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# STABILIZATION OF VITAMIN C IN EMULSIONS OF LIQUID CRYSTALLINE STRUCTURES

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## Highlights:

An emulsion of  $\alpha$ -gel liquid crystal stabilizes vitamin C for several months

## ABSTRACT

Emulsified systems are widely used for topical delivery with the aim of optimizing cutaneous absorption and offering a pleasant sensory. They also may provide a protection of the active molecule against oxidation and/or degradation. The oil phase of o/w emulsions may consist of liquid crystalline structures, especially lamellar structures which are similar to those found in the *stratum corneum* lipids. In the present work, o/w emulsions containing liquid crystals of mixed cetyl alcohol and Polysorbate 60 were developed for topical delivery of vitamin C, a potent antioxidant with several applications in the cosmetic and pharmaceutical fields. In addition to the well-documented lipid supplementation of the *stratum corneum*, the liquid crystal emulsions provide a significant chemical stabilization of vitamin C against its degradation. Emulsions were characterized by X-ray diffraction, polarized optical

microscopy, and transmission electron microscopy. The stability of vitamin C in the formulations was evaluated upon storage in different conditions of temperature. The emulsions contain a complex colloidal structure, consisting of lamellar liquid crystalline ( $L\alpha$ ) and crystalline lamellar gel ( $L\beta$ ) phases, that provide a very efficient protection of vitamin C against its degradation.

**Key words:** vitamin C; liquid crystal; emulsions; chemical stability.

## 1. INTRODUCTION

Emulsions are complex multiphase formulations used for optimizing various end-use properties of cosmetic and dermatological compounds such as stability of active substances, bioavailability, sensory properties [Santos and Rocha-Filho, 2007]. The basic formulation of a classical o/w emulsion includes at least water as continuous phase, oil as dispersed phase, and emulsifiers that ensure the stability of the dispersion of oil droplets.

Some amphiphilic components of the emulsions may self-associate as lyotropic liquid crystals (LC) in the presence of water [Otto *et al.*, 2009]. These structures have characteristics and intermediate properties between solids and liquids [Tyle, 1989]. A large variety of LC structures can be encountered; the three most typical are lamellar, cubic or hexagonal phases. In addition, the hydrophobic chain(s) of the surfactant in a lamellar phase may be in molten ( $L\alpha$ ) or crystalline state ( $L\beta$ ,  $P\beta$ ). The type of supramolecular structure is related with the geometry of the amphiphilic molecule and its critical packing parameter [Iwai *et al.*, 1998; Suzuki, 2017; Otto *et al.*, 2009; Terescenco *et al.*, 2018a].

The use of emulsions containing lamellar LC seems advantageous because this supramolecular organization is similar to that of *stratum corneum* (SC) lipids. This generates a higher affinity of this system for the epidermis [Iwai *et al.*, 1998; Souza *et al.*, 2017], and absorption of the components of lamellar phase into SC restores the barrier function of SC by supplementing a possible deficiency of lipids. Besides improving water retention by the SC barrier, LC emulsions may behave as suitable solubilization medium for hydrophilic substances like Vitamin C (Vit C), thereby providing prolonged release and protection against photochemical, oxidative and thermal degradations [Santos *et al.*, 2006; Masson *et al.*, 2005; Oliveira *et al.*, 2013].

Lamellar LC may be used either as stabilizers of the o/w emulsion, or as the dispersed phase in place of the oil phase. It is presently used as the dispersed phase for Vit C, allowing its dissolution and stabilization. A specific structure known as  $\alpha$ -gel is of particular interest in cosmetic skin care because of its soft touch [Okamoto *et al.*, 2016; Datta *et al.*, 2020]. Its basic ingredients are a fatty alcohol, namely cetyl and stearyl alcohols and their mixtures, and a

surfactant that self-assembles as bilayers in association with the fatty alcohols. The surfactant bilayers form lamellar phase and spherulites, and part of the fatty alcohol crystallizes as its rotator phase (also called  $P\beta$  or  $\alpha$ -gel). Crystals of fatty alcohol act as a thickening agent of the dispersed phase [Jung *et al.*, 2016; Suzuki, 2017]. The structure is quite complex and not fully understood so far. It is a current challenge to understand the interaction between the ingredients in the system that drive the end-use properties [Terescenco *et al.*, 2018b].

