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Shelf life and rheology of emulsions containing vitamin C and its derivatives

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ABSTRACT

Ascorbic acid (AA), or vitamin C, is widely used in the pharmaceutical, cosmetic and food industries as an antioxidant and cosmetics containing AA have been gaining popularity in the last few years for the treatment of photoageing. To solve the problem of its low stability, some esters have been synthesised, such as magnesium ascorbyl phosphate (MAP), also available encapsulated in collagen-based microspheres (EMAP). The aim of this research was to study the physical and chemical stability of O/W emulsions containing AA, MAP or EMAP, by rheological and HPLC analysis, respectively. These emulsions were stored at 25, 37 and 45°C for 28 days and samples tested weekly during storage. It was concluded that all the formulations showed pseudo-plastic behaviour. The presence of MAP provoked an initial rise in thixotropy. The formulation containing AA did not show any marked change in rheological behaviour. In the chemical analysis, the formulation with EMAP was more stable than those with MAP and AA. Hence, replacement of AA with EMAP in this cosmetic formulation may be a viable way to enhance the stability of the active principle.
Keywords: Ascorbic acid. Magnesium ascorbyl phosphate. Shelf-life. Stability. Rheology.

INTRODUCTION

To keep the skin looking young and healthy is the constant desire of many people and a great deal of research effort has gone into the search for effective and safe active principles. Topical L-ascorbic acid functions as an antioxidant and free radical scavenger and as a cofactor for collagen synthesis and skin firmness (Silva & Maia Campos, 2000b; Nusgens, 2001; Jentzsch et al., 2001; Pinnell, 2001; Gaspar & Maia Campos, 2003; Campos et al., 2008; Yilmaz et al., 2008; Campos et al., 2009). However, the development of topical formulations containing ascorbic acid (AA) is complicated by its instability and its autoxidation to dehydroascorbic acid, though this is a reversible reaction. Some ascorbic acid derivatives have been synthesized for topical use, in an attempt to increase the chemical stability of AA while maintaining its desired properties (Campos et al., 2008).

The development process is fundamental to achieving the expected effects of a formulation. Chemical and physical stability studies are essential during development, to ensure selection of the most stable and effective formulation (Silva & Maia Campos, 2000a). In tests to predict the stability of a formulation, it is subjected to challenging conditions such as high temperatures (Gaspar & Maia Campos, 2003). To estimate the shelf-life of a product in relation to a specific component, its chemical degradation is monitored over time and the chemical kinetics of this component is studied (Silva & Maia Campos, 2000a).

Regarding the physical stability of an emulsion, it is known that its degradation by heating is a consequence of changes in the solubility of the components of the emulsion or facilitation of the coalescence phenomenon. Hence, study of the rheological behaviour during the development of cosmetic formulations is fundamental to assessing their stability. Such studies also enable the behaviour of the product during use to be predicted, since rheological properties may be correlated with the effectiveness of the product.

Drug encapsulation is widely used in the pharmaceutical industry as it offers many advantages. One such advantage is the physical protection of the drug molecule, so that there is a tendency for the shelf-life of formulations that employ encapsulated forms to be longer. In the case of vitamin C derivatives, this encapsulation could further improve chemical stability and make the commercialization of products supplying the benefits associated with this vitamin viable.

In Brazil, AA is supplied to compounding pharmacies in the form of vitamin C and derivatives, which are esters of ascorbic acid (magnesium ascorbyl phosphate, ascorbyl tetraisopalmitate, etc). These ingredients are also available encapsulated in liposomes or other vesicles, such

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as nanoparticles. The technology of production of these alternative forms of vitamin C can raise the cost of the final product. However, these new forms may be considered of great potential value, given the low stability of ascorbic acid dissolved in aqueous vehicles.

In this paper we discuss the physical stability of O/W emulsions containing ascorbic acid (AA), its ester magnesium ascorbyl phosphate (MAP) or MAP encapsulated in collagen-based microspheres (EMAP), determined by rheological testing of the formulations, and the chemical stability of the active substances, determined by HPLC analysis, in order to make a comparative assessment of these active principles.

