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Physicochemical study of aqueous dispersions of organogel particles: Role of the ingredients and formulation process leading to colloidal hydrogels

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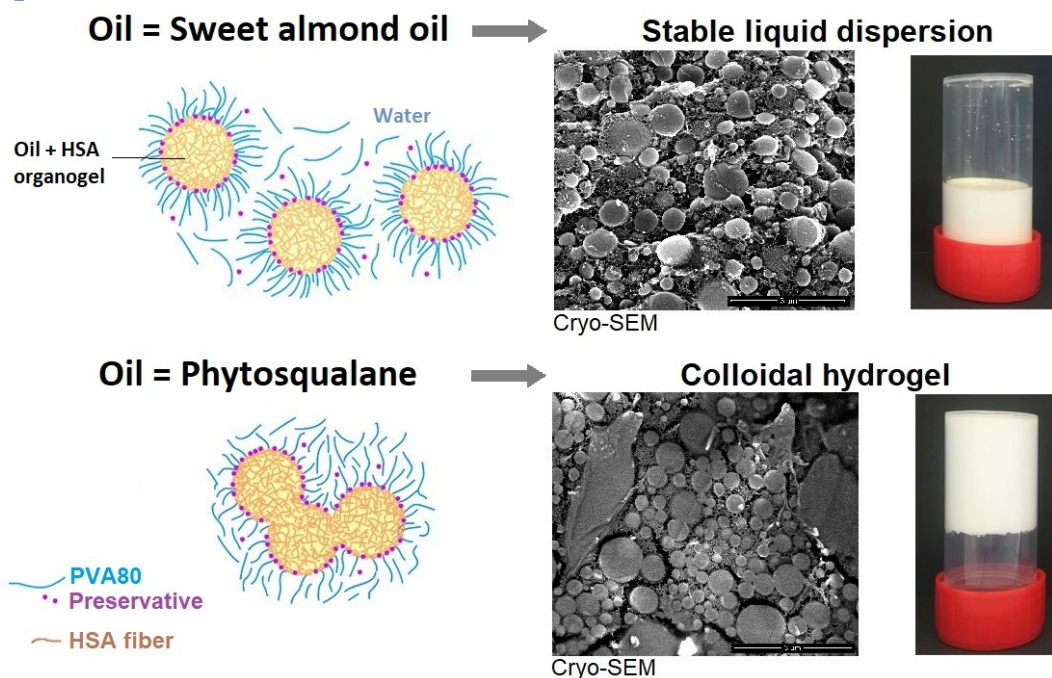
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Highlights

- Organogel particles of sweet almond oil produce a stable dispersion
- Dispersion of organogel particles of phytosqualane produces a colloidal hydrogel.
- Organogels wettability indicates oil-dependent surface organization.
- Adsorption of the preservative agent Saliguard® COS at the water/oil interface.
- Preservative leads to PVA80 desorption during squalane gelation and destabilization.

Graphical abstract



Abstract

The cosmetics industry is always looking for new textures, both from a performance and sensory point of view. In this field, emulsions remain an important source of inspiration. In this study, aqueous

dispersions of organogel particles were obtained by hot emulsification ($T^\circ > T_{\text{gel}}$), and cooling ($T^\circ < T_{\text{gel}}$). Two oils were tested, sweet almond oil and phytosqualane, using 12-hydroxystearic acid (HSA) as an organogelator, and polyvinyl alcohol (PVA80) as a stabilizer. A preservative agent (Saliguard® COS) and an antioxidant (Tocopherol) were also used. Under the same preparation conditions, two completely different textures were obtained depending on the oil: a stable dispersion of organogel particles for sweet almond oil and a colloidal hydrogel for phytosqualane. In this study, we were able to understand the impact of the ingredients on these differences in texture, and propose different mechanisms of formation. The important role played by the preservative agent at the water/oil interface of the droplets, during the gelation step, was demonstrated. Such adsorption, depending on the polarity of the oil, can modify the anchoring of the dispersing agent (PVA80) and lead during the gelation to a destabilization of the droplets by coalescence and to the formation of a colloidal hydrogel. These results highlight the possible formation of colloidal hydrogels from particles of organogels and open the way to new textures in the cosmetic or pharmaceutical field.

Keywords

Organogel; Colloidal hydrogel; 12-hydroxystearic acid; Polyvinyl alcohol; Phytosqualane; Preservative.

1. Introduction

Hydrolipidic formulations have always been part of our daily lives, and in many areas. There are many cosmetic, food or pharmaceutical formulations based on water and oil. There are emulsions [1,2], which are the basis for many formulations (creams and sauces), nanoemulsions [3,4], microemulsions [5,6], high internal phase emulsions (HIPEs) [7], or emulsion gels [8,9] in which the external phase of the emulsion is gelled. In this case, gelation improves stabilization of the emulsion by increasing the viscosity of the external phase, and at the same time provides better control over the final texture. A different approach is to gel the dispersed phase instead of the external one.

In this study, we investigated dispersions based on droplets of gelled oil (particles of organogel) in an aqueous phase.

Organogels are organic solvents or oils that have been gelled using an organic gelator [10–12]. Most of the organic gelators are low molecular weight compounds which, at low concentrations, promote the non-covalent self-assembly of generally fibrous structures to form a three-dimensional network in a solution [13,14]. Among the low molecular mass organic gelators, 12-hydroxystearic acid (HSA) has been largely investigated because it is a biocompatible commercial gelator able to gelify a large variety of organic solvents and oils [15–17]. The fiber-like organization of HSA in various solvents is well established and largely documented [18,19]. The preparation of the particles is based on the characteristic sol–gel phase transition of the organogel. Aqueous dispersion of these particles is obtained by hot emulsification ($T^\circ > T_{\text{gel}}$) in the presence of an emulsifying or stabilizing agent, and cooling ($T^\circ < T_{\text{gel}}$), leading to nanoparticles in water as stable dispersions [20,21], which under some conditions can produce colloidal hydrogels.

Colloidal gels are an important class of soft materials that are found throughout nature and are used in many industrial applications. They typically consist in a suspension of colloidal particles that undergoes dynamical arrest through the formation of an interconnected fractal-like network [22–24]. Colloidal gelation occurs in a system with short range attractive interactions, at high interaction strength for a wide range of density. Upon increasing the attraction, the system falls out of equilibrium, forming a network of particles with voids, which spans through the system [25–27].

