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On the stability of ascorbic acid in emulsified systems for topical and cosmetic use

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Abstract

Several O/W microemulsions, O/W and W/O emulsions and a W/O/W multiple emulsion were prepared using non-ionic, non-ethoxylated, skin compatible emulsifiers. Ascorbic acid (vitamin C) was added to the emulsified systems and its stability against oxidation was studied at 45.0°C in aerobic conditions and compared with that in aqueous solutions at different pH values. All emulsified systems provided protection to ascorbic acid, as its degradation rate, which increased with increasing pH, was slower in emulsified systems than in aqueous solutions. The highest protection of ascorbic acid was when it was dissolved in the inner aqueous phase of the W/O/W multiple emulsion, both at 45 and at 20°C for long storage. A pseudo first-order mechanism was hypothesised for ascorbic acid degradation in the experimental conditions for as long as abundant dissolved oxygen was present.

Keywords: Multiple emulsions; Ascorbic acid; Oxidative degradation

1. Introduction

Ascorbic acid (vitamin C) is an essential nutrient involved in many physiological functions. It readily undergoes two consecutive (yet reversible), one-electron oxidation processes to form the ascorbate radical, a relatively unreactive free radical, and is therefore considered an excellent reducing agent (Buettner and Jurkiewicz, 1996). In living organisms, ascorbic acid can protect tissues and cells against oxidative damage by free radicals and reactive oxygen-derived species. It is said to act synergistically with α-tocopherol (vitamin E) (Rousseau-Richard et al., 1991), as it serves as a donor antioxidant to restore the tocopheroxyl radical (Buettner and Jurkiewicz, 1996). The reaction of the tocopheroxyl radical with ascorbate is one way to export oxidative free radicals from the membranes. Thus α-tocopherol protects the membranes by stopping the propagation reactions of lipid peroxyl radicals, while ascorbic acid simultaneously recycles α-tocopherol (Frei et al., 1990).

Unfortunately, ascorbic acid in solution can undergo oxidation (Tsao and Young, 1996) and produce dehydro-L-ascorbic acid as well as many degradation products. Several factors can negatively influence ascorbic acid degradation (Roig et

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al., 1995; Tsao and Young, 1996), such as high storage temperatures, light, high pH values and the presence of dissolved oxygen, although the reaction mechanism of ascorbic acid with an oxygen molecule has not yet been fully elucidated (Miyake et al., 1997). Moreover, the reaction of ascorbic acid with oxygen is strongly catalysed by metal ions, particularly cupric and ferric ions (Niki, 1991; Buettner and Jurkiewicz, 1996).

In recent years, thanks to the above-mentioned synergistic effect with vitamin E, ascorbic acid has been successfully introduced into a number of cosmetic and dermatological formulations also containing α-tocopherol; topical use is also indicated because of its skin-depigmenting activity and its well-known ability to take part in proline and lysine hydroxylation in collagen biosynthesis.

The aim of this study was to formulate O/W microemulsions, O/W and W/O emulsions, and W/O/W multiple emulsions as potential cosmetic or topical vehicles for ascorbic acid, using non-ionic, non-ethoxylated, glucose or saccharose-derived and skin-compatible emulsifiers, and to evaluate the oxidative stability of the vitamin over time in these formulations.

Multiple W/O/W emulsions are vescicular systems in which very small water droplets are entrapped within oil drops, themselves dispersed in an aqueous phase (Raynal et al., 1993). They have potential uses as drug and cosmetic vehicles (Terrisse et al., 1993), as they might protect encapsulated substances and enable incompatible substances to be incorporated in the same formulation; moreover, prolonged action after administration can be expected.

In the present study, ascorbic acid was dissolved in the inner aqueous phase of a W/O/W emulsion at a pH value at which the vitamin is known to be reasonably stable, and its stability was compared with that provided by other emulsified systems.

2. Materials and methods

2.1. Materials

Ascorbic acid (AA), silybin (2,3 dihydro-3[4-hydroxy-3-methoxy-phenyl]-2-[hydroxymethyl]-6-[3,5,7-trihydroxy-4-oxobenzopyran-2-yl] benzodioxin) were from Sigma; 2 ethyl,1,3-hexanediol was from Fluka; isopropyl palmitate (IPP), fluid paraffin, cetyl palmitate, and D-glucose were from Merck; soya phosphatidylcholine (96% w/w) Phospholipon G 100® was a gift from Rhone Poulenc; cetearyl octanoate was from Alzo; caprylyl-capryl glucoside was from Sinerga; cetearyl glucoside was from Seppic; mineral oil and polyethylene were from Hansen & Rosenthal KG; polyglyceryl-2-sesquiisostearate, polyglyceryl-2-sesquiisostearate and beeswax and mineral oil and magnesium stearate and aluminium stearate (W/O emulsifying mixture) were from Hoechst Farbwereke; octyl palmitate was from Alzo, saccharose monolaurate and saccharose monostearate were from Biochim; Brilliant Blue C.I. 42090 (BB) was from Variati; xanthan gum was from Kelco; dodecylglucoside and cocoamide propylbetaine were from Tego Goldschmidt; methylglucose dioleate was from Amerchol; and octyloctanoate was from Dragoco.