MATERIAL AND METHODS

Material and sample preparation

The O/W formulations were prepared as shown in Table 1 and mixed in a Heidolph RZR 2021 shaker at approximately 625 rpm, either without vitamin C (control) or supplemented with 2% AA (*Roche)*, 2% MAP (*Nikko Chemicals Co*) or 10% EMAP (*Coletica, France*). After preparation, the formulations were stored in suitable plastic containers, previously cleaned with 70% alcohol.

Table 1. O/W emulsion formulations studied

Ingredients (INCI nomenclature)	Formulations			
	C % w/w	AA % w/w	MAP % w/w	EMAP % w/w
Glyceryl stearate (and) ceteareth-20 (and) ceteareth-12 (and) cetearyl alcohol (and) cetyl palmitate	7.0	7.0	7.0	7.0
Cetearyl alcohol	1.0	1.0	1.0	1.0
Phenoxyethanol (and) methylparaben (and) ethylparaben (and) propylparaben (and) butylparaben	0.5	0.5	0.5	0.5
Propylene glycol	6.0	6.0	6.0	6.0
Cetyl lactate	2.0	2.0	2.0	2.0
Coco-Caprylate/Caprate	2.0	2.0	2.0	2.0
Polyacrylamide (and) C13-14 Isoparaffin (and) Laureth-7	2.0	2.0	2.0	2.0
Disodium EDTA	0.2	0.2	0.2	0.2
Sodium dithionite	0.6	0.6	0.6	0.6
Ascorbic acid	-	2	-	-
Magnesium Ascorbyl Phosphate	-	-	2	-
Marine color Vitamin C PMG Spheres	-	-	-	10
Water	to 100	to 100	to 100	to 100

INCI = International Nomenclature of Cosmetic Ingredients
Vitamin C PMG Spheres = encapsulated magnesium ascorbyl phosphate microspheres

Formulation storage

Formulations were stored at room temperature (25°C approximately), 37°C and 45°C, for a period of 28 days. Samples were taken at 7-day intervals for physical and chemical stability studies.

Viscosity and rheological behaviour

The viscosity and the rheological behaviour of the formulations were studied with a Brookfield DV-III Cone-Plate rheometer, using a CP52 spindle and operated with the program Rheocalc 1.01. Rheograms and viscosity measurements were made at 25°C on 0.5g samples. To produce the ascending curve, rotation speeds were progressively raised (10-80 rpm) and then the procedure was reversed, with gradually decreasing speeds (80-10 rpm), for the descending segment. The rheograms obtained were mathematically analysed by the Ostwald Law, to calculate the apparent viscosity and flow index (related to the degree of pseudoplasticity of the sample). Numerical integration of the rheogram curves was performed by the program Origin (Microcal Software Inc), giving the area under the ascending and descending curves (to obtain the hysteresis loop area and degree of thixotropy). The ratios of the viscosity, flow index and thixotropy values observed at various times during the test to the initial values were calculated, in order to analyse the rheological behaviour of the formulations in terms of the following model:

$$\delta = k.D^n ,$$

where δ = shear stress, D = shear rate, k = consistency index and ^{n} = flow index (Martin et al., 1993).

Viscosity, flow index and thixotropy data, and their ratios, were analysed by the Kruskal-Wallis nonparametric test (Yilmaz et al., 2008).

Analysis of chemical stability by HPLC

High performance liquid chromatography (HPLC) was used for quantitative analysis of the active substances under study. The HPLC system was a Shimadzu liquid chromatograph, equipped with a variable UV detector, connected to a computer running LC-10 software (Shimadzu), and a manual injection valve fitted with a 20-µL sample loop.

