

Formulation of lamellar phases using a natural surfactant to understand the effects of fragrance on cosmetic emulsions: from microstructure to sensory perception

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Abstract

Lamellar emulsions are widely used in cosmetics for their textural and non-irritant properties[1]. In previous works, our team was able to control the formulation of lamellar emulsions, using a natural surfactant[2].

There is a growing demand for natural and multifunctional cosmetic ingredients. Fragrances, complex blends of mostly synthetic molecules, are decried. Essential oils are of interest, as they can be used as fragrance and active[3]. However, the impact of these ingredients on the microstructure and sensorial properties of lamellar emulsions is poorly documented.

This study aims to understand the interactions between fragrance and lamellar emulsion matrices, to optimize formulation and fragrance release.

Lamellar emulsions were formulated using a natural surfactant. Emollients exhibiting different chemical structures were used as the dispersed oil phase, and fragrance molecules with different polarities were selected. Emulsions microstructure was characterized by optical microscopy and static light scattering (SLS), textural properties were investigated using rheology and texture analysis, and fragrance release was monitored by gas chromatography (GC).

The results suggest a significant impact of fragrance on emulsions properties. Differences in microstructure and droplet size were observed when adding fragrance. Viscoelastic and textural properties were also impacted. Moreover, gas chromatography evidenced the influence of fragrance-emulsion interactions on fragrance release.

This work is based on an innovative methodology combining the control of lamellar phases in emulsions, their interactions with fragrance components and extensive analytical analyses to elucidate them at microscopic and macroscopic scales. This study will provide new knowledge for optimal control of fragrance incorporation in future emulsions.

Keywords: Fragrance; lamellar emulsion; microstructure; texture; sensory

1. Introduction

Lamellar emulsions are widely used in applied domains like pharmaceutics, food, and cosmetics. Their high viscosity at rest and their shear-thinning properties during application are ideal for skin application. These particular rheological properties are due to the presence of lamellar liquid crystal phases [4]. Lamellar phases are composed of surfactant bilayers, regularly spaced by interlamellar water layers (**Figure 1**). A co-surfactant, often a fatty alcohol, is added to facilitate lamellar phases formation. These structures present numerous advantages for cosmetic use. Layers of surfactant and co-surfactant stabilize the oil droplets, and the lamellar phases create a 3-dimensionnal network that stabilizes the emulsion. Surfactants are non-ionic, and their lamellar organization is similar to the skin lipids organization [1], making lamellar emulsion far less irritant than conventional cosmetic emulsions. Finally, interlamellar water layers provide enhanced hydration and actives encapsulation potentials [5].

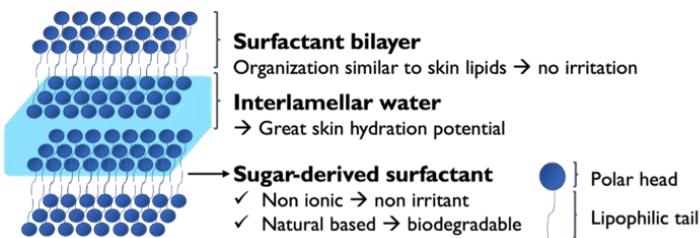


Figure 1. Scheme of the organization and advantages of lamellar phases

Fragrance is added to most cosmetic products. It is one of the most important ingredients of a formulation, as it has been found to be a key driver in consumer choice and contributes to the perception of products performances [6]. Fragrance is a complex blend of numerous natural and/or synthetic chemicals. Fragrance raw materials are characterized by great chemical diversity; molecules of various natures - alcohols, aldehydes, ketones, esters, lactones - are used [7], making the total mixture extremely complex.

Thus, it can be really challenging for the formulation chemist to obtain a formulation performing well with the fragrance of choice at its proper use level. Knowledge of the chemical properties of the individual chemicals in the fragrance composition is essential. But unlike other ingredients, fragrance compositions are considered trade secrets and components that make up the fragrance are not revealed on products labels. Furthermore, due to its elevated cost, fragrance is often the last ingredient added to the base formulation when everything else in the development process is complete. When stability is affected, it provokes the idea that fragrance is the cause of the instability. Indeed, depending on their physicochemical properties, fragrance molecules partition between the different phases of the emulsion and in the lamellar network, and can interact with other ingredients of the emulsion matrix, leading to destabilization, discoloration and odor change of the final product [8,9]. Finally, fragrance is known to have an allergenic potential. It is thus of capital importance to be able to predict and control its release.