Yogurt and many dairy products are colloidal gels, where the particles are large proteins or protein aggregates [28,29].

Aqueous dispersions of gelled oil particles already have some applications in the pharmaceutical field [20,21], but are still very seldom employed. Here, we aim at developing dispersed organogel particles for a topical application, for its potential benefits concerning texture and its application profile on skin. This application implies appropriate ingredients and preparation method. This is particularly the case in the choice of oils, and consequently two different oils were used. These are sweet almond oil and phytosqualane, two oils with emollient properties, which also differ in their physicochemical characteristics. This last point is important since it has been demonstrated the importance of some of them on the crystallization temperature and the hardness of the organogel. Thus it has been observed that for oils of the same chemical family, these properties are directly related to the polarity of the oil [30]. Another important parameter is the viscosity of the oil. Indeed, it has been shown that it can affect the gelation rate and the final hardness of the organogel, by playing on the arrangement of the gelator molecules [31].

Dispersions were realized using two different emollient oils combined with HSA as a gelator, polyvinyl alcohol polymer (PVA80) as a stabilizing agent, and a preservative agent (a mixture of Phenoxyethanol, Caprylyl Glycol and Chlorphenesin). Formulations were characterized in terms of texture, microstructure and interface interactions, and their profiles were compared according to their composition and formulation process.

The aim of this study is to explore new formulations and new textures based on organogels. The applications sought here are for the cosmetic field, where there is a strong demand of new oily textures.

2. Materials and Methods

2.1. Chemicals

12-Hydroxystearic acid (HSA) at cosmetic grade was obtained from Vertellus (Indianapolis, USA) and Poly(vinyl alcohol) (PVA80, with a hydrolysis degree of 88% and a molecular weight of around 50000g/mol) from Kuraray (Tokyo, Japan). Phytosqualane was provided by Sophim (Peyruis, France) and sweet almond oil by Bertin (Le Plessis Belleville, France) and Symrise (Holzminden, Germany). Tocopherol was bought from BASF (Ludwigshafen, Germany) and Saliguard[®] COS (a mixture of Phenoxyethanol, Caprylyl Glycol and Chlorphenesin) from Salicylates and Chemicals (Mumbai, India). Distilled water was used for the preparation of aqueous solutions and the corresponding dispersions.

2.2. Preparation of organogels and aqueous dispersions of gelled-oil nanoparticles

Organogels were prepared by dissolving HSA (10 and 20 wt.% of oil phase) in phytosqualane or sweet almond oil. For a complete dissolution of HSA in the oil, the mixture was heated and stirred with a magnetic stirrer (85°C, 300 rpm, 10 min), and cooled at room temperature to form a solid, white or yellow, gel (with phytosqualane and sweet almond oil respectively). Tocopherol was used (0.2 wt.% of oil phase) to minimize the oxidation of the sweet almond oil.

Aqueous solutions were prepared by dissolving PVA80 (1, 3 and 5 wt.%) in water, with a pre-dissolving step consisting in heating (85°C) and mechanically stirring (500 rpm) for one hour. Pre-dissolved solutions were then homogenized with a high shear mixer (Silverson, Model L5 series, High Shear Laboratory Mixer, 5000 rpm, 30 min), cooled at room temperature and stored for 24h before use. Saliguard® COS (1 wt.%) was added in water as a preservative agent for the concerned formulations.

Organogel and aqueous solution were then introduced together in a beaker (in 10:90, 15:85, 20:80 and 25:75 ratios), and placed in a oven (85°C, 1h) to melt the organogel. The hot mixture was emulsified by sonication (Sonic Dismembrator Ultrasonic Processor-Fisher scientific, 10 min, amplitude 100%, 3 seconds of pulsation, 2 of rest), and the obtained dispersion was left to cool at room temperature or in ice, and stored for 24h before characterization.

2.3. Instrumental characterization

2.3.1. Texture analysis

Texture analysis was performed using a Texture Analyzer TA.XT Plus (Stable Micro Systems, Surrey, UK) in compression traction mode. Penetration tests were carried out according to previous work [32]. All measurements were run at room temperature (~20°C) with a load cell of 5kg, a P/0.5R cylindrical probe (½" diameter (12.7 mm), Radius Edge BS: 757 and ISO: 9665 Gelatine Bloom Cylinder probe, Delrin). 200g of each sample (two repetitions per formulation) were loaded in 500mL containers, directly after homogeneization and before the cooling of the dispersion. No air bubbles removal was necessary, considering the very low apparent viscosity of sweet almond oil based systems, and the fragile state of the phytosqualane based ones. Each sample was penetrated three times (3 holes at equal distance from each other and container sides). The penetration depth was 15 mm with a velocity of 1 mm/s and a trigger force of 0.1g. After penetration, the probe returned to the initial position. The "Force vs time" curve was recorded and different parameters were collected: the maximum penetration force (g), the work of penetration corresponding to the penetration area under the curve from the product surface until the maximum penetration depth and the work of the probe's removal corresponding to the area under the curve during the withdrawal of the probe from the product, from maximum penetration depth until initial position. As evidenced in [32], those parameters are generally well correlated with each other, and penetration tests are generally well correlated to sensory attributes linked with consistency and hardness. In this study, it was decided to mainly analyze the maximum penetration force. Moreover, analysis of the curve profile gave relevant indication about the texture of the aqueous dispersion and the internal macroscopic structuration.

2.3.2. Droplet size distribution

The refractive index of the oil phase was determined using an electronic refractometer Refracto 30 (Mettler Toledo, Schwerzenbach, Switzerland). Phytosqualane and sweet almond oil presented values of 1.457 and 1.469 respectively. Particle size analysis was performed by dynamic light scattering (DLS) using a Malvern Zetasizer Nano ZS, using a He-Ne laser (633 nm) at a scattering angle of 170°C and at $25 \pm 1^\circ\text{C}$. All samples of aqueous dispersions were previously diluted in 40 mL of ultra-pure water and homogenized in an ultrasound bath for 10 minutes to break the colloidal hydrogels and obtain isolated particles. Mean hydrodynamic diameter (Z-average) was obtained using Malvern software with the Contin Model and by calculating the average of nine runs.