All reagents and solvents were of analytical or HPLC grade. A Shimadzu Shim-pack CLC-ODS (m) column was used, with particle size 5 mm, 250 x 4.6 mm i.d., mobile phase: water/methanol (95:5 *v/v*) flowing at 1.0 mL/min. Detector wavelength was set at 254 nm, sensivity at 0.01a.u.f.s. All solutions were filtered through a Millipore filter membrane, pore size 0.45 mm, and vacuum degassed by sonication before use. Standard solutions containing L-ascorbic acid (*Roche*) or magnesium ascorbyl phosphate (*Nikko Chemicals Co*.) at concentrations of 25,50 and 100 µg/mL were prepared at the moment of use.

Samples of 0.5g of each formulation were subjected to extraction of the active substances in distilled water and ultrasonic treatment for 15 min and then filtered and transferred to a 10 mL volumetric flask and diluted to the mark with water purified by Milli-Q. 20 µL of each standard solution and sample (four injections) were injected into the chromatograph. This method has been previously standardized (Austria et al, 1997).

RESULTS

Rheograms of each formulation are shown for various storage times and temperatures in Figures 1, 2 and 3.

Table 2 shows the minimum apparent viscosity of the formulations, Table 3 their flow index and Table 4 their hysteresis loop area. The results refer to the time before storage and after 7, 14, 21 and 28 days at atmospheric temperature, 37°C and 45°C.

