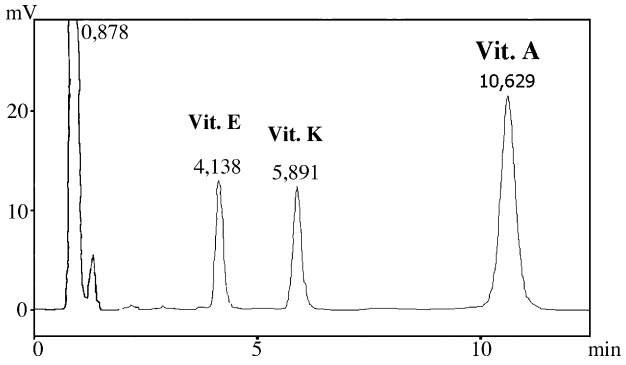

Fig. 1. HPLC chromatograms of Vitamins A (Vit. A) and E (Vit. E) extracted from the gel-cream formulation (containing 137.5 and 500 μg g^{-1}, respectively), with Vitamin K_1 (Vit. K) as an internal standard.

Table 1
Absolute recovery, intra- and inter-days precision and accuracy in quantification of retinyl palmitate extracted from experimental formulations ($n = 5$)

Concentration (μg g^{-1})	Recovery (%)	Precision (CV%)		Accuracy (%)	
		Intra-days	Inter-days	Intra-days	Inter-days
165	100.82	1.9	0.1	0.2	1.2
137.5	102.52	1.2	0.5	1.1	0.8
110	99.17	1.6	1.4	0.4	1.1
82.5	99.01	0.8	1.3	0.4	2.1

Table 2
Absolute recovery, intra- and inter-days precision and accuracy in the quantification of tocopheryl acetate extracted from experimental formulations ($n = 5$)

Concentration (μg g^{-1})	Recovery (%)	Precision (CV%)		Accuracy (%)	
		Intra-days	Inter-days	Intra-days	Inter-days
600	97.28	0.5	1.9	0.9	1.5
500	99.56	1.1	1.0	0.6	1.3
400	100.06	0.8	0.9	0.6	0.2
300	95.64	1.0	1.6	1.9	2.4

lower concentrations often requires more sensitive methods (Guaratini et al., 2004), the high recovery in this case indicates that the procedure used accounts for the entire amount of analyte present in the sample (Causon, 1997). The values for both precision and accuracy in intra- and inter-assays were all less than 2.5%, which is satisfactory for the level of active principles in this formulation.Chemical stability was also analyzed by vitamin degradation kinetics. Retinyl palmitate had a lower stability than tocopheryl acetate (Fig. 2), indicating Vitamin A as the limiting factor in the calculation of product shelf-life.

Previous reports (Kim et al., 2000) show that retinyl palmitate degradation in emulsions follows a first-order reaction, which is in agreement with the results here presented. According to activation energy calculations through the Arrhenius equation, it was considered that 85% of retinyl palmitate is theoretically maintained for approximately 77 days at room temperature. The same result was obtained experimentally in formulations maintained at room temperature, which validates the accelerated method employed for chemical stability determinations. Although it is commercially interesting to develop products with the highest possible shelf-life it is known that the stability of substances is a function of its vehicular system (Gallarete et al., 1999). Already Kennon (1964) had reported that the vehicle might change the heat of activation of a degradative reaction, bringing about a different decomposition mechanism. Although Sepigel™ 305 skin creams with many properties are widely manufactured (Anchisi et al., 2001), it is known that Vitamin A is rapidly degraded in this vehicle. Furthermore, if retinyl palmitate decomposition leads to a complex mixture of products (Samokyszyn and Marnett, 1990), its toxicity must also be considered (Fu et al., 2003). Thus, shelf-life determination is essential not only for efficacy aspects, but also to evaluate formulation toxicity.