2.3.3. Cryo-scanning electron microscopy (Cryo-SEM)

The electron microscopy images were performed using an FEI Quanta 250 FEG scanning electron microscope at an accelerating voltage of 5kV. Samples were observed by Cryo-scanning electron microscopy (Cryo-SEM) after high-pressure freezing (HPF). Samples were inserted between two HPF specimen carriers dedicated for cryofracture (Leica) and were immediately loaded into a HPM100 HPF machine (Leica). They were then fixated and frozen within 5 ms at 2050 bars and transferred to cryovials under liquid nitrogen. Finally, HPF specimen carriers with freeze samples were inserted in the Quorum PP3000T preparation chamber. For insertion, specimen shuttle cryo stubs and a suitable HPF specimen carrier adapter was used following the B. Payre and coll. protocol [33]. After the quick transfer in a vacuum inside the preparation chamber, the samples were fractured at -140°C, sublimed at -95°C for 30 min and then coated by platinum sputtering. They were at last transferred to the cryo-SEM Quanta 250 FEG chamber and kept at -140°C for observation.

2.4. Interaction modeling at the oil/water interface

2.4.1. Superficial and interfacial tension

A GBX Digidrop tensiometer was employed to measure surface tension as well as interfacial tension between the oil and aqueous phases, using the pendant drop method. A drop of aqueous solution, hung at the tip of a needle connected to a micrometric syringe, was photographed in the air or in a glass cuvette containing the oil. The volume of the drop was adapted for each interface, corresponding to the maximal volume before the drop fell. The photograph was then analyzed and the surface/interfacial tension was obtained using a calculating model based on an approximation of the Young-Laplace equation (dE/dS). The method is explained with a schematic diagram in Fig. S1. Each drop was photographed and analyzed ten times, and measurements were carried out in triplicate.

2.4.2. Contact angle measurements

Flat organogel surfaces were prepared by placing 4g of solid organogel in 50ml beakers, together with 6g of different aqueous solutions, with or without PVA80 and preservative agent. The beakers were placed in a heat chamber (85°C) for 1 hour, after what the hot biphasic systems were cooled at room temperature to form thin disks of organogel (~3mm high) inside the beaker. The disks presented two interesting sides: one in contact with air, and one in contact with the aqueous solution during gelation. They were removed from the beakers and air dried before contact angle measurements. For the surfaces in contact with PVA80 or preservative solutions during gelation, contact angle measurement was carried out right after air drying.

Advancing contact angle measurements were performed using a portative goniometer PGX+ (ScanGaulle, Gravigny, France) connected to PGPlus software. It was equipped with a high-resolution camera to acquire images, and a specific lighting system to visualize the droplet. A syringe ($\varnothing=0.77\text{mm}$) was used to deposit a first drop on the organogel surface and its volume was increased by the addition of five successive drops until a total volume of approximately 7 μL . This method was adapted from a protocole for contact angle measurements on skin [34], and used as it limits the effect of the substrate roughness, considering the difficulty to obtain perfectly flat organogel surfaces. A photograph was taken after each addition and the contact angle was measured by the software for each photograph. The maximum contact angle value among the five pictures was selected, and the measurement was performed at least in triplicate for each surface.

3. Results and discussion

3.1. Textures and ingredients

In the field of cosmetics and products for topical applications, emulsion textures are very important for stability, but also for sensory aspects and efficacy. For this study, we selected ingredients and additives that are commonly used in the cosmetics field. Sweet almond oil and vegetable squalane (phytosqualane) were chosen for their emollient properties, but also for their very different polarity and viscosity. Their compositions and main characteristics have been summarized in Table S2. HSA and PVA80 are ingredients that have already been used to formulate organogel dispersions [20,21], but they are also found in cosmetics. Finally, for preservation reasons and potential *in-vivo* application, the systems were secured using the preservative agent Saliguard® COS (a mixture of Phenoxyethanol, Caprylyl Glycol and Chlorphenesin; 1 wt.%), often used in cosmetic formulations [35]. In the case of sweet almond oil, an antioxidant (Tocopherol, 0.2 wt.%) was also added.

The obtained dispersions showed very different textures with a hydrogel and a stable liquid dispersion, so texture analysis was first carried out.

Figure 1 shows the influence of the nature of the oil on the final texture, even when prepared under the same conditions. For clarity, only one repetition of the triplicate measurement is presented here for each sample, the standard deviation will be presented thereafter (Fig.2). The upper curve in Figure 1 shows the penetration profiles obtained with an aqueous dispersion of phytosqualane organogel particles, at 10 wt.% of the inner phase containing 10 wt.% of HSA and stabilized with 3 wt.% of PVA80 in the aqueous phase. The force applied by the probe to penetrate the sample to the maximum depth induced a series of sharp ruptures (black arrows on Fig. 1) followed by constant or increased force, suggesting a collapse in the internal structuration. This profile indicates behavior characteristic of a brittle hydrogel, in agreement with the observed macroscopic texture. The obtainment of such a texture is hypothesized to be attributed to connections between the gelled oil particles, leading to a colloidal hydrogel. To date, there have been no examples in the literature of colloidal hydrogels obtained from particles of organogels.

The texture obtained in the case of sweet almond oil presents a totally different profile. For this oil, a liquid, corresponding to a stable dispersion of organogel particles is obtained. The allure of the penetration test curve (lower curve) is typical of an emulsion with a liquid behavior [32]. This behavior is in agreement with what was already observed with other vegetable oils [20,21].

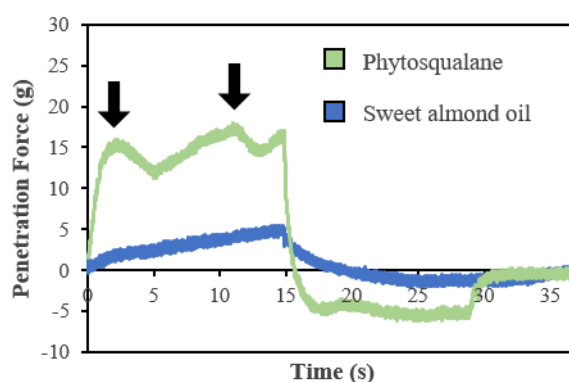


Fig. 1. Penetration test curve data of gelled oil aqueous systems, composed of phytosqualane or sweet almond oil; 10 wt.% of oily phase containing 10 wt.% of HSA and stabilized with 3 wt.% of PVA80.