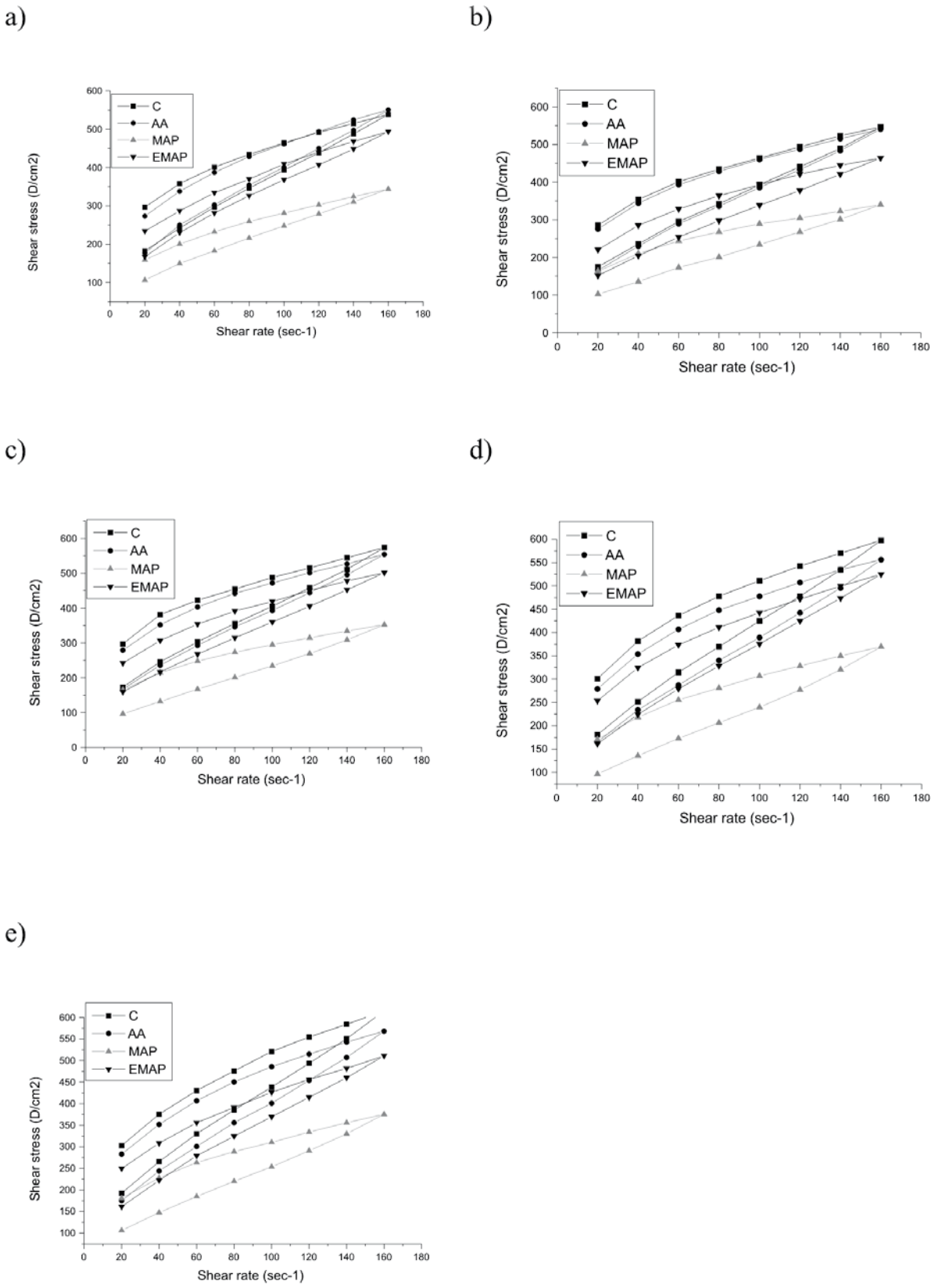

Figure 1. Rheological behaviour of formulated emulsions: control (C), formulation containing ascorbic acid (AA) or magnesium ascorbyl phosphate (MAP) or encapsulated magnesium ascorbyl phosphate (EMAP), (a) initially and stored at 25°C for (b) 7, (c) 14, (d) 21 and (e) 28 days.

