J. Cosmet. Sci., 62, 405–415 (July/August 2011)

Vitamin A palmitate and α-lipoic acid stability in o/w emulsions for cosmetic application

M. A. MOYANO and A. SEGALL, *Cátedra de Control de Calidad de Medicamentos, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Junín 956, 1113 Buenos Aires, Argentina.*

Accepted for publication February 28, 2011.

Synopsis

Skin becomes thin, dry, pale, and finely wrinkled with age. Retinoids are a large class of compounds that are important in modern therapy for dermatological treatment of wrinkled skin. Of the retinoids, retinol and vitamin A palmitate are thought to induce thickening of the epidermis and to be effective for treatment of skin diseases. Accordingly, α-lipoic acid or the reduced form, dihydrolipoate, are potent scavengers of hydroxyl radicals, superoxide radicals, peroxyl radicals, singlet oxygen, and nitric oxide with anti-inflammatory properties (1).

Cosmetic ingredient stability prediction relies on kinetic quantitative chemical analysis of active components at different temperatures. Vitamin A palmitate and α-lipoic acid, are known to be unstable to light or heat (2).

The aims of this study were to evaluate the stability of α-lipoic acid and vitamin A palmitate in the presence of vitamin E (acetate) and other antioxidants in lipophilic/hydrophilic medium (O/W emulsions) at pH 3.0, 5.0, and 7.0. The formulations that were investigated contained 0.12% (w/w) vitamin A palmitate, 0.4% (w/w) vitamin E acetate, and 0.5 % α-lipoic acid (formulation A), supplemented with ascorbyl palmitate, magnesium ascorbyl phosphate and vitamin C (formulation B) or with butylhydroxytoluene (BHT, formulation C) or ascorbyl palmitate (formulation D). The chemical analyses of α-lipoic acid and vitamin A palmitate were carried out by HPLC. Formulations C and D at pH 7.0 were selected as the most stable for these components.

The purpose of this paper is the selection of the most stable formulations for their application in *in vivo* studies.

INTRODUCTION

Vitamin A and its esters (Figure 1) are widely used as active components in cosmetic and dermatological preparations. They take part in the regulation of epidermal cell growth, inhibit the final step of keratinization, participate in the collagen synthesis process, prevent atrophy of connective tissue, enhance glycosaminoglycane synthesis, and are essential in the reproduction of basal membrane cells. A characteristic feature of retinoids is their sensitivity to ultraviolet radiation. Both UVB and UVA radiation reduce the vitamin A content of the human epidermis. The chemical nature of retinoids, consisting of polyunsaturated polar lipids, makes them able to interact with oxygen and UV or visible light to produce reactive oxygen species and free radicals (3,4).

Address all correspondence to A. I. Segall

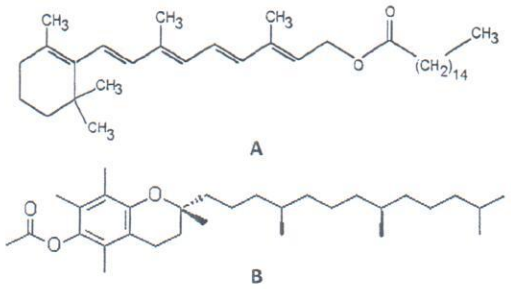

Figure 1. (A) Vitamin A palmitate. (B) Vitamin E acetate.

The vehicle used influences the *in vivo* skin absorption of vitamin A. Percutaneous absorption is modulated by the skin's pH and integrity, hydratation conditions, the mode of application, the oil/water partition coefficient, the ionization state, and Vitamin A palmitate concentration. (5)

Most of the stability studies for vitamin A palmitate described in the literature are based on the liquid chromatographic determination of this drug in cosmetic formulations alone or in the presence of antioxidants, sunscreen, or encapsulated liposomes (2–4, 6–13).

BHT is important for the correct protection of o/w emulsions over time. BHT provides good protection for vitamin A palmitate, which might suggest that the photodegradation mechanism is an oxidative one. BHT provides better protection under UVA than under UVB. (3,4,7,13,17).

In 1951 Reed and coworkers isolated α-lipoic acid (Figure 2). Lipoic acid or the reduced form, dihydrolipoate, are potent scavengers of hydroxyl radicals, superoxide radicals, peroxyl radicals, singlet oxygen, and nitric oxide. Lipoic acid plays an important role in the mitochondrial dehydrogenase processes and as a modulator of the inflammatory response (1).