Vit C or ascorbic acid is widely used for its various biological activities in cosmetic and dermatologic products. Vit C is an anti-aging molecule [Colven and Pinnell, 1996; Gu *et al.*, 2016; Caritá *et al.*, 2020] and a potent antioxidant, able to neutralize oxidative stress triggered by several factors, such as exposure to UV radiation [Telang, 2013; Al-Niaimi and Chiang, 2017]. Another activity of Vit C is on collagen biosynthesis [Zhou *et al.*, 2014]. Vit C is an enzymatic cofactor for lysyl and prolyl hydroxylases, key enzymes for the stabilization and cross-linking of collagen fibers. In addition, Vit C directly activates collagen synthesis by enhancing the transcription of its mRNA [Nusgens *et al.*, 2001]. Vit C is also a skin-whitening ingredient, able to interfere with the tyrosinase active site, a fundamental enzyme in melanin production [Pullar *et al.*, 2017]. Laboratory and clinical studies showed the successful application of this molecule in the treatment of melasma and hyperpigmentation [Hwang *et al.*, 2009; Stamford, 2012].

However, Vit C must be kept stable in its free form to keep its biological activity and play a relevant role in preventing aging process [Humbert *et al.*, 2003; Farris, 2005; Gu *et al.*, 2016]. In this sense, release systems with LC structures are interesting because they can promote protection against oxidation, delay Vit C degradation and control the drug release [Santos *et al.*, 2006; Chorilli *et al.*, 2011]. LC containing emulsions were already investigated for the skin delivery of vitamins derivatives, such as retinyl palmitate and 3-O-ethyl-ascorbic acid, and showed promising results. It was reported that the presence of LC in the formulation enhanced skin retention, drug activity and rheological properties [Chorilli *et al.*, 2011; Li *et al.*, 2016].

The aim of the present study was the development of emulsions with liquid crystalline structures for topical delivery of Vit C. To that end, emulsions

composed of cetyl alcohol and Polysorbate 60 were investigated. The presence of colloidal structures, consisting of liquid crystalline and  $\alpha$ -gel phases, was confirmed by polarized microscopy, transmission electron microscopy and X-ray scattering techniques. The stability of Vit C in the formulations was assessed upon storage in different conditions.

## **2. MATERIALS AND METHODS**

### **2.1 Materials**

Cetyl alcohol, L-ascorbic acid, ethylenediaminetetraacetic acid disodium salt (EDTA) and sodium metabisulfite were purchased from Alfa Aesar (Haverhill, MA). Propylene glycol was purchased from Sigma-Aldrich (Saint Louis, MO). Polysorbate 60 was supplied by Croda (Campinas – SP, Brazil). Phenochem™ (methylparaben (and) ethylparaben (and) propylparaben (and) butylparaben (and) isobutylparaben (and) phenoxyethanol) were purchased from Sharon Laboratories (Ashdod, Israel). All other chemicals were of the reagent grade. Deionized water of 18 M $\Omega$ ·cm resistivity was used throughout the whole work.

### **2.2 Methods**

#### **2.2.1 Preparation of samples**

Mixtures of cetyl alcohol and Polysorbate 60 were prepared in different ratios, ranging from 10 % to 90 % (w/w) by heating them together at 70 °C under magnetic stirring. Emulsions were prepared by heating the oily phase (9 % of cetyl alcohol and 6 % of Polysorbate 60) and the water phase separately at 75  $\pm$  5 °C. The water phase was introduced into the oily phase with agitation speed of 10,000 rpm using a rotor-stator device Ultra-Turrax T25 equipped with a S25N-8G shaft (IKA, Freiburg, Germany) until cooling to below 40 °C. Vit C (5 % w/w) was dissolved separately in water and added into the system when the temperature reached 40 °C. For stability studies, two formulations (F1 and F2) presented in Table 1 were developed. The pH of both formulations was 3.5.

Table 1: Composition of the formulated systems.

Component	Ratio (% w/w)	
	F1 with sodium metabisulfite	F2 Antioxidant free
Cetyl alcohol	9	9
Polysorbate 60	6	6
Vitamin C	5	5
Propylene glycol	5	5
Phenoxyethanol + parabens	0.5	0.5
Sodium metabisulfite	0.3	–
EDTA	0.1	0.1
De-ionized water	q.s. 100	q.s. 100

### 2.2.2 Characterization of the emulsions

*Wide angle X-ray scattering.* WAXS diffraction patterns of samples were obtained using a Siemens D5000 diffractometer with copper tube at the Centre de Diffractométrie Henry Longchambon facility (<http://cdalpha.univ-lyon1.fr/>) of University of Lyon, France. The powder scattering patterns were collected for  $\theta$  values from 1 ° to 50 °.