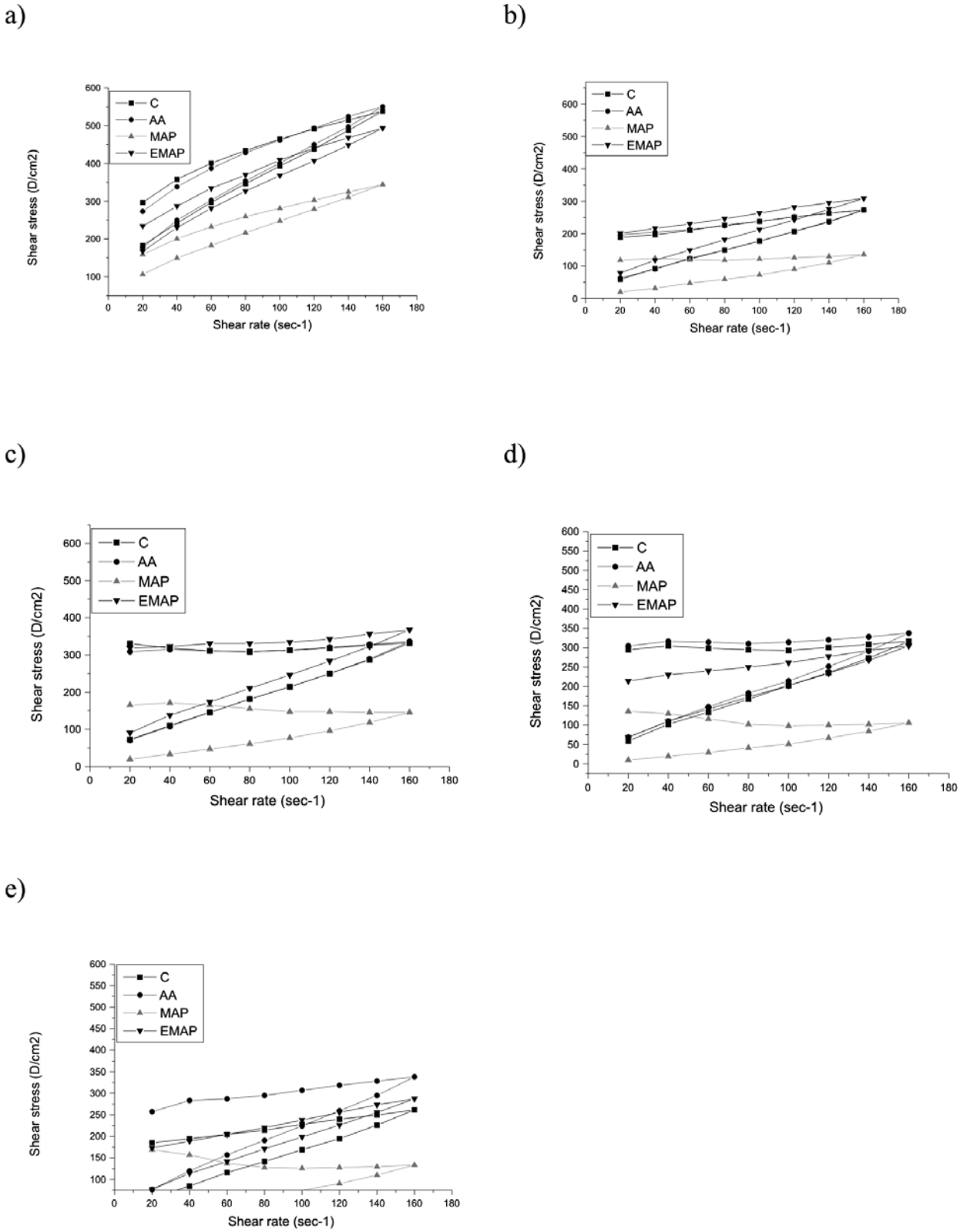

Figure 2. Rheological behaviour of formulated emulsions: control (C), formulation containing ascorbic acid (AA) or magnesium ascorbyl phosphate (MAP) or encapsulated magnesium ascorbyl phosphate (EMAP), (a) initially and stored at 37°C for (b) 7, (c) 14, (d) 21 and (e) 28 days.