The purpose of this paper is to study the stability of lipoic acid in the presence of vitamin A (as palmitate) and E (as acetate) (Figure 1) and other antioxidants in semisolids for cosmetic use. Previous studies have shown that lipoic acid was not very stable in these formulations, but the presence of vitamin A favors its chemical stability (2). We have included vitamin C derivatives to enhance vitamin A stability. The results reported demonstrate that phosphate esters of vitamin C formulations are more stable than ascorbyl palmitate formulations. In particular, esterification with palmitic acid in the 6 position (Figure 3) reduces

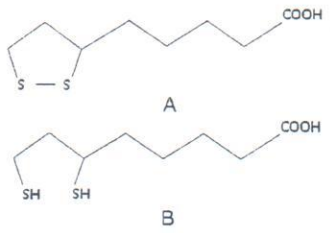

Figure 2. (A) α-lipoic acid. (B) α-dihydrolipoic acid (reduced form).

the hydrolysis of ascorbic acid but does not guarantee satisfactory stability levels in finished products. Instead, the introduction of phosphoric group in the 2 position (Figure 3) protects the molecule from break-up of the enediol system, confirming that phophate esters of vitamin C as stable derivatives of vitamin C may be easily used in cosmetic products (14). Although we have included vitamin C derivatives in the original formulation, the stability of vitamina A was enhanced but not the stability of lipoic acid. We supposed there was an incompatibility between lipoic acid and components in the formulation.

As a part of the ongoing project on the development of formulations containing lipoic acid, techniques of thermal analysis (DSC), isothermal stress testing (IST), and IR were utilized for drug-excipient compatibility testing (15). In the first phase of the study, DSC was used as a tool to detect any interaction. Use of DSC has been proposed as a rapid method for evaluating the physicochemical interaction between two components. Based on the DSC results alone, methyl p-hydroxybenzoate, propyl p-hydroxybenzoate, butylated hydroxytoluene, non-ionic self-emulsifying wax, propylene glycol, and acetylated lanolin were found to exhibit interaction with lipoic acid. Stressed binary mixtures (stored at 50°C for one week) of lipoic acid and excipients were evaluated by HPLC. Binary mixtures were evaluated by IR spectroscopy. The results obtained with the thermal analysis were confirmed by HPLC and FT-IR studies (15).

Based on these results, we proposed some new formulations which were stored in three different packaging materials—polyethylene, polypropylene, and glass—in order to evaluate if there is a significative difference of stability in these formulations. There were not significant differences between the stability of vitamin A and lipoic acid in the different packaging materials (16).

MATERIALS AND METHODS

MATERIALS AND REAGENTS

Ascorbyl palmitate was provided by Hoffmann La Roche (Switzerland), magnesium ascorbyl phosphate by Merck (Germany), butylhydroxytoluene by Eastman Chemical

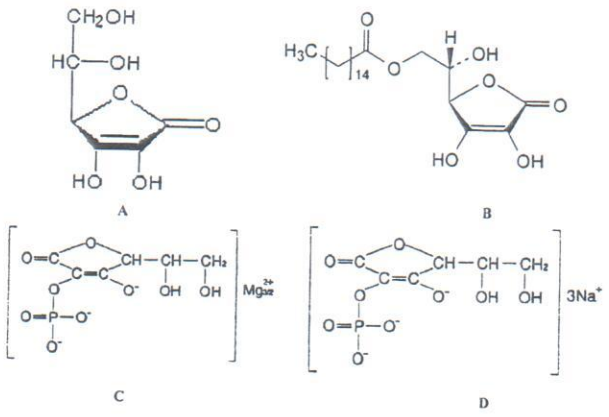

Figure 3. (A) Vitamin C. (B) Ascorbyl palmitate. (C) Magnesium ascorbyl phosphate. (D) Sodium ascorbyl phosphate.

Company (USA), and vitamin C by Kromberg (Argentina). Vitamin A (as palmitate) was provided by DSM (Netherlands), vitamin E (as acetate) by Merck (Germany), and lipoic acid by Labochim (Laboratorio Chimico Internazionale, Italy).

The emulsions consisted of silicone fluid (Dow Corning, Brazil); mineral oil and petrolatum (R.A.A.M., Argentina) as oil phase; anionic self-emulsifying wax (Flamacer SX, Flamaquímica, Argentina) as surfactant; imidazolidinyl urea (ISP, United Kingdom) as preservative; and sorbitol 70% (water solution) (Unión Química Argentina, Argentina) 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 anionic emulsifier was melted in a stainless steel container; then silicone fluid and mineral oil were added. The emulsion was mixed by slow agitation, avoiding the incorporation of air and keeping the temperature between 72°C and 74°C. Lipoic acid and butylhidroxytoluene were then added. The emulsion was stirred, maintaining the temperature until a full dispersion was obtained.