The aim of this paper is thus to understand the interactions between fragrance molecules and lamellar emulsion matrices, to predict how fragrance can alter the physicochemical properties of emulsions, and to optimize fragrance release.

In the past, studies were conducted on the behavior of fragrance molecules incorporated in different ternary model systems [10–14]. Depending on their nature and structure, fragrance molecules

partition in the lamellar network, either between the polar heads of the surfactants (zone A on **Figure 2**), alongside the hydrocarbon chains of the surfactants (zone B), or between the lamellae (zone C). However, these studies deal with model ternary water/ surfactant/fragrance systems, and do not allow for the comprehension and prediction of the effects of fragrance when incorporated in complex cosmetic emulsions.

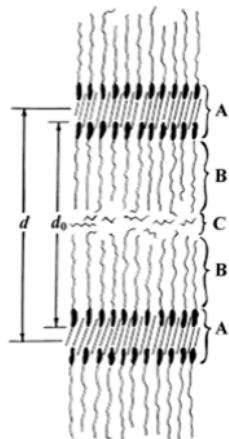


Figure 2. Possible locations of fragrance molecules in lamellar phases[11]

In previous works, our team was able to control the formulation of non-fragranced model lamellar emulsions using an alkyl polyglucoside (APG) / fatty alcohol (FA) mix as the emulsifier [2,15]. This sugar-derived emulsifier is of natural origin and responds to the growing concern from consumers to use new natural and multifunctional cosmetic ingredients. Emollients exhibiting different chemical structures were used as the dispersed oil phase. Through a combined approach of WAXS and viscoelastic analyses correlated with specific tests modelling application on the skin surface, we highlighted a link between emulsion composition, microstructure, and type of lamellae with its texture and applicative properties. Especially, emollient polarity was found to influence the nature of the lamellar phases formed.

The challenge of this work is to incorporate fragrance in these model lamellar emulsions, and to study the impact of fragrance on emulsions structural and sensorial properties. To do so, we used a C₁₆/C₁₈ APG/FA mix, as the emulsifier. Emollients exhibiting different polarities were selected to formulate lamellar emulsions with different structures. Individual fragrance molecules, widely used in perfumery, having different physicochemical properties were also selected to be incorporated in the emulsions. Fragranced emulsions were then characterized to study the impact of fragrance on emulsions microstructure and macroscopic properties. The impact of emulsion structure on fragrance release was also studied.

2. Materials and Methods

2.1 Materials

Cetearyl alcohol and cetearyl glucoside, used as emulsifier, and Phenoxyethanol and Methylparaben and Ethylparaben and Propylparaben and Butylparaben, used as preservative, were provided by SEPPIC, France. Three emollients of croissant polarities, namely phytosqualane

(SQUA), ethylhexyl stearate (EHS), and caprylic/capric triglycerides (CCT), were used as the dispersed oil phase, and are gathered in **Table 1**. Three individual fragrance molecules with different chemical properties, namely phenylethyl alcohol (APE), linalool (LNL) and limonene (LMN) were also selected (**Table 1**).

Table 1. Supplier and physicochemical properties of selected emollients and fragrance molecules

	Supplier	M _w (g/mol)	Log P	Water solubility at 25°C (mg/L)
SQUA	Comercial Química Massó	422,8	12,86	2,04.10 ⁻¹⁰
EHS	Stearinerie Dubois	396,7	10,31	0,05
CCT	Stearinerie Dubois	387,5	3,59	0,07
APE	Merck	122,2	1,36	22000
LNL	Fischer Scientific	154,3	2,97	1590
LMN	Merck	136,2	4,57	13,8

2.2 Methods

2.2.1 Sample preparation

The formulation and characterization protocols were adapted from the previous work of our team on the study of model lamellar emulsions. Emulsions were formulated using 10% APG/FA mixture as the emulsifier, 20% emollient as the dispersed oil phase, 1% preservative, 1% fragrance and q.s.p deionized water. Three different emollients were used, namely SQUA, CCT, and EHS. Emulsions without emollient were also formulated, the FA from the surfactant playing the role of the dispersed oil phase; such systems are called “auto-emulsions”. Three isolated fragrance molecules, namely APE, LNL and LMN were added to the different emulsions at the end of the formulation process. Control non-fragranced emulsions were also formulated. Relatively high percentages of surfactant and fragrance were used to emphasize the interactions between fragrance molecules and emulsion matrices.