In this study, the two oils differ in terms of polarity (phytosqualane and sweet almond oil present water/oil interfacial tensions of 51 and 20mN/m at 25°C respectively, determined with the pendant drop method (2.4.1)) and viscosity (31 mPa.s for phytosqualane [36] and 69-76 mPa.s for sweet almond oil [37] at 25°C). Starting from fact that the two oils produce different textures, we studied for each of them the influence of the amount of gelling agent (HSA), inner phase, and stabilizer (PVA80) on the maximum peak force measured by texture analysis (Fig. 2). Each ingredient was individually varied with respect to the following reference formulation: 25 wt.% inner phase, containing 10 wt.% HSA, 3 wt.% (of aqueous phase) PVA80.

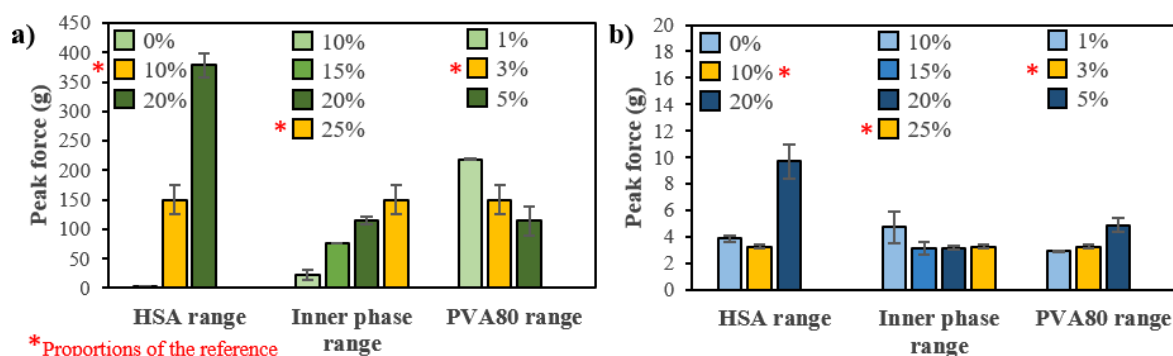


Fig. 2. Influence of HSA, inner phase and PVA80 (from left to right) percentages on the hardness of gelled oil aqueous systems, composed of a) phytosqualane or b) sweet almond oil.

Figure 2a shows the results obtained for phytosqualane, leading to a hydrogel. The study shows that the amount of gelator (HSA) plays a major role in the hardness of the mixture, with peak force increasing very strongly as we increase the HSA ratio. This observation is expected, and reflects a greater cohesion and density of the gelled particles. This behavior is classically observed with bulk organogels obtained with low-molecular weight gelators [38]. The maximum penetration peak of the mixture increases sharply when the proportion of oil is increased from 10 to 25 wt.%. On the other hand, it is observed that when the stabilizer content increases, the peak force decreases. These phenomena can be explained by the formation of a colloidal hydrogel. An increase of the hardness with the inner phase ratio indicates the formation of a denser hydrogel due to an increased number of particles available to form the network. Conversely, as we increase the amount of stabilizing agent (PVA80), we improve the dispersion of the gelled particles, limiting the connections between particles and leading to a less cohesive network and a less firm hydrogel.

In the case of sweet almond oil, the behavior in texture analysis is different, because a stable liquid dispersion of organogel particles is obtained. Maximum penetration peak force values are comparatively much lower than for phytosqualane, and not very sensitive to the amount of oil and stabilizer. Only the amount of HSA has a significant effect at 20 wt.%, with an increased value, compared to the emulsion (0wt.% HSA) or 10 wt.% of HSA. This effect on the consistency and cohesion of the liquid dispersion can again be correlated to a strengthening of the gelling agent network inside the particles.

Figures 3 illustrates with different pictures the visual aspect of the textures obtained according to the nature and the proportion of the oil phase. The visual aspect of formulations with varying proportions of HSA and PVA80 are presented in S3 and S4.

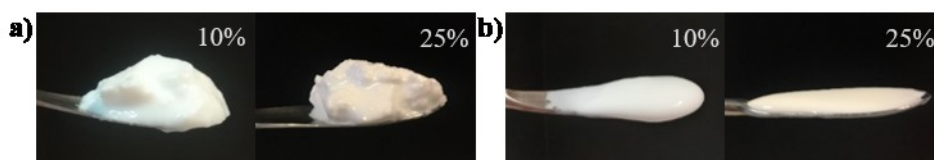


Fig. 3. Pictures of formulated systems, composed of a) phytosqualane or b) sweet almond oil; 10-25 wt.% of oily phase containing 10 wt.% of HSA and stabilized with 3 wt.% of PVA80.

The obtained textures, hydrogel or fluid liquid, are based on dispersions of particles and are therefore related to the microstructure of the systems. In order to better understand the interactions involved, the influence of the ingredients and their amounts on the average diameter of the obtained particles was studied. Each ingredient was individually varied with respect to the following reference formulation: 25 wt.% inner phase containing 10 wt.% HSA, 3 wt.% PVA80 in the aqueous phase. The measurements were carried out by dynamic light scattering (DLS) at 25°C after 24 hours. Figure 4 summarizes the obtained results. An ANOVA statistical study on these results is presented in Table S6.

Figure 4a shows the influence of the proportion of the oil phase on the mean particle diameter. The size of the particles in the studied range varies from 230nm to 250nm with phytosqualane, and from 230nm to 260 nm for sweet almond oil. The particles obtained with the two different oils are globally in the same size range, showing neither significant nor systematic impact of the nature and ratio of the oil on the particle size, apart from samples with sweet almond oil and with 10 and 15wt.% of oily phase. Regarding the influence of the HSA content in the oily phase (Fig. 4b), it is almost the same with both oils. A slight decrease in the size of the droplets can be observed in the presence of HSA, compared to the corresponding emulsion (0wt.% HSA). This phenomenon can be attributed to a potential destabilization of the emulsion, which is avoided when HSA is used. Moreover, the amphiphilic character of HSA, induced by its carboxylic acid function [39], could promote the formation of slightly smaller particles. We can conclude that concerning the nature and amount of oil, or the percentage of HSA, there is no clear influence on the average size of the gelled nanoparticles. On the contrary, figure 4c shows a more significant impact of the stabilizer amount (PVA80) on the mean particle diameter. At a 1wt.% of PVA80 in the aqueous phase, the size of the droplets are higher than with 3wt.% or 5wt.% of PVA80 for both oils. This result reveals that at this concentration, there is not enough stabilizing agent to obtain an emulsion with a small size distribution. Moreover, at 1wt.% of PVA, there is a large difference between phytosqualane and sweet almond oil particle sizes, which can be attributed to the difference in oil viscosity. Sweet almond oil, which is much more viscous than phytosqualane (66-76 and 31 mPa.s respectively, at 25°C) produces larger particles (400 nm). From 3wt.% of PVA80, the dispersing effect is sufficiently powerful, so the difference in viscosity no longer affects the diameter of the particles (~250 nm).