Demineralized water, sorbitol 70%, and imidazolidinyl urea were mixed in another stainless steel container. This mixture was heated to 75°C.

Both phases were filtered by gravity filtration. Then mixture 1 was incorporated into mixture 2 and stirred at 900 rpm for five minutes. Then cooling was started and stirring was slowed down.

Vitamins A and 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 15 months at room temperature, and were analyzed under the same conditions in all cases. The quantitative compositions of the formulations are shown in Table I. The pHs were corrected, adding 1 N phosphoric acid or 1 N sodium hydroxide if needed.

ANALYSIS OF THE ACTIVE INGREDIENTS

The analyses of lipoic acid and vitamin A were made by HPLC.

Materials and reagents. The working standards employed for lipoic acid and vitamin A were the same as those used in the preparation of the creams. The solvents were HPLC grade. Water (HPLC grade) was obtained by distillation and passed through a 0.45-micron membrane filter.

Instrumentation. The HPLC system consisted of a dual-piston reciprocating Spectra Physics pump (model ISO Chrom. LC pump), a UV-Vis Hewlett Packard detector (Model 1050), a Hewlett Packard integrator (Series 3395), and a Rheodyne injector (Model 7125).

HPLC conditions. The experiment was performed on a LiChroCART^{R} 125*4 mm HPLC Cartridge LiChrospher^{R} 100 RP-18 (5 μm) (Merck, Darmstadt, Germany) for vitamin A.

Table I
Composition of Emulsions

Materials (g/100 g) INCI		System A	B	C	D
Cetearyl alcohol/sodium lauryl sulfate/sodium cetearyl sulfate	Anionic self emulsifying wax	9.000	9.000	9.000	9.000
Dimethicone	Silicone fluid	0.750	0.750	0.750	0.750
Paraffinum liquidum	Petrolatum	5.750	5.750	5.750	5.750
Imidazolidinyl urea	Imidazolidinyl urea	0.200	0.200	0.200	0.200
Sorbitol	Sorbitol 70%	9.000	9.000	9.000	9.000
Retinyl palmitate	Vitamin A palmitate	0.120	0.120	0.120	0.120
Tocopheryl acetate	Vitamin E acetate	0.400	0.400	0.400	0.400
Thioctic acid	Lipoic acid	0.500	0.500	0.500	0.500
BHT	Butylated hydroxytoluene	—	—	0.020	—
Ascorbyl palmitate	Ascorbyl palmitate	—	0.200	—	0.200
Magnesium ascorbyl phosphate	Magnesium ascorbyl phosphate	—	0.500	—	—
Ascorbic acid	Vitamin C	—	0.500	—	—
Aqua	Demineralized water	100.000	100.000	100.000	100.000

For lipoic acid it was performed on a Microsorb-MV® 100Å C18 (5 µm) (Varian Analytical Instruments, Walnut Creek, United States).

The mobile phase was methanol for vitamin A and methanol:water (80:20, v/v), pH 3.0, adjusted with 85% phosphoric acid, for lipoic acid. Both were filtered and degassed under reduced pressure prior to use. Separation was isocratically carried out at room temperature (20 ± 2°C). The flow rate was 1.8 ml/min, with UV detection at 325 nm. The flow rate was 0.6 ml/min, with UV detection at 332 nm for lipoic acid. The volume of each injection was 20 µl. In these conditions vitamin A and lipoic acid retention times were nine and six minutes, respectively.

Procedure. Solutions of the vitamins and lipoic acid were prepared on a weight basis with volumetric flasks to minimize solvent evaporation. Prior to injecting the solutions, the column was stabilized for at least 30 min, with the mobile phase flowing through the system. Quantification was accomplished using an external standard method. Each solution was prepared in duplicate and was injected in triplicate, and the relative standard deviation (RSD) was below 2.0%.

Working standard solutions. Twenty milligrams of vitamin A were placed into a 50-ml volumetric flask, dissolved in 40 ml of isopropyl alcohol, shaken for about five minutes, and then diluted to volume with isopropyl alcohol. The standard preparation was obtained by diluting 4 ml of the vitamin A stock solution with the mobile phase to yield a concentration of 0.016 mg/ml.

Twenty-five milligrams of lipoic acid were taken in a 25-ml volumetric flask, dissolved in 20 ml of methanol, shaken for about five minutes, and then diluted to volume with methanol. The standard preparation was obtained by diluting 8 ml of this acid stock solution with the mobile phase to yield a concentration of 0.08 mg/ml.