2.2. Apparatus

The apparatus used included Ultra Turrax Homogenizer T25 (IKA Janke and Kunkel IKA Labortechnik); optical microscope Labowert with photocamera Wild MPS – 46 Fotoautomat (Leitz); conducimeter Conductivity meter mod.101 (Orion Research); laser light scattering Model N4 MD Submicron particle analyzer (Coulter); and HPLC apparatus consisting of a UV detector SPD-2A, a pump unit control LC 6A and a C-R3A chromatopac integrator (Shimadzu).

2.3. Preparation of O/W microemulsions

Several O/W microemulsions were prepared using IPP or cetearyl octanoate as oils. A glucose derivative, dodecylglucoside and cocoamide propylbetaine, was used as surfactant and 2-ethyl-1,3-hexanediol was chosen as cosurfactant. Mi-croemulsions were also obtained by partially substituting the primary surfactant with phosphatidylcholine.

An amount of oil corresponding to 4.0% w/w

was added to a surfactant (or surfactant-PC) aqueous dispersion and then titrated with cosurfactant until transparency was reached. Microemulsions with AA were prepared as above by dissolving AA in water to a final concentration of 1.00% w/w. The pH of each microemulsion was corrected to 5.0, 6.0, and 7.0.

2.4. Laser light scattering measurements

Mean diameters of microemulsions oil droplets were determined at 25.0 ± 0.1°C by quasi-elastic light scattering technique (Trotta et al., 1991).

2.5. Preparation of O/W and W/O emulsions

An O/W emulsion containing 1.00% w/w AA was prepared as follows: the mixture of oil and O/W emulsifier was heated to 65°C and then added to water at 70°C using an ultra turrax homogenizer. AA was added at room temperature. pH was corrected to 5.0 or 7.0.

A W/O emulsion containing 1.00% w/w AA was prepared mixing oil and emulsifiers at 40°C; the mixture was then cooled and added to an aqueous solution of AA at pH 5.0 by means of an ultra turrax homogenizer. A W/O emulsion containing 0.02% w/w silybin and 7.1% w/w ethanol was also prepared.

2.6. Preparation of W/O/W multiple emulsions

The W/O/W emulsion was prepared by a two-step procedure (Florence and Whitehill, 1982): in the first step the primary W/O emulsion was obtained at 25°C, adding a pH 3.0 AA aqueous solution to the pre-heated dispersions of oils and emulsifiers under vigorous, 1-h mixing. In the second step the primary emulsion was slowly poured into a pH 7.0 aqueous dispersion of O/W emulsifiers under gentle stirring. The percentage of AA in the multiple emulsion was 0.25% w/w.

2.6.1. Characterisation of multiple emulsion

The W/O/W multiple emulsion was characterised by optical microscopy, by pH and conductivity measurements and by the addition of the hydrophilic dye Brilliant Blue (BB).

2.7. AA degradation studies

The degradation of AA at 45.0 ± 0.1°C was studied in:

1. aqueous solutions at pH 3.0, 4.0, 5.0, 7.0 (AA = 1.00% w/w)
2. aqueous solutions at pH 3.0, 4.0, 5.0, 7.0 (AA = 0.25% w/w)
3. O/W emulsion at pH 5.0 and 7.0 (AA = 1.00% w/w)
4. W/O emulsions with pH of the aqueous phase = 5.0; (1) without silybin; (2) with silybin (AA = 1.00% w/w)
5. O/W microemulsions at pH 5.0, 7.0 (AA = 1.00% w/w)
6. W/O/W emulsions (pH of inner aqueous phase = 3.0; pH of outer aqueous phase = 7.0) (AA = 0.25% w/w)

Samples were placed in sealed glass bottles and maintained in a thermostated bath at 45.0 ± 0.1°C for 24 h. At prefixed times, each sample was opportunely treated for AA extraction and then analysed by HPLC for AA determination. Analytical conditions were: column: Spherisorb S5 C8 (15 cm × 4.6 mm); mobile phase: CH_3OH:H_3PO_4 (0.1% in H_2O) (10:90); flux: 0.6 ml min^{-1}; detector: UV $\lambda = 245$ nm; RT: 3.23 min (Howard et al., 1988).