*Small angle X-ray scattering.* SAXS measurements were performed on the SAXS beamline of the Brazilian Synchrotron Light Laboratory. Emulsions were placed in stainless steel sample holders and thermostated at 25 °C, 37 °C and 60 °C with accuracy of  $\pm 0.5$  °C. A linear detector was used at 43.5 cm and the wavelength of incident beam was 1.605 Å. SAXS data were corrected for sample attenuation. The repeat distances of lamellar structures were calculated using Bragg relationship:  $d = 2\pi/(nq)$ , with  $q = (4\pi/\lambda) \sin(\theta/2)$  the wave vector of maximum intensity of the Bragg reflection and  $n = 1, 2, 3, \dots$ , the diffraction order.

*Optical microscopy.* Emulsion droplet size measurements were obtained using a Leica™ DMLM (Germany) optical microscope equipped with a video camera. The sample holder was made of a classical glass plate and cover slip. The size

distribution of droplets was analyzed by image analysis using the software AnalySIS™. The presence of anisotropic phases in the samples was checked by polarized microscopy with the sample placed between crossed polarizer and analyzer.

*Transmission electron microscopy.* TEM images of emulsions were obtained at the Centre Technologique des Microstructures facility (<http://microscopies.univ-lyon1.fr/>) of the University of Lyon using a Philips CM 120 TEM instrument working at 80 kV acceleration. The samples were placed on formvar/carbon-coated copper TEM grids and dried under open air before their observations.

*Quantification of Vit C by High Performance Liquid Chromatography (HPLC) analyses.* Injections of 20  $\mu\text{L}$  of sample were performed under elution at 1  $\text{mL}\cdot\text{min}^{-1}$  flow rate of mobile phase through a column XTerra MS C18 Cartridge 4.6x250 mm 5  $\mu\text{m}$  from Waters (St Quentin en Yvelines – France). Experiments were performed at room temperature ( $\sim 22\text{ }^{\circ}\text{C}$ ), using a HPLC set up from Waters, composed of a Waters 717 injector, a Waters 600 pump and a Waters 2996 photodiode 176 array UV detector ( $\lambda = 254\text{ nm}$ ). An isocratic mobile phase consisting of water and 0.1 % of trifluoroacetic acid (TFA) was used [Maia *et al.*, 2007; Bansal *et al.*, 2015]. The calibration curve for the quantification of Vit C was linear over the range of concentrations used, ranging from 5  $\mu\text{g}\cdot\text{mL}^{-1}$  to 200  $\mu\text{g}\cdot\text{mL}^{-1}$  ( $R^2 = 0.999$ ). All analytical data have been validated.

*Stability assay.* To determine the stability of Vit C in the emulsions, two formulations were developed, as showed in Table 1: (F1) formulation with sodium metabisulfite, an antioxidant molecule frequently used to stabilize Vit C [Maia *et al.*, 2006] and (F2) free-antioxidant formulation, to evaluate the capacity of the system to protect Vit C against degradation. An aqueous solution of Vit C was used as a control group. Samples were stored in the dark at 25  $^{\circ}\text{C}$  and 45  $^{\circ}\text{C}$ . For conducting the experiments, an aliquot of the sample was weighed, diluted with water until the desired concentration was reached for HPLC analysis and filtered on 0.45  $\mu\text{m}$  syringe filters. HPLC analyzes were performed in triplicate at regular time intervals during four months.



### 3. RESULTS AND DISCUSSION

The present study began with the screening of emulsions with cetyl alcohol and Polysorbate 60 in different proportions. The cetyl alcohol concentration ranged from 6 % to 13.5 % (w/w) and the Polysorbate 60 from 1 % to 6 % (w/w). All the formulations were stored at room temperature for 6 months. The presence of liquid crystalline structures was verified by polarized light microscopy. Formulations with less than 4 % of Polysorbate 60 presented instability manifested by macroscopic phase separation. The presence of LC was predominant in emulsions containing 9 % of cetyl alcohol, 6 % of Polysorbate 60 and 85 % of water. Therefore, this formulation was chosen for the incorporation of Vit C. Next, we present the characterization of this emulsion by optical and polarized microscopy, TEM, SAXS and WAXS. Finally, the stability of Vit C is assessed upon storage in different conditions of temperature and is compared to that in an aqueous solution.

#### 3.1 Characterization of the emulsions

In this section, characterizations of emulsions composed of 9 % cetyl alcohol, 6 % Polysorbate 60, 5 % Vit C and water (q.s. 100) are presented.

##### 3.1.1 Microscopy analysis

Optical microscopy of o/w emulsions of cetyl alcohol and Polysorbate 60 revealed a collection of spherical droplets (Figure 1A). Statistical analysis of 100 droplets using the Image J software yielded a mean diameter of  $2.2 \pm 0.1 \mu\text{m}$  and quite a narrow width of the diameter distribution with a standard deviation of  $0.9 \mu\text{m}$ .