Emulsions were prepared by heating separately the oil phase containing the surfactant and the emollient up to 85°C under mechanical stirring (250 rpm), and the water phase up to 85°C. The oil phase was then added to the water phase under mechanical stirring (250 rpm), and the mixture was homogenized at 10 000 rpm for one minute using a T25 digital Ultra-Turrax (IKA, Freiburg, Germany). Next, the mixture was cooled down to 25°C under mechanical stirring (450 rpm) using a Turbotest (Rayneri, VMI Mixing), then preservative and fragrance were added. Samples were left at room temperature for 48h, then stored at 4°C.

2.2.2 Optical microscopy

Emulsions microstructure was investigated using a light photomicroscope (ECLIPSE Ni-U, Nikon) equipped with a camera, under bright light then under polarized light using cross-polarizers to

visualize the presence of liquid crystalline phases. Indeed, lamellar phases are liquid crystalline phases. Their semi-crystalline nature is responsible for their birefringent properties, allowing the observation of Maltese crosses under polarized light [4]. Nikon software (NIS Element Viewer) was used to analyse the obtained images.

2.2.3 Droplet size distribution

Droplet size distribution of emulsions was determined by static light scattering (SLS) using a laser diffraction particle size analyser SALD-7500 nano (Shimadzu Co., Ltd., Japan) equipped with a violet semiconductor laser (405 nm) and a reverse Fourier optical system. The samples were diluted in deionized water prior to measurement to achieve the adsorption parameter equal to 0,2. When introduced in the batch cell, samples were stirred during measurements to ensure homogeneity. Three samples were collected and analysed for each emulsion, and the measures were carried out in triplicate for each sample. Wind SALD II software was used to analyse data.

2.2.4 Surface and interfacial tension

Surface tension of raw materials (emollients and fragrance molecules) was measured using a K11 Tensiometer (Krüss), equipped with a platinum Du Nouy ring (RI02, 9,7 mm diameter, Krüss). A sufficient volume of material was poured in a crystallizer to ensure that the height of the liquid is above 11mm, to make a correct surface tension measurement. Surface tension of each fragrance molecule dissolved in the different media (deionized water, emollients) was also measured. Interfacial tension measurements of fragrance molecules dissolved in emollients *vs* water were also measured. A minimum height of 11 mm of water was covered with a minimum height of 11 mm emollient to allow the formation of a plane interface. Each measurement was repeated 5 times to ensure reproducibility. Measurements were made at different times ($t = 0$ and $t = 2\text{h}$) to follow the evolution of interfacial properties with time.

2.2.5 Rheological properties

Rheological properties of emulsions were determined by continuous and oscillatory measurements, using a controlled stress rheometer (HR1, TA Instruments). The measurements were performed at 25°C using a cone-plate aluminium geometry with a 40 mm diameter, a cone angle of 2°00'18" and a gap of 54 µm. Once loaded, samples were left at rest for 120 s prior to measurement. Flow properties were determined by continuous ramp testing, recording viscosity value as shear rate was increased from 0,001 to 1200 s^{-1} (logarithmic mode) for 300 s. Oscillatory measurements were conducted at 1 Hz with increasing strain from 0,01% to 100%, to determine samples linear viscoelastic region. Frequency sweep ramp was performed from 0,01 to 10 Hz at a fixed strain comprised in the linear viscoelastic region of the samples. Storage (G') and loss (G'') moduli were recorded to characterize viscoelastic properties of emulsions. Measurements were carried out in triplicate.

2.2.6 Texture analysis

Analysis of emulsions consistency and spreading properties was carried out using a texture analyser TA.XT Plus (Stable Micro Systems, Cardiff, UK). Compression tests were conducted at room temperature. A volume of 1mL of sample was disposed on the apparatus base and compressed by a cylindrical P/35 probe (35 mm diameter, aluminium) at a constant speed of 1mm/s before returning to its start position. Penetration tests were conducted at room temperature, using a cylindrical P/0.5R probe (12,7 mm diameter). The probe penetrated the samples loaded in 250 mL containers, at a constant speed of 1mm/s to a depth of 10 mm before returning to its start position. For each test, the curve force = f(time) was recorded, and several parameters were collected: the maximum and minimal forces (g), the penetration area (g.s) corresponding to the compression/penetration work, and the withdrawal area (g.s) corresponding to the withdrawal work. Spreading tests were performed at room temperature in traction mode, with the apparatus equipped with an A/FR friction module consisting of a 50x50 mm² PMMA plate fixed to a sled. Four parallel lines of 50 µL each of sample were deposited on the PMMA plate, parallel to the movement of the sled. The sled was then pulled across a polypropylene sheet having a roughness similar to the skin, at a constant speed (3 mm/s) on 120 mm. The positive area (g.s) corresponding to the difficulty of product spreading was collected.