Preparation of o/w samples. Around 450 mg of cream was exactly weighed, placed into a 25-ml volumetric flask, taken to volume with methanol, and shaken for about five minutes for vitamin analysis. Approximately 450 mg of cream was exactly weighed and placed into a 25-ml volumetric flask, taken to volume with the mobile phase, and shaken for about five minutes for lipoic acid analysis. The solutions were passed through a 0.45-micron membrane filter before injection.

PHYSICAL STABILITY OF THE SYSTEMS

The centrifuge model was performed to study the physical stability of the systems. The centrifuge technique, based on theorical principles reflected in the Stokes formula, was used as one of the ways for predicting the vulnerability of the emulsion-to-oil coalescence (18). Centrifugation was performed for 30 minutes at 3500 rpm at room temperature on a Rolco (Argentina) centrifuge. Ten-milliliter samples in graduated centrifuge tubes were used. The classification adopted was:

- Good: no creaming or phase separation was observed.
- Poor: a considerable creaming and/or phase separation was observed.

PH DETERMINATIONS

The pH data for all the systems were obtained with model Altronix TPX I (Saen S.R.L., Buenos Aires, Argentina). The pH was measured as directed in USP 31 <791>, using an indicator glass electrode. The buffer solutions for standardization were from Merck (Darmstadt, Germany) at pH 4.00 and 7.01.

RESULTS AND DISCUSSION

Based on the results obtained in reference 16, we proposed the second series of emulsions (Table I) without acetylated lanolin, which was incompatible with lipoic acid (15), and varying the pH. We developed a new base emulsion and prepared the formulations from A to D at pH 3, 5, and 7. All the o/w formulations were stable under centrifugation. The pH variations in the period are indicated in Table II; generally, the pH decreased.

Percentages of vitamin A calculated against time are shown in Figures 4–7 in systems A to D during their storage at ambient temperature. It can be seen that initially the different batches conform to the regulations laid down by USP 32 /NF 27 (19) with regard to vitamin A content. However, during storage a diminution in mean vitamin A content was observed in the formulations: the lower the pH, the greater the loss.

In these systems, the content of lipoic acid decreased approximately 14% during the preparation of the emulsions, not conforming to the regulations according to USP 32 /NF 27. Lipoic acid could be quantified in system B at initial time and in system A at pH 3 and 5 until 259 days. In the formulations A, C, and D at pH 3 and 5, the loss of content was considerable, reaching 65 % for formulation A. During fifteen months of storage, lipoic acid was quantified with a significative difference in its stability at pH 7 (Figures 8–10).

Table II
pH Variations in System A–D

	Time (days)						
	0	43	83	111	195	321	438
System A (pH 3)	3.00	2.98	2.83	2.79	2.79	2.84	2.67
System A (pH 5)	5.00	5.04	4.57	3.84	3.76	3.54	3.34
System A (pH 7)	7.00	7.01	6.83	6.76	6.74	6.63	6.58
System B (pH 3)	3.00	3.08	3.00	2.81	2.89	2.94	2.77
System B (pH 5)	5.00	5.27	5.35	5.30	5.21	5.02	4.91
System B (pH 7)	7.00	6.96	6.89	6.88	6.75	6.60	6.70
System C (pH 3)	3.00	2.96	3.01	2.94	2.99	2.97	2.80
System C (pH 5)	5.00	4.97	5.32	5.25	5.30	5.14	5.18
System C (pH 7)	7.00	6.98	7.02	7.15	7.15	7.08	6.87
System D (pH 3)	3.00	2.98	3.04	2.96	3.03	3.04	2.90
System D (pH 5)	5.00	5.01	5.05	4.92	4.92	4.86	4.76
System D (pH 7)	7.00	6.93	6.84	6.84	6.85	6.83	6.70

It can be deduced from the results that the absence of antioxidants favors the degradation of vitamin A. The presence of ascorbyl palmitate, magnesium ascorbyl phosphate, and vitamin C seem to protect vitamin A as well as BHT at pH 5 and 7. Ascorbyl palmitate (system D) seems less to protect vitamin A palmitate and more to protect lipoic acid in these conditions. Although we have determined an interaction between lipoic acid and BHT (15), the addition of vitamin A in the proportion indicated improves the stability of these preparations at pH 5 and 7. Content was observed in these formulations: the higher the pH, the greater the stability. Based on these results, we propose systems C and D at pH 7 as the most stable for formulations containing vitamin A and lipoic acid.