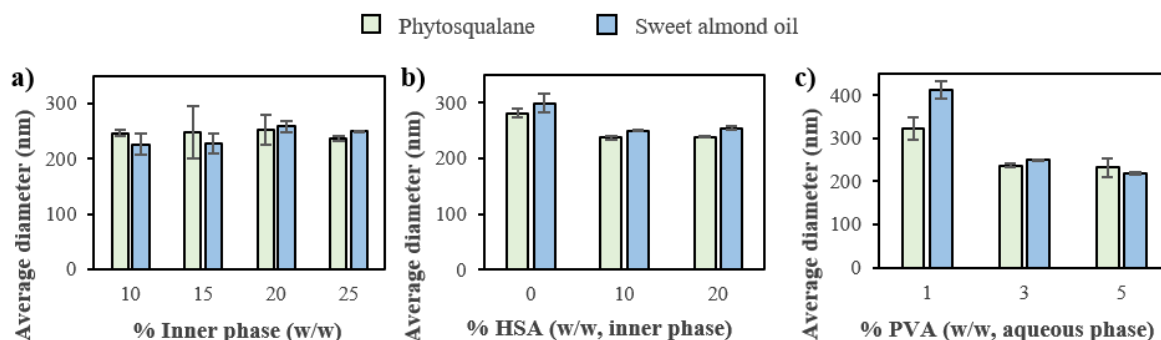


Fig. 4. Influence of (a) inner phase, (b) HSA and (c) PVA80 percentages on the average diameter of gelled oil particles.

3.2. Influence of the preservative agent and cooling rate

All of the measurements and variations in the amount of ingredients seem to indicate that the formation of a colloidal hydrogel essentially depends on the nature of the oil. However, this conclusion does not take into account all of the present ingredients. The Tocopherol used as an antioxidant in sweet almond oil is introduced in very small amounts (0.2 wt.%) and had no effect on the obtained textures. However, although it is in a very small proportion (1 wt.% in the aqueous phase) and essential for preservation, the possible role of the preservative agent should not be neglected. The preservative agent is a mixture of three ingredients: phenoxyethanol, caprylyl glycol, chlorphenesin; which could possibly affect some interfacial phenomena [40,41]. Consequently, it seemed to us important to test the influence of this ingredient on the observed textures.

Different formulations have been prepared with and without a preservative agent for the two oils, with varying amounts of stabilizing agent. For each oil, different PVA80 contents (1 and 5 wt.%) were tested for 25 wt.% of oily phase containing 10 wt.% of HSA.

Phytosqualane-based formulations showed a drastic effect of the preservative agent on the obtained texture (S4a). Indeed, without a preservative agent, there is no formation of a hydrogel, but only a liquid dispersion similar to that obtained with sweet almond oil. On the other hand, for sweet almond oil, a fluid dispersion with or without a preservative agent is retained (S4b). For both oils, the proportion of PVA80 (1 or 5 wt.%) does not obviously affect the texture in the absence of a preservative agent. This study shows the crucial role played by the preservative agent in the textures obtained with phytosqualane.

In general, the cooling rate of an organogel is also an important experimental parameter [42–44]. Rapid cooling affects the organization of the gelling agent network and here could have an effect on the final texture of the gelled particle dispersions. A rapid cooling at 0°C (ice bath) was therefore carried out on the dispersion of phytosqualane obtained just after hot sonication. Under these conditions, even with the preservative agent, it does not form a hydrogel, but a fluid dispersion of organogel particles (S5). To explain this result, we can make the following hypothesis. The cooling rate can affect the stability of the system by acting at two levels; gelation rate and stability of the dispersion (Brownian motion). When the cooling is slow, the gelation of the droplets is slowed down, and a more important thermal motion allows greater destabilization of the droplets. The conjunction of the two phenomena leads to a partial coalescence of the droplets and their connection by gelation, and could lead to the formation of colloidal hydrogel.

This mechanism underlines an important role played by the interfaces between droplets, before and after gelation. A mastery of the textures and especially the formation of a possible colloidal hydrogel implies understanding the mechanisms involved.

3.3. Interfacial studies

In order to better understand the role played by the preservative agent, the other ingredients and their interactions, the surface (with the air) and interfacial (with phytosqualane and sweet almond oil) tensions of different aqueous solutions were measured at 25°C, with or without PVA80, and with or without a preservative agent (Fig. 5). The measurements of surface tension in the air underline the surfactant activity of PVA80, which lowers the surface tension of water to 53 \pm 1 mN/m. This surface tension decrease is further reinforced by the addition of the preservative agent (37 \pm 1 mN/m), and

demonstrates its interfacial effect. The result obtained with the preservative agent alone, of the same order of magnitude as the PVA80+preservative agent mixture, and lower than the PVA80 alone in water, confirms a surface activity specific to this preservative agent, which drives the results when incorporated in a solution.

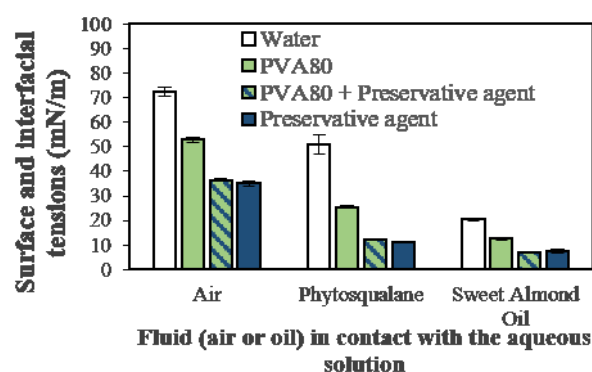


Fig. 5. Surface and interfacial tensions of PVA80 (1%, w/w) and preservative (1%, w/w) aqueous solutions, in the air and in phytosqualane or sweet almond oil.