2.2.7 Headspace gas chromatography

A CombiPAL gas chromatograph (Agilent Technologies) equipped with a flame ionization detector and an automatic headspace sample injector was used, with helium carrier gas at a flow rate of 1 ml/min. Samples were injected in split mode (ratio 25:1) onto a ZB-5MS (30 m x 0.25 mm i.d., 0.25 µm film thickness, Zebron) capillary column. The injector and detector were maintained at 250°C, and the oven was heated at 50°C.

A volume of 2 ml of fragranced emulsions was added to sealed glass vials and incubated at 32°C for two hours until an equilibrium was reached. A sample (1 ml) of the headspace above the sample was automatically collected with a heated gas-tight Hamilton syringe and injected into the gas chromatograph. The separation program consisted in maintaining the column at 50°C for 2 minutes, then the temperature was increased to 200°C at a rate of 10°C/min, then to 250°C at a rate of 20°C/min, and finally to 280°C at a rate of 20°C/min. The total program lasted for 26 minutes and enabled clear separation of fragrance molecules and emulsion ingredients.

Fragrance release was studied by calculating the corresponding normalized peak areas on the obtained chromatographs.

2.3 Statistical analysis

The statistical analyses of collected data were performed using XLSTAT software (Addinsoft, Paris, France). A two-way analysis of variance (ANOVA) was applied to granulometry, rheology and texture analysis data to determine the discriminating attributes and to investigate the effect of

fragrance on these measurements. When significant differences existed between products ($P < 0,05$), Fisher multiple comparison test was applied to highlight different groups of samples.

3. Results

3.1 Impact of fragrance on emulsions properties

3.1.1 Impact of fragrance on emulsions microstructure

All the emulsions were observed by means of optical microscopy, under bright field then under polarized light. The presence of Maltese crosses was confirmed for all the samples, attesting the formation of lamellar phases. When compared visually, the images of control emulsions and emulsions fragranced with APE and LNL appeared very similar (**Figure 3**). For some of the systems containing LMN, observed objects were different in nature (onion rings were observed), and less numerous. The incorporation of LMN seemed to impact the formation of lamellar phases, or their nature. However, microscopic observations alone do not allow to conclude on the impact of fragrance on emulsions microstructure.

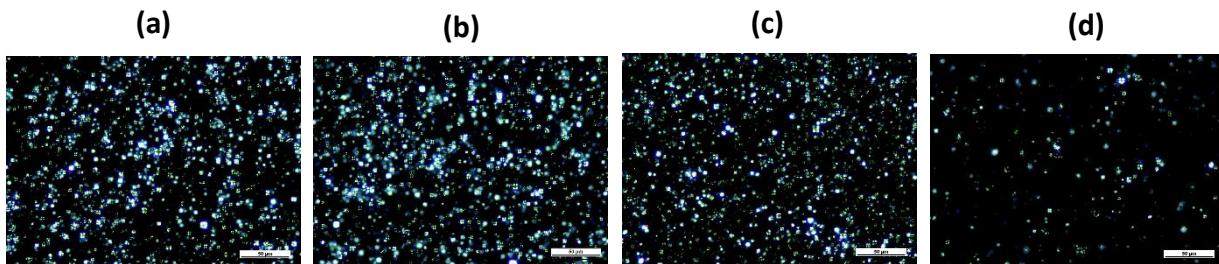


Figure 3. Examples of microscopic observations of (a) control, (b) APE, (c) LNL and (d) LMN samples under polarized light (x400)

Thus, emulsions granulometry was determined by SLS, to study the impact of fragrance on the size and distribution of emulsions droplets. The results illustrated by **Figure 4** showed that control and APE samples presented a similar monodisperse distribution with comparable mean diameters. Samples containing LNL presented a wider monodisperse distribution, whereas samples containing LMN presented a polydisperse distribution. According to the results of the ANOVA test, both systems exhibit a significantly higher mean diameter than the control samples.