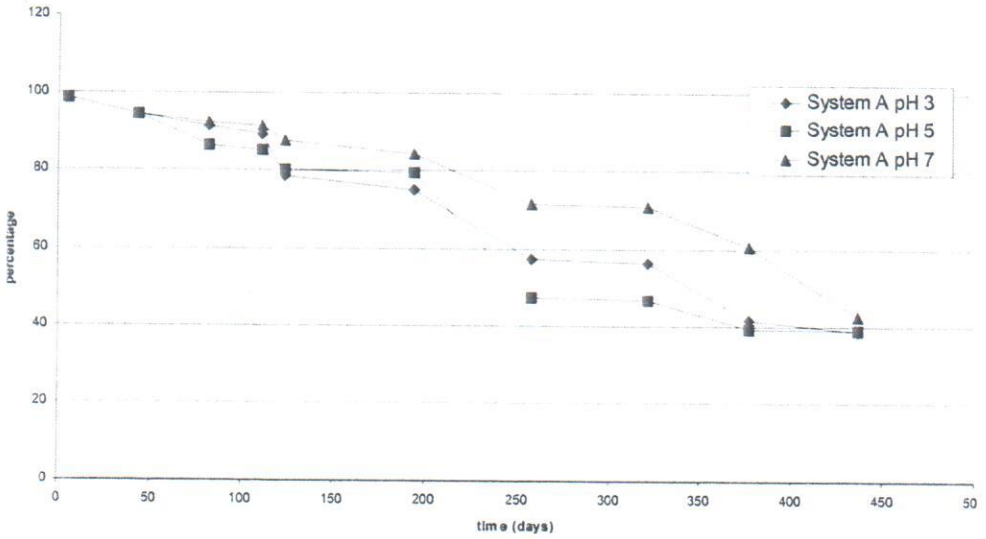

Figure 4. Percentage of vitamin A palmitate vs time in system A.

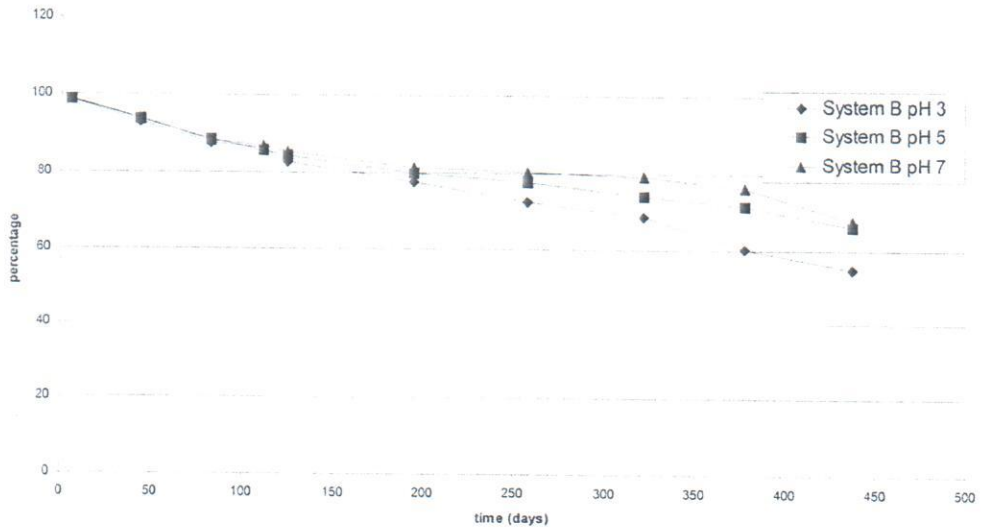

Figure 5. Percentage of vitamin A palmitate vs time in system B.