The results obtained at the aqueous phase/phytosqualane interface are also very instructive and are similar to those obtained at the aqueous phase/air interface. This oil is very apolar (C30 hydrocarbon) and has a high interfacial tension against water (51 \pm 4 mN/m), illustrating that a certain number of interactions between both media are not satisfied. When PVA80 is added to the solution, the interfacial tension drops to 25 \pm 1 mN/m, and this decrease is emphasized when the PVA80 is paired with the preservative agent (12 \pm 0.2 mN/m). Besides, the preservative agent alone, generating a similar fall in interfacial tension, again shows a significant effect at the aqueous phase/oil interface. In comparison to the values of surface tension obtained against the air, the lowering of interfacial tension against phytosqualane with additives is stronger. This indicates for the PVA80, the preservative agent or their mixture, a good affinity for the interface with that particular oil.

In the case of sweet almond oil, which is more polar, the decrease in interfacial tension (water/sweet almond oil) is greater compared to phytosqualane (20 \pm 1 mN/m vs 51 \pm 4 mN/m). In this case, due to the chemical composition of both media (water and sweet almond oil), more interactions are satisfied. More precisely, sweet almond oil composition indicates saturated fatty acids, mono-unsaturated fatty acids, poly-unsaturated fatty acids and phytosterols (essentially B-sitosterol) [45]. Plants sterols are amphiphilic, and this property could help to better emulsify this oil [46]. Consequently, the differences between the mixtures tested are less marked, and the effect of the preservative agent much lower. Nevertheless, the same trend as in the aqueous phase/air and aqueous phase/phytosqualane is obtained when PVA80, PVA80/preservative or preservative are added. These last observations are important and could partly explain the differences in textures obtained when the preservative agent is used, but the effect of gelation should also be taken into account.

In order to assess the interfacial interactions after gelation of the oily phases, organogels were prepared, and contact angles were measured on their surfaces. To do so, a mixture of oil and HSA was heated ($T > T_{gel}$, 85°C) in the presence of aqueous solutions (pure water, PVA80 1 wt.%, preservative agent 1 wt.% and their mixture), and then cooled to room temperature. After gelation, a material was obtained with two different surfaces, a lower face in contact with the aqueous phase and an upper face in contact with the air (Fig.6). After preparation, the disk of organogel was air dried before the contact angle measurement. These experiments were realized to better understand the ingredient interactions at the gelled droplet surface in the formulation.

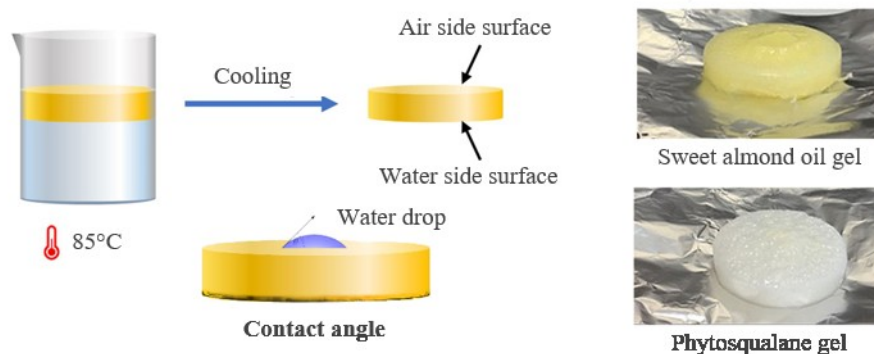


Fig. 6. Schematic illustration of organogel surface preparation for contact angle measurements and images of the materials.

The contact angle measurement is a way to characterize wettability and the affinity of a liquid with a surface, in our case water. The higher the contact angle, the lower the affinity and the more hydrophobic the surface. Figure 7 shows the contact angles obtained with pure water on the surface of an organogel of phytosqualane or sweet almond oil, formed at the air/oil or aqueous phase/oil interface of the material. After gelation, the surface of the material is not essentially composed of oil, but also presents a network of HSA fibers dispersed in the oil [18,19]. These experiments allow us to evaluate, from a macroscopic point of view, interfacial interactions with HSA and oil after gelation.

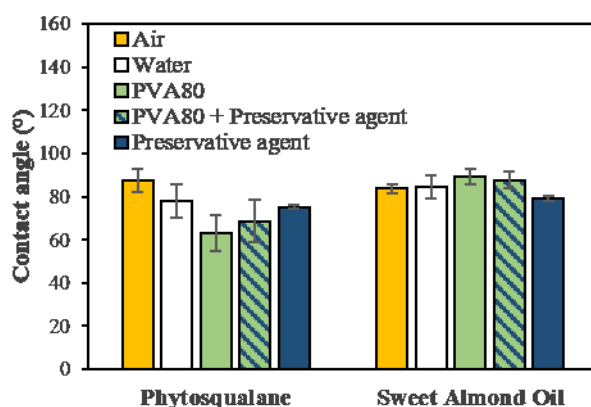


Fig. 7. Contact angle measurements of a drop of pure water on the surface of organogels (containing 10 wt.% of HSA) of phytosqualane and sweet almond oil, formed in contact with the air or with various solutions (1 wt.% PVA80, 1 wt.% preservative agent).

The surface wettability of the organogels of phytosqualane and sweet almond oil presented very different evolutions when varying the composition of the fluid in contact during the gelation. In the case of phytosqualane, the air/organogel interface exhibits a contact angle with water of 92°, indicating poor wettability of the surface. When the organogel is formed against water, wettability is slightly improved, with a contact angle of 81°. This can result from a rearrangement of the HSA inside the organogel, moving closer to the interface, due to its amphiphilic character. Other phytosqualane organogels were formed against solutions of PVA80 or PVA80+ preservative agent (column 3 and 4 respectively). PVA80 accumulates at the interface and increases the wettability of the organogel surface (angle=63°). In the case of the PVA80+ preservative agent mixture, the contact angle is increased (72°) compared to PVA80 alone. Since the wetting effect of the preservative alone is minor (angle=78°), this observation indicates that in the case of the mixture, the preservative agent interferes

with the adsorption of PVA80 at the surface of the organogel and decreases the wettability and therefore the effectiveness of the PVA80 as a stabilizer (negative effect).