Polarized optical microscopy allows the detection of liquid crystals in the formulations [Savic *et al.*, 2005; Oliveira *et al.*, 2013]. Birefringent anisotropic materials are detected under polarized light. The types of liquid crystal are assigned through observation of their structural defects. Those of lamellar phases display oily streaks and Maltese crosses in polarized microscopy [Chorilli *et al.*, 2011]. Many Maltese crosses were present in the pictures of emulsions, and oily streaks were not observed (Figure 1B). Maltese crosses correspond to spherulites made of closed droplets containing stacks of bilayers

similar to multilamellar liposomes. The high density of Maltese crosses may have obscured oily streaks. Indeed, the presence of such defects characteristic of disinclinations of bulk lamellar phase was expected because lamellar stacks have been observed by TEM (Figure 2). The presence of a bulk phase of  $\alpha$ -gel that acts as a binder for spherulites is expected [Boltenhagen *et al.*, 1991]. Otherwise, spherulites would peptize as a dispersion of multilamellar liposomes.

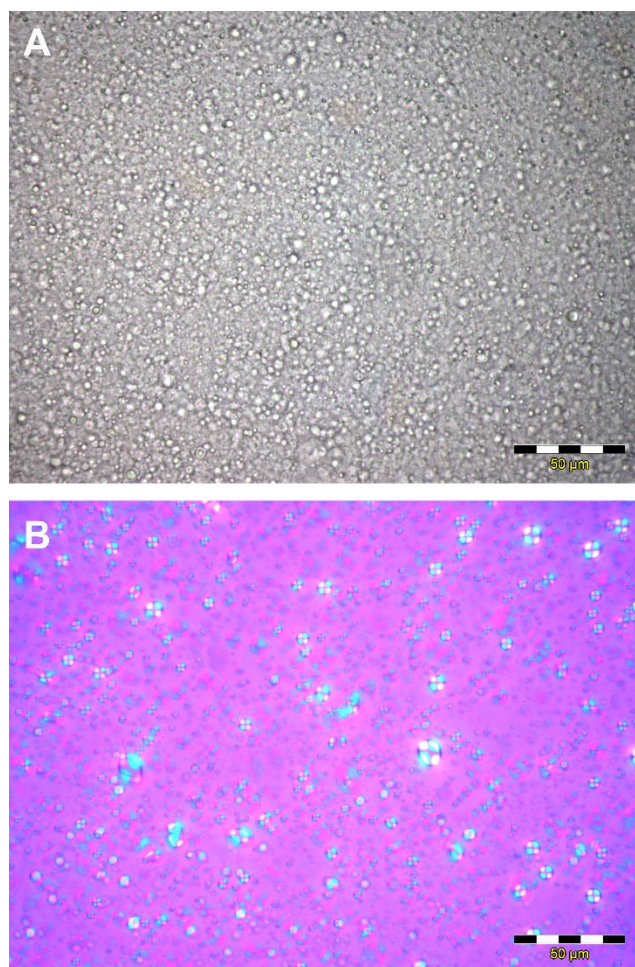


Figure 1: Optical (A) and polarized microscopy pictures of emulsions (B).

Additional observations by TEM were performed [Mondain-Monval, 2005] to confirm the presence of the lamellar liquid crystals. According to Savic *et al.* [2011], some typical features of the lamellar liquid crystalline phase can be detected in samples, particularly of the ordered lamellar gel type. They included planar layers as widespread lamellar sheets with regular and sharp

edges. TEM pictures (Figure 2) present this kind of organization: multilayers of planar arrangement within the continuous phase.