AA degradation was also studied (in two series of experiments) in the following systems containing 0.10% w/w AA:

1. Aqueous solutions at pH 3.0, 4.0, 5.0
2. O/W emulsion at pH 5.0
3. W/O emulsion (pH of the inner phase = 5.0)
4. W/O/W emulsion (pH of the inner phase = 3.0; pH of the outer phase = 7.0)

Samples were stored at 20.0 ± 0.1°C or at 45.0 ± 0.1°C for 20 days and then analysed as described above.

The volumes of the sample and of the headspace air in the experimental vessels were 5.0 and 7.5 ml, respectively, for all systems studied. The experimental conditions were chosen so as to operate in the presence of a volume of oxygen as

close as possible to that of effective storage conditions.

To evaluate the possibility of anaerobic degradation of AA, a 1.00% w/w AA aqueous solution at pH 5.0 was stored at 45.0 ± 0.1°C in a completely filled, sealed bottle. Helion was bubbled into the solution to eliminate the dissolved oxygen.

2.8. Kinetic study of AA degradation

Studied reported in the literature (Singh et al., 1976; Eison-Perchonok and Downes, 1982) indicate that AA oxidation under limited presence of dissolved oxygen follows a second-order mechanism. However, when dissolved oxygen is abundant, the reaction can be considered to follow first order-mechanism (Robertson and Samaniego, 1986).

In a closed water/air system, assuming oxygen dissolution to be faster than its disappearance through any possible reaction (Joslyn and Miller, 1949; Singh et al., 1976), the dissolved oxygen can be maintained at saturation when its partial pressure is sufficiently high to provide an oxygen 'reservoir'.

Provided this assumption is valid, then combining the general solution of the second order rate equation:

$$d[AA]/dt = -k_2[AA][O_2] \quad (1)$$

with the expression for oxygen replenishment:

$$-d[O_2]_1/dt = 0 \quad (2)$$

and solving, yields:

$$\ln([AA_t]/[AA_0]) = -k_2[O_2]t \quad (3)$$

where: k_2 is second order rate constant; $[AA_t]$ is AA concentration at time t; $[AA_0]$ is AA concentration at time $t = 0$; and $[O_2]$ is dissolved oxygen concentration.

Eq. (3) is a pseudo-first order rate equation, and $k_2[O_2]$ can be substituted by the pseudo-first order rate constant k'_1.

Eq. (3) can be re-written as:

$$\ln([AA_t]/[AA_0]) = -k'_1 t \quad (4)$$

In the present study of AA degradation the initial conditions in the experimental vessels were as follows:

O_2 concentration in the headspace air: 9.38×10^{-3} mol l^{-1}

1.00% w/w AA concentration: 5.68×10^{-2} mol l^{-1}

0.25% w/w AA concentration: 1.42×10^{-2} mol l^{-1}

0.10% w/w AA concentration: 5.68×10^{-3} mol l^{-1}

O_2 solubility in water at 45.0°C: 1.76×10^{-4} mol l^{-1} (Eison-Perchonok and Downes, 1982).

For each system in the study, $\ln([AA_t]/[AA_0])$ was calculated and then plotted versus time, to verify whether a first-order mechanism was followed in the experimental conditions.

3. Results and discussion

Table 1 reports the percent compositions of the microemulsions obtained. It can be seen that the

Table 1
Compositions of O/W microemulsions with AA

	No. 1	No. 2	No. 3	No. 4	No. 5	No. 6
IPP	4.0		2.0	4.0		
Cetearyl octanoate		4.0	2.0		4.0	4.0
Dodecylglucoside and cocoamide propylbetaine	12.0	15.0	12.0	6.0	11.0	8.5
2-Ethyl,1,3-hexanediol	3.0	4.0	4.0	4.0	3.5	
Phosphatidylcholine				1.0	1.0	1.0
Water	80.0	76.0	79.0	84.0	79.0	75.5
AA	1.0	1.0	1.0	1.0	1.0	1.0

Table 2
Mean diameters of microemulsions determined by LLS

Microemulsion	Mean diameter (S.D.), nm
No. 1	42.2 (14.1)
No. 2	61.8 (18.7)
No. 3	39.5 (11.3)
No. 4	19.9 (4.3)
No. 5	13.3 (4.0)
No. 6	15.6 (3.9)

Table 3
Composition of W/O/W multiple emulsion

Component	Percent w/w
W/O primary emulsion[a]	36.53
Saccharose monostearate	0.42
Saccharose monolaurate	0.57
Xanthan gum	0.42
Glucose	2.62
Water	59.44

[a] W/O primary emulsion: octyloctanoate = 17.94% w/w; mineral oil and polyethylene = 6.97% w/w; polyglyceryl-2-sesquiisostearate = 3.49% w/w; methylglucose dioleate = 1.69% w/w; water: 67.17% w/w; AA = 2.74% w/w.