For sweet almond oil, the wettability of the air/organogel interface is slightly higher than for phytosqualane, and is not impaired when the organogel is formed against water ($\sim 87^\circ$). These results are in agreement with the less hydrophobic nature of sweet almond oil, in which the HSA does not present a marked interfacial behavior. With PVA80 alone or PVA80+ preservative agent, the interface wettability is almost equivalent to pure water. This observation indicates that the preservative agent has no effect on wettability and on the action of PVA80 at the interface (neutral effect). The preservative alone lowers the contact angle to the same value as with phytosqualane (78°), but in this case improves wetting compared to PVA80 alone or with a preservative. This observation indicates that, after gelation, surface tension is more reduced with the preservative than with PVA80. In conclusion, these macroscopic observations on organogel interfaces could be correlated with the stability of the aqueous dispersions of particles. In the case of phytosqualane the preservative has a negative effect on stabilization by the PVA80, resulting in destabilization of the droplets and the formation of a colloidal hydrogel. For sweet almond oil, the presence of the preservative agent after gelation does not modify the interface, which provides a good stability of the droplets and the formation of stable liquid dispersion.

3.4. Colloidal gel morphology

In order to better correlate the formed textures with the interactions between particles, observations by Cryo-SEM were carried out. Firstly, Cryo-SEM images of liquid dispersions with phytosqualane or sweet almond oil (without a preservative agent) were analyzed. The images in figure 8 clearly show polydispersed spherical organogel particles within a sparse PVA80 network (fine strands in between the particles, red arrows), and a slight anchorage of PVA80 at the surface of the particles is clearly present (textured surface). Two particle populations were observed for phytosqualane samples (Figure 8A), with a diameter range between 250-400 nm for the most significant part of the particle population, and a larger one, in weak ratio, with a diameter range between 0.5 and 1.5 μm . The sweet almond oil sample presents the same kind of images (Figure 8.B). In this case, there are also two populations, but the diameter range is larger for the bigger one, 1.5-3 μm . The most numerous population also has a diameter range of between 250-400nm. Such observations are in accordance with the DLS measurements (Figure 4).

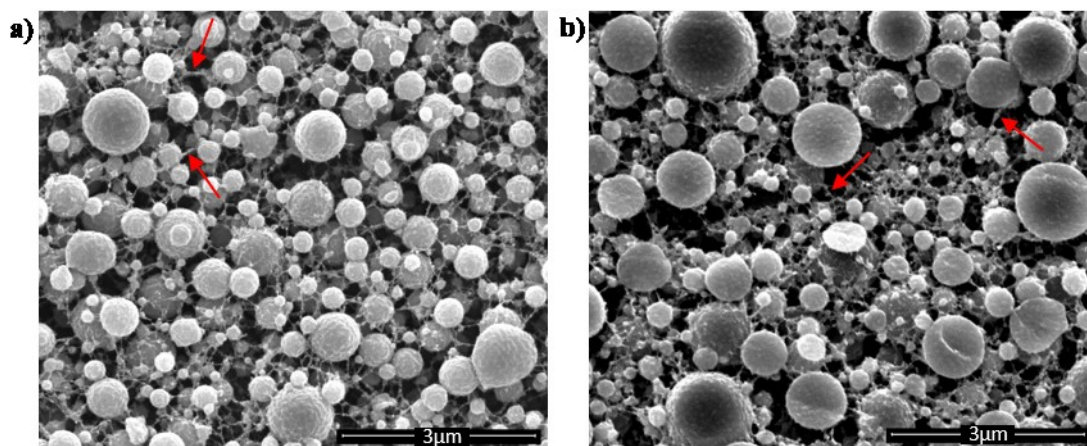


Fig. 8. Cryo-SEM micrograph of samples with phytosqualane (A) or sweet almond oil (B); 25 wt.% of the oily phase containing 10 wt.% of HSA and stabilized with 1 wt.% of PVA80.

The samples obtained with a preservative agent looked different depending on the oil. Phytosqualane systems form a colloidal hydrogel, unlike sweet almond oil ones, for which the liquid dispersion is still present. Cryo-SEM was carried out to correlate and better understand such phenomena. Both for PVA80 and a preservative agent, there is a partitioning between the particle surface and the aqueous phase. PVA80 is a polymer agent, easily recognizable as a strand-like network, contrary to the preservative agent, which is made of small molecules and not detectable by Cryo-SEM.

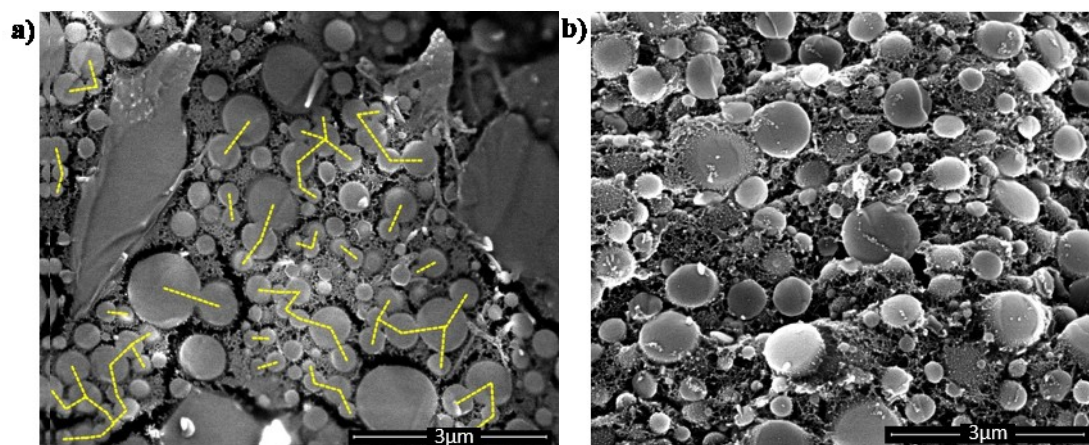


Fig. 9. Cryo-SEM micrograph of samples with phytosqualane (A) or sweet almond oil (B) and with a preservative agent; 25 wt.% of oily phase containing 10 wt.% of HSA and stabilized with 1 wt.% of PVA80. The dotted yellow lines show the connected particles.

For the phytosqualane sample (Fig.9a), particles are much less dispersed than without a preservative agent, and many of them are connected. These connections are not total in the entire volume, and the colloidal hydrogel is formed by free particles trapped in varying sized clusters of connected particles.