Thus, the safety and efficacy the system proposed in this study is acceptable only if the product is to be used in a restricted period of time.

Rheological measurements carried out in parallel with chemical studies represent a complete, rational and necessary approach to predict physical sample behavior during expected shelf-life. Rheological parameters indicated that addition of vitamins to the basic formulation did not compromise its structure but altered some of the rheological parameters as shown in rheogram (A) (Fig. 3).

The flow index in all formulations was below 1 indicating pseudoplasticity, which is a desirable rheological property in these preparations. It was not significantly altered despite a

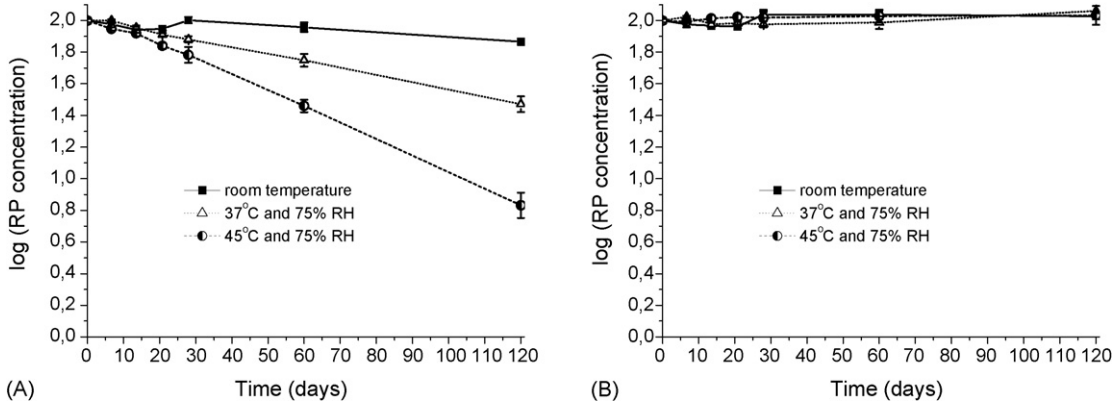

Fig. 2. Quantification of retinyl palmitate (RP) (A) and of tocopheryl acetate (TA) (B), expressed as logs of concentration values over time, in formulations maintained at room temperature (25 °C), 37 or 45 °C with 75% relative humidity (RH).

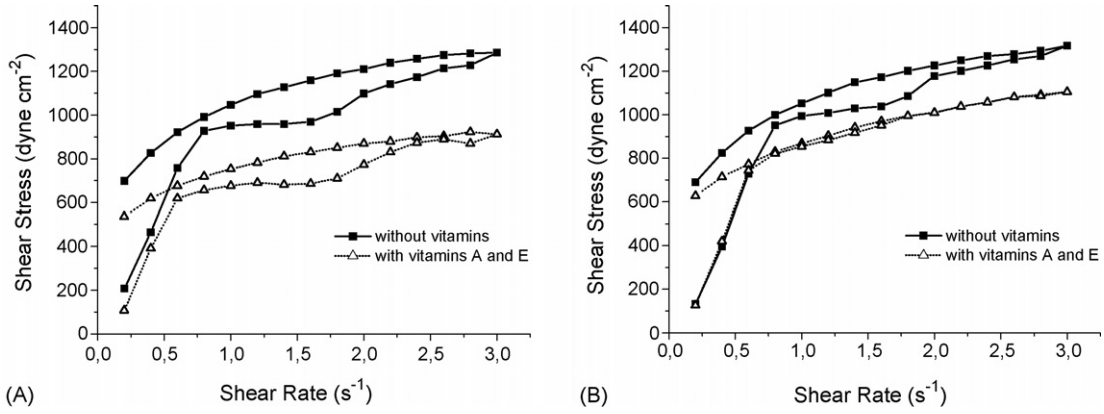

Fig. 3. Formulation rheograms, 24 h after preparation (A) and 120 days after preparation (B) at room temperature. Formulations containing or not vitamins were compared in both time periods.