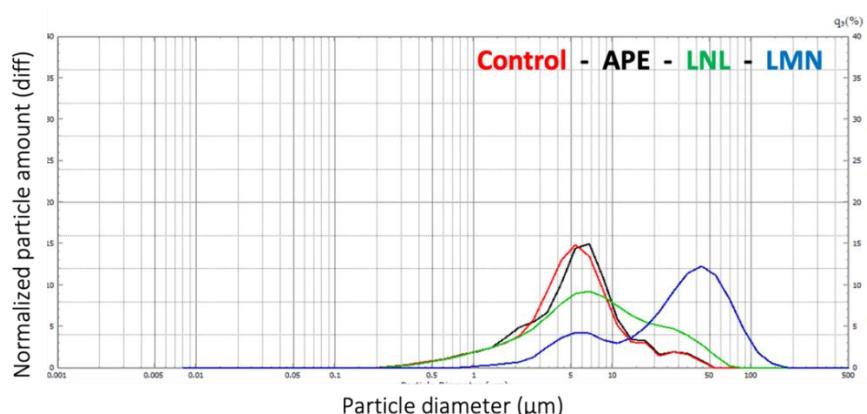


Figure 4. Example of particle size distribution

Results suggest that incorporation of LNL and LMN in the different lamellar systems impacts their microstructure. A possible explanation would be that fragrance molecules interact with the dispersed oil phase, or with the lamellar network.

3.1.2 Impact of fragrance on the macroscopic properties of emulsions

Rheological properties of emulsions were determined by oscillatory deformation tests. Results showed that storage modulus G' and loss modulus G'' were affected by the incorporation of fragrance, suggesting an impact of fragrance on the viscoelastic properties of emulsions. **Figure 5** shows that samples containing APE exhibited similar G' and G'' to the control samples, suggesting no impact of the incorporation of APE on the lamellar network. However, ANOVA tests highlighted that samples containing LNL and LMN had significantly lower viscoelastic moduli than control samples. These results suggest that these fragrance molecules somehow interact with the lamellar network and modify its properties. Indeed, viscoelastic properties of emulsions are intimately related to their microstructure [16]. Previous results suggest that LNL and LMN impact samples microstructure, which could explain the differences in rheological behavior, when compared to the control samples.

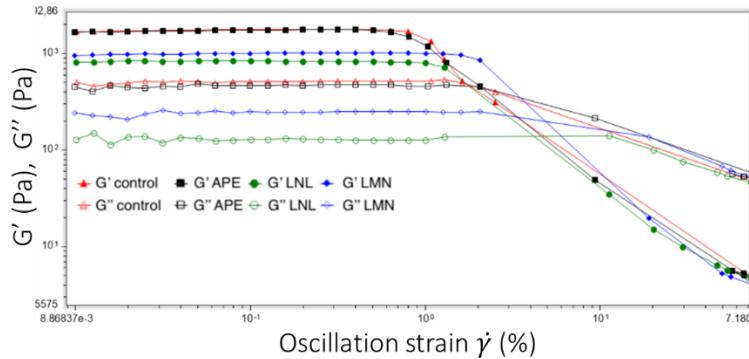


Figure 5. Deformation test. Evolution of viscoelastic moduli as a function of oscillation strain for the samples

Furthermore, visual, and tactile differences between the samples were observed, particularly in terms of glossiness and consistency. Texture analyses were carried, and confirmed the differences observed between samples. Emulsions consistency can be measured during compression tests, as it was found to be represented by the positive penetration area A_+ (g.s) under the curve force=f(time), corresponding to the work of penetration of the probe in the samples. Results of the ANOVA test showed that samples containing APE exhibited significantly higher consistency than other samples. Samples containing LMN and LNL exhibited significantly lower A_+ values. These results are in agreement with the results of microstructure characterization, and of rheological behavior.

3.2 Impact of emulsions structure on fragrance release

Release of APE, LNL and LMN from emulsions exhibiting different microstructures was studied by headspace gas chromatography. Areas of the odorants peaks on the obtained chromatograms were compared.

Results suggest an impact of the presence of emollient and of the nature of the fragrance molecule on its release from the different systems.