Such connections are not observed in the sweet almond oil sample (Fig.9b). For both systems, the gelled nanoparticles with a preservative reveal a smoother surface than without a preservative agent (Fig. 8). Furthermore, the Cryo-SEM micrographs of both systems show a greater density of the PVA80 network in the aqueous phase than without a preservative agent. Such observations can be correlated to a depletion of PVA80 from the surface and a better ability of the preservative agent to interact with the particle surface. Also in this case, images clearly show two populations of spherical particles. For sweet almond oil, the diameter of the smallest particles did not change, with a range between 200-400nm; for the larger one, the diameter range is between 0.5-1μm and is much smaller than without any preservative agent (Fig 9a). The preservative agent in this case acts as a surface agent, and improves the dispersion of droplets. For the phytosqualane sample, there is no effect on the size distribution, with and without a preservative agent, because of its lower viscosity, already allowing a small size.

3.5. Colloidal gel formation mechanism

To summarize all the observations, several mechanisms could be proposed and considered (Fig.10). Depending on the nature of the oil, the preservative agent does not have the same effect on the action of PVA80 at the interface during gelation, and consequently on the final stability of the dispersion. Such phenomenon can be correlated to Cryo-SEM observations. The particle surfaces are smoother in the presence of the preservative agent, confirming its interfacial action on HSA organization. Indeed, the gelation of the oil droplets leads to a strong modification in the water/oil interface and

consequently to the formation of HSA fibers. This phenomenon can lead to a more or less significant destabilization. For sweet almond oil or phytosqualane, at high temperature (Fig. 10a and 10c), the systems are still stabilized by the PVA80 located at the interface of the emulsion droplets. In the case of sweet almond oil, the gelation does not sufficiently disturb the interface (Fig 10b), even in the presence of the preservative agent, and the droplets are well stabilized until the end of the process. At room temperature, a stable dispersion is finally obtained.

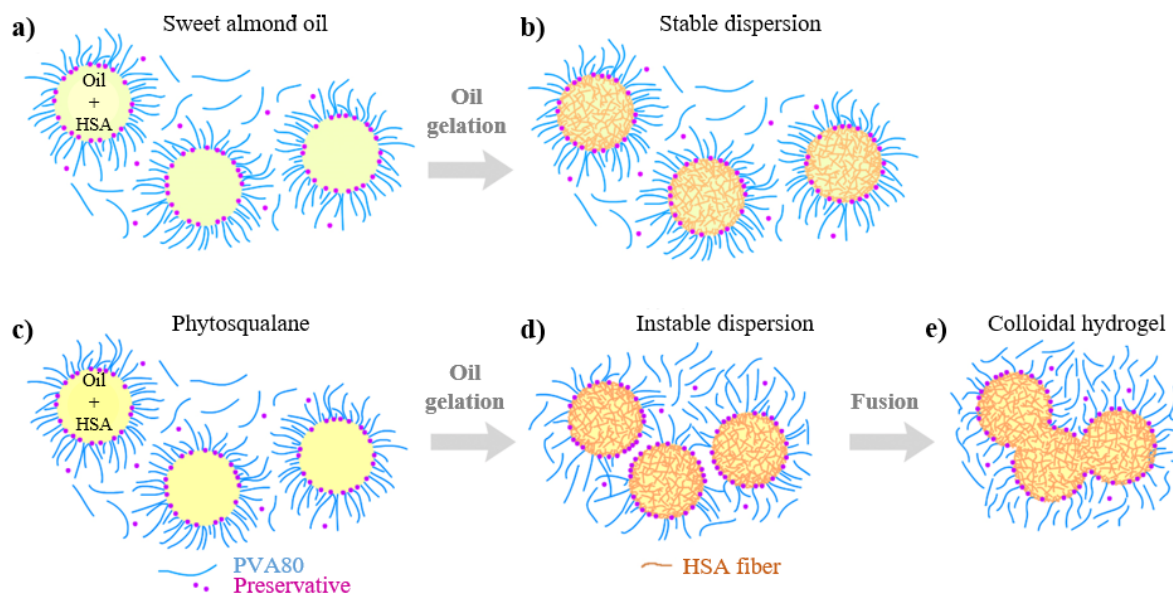


Fig. 10. Scheme illustrating the mechanisms leading to the different textures, depending on the nature of the oil and the interactions at the interfaces.

Phytosqualane is a more apolar oil than sweet almond oil. Nevertheless, at high temperature, before gelation, and even in the presence of the preservative agent, the oil droplets remain well dispersed. After cooling, and during gelation, the interface is disturbed by the HSA fibers, but in the absence of a preservative agent, there is still enough PVA80 at the interface to maintain a stable dispersion and to obtain a stable dispersion, as in Fig. 14b. On the other hand, during gelation in the presence of the preservative agent (Fig. 10d), there is a high level of desorption of the PVA80 in the continuous phase, and the system is greatly destabilized by the coalescence of the droplets. This also results from the fact that the preservative agent still at the interface is not a stabilizing agent. When slowly cooled at room temperature, this instability leads to connected droplets (colloidal hydrogel) within a solution rich in desorbed PVA80 (Fig. 10e).

These proposed mechanisms are in full agreement with the observations made by electron microscopy.

4. Conclusion

As part of a search for new textures for dermo-cosmetic and topical applications, stable dispersions and colloidal hydrogels have been obtained. Aqueous dispersions of organogel particles had already been described for many applications [20,21], but to date colloidal hydrogels obtained from such objects had never been mentioned. The experimental results described in this study have shown the crucial importance of the nature of the oils and some additives leading to a colloidal hydrogel or a stable dispersion of organogel particles. The gelation of the dispersed oil droplets is a critical step that could affect the molecules present at the water/oil interface, depending on their affinity. The preservative used in this study presents an amphiphilic character, and it adsorbs at the interface of the droplets. In agreement with all the results, we can hypothesize that this adsorption, depending on

the polarity of the oil, can in turn modify the action of the stabilizing agent (here the PVA80), and lead during gelation to a destabilization of the droplets and their partial coalescence to form clusters of connected particles. Understanding the phenomena involved will lead to better control over the textures, and in particular the formation of colloidal hydrogels from particles of organogels. These original systems constitute a new family of soft materials which could find applications in cosmetics, but also in the pharmaceutical field.

CRediT authorship contribution statement

Bérénice Duret: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Writing – original draft, Writing – review & editing, Visualization. Emile Perez: Conceptualization, Methodology, Writing – review & editing, Visualization, Supervision. Sarah Arneodo: Conceptualization, Methodology, Validation, Formal analysis, Investigation. Bruno Payré: Methodology, Resources, Investigation. Céline Picard: Conceptualization, Methodology, Writing – review & editing, Visualization, Supervision. Sophie Franceschi: Conceptualization, Methodology, Writing – review & editing, Visualization, Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this paper.

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