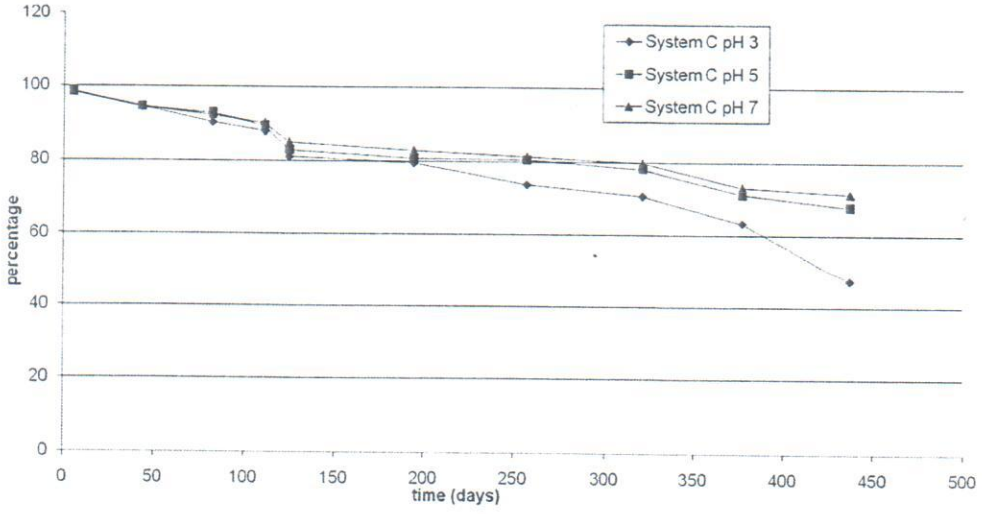

Figure 6. Percentage of vitamin A palmitate vs time in system C.

CONCLUSIONS

The photoirradiation of Vitamin A palmitate with UVA light results in the formation of photodecomposition products and reactive oxygen species through three distinct mechanisms: a UVA-initiated free-radical mechanism, an ionic photodissociation mechanism, and Vitamin A palmitate photosensitization. The photoirradiation of vitamin A palmitate generates singlet oxygen. The presence of antioxidants, such as BHT, ascorbyl palmitate, magnesium ascorbyl phosphate, and vitamin C increases the photostability and stability

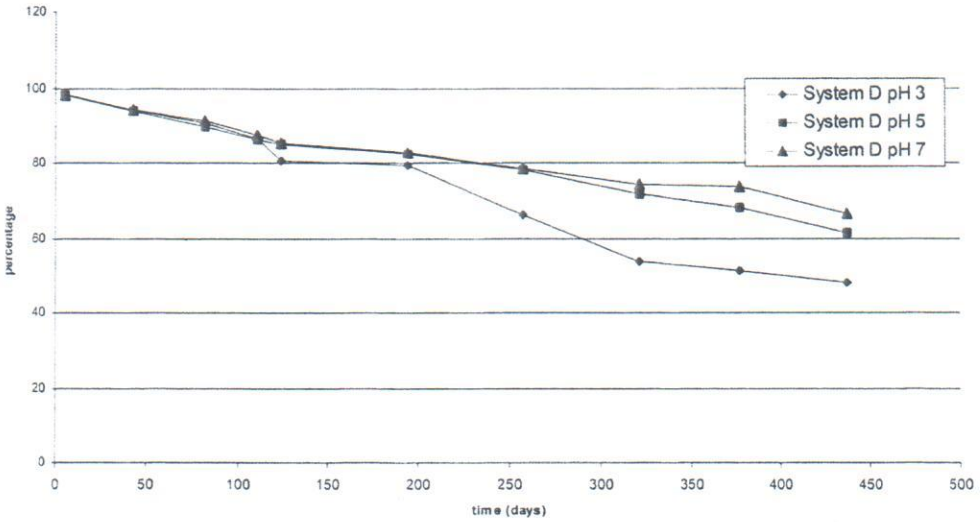

Figure 7. Percentage of vitamin A palmitate vs time in system D.

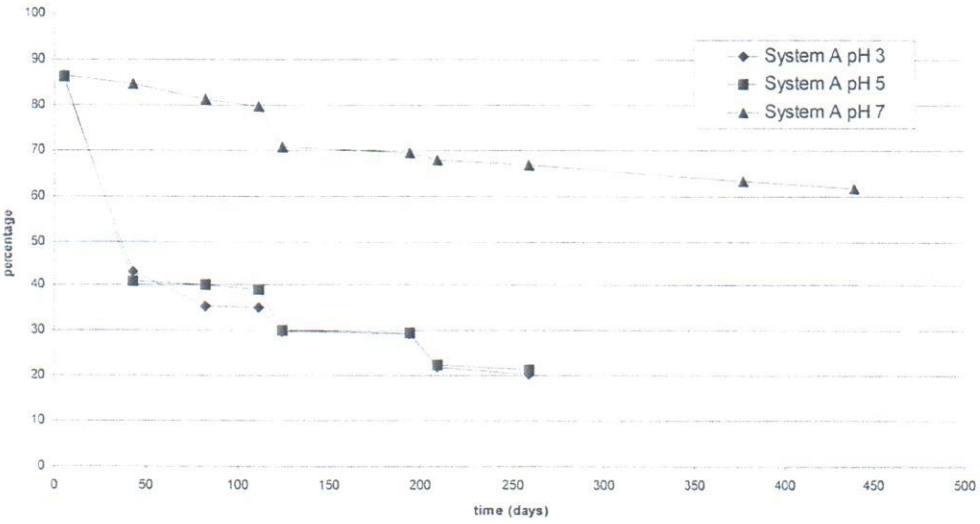

Figure 8. Percentage of lipoic acid vs time in system A.