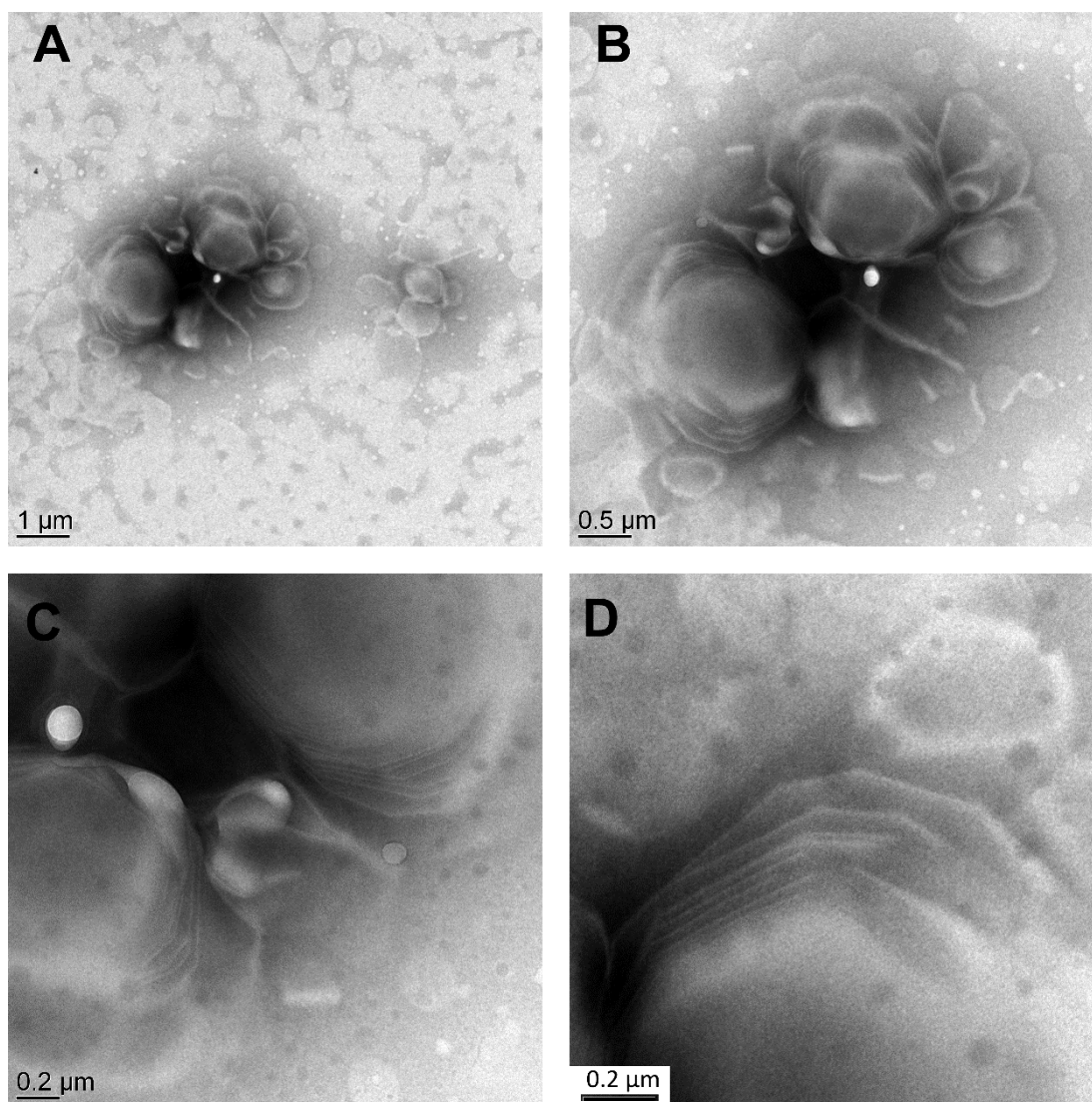


Figure 2: TEM micrographs showing lamellar sheets at increasing magnifications from A to D.

### 3.1.2 X-ray diffraction analysis (SAXS and WAXS)

SAXS and WAXS techniques were used to precisely determine the structure of liquid crystals. SAXS allows the detection of periodic structures with large repeat distances at  $\theta < 10^\circ$ . WAXS in turn, refers to scattering at  $\theta > 10^\circ$  and provides information over short distances for analyzing molecular arrangements [Dong and Boyd, 2011]. Examples of observed X-ray scattering patterns are shown in Figure 3 (SAXS) and Figure 4 (WAXS).

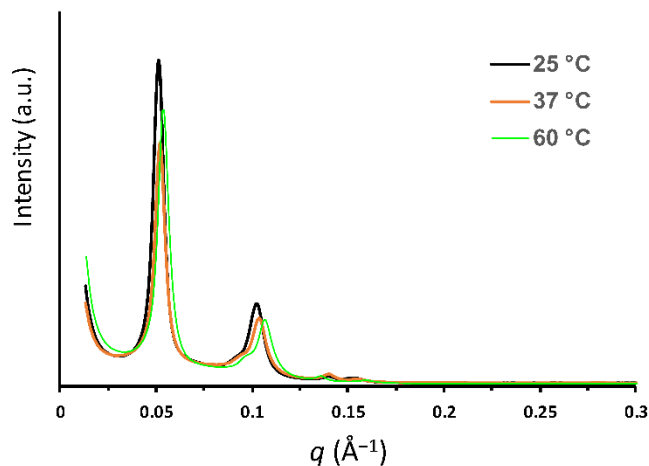


Figure 3: SAXS pattern of the Vit C emulsion at 25 °C, 37 °C and 60 °C.