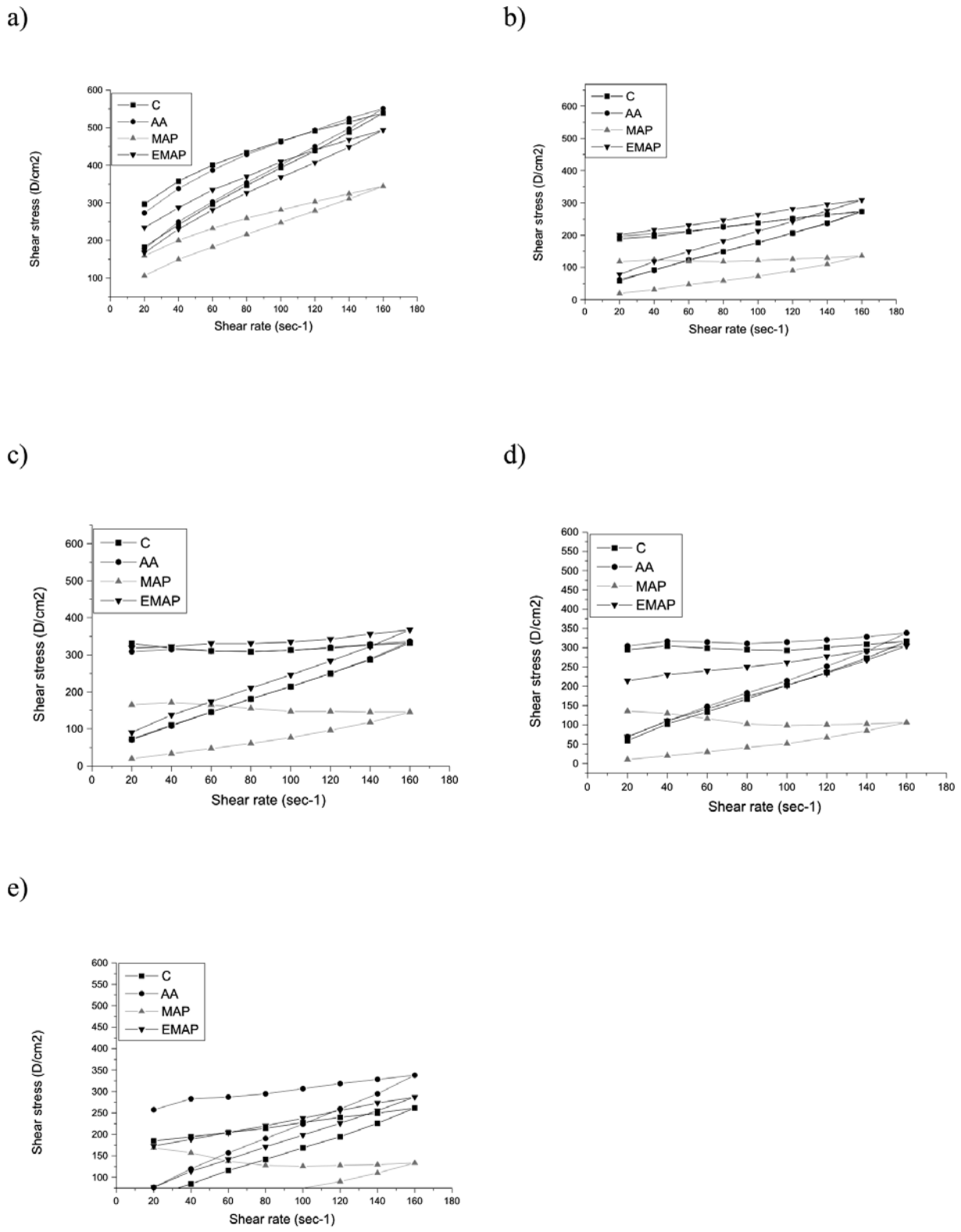

Figure 3. Rheological behaviour of formulated emulsions: control (C), formulation containing ascorbic acid (AA) or magnesium ascorbyl phosphate (MAP) or encapsulated magnesium ascorbyl phosphate (EMAP), (a) initially and stored at 45°C for (b) 7, (c) 14, (d) 21 and (e) 28 days.

Table 2. Minimum apparent viscosity (Pa.s) of the formulations initially and after 7, 14, 21 and 28 days at ambient temperature, 37°C and 45°C. Values calculated at the maximum point of the shear gradient.

	Apparent Viscosity (Pa.s)												
	Ambient					37°C				45°C			
Day:	0	7	14	21	28	7	14	21	28	7	14	21	28
Formulation													
C	14450	23223	24722	25558	24354	1841	17055	15925	15875	7520	12435	7078	7176
AA	15900	16736	17055	17424	14278	17055	15384	15777	14352	9732	12509	2556	9535
MAP	14991	14131	15384	15114	14376	13737	12042	13418	12632	10395	12140	6488	7692
EMAP	10985	12951	13074	13516	11501	12337	11206	10052	9191	10371	10100	8945	4301

C: control; AA: formulation containing L-ascorbic acid; MAP: formulation containing magnesium ascorbyl phosphate; EMAP: formulation containing encapsulated magnesium ascorbyl phosphate

Table 3. Flow index of the formulations initially and after 7, 14, 21 and 28 days at ambient temperature, 37°C and 45°C

	Flow Index												
	Ambient					37°C				45°C			
Day:	0	7	14	21	28	7	14	21	28	7	14	21	28
Formulation													
C	0.36	0.36	0.38	0.34	0.33	0.36	0.34	0.33	0.33	0.43	0.47	0.21	0.25
AA	0.36	0.42	0.39	0.38	0.42	0.33	0.34	0.33	0.35	0.52	0.60	0.32	0.51
MAP	0.38	0.37	0.38	0.38	0.39	0.35	0.45	0.34	0.36	0.59	0.45	0.26	0.39
EMAP	0.37	0.37	0.39	0.06	0.37	0.35	0.36	0.36	0.37	0.50	0.45	0.43	0.36

C: control; AA: formulation containing L-ascorbic acid; MAP: formulation containing magnesium ascorbyl phosphate; EMAP: formulation containing encapsulated magnesium ascorbyl phosphate

Table 4. Hysteresis loop area (D/cm2.s) of the formulations initially and after 7, 14, 21 and 28 days at ambient temperature, 37°C and 45°C