over time of vitamin A palmitate, suggesting that degradation has oxygen as a photodegradation partner. Based on experimental results, we concluded that although lipoic acid in not very stable, the presence of vitamin A and a higher pH favors its chemical stability. Thus, a determining factor in the preformulation of semi-solid mixtures with Vitamin A and lipoic acid is the necessary control of the system's stability against oxidation.

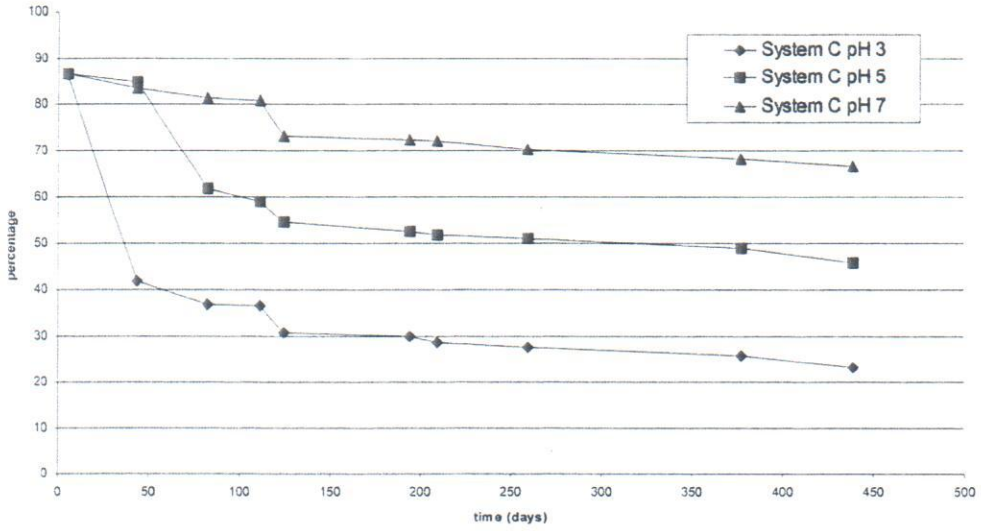

Figure 9. Percentage of lipoic acid vs time in system C.

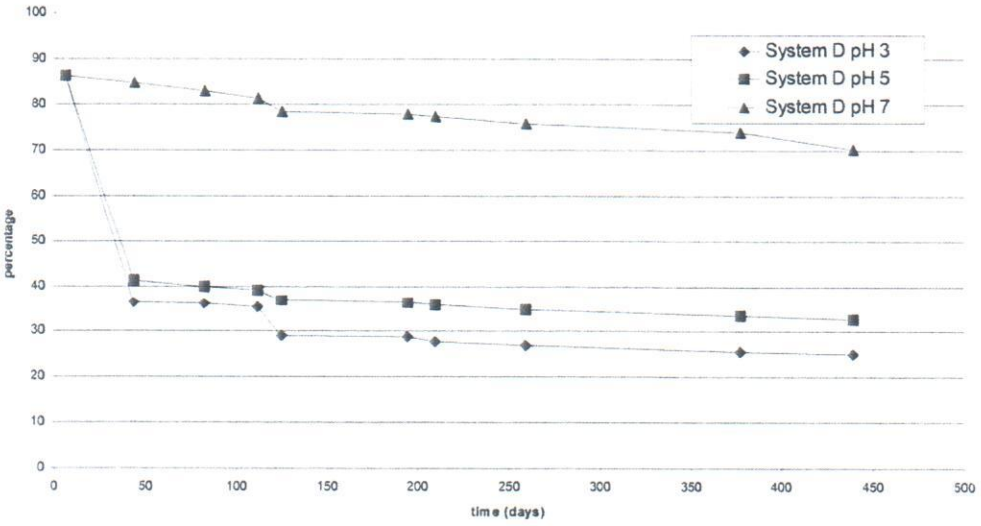

Figure 10. Percentage of lipoic acid vs time in system D.

ACKNOWLEDGMENTS

This work was supported by grant B072 to A. I. Segall from UBA.

REFERENCES

(1) H. Beitne, Randomized, placebo-controlled, double blind study on the clinical efficacy of a cream containing 5% α-lipoic acid related to photoageing of facial skin, *Br. J. Dermatol.*, 149, 841–849 (2003).