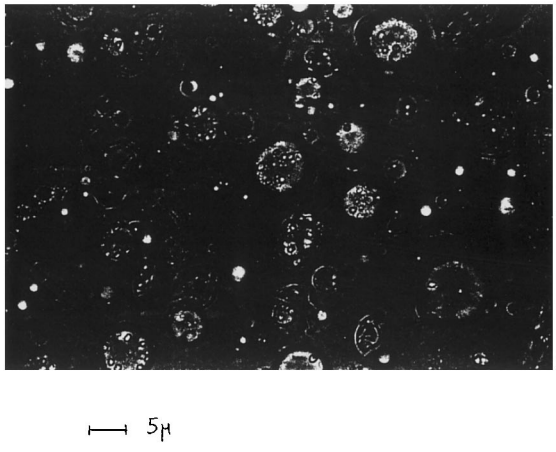

Fig. 1. Microphotograph of W/O/W multiple emulsion containing 0.25% w/w AA.

presence of 1.0% w/w PC in microemulsions 4, 5, 6 allowed us to reduce significantly the amount of surfactant required compared to microemulsions 1, 2, 3. The possibility of partially substituting a synthetic surfactant with a natural-occurring substance like PC — with regard to a potential topical use — provides some advantage due to its skin-compatibility.

All microemulsions were stable over time at pH 5.0, 6.0 and 7.0 and their mean diameters, determined by LLS and reported in Table 2, did not change over 3 months' storage.

The O/W emulsion obtained had the following composition: cetearyl glucoside = 5.0% w/w; octyl palmitate = 20.0% w/w; water = 74.0% w/w; AA = 1.0% w/w.

The compositions of W/O emulsions were as follows: W/O_1: fluid paraffin = 14.0% w/w; mineral oil and polyethylene = 10.0% w/w; polyglyceryl-2-sesquiisostearate = 4.0% w/w; water = 71.0% w/w; AA = 1.0%; W/O_2: fluid paraffin = 11.0% w/w; mineral oil and polyethylene = 10.0% w/w; W/O emulsifying mixture. = 7.0% w/w; ethanol = 7.1% w/w; silybin = 0.025 w/w; water = q.b. 100.0 w/w. Ethanol was added to emulsion W/O_2 to improve water solubility of silybin.

The composition of W/O/W multiple emulsion is reported in Table 3.

The pH conditions of the aqueous phases of the emulsions prepared have already been described in Section 2. Preservatives were not added to any emulsified systems to avoid possible interactions with AA or protective effects against its degradation.

In the W/O/W multiple emulsion, 0.25% w/w AA was the maximum achievable concentration without determining loss of droplet multiplicity. Indeed, Fig. 1 is a microphotograph of W/O/W emulsion (AA = 0.25% w/w): several small water droplets can be seen entrapped within the oil drops. The W/O/W emulsion was similar to that formulated in previous research (Carlotti et al., 1997), although some modifications were necessary to ensure stability in the presence of AA: glucose was added to avoid osmotic diffusion from the outer aqueous phase to the inner one containing AA. The presence of the multiple structure was confirmed by the different chromatic intensities noted when the hydrophilic dye BB was solubilised at the same concentration in O/W, W/O and W/O/W emulsion. Moreover, the pH value of W/O/W emulsions was 6.8, quite

Table 4
Percentages of non-degraded AA in 1.0% w/w aqueous solutions after storage at 45.0 ± 0.1°C[a]

pH	1 h	2 h	4 h	6 h	24 h
3.0	100.0 (0.2)	98.9 (0.2)	97.6 (0.1)	94.9 (0.1)	85.6 (0.2)
4.0	98.7 (0.2)	96.6 (0.1)	92.6 (0.1)	88.7 (0.3)	80.5 (0.3)
5.0	94.0 (0.1)	93.5 (0.4)	89.6 (0.2)	84.3 (0.2)	80.5 (0.2)
7.0	93.4 (0.2)	91.6 (0.3)	86.4 (0.1)	83.2 (0.2)	76.0 (0.3)

[a] Standard deviations in brackets; $n = 4$. $P < 0.00005$.