significant decrease in the consistency index after additions of retinyl palmitate and tocopheryl acetate.Semenzato et al. (1992) demonstrated that Vitamin A chemical stability strictly depends on the physical stability of the formulations. The present study showed that, over time, physical alterations are more prominent in formulations with added vitamins. Accordingly, Fig. 3B illustrates alterations of physical stability in the formulations containing vitamins, such as thixotropy, in addition to flow and consistency indexes, presented in Table 3. This instability was not seen in the formulations without vitamins, suggesting that vitamin instability affected the formulation physical integrity.Rheological parameters were determined and compared in formulations kept at different temperatures after 15% of the Vitamin A was degraded. The rheograms and rheological data obtained in these experiments (77 days of storage at 25 °C, 21 days at 37 °C and 14 days at 45 °C) are shown in Fig. 4 and Table 3, respectively. Rheological characteristics of formulations with approximately 85% of retinyl palmitate at all storage conditions were similar. It is concluded that accelerated physical stability studies by rheological measurements were also validated.

Although flow indexes were not altered by thermal stress consistency indexes increased significantly (Table 3). It is widely known that consistency indexes normally decrease during storage, indicating instability (Martin et al., 1993; Korhonen et al., 2001), but in our results the consistency index increased. It is possible that this was due to the interaction of retinyl palmitate and polyacrilamide, the vehicle polymer, as shown by an artifact when the decomposition products were analyzed by mass spectrometry (data not shown).

Table 3
Consistency (CI) and flow index (FI) values determined in formulations 24 h after preparation and after 15% degradation of retinyl palmitate

	24 h after preparation		After 15% degradation of RP	
	CI	FI	CI	FI
25 °C	64325 ± 102	0.40 ± 0.01	71459 ± 89 a	0.39 ± 0.00
37 °C	64325 ± 102	0.40 ± 0.01	72946 ± 116 b	0.41 ± 0.02
45 °C	64325 ± 102	0.40 ± 0.01	72652 ± 149 c	0.40 ± 0.03

Different symbols (a ≠ b ≠ c) indicate statistically different values ($p < 0.05$).

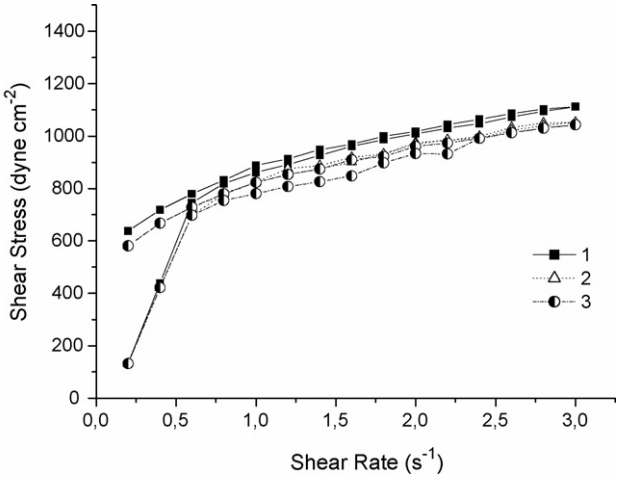

Fig. 4. Formulation rheograms after 15% degradation of Vitamin A. Analyses were performed after (1) 77 days at 25 °C, (2) 21 days at 37 °C and (3) 14 days at 45 °C.

It is concluded that the results in the present study validate chromatographical and rheological methods for analysis of gel-cream stability. Rheology measurements are simple and effective means to compare properties over time and HPLC furnishes accurate quantitative data on different substances added to complex matrixes. In addition, the results contribute to the understanding of cosmetic stability in formulations containing retinyl palmitate and tocopheryl acetate and confirm that chemical and physical stability must be evaluated at the same time since they seem to have related effects.

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