The presence of emollient in the emulsion formulations decreased fragrance release. In the absence of emollient, more water is present in the system, lowering the viscosity. This could facilitate fragrance mass transfer through the continuous phase, thus its release from the system.

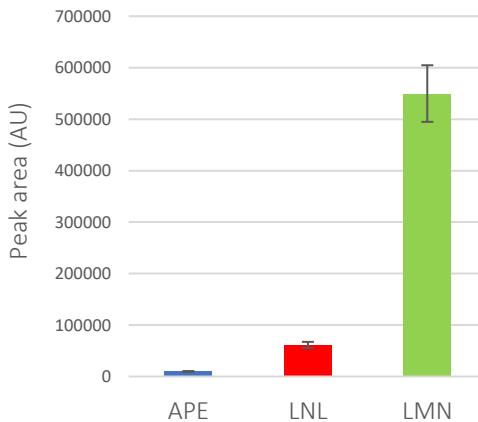


Figure 6. Areas of odorant peaks released from emulsions

Furthermore, LMN was found to be significantly more released than LNL and APE (**Figure 6**), regardless of the emulsion structure. This can be explained by its very poor affinity with water.

4. Discussion

Previous work of our team showed that the nature of the emollient used as the oil phase influences the lamellar organization of the emulsion microstructure [15]. Emollients and fragrance molecules have similar physicochemical properties, such as polarity and hydrophobicity. One could suggest that fragrance would interact with lamellar phases in a similar manner when incorporated in emulsions. Indeed, depending on the liquid crystalline phases present and on their nature, fragrance molecules also partition between the different parts of the emulsion [17].

APE is thought to be solubilized in the continuous water phase [8], due to its high water solubility and low log P. In this case, it would not interact with the lamellar network, explaining the similar behavior of control and APE-containing samples, as evidenced by SLS and rheology measurements. However, APE might have an impact on the viscosity of the continuous phase. Indeed, an increase of continuous phase viscosity could explain the higher consistency of APE-containing samples, as evidenced by texture analysis.

LNL is a terpene alcohol, and as such exhibits amphiphilic properties. It is thought to compete with surfactants at the interface between the lamellae and the water phases [8], leading to increased fluidity of the lamellar phases. When added in a water solution, LNL was found to significantly decrease surface tension of water (results not shown) confirming its surfactant properties. This could explain the significantly lower values of G' and G'', and the lower consistency when compared with control samples.

LMN is an aliphatic hydrocarbon and is the most hydrophobic fragrance molecule involved in this study. When incorporated in emulsions, it is thought to be located between the lamellae, at the heart of the lamellar network [18]. This could induce a swelling of the lamellae, explaining the higher mean diameter of the droplets, and a decrease of their compacity, leading to significantly lower viscoelastic properties and consistency.

Conclusion

This work proposed an innovative methodology combining the use of liquid crystalline phases in emulsions, their interactions with fragrance components and extensive analytical analyses to elucidate them at microscopic and macroscopic scale. Lamellar emulsions were formulated using a natural APG/FA as the emulsifier, three emollients of different chemical structures as the dispersed oil phases, and three odorant molecules of different polarities.

The results highlight a significant impact of fragrance on emulsions. Depending on their nature and physicochemical properties, fragrance molecules partition between the different parts of the lamellar network, imparting microstructure, and macroscopic properties of the samples. Indeed, differences in microstructure organization and droplet size were observed using microscopy and SLS respectively. This impact was also noticeable on viscoelastic properties and texture properties of the emulsions.

Moreover, emulsion microstructure and fragrance nature were found to have a significant impact on fragrance release, as evidenced by kinetics studies using headspace gas chromatography. Interactions between fragrance molecules and lamellar network are thought to be at the origin of the observed differences. This is corroborated by surface tension measurements of odorants incorporated in water and in the emollients.

In the future, X-ray diffraction measurements will be carried out to confirm the location of fragrance in the lamellar network and investigate their potential impact on the nature of the lamellar phases. Sensorial properties of emulsions will also be characterized by trained panellists, to study the impact of fragrance on the perception of emulsions texture, and the impact of emulsions structure on perceived fragrance intensity.

In the end, this work will provide new knowledge for optimal control of fragrance incorporation in the design of future emulsions. Indeed, understanding the interactions between fragrance molecules and dermo-cosmetic emulsion matrices is crucial, to predict how fragrance can alter the physicochemical properties of emulsions, and to optimize fragrance release.

Conflict of Interest Statement

None.

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