similar to the pH of the outer phase (7.0), indicating that no, or at most minimal, diffusion of AA from the inner to the outer aqueous phase had occurred. In fact, the pH of the mixture of both aqueous phases, not containing oils, was 3.5. Also the conductivity value of W/O/W emulsion (1.5 μS), much lower than that of the mixture of both phases (500 μS), could be due to the lack of AA in the outer aqueous phase, as its dissociation could cause an increase in conductivity. The transfer of AA to the outer aqueous phase could take place via total breakdown of the multiple droplets or via diffusion of the non-ionised and part of the ionised drug through the oil layer (Davis, 1981). Several factors can influence the release patterns, such as osmotic gradient, the nature and concentration of the emulsifier, and the rigidity of the interface (Magdassi and Garti, 1986). In the multiple emulsion under study the osmotic balance was guaranteed by the presence of glucose in the outer aqueous phase; moreover, the mineral oil and polyethylene present as components of the oil phase could contribute to give a certain rigidity to the interphase structure.

The aerobic degradation of AA was studied to assess whether the aqueous location of AA in different emulsified systems might influence its stability toward oxidation. A temperature of 45.0°C was chosen to accelerate the oxidation rate: higher temperatures could modify the microstructure of emulsion systems.

The stability of 1.0% w/w AA determined in aqueous solutions in the experimental conditions described decreased with increasing pH (Table 4), in agreement with data reported in the literature (Tsao and Young, 1996).

The molar concentration of AA in aqueous solutions was quite a bit higher than the oxygen concentration in the headspace air and in the water (Section 2); however, the high rate of oxygen water solubilisation allowed us to consider its water concentration as constant, almost as long as the oxygen headspace concentration remained sufficiently high.

Plotting $\ln([AA_t]/[AA_0])$ versus time, pseudo-first order plots were obtained for storage times

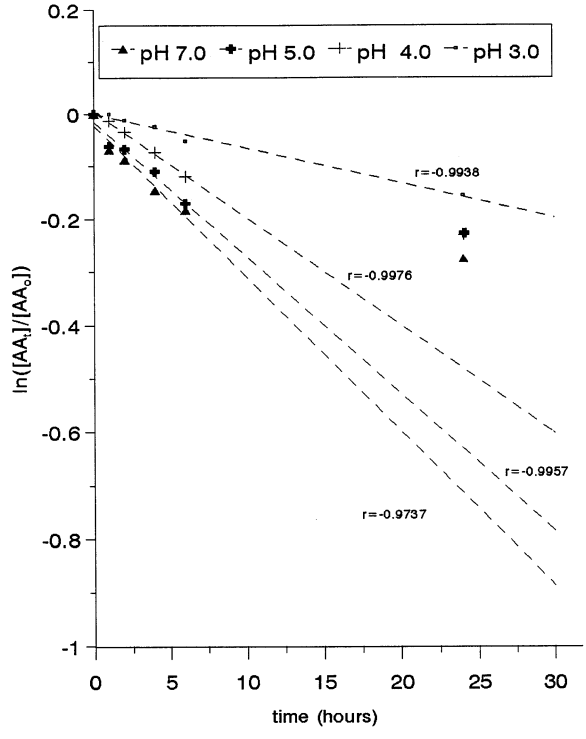

Fig. 2. Pseudo first-order plot of AA degradation at 45 ± 0.1°C in aqueous solutions at different pH values (AA = 1.00% w/w).

Table 5
Oxygen concentrations (mol l^{-1}) in the headspace air over aqueous solutions of AA at different pH values at different storage times

Time	Sol. pH 3.0	Sol. PH 4.0	Sol. pH 5.0	Sol. pH 7.0
6 h	4.20×10^{-3}	3.62×10^{-3}	3.20×10^{-3}	3.10×10^{-3}
24 h	3.87×10^{-3}	2.86×10^{-3}	2.86×10^{-3}	2.43×10^{-3}

up to 6 h for all the solutions studied, and up to 24 h for the solution at pH 3.0 (Fig. 2). It was clear that when dissolved oxygen was present in sufficient quantities, the reaction could be considered to follow a first-order mechanism with regard to AA; on the other hand, with a limited presence of oxygen, (i.e. after 24 h at pH 4.0, 5.0, 7.0) the data did not fit a straight-line plot. Pseudo-first order constants k'_1, calculated from Eq. (4) were: pH $3.0 = 0.66 \times 10^{-2}$ h^{-1}; pH $4.0 = 2.02 \times 10^{-2}$ h^{-1}; pH $5.0 = 2.56 \times 10^{-2}$ h^{-1}; pH $7.0 = 2.88 \times 10^{-2}$ h^{-1}. These results appeared to be emphasised by the fact that the oxygen concentration (partial pressure) in the headspace remained sufficiently high to provide an oxygen reservoir to maintain the dissolved oxygen at saturation for up to 6 h at pH 4.0, 5.0, 7.0 and for up to 24 h at pH 3.0. Table 5 reports the oxygen headspace concentration at 6 and 24 h, calculated assuming that one mole of AA consumed one mole of oxygen (Buettner and Jurkiewicz, 1996).