	Hysteresis area (D/cm².s)												
	Ambient					37°C				45°C			
Day:	0	7	14	21	28	7	14	21	28	7	14	21	28
Formulation													
C	-103	-36	-302	42	-78	401	390	478	349	855	2693	1810	1400
AA	38	-200	221	76	-30	304	400	401	361	1374	2415	1302	199
MAP	112	99	49	27	77	393	49	478	435	1334	355	1378	794
EMAP	36	53	29	222	214	496	438	501	565	1114	588	816	1329

C: control; AA: formulation containing L-ascorbic acid; MAP: formulation containing magnesium ascorbyl ahosphate; EMAP: formulation containing encapsulated magnesium ascorbyl phosphate

The estimated shelf-lives of each of the formulations tested are shown in Table 5.

Table 5. Estimated shelf-lives of the emulsion formulations, assuming first-order kinetics.

Formulation	Shelf life (days)
Formulation containing AA	119
Formulation containing MAP	200
Formulation containing EMAP	299

DISCUSSION

The apparent viscosities at the loop apex analysed 24 h after preparation and storage of the formulations at room temperature showed that the viscosity of the vehicle was low, with values around 14,000 Pa.s. None of the formulations showed significant variations in the viscosity during the experiment, in congruence with the absence of change in the visual observations. The rheological behaviour of these formulations can be assessed from their rheograms and the data presented in Figures 1 to 3 and Tables 2 to 4. According to the rheograms, all the formulations containing active substances showed rheological behaviour similar to that of the vehicle and there were no great differences in their ascending and descending curves. The ascending and descending flow curves showed hysteresis, usually referred to as a 'thixotropic loop'. These rheograms also had no peak.

The graphs were different for each formulation but there were no major changes over time. The main variation was seen among the results for each storage temperature. The storage conditions influenced the formulations under study. An important observation is that the rheograms start above the origin, suggesting a positive yield stress, which is desirable for the stability of a semi-solid.

In relation to the thixotropy, the formulations exhibited hysteresis areas, with some negative values (Table 4). This was also observed by Viseras et al. (1999) in suspensions with particles of various formats. Those authors reported that this behaviour can be reduced by agitation before the rheological analysis and they stated that it does not indicate loss of stability. Budtova et al. (2001) suggested that this flow is caused by the break-up of complexes formed as a result of molecular interactions in the solution. Other authors have found that thixotropy varies with the intensity and duration of the force applied to the solution (Bautista et al., 1999). Therefore, the values of negative thixotropy and the variations in the rheograms do not necessarily indicate lack of stability of the formulations, as they may be characteristic of the polymer used.

Thixotropy is desirable in topical formulations because they are deformed during application and become fluid, facilitating spreading. The recovery of the initial viscosity after application prevents the product from dripping (Correa et al, 2005).

The formulations showed pseudoplastic behaviour, with a flow index (n) below 1 (Table 3) (Gaspar & Maia Campos, 2003). This is generally a useful characteristic in emulsions, since the viscosity decreases with increasing

shear stress, again making them easy to spread on the skin and facilitating production. In general, none of the formulations showed significant variation in these values.

Thus, according to the rheological study, the formulations remained stable throughout the test period, especially at the lower temperatures. The storage temperature can alter the viscosity of the product, and higher temperatures lead to precocious instability, by reducing the continuous phase viscosity and favouring an increase in particle motility and the interactions between the phases.

The thixotropy of a material can vary according to its molecular orientation and the flow direction, and it is influenced by the shear stress and is time-dependent. Thus, an increase in the shear stress disorganises the three-dimensional network of molecules, by temporary disruption of the hydrogen bonds by the shearing force, and alters the viscosity (Tadros, 2004). It has been speculated that thixotropy may favour the penetration of the active substances into the skin, owing to disorder in the system structure. However, further studies need to be done before the role of thixotropy in drug release can be firmly established and documented.

The chemical stability was tested by HPLC and the results suggested that AA and MAP degradation follows first-order kinetics, corroborating the results of Gallarate et al (1999).