The SAXS pattern (Figure 3) showed Bragg peaks at  $q_1 = 0.051 \text{ \AA}^{-1}$ ,  $q_2 = 0.103 \text{ \AA}^{-1}$  and  $q_3 = 0.154 \text{ \AA}^{-1}$  at 25 °C. This pattern is typical of lamellar phase with 1:2:3 order of the positions of first, second and third order diffraction peaks. An additional small feature is seen as an elbow around  $0.09 \text{ \AA}^{-1}$  and a small broad peak at  $0.14 \text{ \AA}^{-1}$ , which might indicate the presence of a minor contribution from another type of ordered structure. The lamellar repeat distance, which is clearly the preponderant structure at all temperatures, is  $123 \text{ \AA}$  at 25 °C and 37 °C and decreases to  $116 \text{ \AA}$  at 60 °C. This small decrease in the repeat distance most probably reflects a decrease in the bilayer thickness, compatible with the chain melting expected to happen around 50 °C, whereas the water layer may also change with temperature [Wunsch *et al.*, 2015; Bahadur *et al.*, 2019]. The fact that the lamellar structure is preserved in the whole temperature range explored is an important factor, since the emulsion is prepared at 75 °C, stored at room temperature and applied to the skin surface that has been shown to be around 32 °C [Shusterman *et al.*, 1997; Borowiec *et al.*, 2013]. The thermal stability of the lamellar phase is important to ensure the similarity with the *stratum corneum* lipids and promote the skin retention of droplets in various conditions [Souza *et al.*, 2017].

WAXS experiments (Figure 4) showed one single sharp peak at  $q = 1.52 \text{ \AA}^{-1}$  corresponding to a repeat distance of  $4.2 \text{ \AA}$ . Two broad peaks, around  $2.1 \text{ \AA}^{-1}$  and  $2.9 \text{ \AA}^{-1}$ , are related to the disordered matrix of the sample in the

fluid phase. The powder scattering pattern with a unique Bragg peak is different of that of the monoclinic crystalline structure of cetyl alcohol displaying many Bragg reflections [Métivaud *et al.*, 2005]. The presence of cetyl alcohol together with Polysorbate 60 and water in the emulsified system caused the formation of a lamellar phase with periodic structure in the direction parallel to the bilayers. It comes from crystallization of the fatty chains of mixed cetyl alcohol and Polysorbate 60. The position of the unique Bragg peak is typical the hexagonal packing of hydrophobic chains [Savic *et al.*, 2011; Oliveira *et al.*, 2013; Wunsch *et al.*, 2015; Terescenco *et al.*, 2018c]. This phase has been called  $\alpha$ -gel (or rotator phase, or  $L\beta$ ).

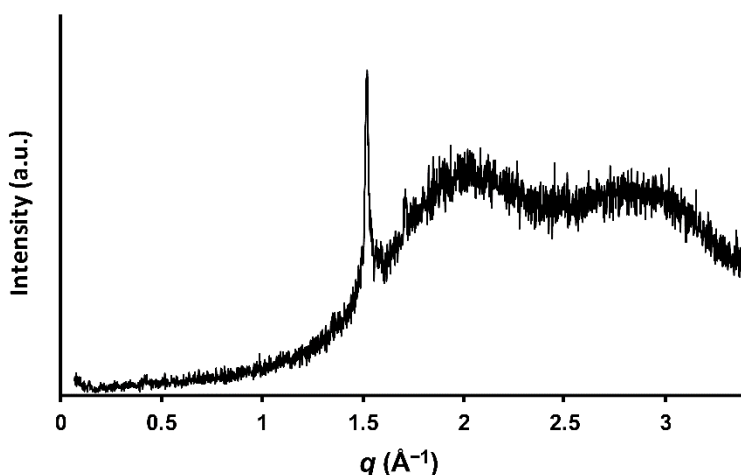


Figure 4: WAXS pattern of the Vit C emulsion at room temperature (22 °C).

The combined results of SAXS and WAXS reveal a complex structure of the emulsion with a coexistence of lamellar liquid crystalline ( $L\alpha$ ) and lamellar crystalline gel phase ( $L\beta$ ). In the  $L\alpha$  phase, the hydrocarbon chains are in a liquid-like state while in the  $L\beta$  phase the hydrocarbon chains are organized on a 2D crystalline array with their axes organized on a hexagonal lattice. The relative amounts of  $L\alpha$  and  $L\beta$  phases cannot be known from the present experiments.