The same trend of AA degradation versus pH was observed in emulsified systems (Table 6). Indeed, in microemulsion no. 4, as well as in the O/W emulsion, AA degradation proceeded somewhat faster at pH 7.0 than at pH 5.0 ($P < 0.03$). Both O/W emulsion and microemulsion no. 4 provided a certain protective effect against AA degradation in the experimental conditions compared with aqueous solutions at the same pH ($P < 0.0005$). Some difference was seen between the emulsified and microemulsified systems both at pH 5.0 ($P < 0.01$) and at pH 7.0 ($P < 0.05$). The protective effect of emulsions and microemulsions was particularly pronounced since oxygen solubility is generally considered to be higher in emulsified systems than in water. A significant protection of AA against degradation was also noted in both W/O_1 and W/O_2 emulsions with respect to the aqueous solution at pH 5.0 ($P < 0.0005$). Increased protection with respect to O/W emulsion at pH 5.0 was noted in W/O_1 ($P < 0.05$) and in W/O_2 ($P < 0.05$) emulsions. The presence of the continuous oil phase, in which AA is insoluble, probably affords a further protection, despite the fact that oxygen solubility in oils is generally considered to be roughly one-order of magnitude higher than in water; indeed, the W/O interface could act as a physical barrier to oxygen diffusion into the inner aqueous phase. Silybin, which is a naturally-occurring flavolignan (*Sylibum marianum*) was added to W/O_2 emulsion, as it is known to be a powerful natural antioxidant and could therefore probably inhibit AA degradation. The protective effect of sylibin prolonged the induction time before AA degrada-

Table 6
Percentages of non-degraded AA in emulsified systems after storage at 45 ± 0.1°C[a]

	1 h	2 h	4 h	6 h	24 h
O/W pH 5.0	100.0 (0.2)	97.8 (0.2)	96.0 (0.1)	93.2 (0.1)	87.5 (0.2)
O/W pH 7.0	98.4 (0.3)	98.0 (0.1)	94.6 (0.1)	90.9 (0.1)	85.4 (0.3)
O/W microemulsion pH 5.0	95.4 (0.1)	95.2 (0.2)	93.7 (0.3)	89.6 (0.1)	82.2 (0.3)
O/W microemulsion pH 7.0	96.4 (0.2)	94.8 (0.1)	92.3 (0.1)	91.1 (0.3)	84.2 (0.3)
W/O_1	100 (0.4)	98.7 (0.2)	97.7 (0.1)	96.3 (0.1)	89.6 (0.1)
W/O_2	100 (0.2)	100 (0.3)	99.6 (0.2)	99.6 (0.2)	91.0 (0.2)

[a] AA = 1.00% w/w. Standard deviations in brackets; $n = 4$.

tion took place (almost 4 h in W/O_2 compared to 1 h in W/O_1).

Plotting $\ln([AA_t]/[AA_0])$ versus time, pseudo-first order plots were obtained for storage times up to 6 h for O/W emulsions and microemulsions and up to 24 h for both W/O emulsions. In Fig. 3, pseudo first-order plots are reported for the systems at pH 5.0. Pseudo first-order constants k'_1 calculated from Eq. (4) were: O/W emulsion pH $5.0 = 1.33\ h^{-1}$; O/W emulsion pH $7.0 = 1.58 \times 10^{-2}\ h^{-1}$; microemulsion pH $5.0 = 1.55 \times 10^{-2}\ h^{-1}$; microemulsion pH $7.0 = 1.58 \times 10^{-2}\ h^{-1}$; W/O_1 emulsion $= 0.55 \times 10^{-2}\ h^{-1}$; W/O_2 emulsion $= 0.55 \times 10^{-2}\ h^{-1}$.

W/O/W emulsion was prepared to vehicle AA in the inner aqueous phase at pH 3.0 to guarantee its stability, in the hope that the W/O and the O/W interphases would act as a fairly good barrier to the diffusion of AA towards the outer aqueous phase at pH 7.0. The experimental results met our expectations, as no degradation of AA took place in 6 h at 45.0 ± 0.1°C. Fig. 4 compares