The Arrhenius method was used to estimate the chemical expiry dates (Silva & Maia Campos, 2000a). The longest shelf life was obtained for the EMAP formulation, followed by the MAP formulation and then the AA formulation. The formulation containing EMAP was, in fact, the most stable because its initial concentration was maintained throughout the study. This result was expected in view of the protective encapsulation of the MAP, which reduces its interaction with the other ingredients of the formulation. Thus, the other formulations were less stable, especially that containing AA, and these results are consistent with the rheological study.

The Arrhenius method has been used to estimate the shelf life of a product or to perform a selection among different products. A long-term stability study is also performed to confirm the result. Samples are kept in controlled temperature and humidity, close to the intended environment, and monitored during the proposed shelf-life (Manfio et al, 2007).

The concentrations of active substances used in each formulation were based on previous work (Silva & Maia Campos, 2000b). In the case of AA and MAP, 2% of each substance was used, but the concentration of EMAP was 10%, as the encapsulation efficiency was 20%, so that this formulation also had 2% MAP. The use of the same concentration of active substance in all emulsions was important for this research, because the decomposition reaction follows first-order kinetics and the conditions of study should be standardized.

When comparing the cost of the active substances and the differences between their shelf-lives, it is clear that the main advantage of the encapsulated form is the potential for combination with substances that would not be compatible with MAP or with formulations of pH below 7. An acid pH could promote the hydrolysis of the ester, which is protected in the encapsulated form.

In another study by the same authors, the effects of formulations containing AA or MAP were measured directly on human skin by means of biophysical skin tests. The vitamin C derivatives did not show the same effects as AA on human skin, but MAP showed other significant effects in improving skin hydration, which is very important for normal cutaneous metabolism and also to prevent skin alterations and early aging.

Innovation is an essential requirement for survival in the cosmetic industry, a very competitive market. Technological advances have made available in new products with more and better safety and efficacy. Cosmetics claim to promote healthy skin, but it is essential to ensure that they are effective and accessible. This is a crucial first step towards producing a stable product. The use of AA has been a challenge to cosmetic formulators, because of the special resources needed to improve the product stability. Introduction of the phosphate group into the enediol system, in the MAP molecule, protects it against hydrolysis. Besides, it significantly increases the stability of the formulation and shows the importance of alternative uses of this substance in cosmetic formulations.

We conclude that, under the present experimental conditions, the HPLC analysis showed the EMAP formulation to be the most stable chemically, as expected. In relation to physical stability, all formulations exhibited pseudoplastic behaviour and the presence of MAP provoked an initial rise in thixotropy. The most stable formulation was that containing EMAP, followed by those with MAP and AA, respectively.

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RESUMO

Prazo de validade e reologia de emulsões contendo ácido ascórbico e derivados

Cosméticos contendo ácido ascórbico (AA) têm sido muito empregados nos últimos anos na prevenção e tratamento do fotoenvelhecimento cutâneo. Para solucionar o problema de sua baixa estabilidade, alguns derivados têm sido utilizados como substitutos dessa vitamina e dentre eles o Magnesium Ascorbyl Phosphate (MAP), disponível também em microesferas à base de colágeno (EMAP). O objetivo desta pesquisa foi estudar a reologia e a estabilidade química de emulsões contendo AA, MAP ou EMAP. Assim, formulações contendo AA, MAP ou EMAP foram armazenadas a 25, 37 e 45°C durante 28 dias e avaliadas. Concluiu-se que todas as formulações apresentaram comportamento pseudoplástico. A presença de MAP provocou um aumento inicial da tixotropia e a formulação contendo AA não apresentou grande alteração no comportamento reológico. Nos estudos químicos a formulação contendo EMAP foi mais estável que o MAP e AA, com o maior prazo de validade. Desta forma, a substituição de AA pela EMAP em cosméticos pode ser uma alternativa viável do ponto de vista da estabilidade da formulação.

Palavras-chave: Ácido ascórbico. Magnesium Ascorbyl Phosphate. Prazo de validade. Estabilidade. Reologia.

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