In this specific organization related to the presence of cetyl alcohol in the emulsion, the long-chain alcohol molecules get hydrated as they mix with the solutions of hydrophilic surfactant at 25 °C and form of mixed bilayer structure. Stacks of bilayers are organized in a lamellar structure as observed by SAXS

analysis and the alkyl chains of mixed Polysorbate 60 and cetyl alcohol are laterally ordered on a 2D crystalline hexagonal array known as  $\alpha$ -gel that evidence is given by one single Bragg peak in WAXS measurements ( $q = 1.52 \text{ \AA}^{-1}$ ). Other authors also reported similar behavior in emulsions including fatty alcohols [Savic *et al.*, 2011; Wunsch *et al.*, 2015; Valoppi *et al.*, 2016; Terescenco *et al.*, 2018a,b]. Both  $L\alpha$  and  $L\beta$  phases entrap a significant amount of water inside their interlamellar spaces, which is interesting for Vit C encapsulation, due to its hydrophilic character.

### 3.2 Chemical Stability of Vit C

The major challenge for the utilization of Vit C is to maintain its chemical stability. Vit C is easily oxidized in aqueous media, in alkaline pH (higher stability is obtained at  $\text{pH} \leq 4$ ) and in the presence of light, oxygen and metal ions [Stamford, 2012, Comunian *et al.*, 2013, Sheraz *et al.*, 2014, Casanova and Santos, 2016]. This process is frequently accompanied by a color change of the formulations, which gradually become yellowish. Different strategies have been developed to limit these processes, between them: controlling the presence of oxygen and light exposition during formulation and storage, acidic pH and reduction of water content through the use of anhydrous/nonaqueous formulations [Ziming and Parr, 2006; Stamford, 2012; Parhizkar *et al.*, 2018, Caritá *et al.*, 2020]. The physicochemical properties of the formulation, such as the dielectric constant and viscosity can also impact the Vit C stability. In general, higher viscosity formulations and multiple emulsified systems offer greater protection against oxidation [Ahmad *et al.*, 2011, Khan *et al.*, 2016]. The addition of preservatives such as antioxidants and anti-chelating agents also prevents the degradation of Vit C. In this context, the sodium metabisulfite antioxidant has shown good results [Maia *et al.*, 2006; Sheraz *et al.*, 2011; 2014].

The concentration of the non-oxidized Vit C in the formulations has been monitored over storage time. Two groups of formulations were prepared for this purpose as showed in Table 1: (F1) is a formulation with sodium metabisulfite and (F2) is an antioxidant-free formulation. Samples were stored at 25 °C and 45 °C and analyzed by HPLC. A solution of Vit C in water, kept at 25 °C, was used as a control group. The stability of Vit C in F1, F2 and aqueous

solution are presented in Figure 5. All measurements were performed in triplicate.

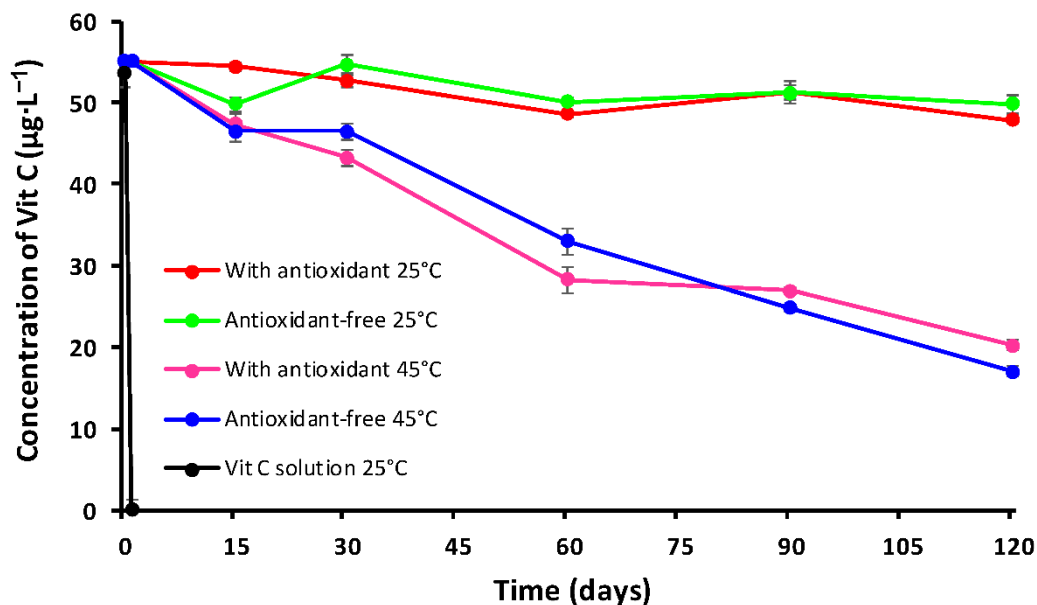


Figure 5: Chemical stability of Vit C in the formulations over storage time at 25 °C and 45 °C. The formulation with sodium metabisulfite (F1) and the antioxidant-free formulation (F2) are shown. An aqueous solution of Vit C was used as a control.