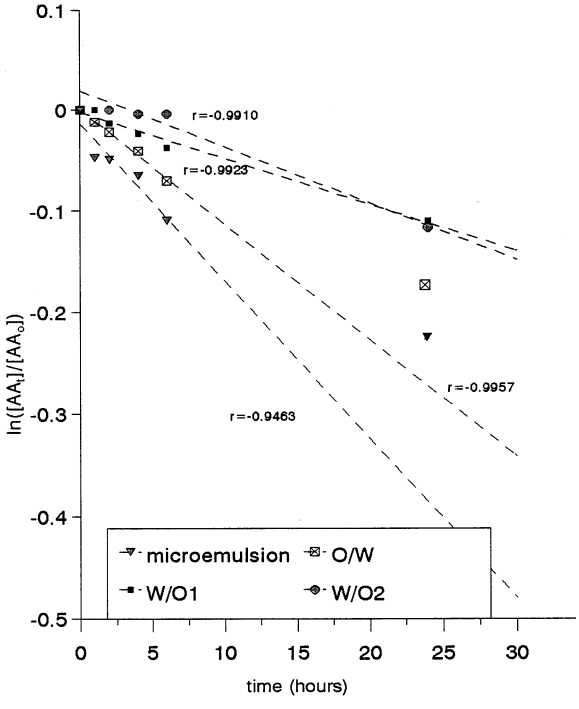

Fig. 3. Pseudo first-order plot of AA degradation at 45 ± 0.1°C in emulsified systems at pH 5.0 (AA = 1.00% w/w).

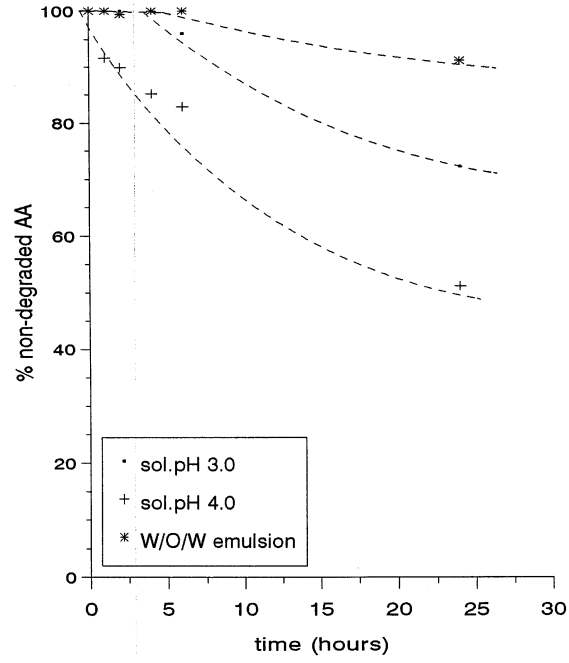

Fig. 4. Degradation of AA at 45 ± 0.1°C in aqueous solutions at different pH values and in W/O/W multiple emulsion (AA = 0.25% w/w).

the degradation trend of 0.25% w/w AA in W/O/W emulsion with those in aqueous solutions at pH 3.0 (pH of the inner aqueous phase of the multiple emulsion) and at pH 4.0 (pH obtained by roughly mixing inner and outer phase of the multiple emulsion). After 24 h non-degraded AA was 91.0% w/w (S.D. = 0.3) in W/O/W emulsions, 72.2% w/w (S.D. = 0.2) in aqueous solution at pH 3.0 and 51.1% w/w (S.D. = 0.4) in that at pH 4.0. Plotting $\ln([AA_t]/[AA_0])$ versus time, pseudo-first order plots were obtained for storage times up to 24 h for all the systems in the study. Pseudo first-order constants k'_1 calculated from Eq. (4)were: W/O/W emulsion $= 0.46 \times 10^{-2}\ h^{-1}$ ($r = -0.9959$); solution at pH $3.0 = 1.61 \times 10^{-2}\ h^{-1}$ ($r = -0.9997$); solution at pH $4.0 = 2.64 \times 10^{-2}\ h^{-1}$ ($r = -0.9995$).

The results obtained in aqueous and emulsified systems containing 0.10% w/w AA, tested for long-term stability, confirmed the enhanced stability of AA in the W/O/W multiple emulsion under study: after 20-day storage at 45.0 ± 0.1°C no AA could be detected in any of the systems examined

except in the multiple emulsion, which still contained 20.0% (S.D. = 0.1) of the initial AA. After 20-day storage at 20.0 ± 0.1°C, no AA could be detected in aqueous solutions at pH 4.0 and 5.0. The aqueous solution at pH 3.0 still contained 17.0% w/w AA (S.D. = 0.2), O/W emulsion 21.3% w/w (S.D. = 0.2), W/O_1 emulsion 27.4% w/w (S.D. = 0.1), and W/O/W emulsion 96.7% w/w (S.D. = 0.2). The low concentration of AA was chosen to be comparable with the saturation concentration of oxygen in water at 45 and 20°C, so as to promote oxidative degradation.