(2) A. Segall, M. Sosa, A. Alami, C. Enero, F. Hormaechea, M. T. Pizzorno, C. Bregni, and R. Serrao, Stability study of lipoic acid in the presence of vitamins A and E in O/W emulsions for cosmetic application, *J. Cosmet. Sci.*, **55**, 449–461 (2004).
(3) M. E. Carlotti, V. Rossatto, M. Gallarate, M. Trotta, and F. Debernardi, Vitamin A palmitate photostability and stability over time, *J. Cosmet. Sci.*, **55**, 233–252 (2004).
(4) M. E. Carlotti, V. Rossatto, and M. Gallarate, Vitamin A and vitamin A palmitate stability over time and under UVA and UVB radiation, *Int. J. Cosmet. Sci.*, **240**, 85–94 (2002).
(5) V. Jenning, M. Schäfer-Korting, and S. Gohla, Vitamin A-loaded solid lipid nanoparticles for topical use: Drug release properties, *J. Controlled Release*, **66**, 115–126 (2000).
(6) A. Akhavan and J. Levitt, Assessing retinol stability in a hydroquinone 4%, retinol 0.3% cream in the presence of antoxidants and sunscreen under simulated-use conditions: A pilot study, *Clin. Ther.*, **30**, 543–547 (2008).
(7) L. R. Gaspar and P. M. B. G. Maia Campos, Photostability and efficacy studies of topical formulations containing UV-filters combination and Vitamins A, C and E, *Int. J. Pharm.*, **343**, 181–189 (2007).
(8) Q. Xia, J. J. Yin, S. H. Cherng, W. G. Wamer, M. Boudreau, P. C. Howard, and P. P. Fu, UVA photoirradiation of retinyl palmitate: Formation of singlet oxygen and superoxide, and their role in induction of lipid peroxidation, *Toxicol. Lett.*, **163**, 30–43 (2006).
(9) T. Guaratini, M. D. Gianeti, and P. M. B. G. Maia Campos, Stability of cosmetic formulations containing esters of Vitamin E and A: Chemical and physical aspects, *Int. J. Pharm.*, **327**, 12–16 (2006).
(10) S. H. Cherng, Q. Xia, L. R. Blakenship, J. P. Freeman, W. G. Wamer, P. C. Howard, and P. P. Fu, Photodecomposition of retinyl palmitate in ethanol by UV light, formation of photodecomposition products, reactive oxygen species, and lipid peroxides, *Chem. Res. Toxicol.*, **18**, 129–138 (2005).
(11) R. Gatti, M. G. Gioia, and V. Cavrini, Analysis and stability study of retonoids in pharmaceuticals by LC with fluorescence detection, *J. Pharm. Biomed. Anal.*, **23**, 147–159 (2000).
(12) A. K. Singh and J. Das, Liposome encapsulated vitamin A compounds exhibit greater stability and diminished toxicity, *Biophys. Chem.*, **73**, 155–162 (1998).
(13) L. Halbaut, C. Barbé, M. Aróztegui, and C. de la Torre, Oxidative stability of semi-solid excipient mixtures with corn oil and its implication in the degradation of Vitamin A, *Int. J. Pharm.*, **147**, 31–40 (1997).
(14) M. A. Moyano and A. I. Segall, Stability of vitamin C derivatives in topical formulations containing lipoic acid, vitamins A and E, *Int. J. Cosmet. Sci.*, **30**, 453–458 (2008).
(15) M. A. Moyano, A. M. Broussalis, and A. I. Segall, Thermal analysis of lipoic acid and evaluation of the compatibility with excipients, *J. Therm. Anal. Calorimetry*, **99**(2) 631–637 (2010).
(16) M. A. Moyano and A. I. Segall, Estudios de estabilidad de nuevas formulaciones y en diversos envases conteniendo Vitaminas A, E y ácido lipoico, *Ciencia Cosmética*, **2**, 19–23 (2009).
(17) M. E. Calotti, E. Ugazio, S. Sapino, E. Peira, and M. Gallarate, Photodegradation of retinol and anti-aging effectiveness of two comercial emulsions, *J. Cosmet. Sci.*, **57**, 261–277 (2006).
(18) *Cosméticos, Guía de Estabilidad de Productos Cosméticos*, Editora Agencia Nacional de Vigilancia Sanitaria (ANVISA, 2005), Vol 1, pp 17.
(19) *The United States Pharmacopeia*, 32th ed. (U.S. Pharmacopeial Convention, Rockville, MD, 2009).