Both formulations were able to protect the Vit C against degradation at 25 °C. In the end of the assay, after 4 months, the Vit C concentration obtained from the emulsions samples F1 and F2 have decreased to 87 % and 91 % respectively. These results are remarkable owing to the instable character of Vit C [Maia *et al.*, 2006]. Indeed, the Vit C concentration in the aqueous solution showed a dramatic decline in less than 24 h.

At 45 °C, an important decrease in the Vit C content was observed in both formulations, confirming that the increase in temperature is a determinant factor on the degradation process of Vit C [Sheraz *et al.*, 2014]. At the end of the experiment (after 4 months), the Vit C content obtained from the samples F1 and F2 were 28 % and 33 % respectively.

Sodium metabisulfite is one of the best antioxidants for the protection of Vit C. However, even with the addition of this antioxidant, the degradation of Vit C may occur over time due to its high instability [Maia *et al.*, 2006; 2007]. It was noticed that the antioxidant-free formulation (F2) presented a stability



profile similar to the formulation with sodium metabisulfite (F1), which indicates that the LC is able to protect the Vit C with such high efficiency that the effect of an antioxidant is no more detected.

The literature reports several limitations and challenges in the utilization of Vit C due to its unstable character. For instance, Margolis and Park, 2001 published a technical account about the degradation of Vit C in autosampler vials during the course of several experiments. In another study, it has been reported that the peak area of a Vit C (in an aqueous solution pH 2.0, 0.1 % phosphoric acid) chromatogram decreases by 27 % in 50 min [Golubitskii *et al.*, 2007].

Concerning topical preparations, several publications report the development of drug delivery systems for Vit C; but the chemical stability of this molecule still remains a major concern [Rozman and Gasperin, 2007; Sheraz *et al.*, 2014; Duarah *et al.*, 2017; Maione-Silva *et al.*, 2019]. Derivatives of Vit C, such as sodium ascorbyl phosphate, magnesium ascorbyl phosphate, sodium ascorbate and ascorbyl-6-palmitate have been employed in cosmetic and pharmaceutical formulations in order to remedy this problem [Segall and Moyano, 2008; Serrano *et al.*, 2015]. However, despite being more stable, their permeation efficiencies and biological activities are still limited [Stamford, 2012; Starr *et al.*, 2019; Caritá *et al.*, 2020]. Results disclosed in patents also show limited stabilization effects of anhydrous formulations [Thomas, 2014].

Therefore, the stabilization of Vit C presented here appears very promising. The emulsion F2 was able to protect Vit C against oxidation at 25 °C at least for four months without the need for an antioxidant. This indicates that the organization of the system is playing a key role in the protection of Vit C, which might be related to the partition and diffusion of oxygen in fluid and crystalline bilayers. The contributions of the complex structure are difficult to figure out because several opposite effects are operating. Thus, experiments on hydrated phospholipid bilayers [Möller *et al.*, 2016] showed that the concentration of oxygen was almost three times larger in fluid phospholipid bilayers than in the interlamellar aqueous solution whereas the reverse trend was observed for crystalline bilayers below the chain melting temperature. Such large differences of oxygen partition coefficient did not have significant influences on the transport of oxygen by



passive diffusion. It is presumed from these literature data that Vit C entrapped in emulsion droplets was protected against the contact with oxygen because of the presence of the crystalline  $\alpha$ -gel bilayers within the structure.

#### 4. CONCLUSION

In the present study, emulsions composed of cetyl alcohol and Polysorbate 60 were developed for the encapsulation of Vitamin C. The presence of a complex colloidal structure, consisting of lamellar liquid crystalline ( $L_\alpha$ ) and lamellar gel crystalline ( $L_\beta$ ) phases, in the oil-water interface of emulsions was confirmed by SAXS, WAXS, TEM and polarized microscopy techniques.

Encapsulation inside droplets of  $\alpha$ -gel phase stabilizes Vitamin C for more than 4 months. Such high stabilization together with the known good properties of  $\alpha$ -gel phase in cosmetic products makes the present formulation quite promising. Dermatological applications or transdermal delivery may also benefit from such stabilization.

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