In contrast, no AA degradation was observed in the aqueous solution stored at 45.0 ± 0.1°C in anaerobic conditions. Some researchers (Nagy, 1980) report on AA anaerobic degradation process in fruit juices taking place after oxygen has been consumed, probably due to the presence of enzymatic systems in natural juice.

The results of the present study underline the possibility of increasing the stability of AA as a function of the kind of emulsified system in which it is vehicled. The W/O/W multiple emulsion prepared appears to be an interesting topical vehicle for AA, as it provides better stability of the vitamin over time than that given by W/O emulsions. Moreover, W/O/W multiple emulsions offer further advantages, such as low viscosity and non-oiliness, typical of the aqueous external phase, which make them easier to handle and use.

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References

Buettner, G.R., Jurkiewicz, B.A., 1996. Chemistry and biochemistry of ascorbic acid. In: Cadenas, E., Paker, L. (Eds.), Handbook of Antioxidants. Marcel Dekker, New York, pp. 91–115.

Carlotti, M.E., Gallarate, M., Morel, S., Pattarino, F., 1997. Preparazione di emulsioni multiple cosmetiche contenenti sostanze funzionali. Acta Techn. Legis Medicamenti VII, 169–190.

Florence, A.T., Whitehill, D., 1982. The formulation and stability of multiple emulsions. Int. J. Pharm. 11, 277–308.

Davis, S.S., 1981. Liquid membranes and multiple emulsions. Chem. Ind. 19, 670–683.

Eison-Perchonok, M.H., Downes, T.W., 1982. Kinetics of ascorbic acid autoxidation as a function of dissolved oxygen concentration and temperature. J. Food Sci. 47, 765–773.

Frei, B., Stocker, R., England, L., Ames, B.N., 1990. Ascorbate: the most effective antioxidant in human blood plasma. In: Emerit, I., Paker, L. (Eds.), Antioxidants in Therapy and Preventive Medicine. New York, Plenum.

Howard, R.R., Peterson, T., Kasil, P.R., 1988. High performance liquid chromatographic determination of ascorbic acid in human tears. J. Chromatogr. 41, 434–439.

Joslyn, M.A., Miller, J., 1949. Effect of sugars on oxidation of ascorbic acid. 1. Kinetics of autoxidation of ascorbic acid. Food Res. 14, 325–330.

Magdassi, S., Garti, N., 1986. A kinetic model for release of electrolytes from W/O/W multiple emulsions. J. Controlled Release 3, 273–277.

Miyake, N., Miok, K., Kurata, T., 1997. Formation mechanism of mono dehydro-L-ascorbic acid and superoxide anion in the autoxidation of L-ascorbic acid. Biosci. Biotech. Biochem. 61, 1693–1695.

Nagy, S., 1980. Vitamin C contents of citrus fruit and their products: a review. J. Agric. Food Chem. 28, 8–18.

Niki, E., 1991. Vitamin C as an antioxidant. World Rev. Nutr. Diet 64, 1–30.

Raynal, S., Grossiord, J.L., Seiller, M., Clausse, D., 1993. A topical W/O/W multiple emulsion containing several active substances: formulation, characterisation and study of release. J. Controlled Release 26, 129–140.

Robertson, G.L., Samaniego, C.M.L., 1986. Effect of initial dissolved oxygen levels on the degradation of ascorbic acid and the browning of lemon juice during storage. J. Food Sci. 51, 184–192.

Roig, M.G., Rivera, Z.S., Kennedy, J.F., 1995. A model study on rate of degradation of L-ascorbic acid during processing using home-produced juice concentrates. Int. J. Food Sci. Nutr. 46, 107–115.

Rousseau-Richard, C., Richard, C., Martin, R., 1991. Effets de synergie de la vitamine C et d'acides aminès sur les propriètès antioxydantes de la vitamine E. New J. Chem. 15, 283–291.

Singh, R.P., Heldman, D.R., Kirle, J.R., 1976. Kinetics of quality degradation: ascorbic acid oxidation in infant formula during storage. J. Food Sci. 41, 304–308.

Terrisse, I., Seiller, M., Rabaron, A., Grossiord, J.L., 1993. Rheology: how to characterise and to predict the evolution of W/O/W multiple emulsions. Int. J. Cosmet. Sci. 15, 53–62.

Trotta, M., Gasco, M.R., Morel, S., 1991. Behaviour of oil/water microemulsions upon dilution with water. J. Dispersion Sci. Tech. 12, 239–255.

Tsao, C.S., Young, M., 1996. A stabilised ascorbic acid solution. Med. Sci. Res. 24, 473–475.