Design of a novel melatonin Pickering emulsion sunscreen

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INTRODUCTION

Melatonin, the main secretory product of the pineal gland, was recently found to have a protection effect against photocarcinogenesis based on its free radical scavenger and antioxidant activity. On the other hand, the daily use of sunscreens is particularly recommended for patients treated with immunosuppressive drugs and submitted to surgeries, chemotherapy and radiotherapy. Therefore, melatonin associated with sunscreens may be the key strategy in order to prevent UV-radiation induced skin damages, such as pre-cancerous and cancerous skin lesions. Given the increase in skin cancer incidence and the existence of an emerging group of high risk patients treated with immunosuppressive drugs submitted to oncological therapy the application of sunscreens with a high sun protection factor is one of the main strategies strongly recommended to prevent UV-induced cell damage. This work arose from the necessity to fill the gap of photoprotection market, by developing a formulation with high SPF and a lower content of substances of synthetic origin, i.e., less aggressively to the skin.

The aims of this study were the design and stabilization of melatonin in a novel sunscreen Pickering emulsion and its morphological characterization, droplet size distribution, contact angle and sun protection factor.

Keywords: Melatonin, Pickering Emulsion System, UV protection, Physical filters.

1 MATERIALS AND METHODS

1.1 Materials

Melatonin was purchased from Alfa Aesar (Ward Hill, USA). Ethanol was obtained from Merck® (Kenilworth, USA). Green coffee oil was supplied by COOXUPE´ – Cooperativa de Cafeicultores de Gauxupé, (Minas Gerais, Brazil). Titanium dioxide was a gift from Sensient (Milwaukee, USA). Liquid paraffin was obtained from José Vaz Pereira, S.A. (Lisbon, Portugal). All other reagents were HPLC grade and used as received. Purified water was obtained by inverse osmosis (Millipore, Elix® 3).

1.2 Methods

Solid Particles

Wettability measurements

The measurement of contact angles of water and liquid paraffin on TiO_2 in air was performed at room temperature by using ConAnXL - a Microsoft Excel based workbook and Add-in software. TiO_2 surface were prepared by compressing suitable amounts of particles size under a pressure of 10 metric tons applied by a KBr press. Briefly, a drop (approximately 10 µL) of water, paraffin and green coffee oil was carefully deposited on the solid surface. The contact angle was defined as the angle between the PS surface and the tangent to the surface of the drop. All measurements were performed in triplicate.

Particle size distribution

Particle size distribution was determined using a Malvern Mastersizer 2000 (Malvern Instruments, UK), coupled with a Hydro S accessory. The refractive index used was 1.52 (default). For a correct turbidity, about 0.1-0.5 g of TiO_2, corresponding to an obscuration between 1-5%, was added to the sample chamber containing 150 mL of purified water. The readings were performed under mechanical stirring (3500 rpm). Data was expressed in terms of relative distribution of volume of particles in the range of size classes, and given as diameter values corresponding to percentiles of 10, 50 and 90. The Span value is a useful statistical parameter to characterize the particle size distribution, and it was calculated by using the **(1**.

$$Span = \frac{d(90) - d(10)}{d(50)} \qquad (1)$$

Preparation of the Melatonin SunScreen (MSS)

According to the pre-formulation studies, four final formulations were selected (**Erro! A origem da referência não foi encontrada.**) based on macroscopic appearance, stability and SPF value. The continuous oil phase (Phase A) consisted of green coffee oil, and the aqueous phase (Phase B) was composed by purified water, ethanol and melatonin. TiO_2 was firstly dispersed in the oil phase. The oil and aqueous phases were then mixed using an UltraTurrax® homogenizer (IKA-Werke GmbH & Co. KG, Germany) at room temperature (cold process).

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Ingredients	**Quantitative Composition (%)**			
	MSS 1	**MSS 2**	**MSS 3**	**MSS 4**
Phase A (external)				
TiO2	7.5	10	15	25
Green Coffee Oil	62,5	60	55	45
Phase B (internal)				
Melatonin	1	1	1	1
Ethanol	6	6	6	6
H2O	23	23	23	23

Table 1: **Qualitative and Quantitative composition of the final formulations**

Characterization Studies of MSS

Emulsion Type

The emulsion type was identified by the dilution method and staining technique.

In the first, a drop of emulsion was added to a certain volume of pure water and another to pure oil (liquid paraffin). If it disperses in one of them, then that liquid is the continuous or external phase of the emulsion. The liquid in which it remains as drops, is the discontinuous or internal phase.

In the second, a drop of methylene blue (hydrophilic dye) was added to the emulsion and then the emulsion was inspected in an optical microscope to detect whether or not the water phase was the continuous one.

Droplet Size

After one day of storage at room temperature, the emulsions were investigated using an optical microscope (Olympus CX40, Japan), which was equipped with a video camera. One drop of the emulsion was added on a glass plate without cover glass and diluted with two drops of paraffin for microscope observation and photographed for posterior size analysis. The droplet size was determined using the image analysis software Olympus Stream Essentials®. The size data was expressed in terms of relative size distribution of particles, and given as diameter values corresponding to percentiles of 10%, 50%, 90%. The Span value was also taken into consideration since it is a statistical parameter useful to characterize the particle size distribution, and is calculated using the equation 1.

Efficacy of the Sunscreen Formulations

Theoretical SPF

The algorithm indicated in the BASF sunscreen simulator was used in order to find a sunscreen with a sun protection factor (SPF) of 50+ that protects the skin against both UVA and UVB radiation. The European Legislation (CE) N.º 1223/2009 - Annex VI was also considered.

The BASF Sunscreen Simulator is a tool that estimates the SPF as well as various common UVA-Metrics. This tool helps to plan the composition of active ingredients in sunscreen formulations. However, it does not replace the formulation study and *in vivo* SPF determination of the final product.

2 RESULTS AND DISCUSSION

2.1 Solid Particles

Wettability Studies

In this type of emulsions, one of the liquids will probably wet the solid more than the other liquid, with the more poorly wetting liquid becoming the disperse phase. The importance of the wettability of the particles at the oil-water interface is quantified by the contact angle, θ, that the particle makes with it, which will determine the type of emulsion. If the contact angle, measured through the aqueous phase, is inferior to 90º the emulsion will be o/w and, by contrast, if the contact angle is greater than 90º the emulsion will be w/o [1, 2, 3].

TechConnect Briefs 2015, TechConnect.org, ISBN 978-1-4987-4729-5

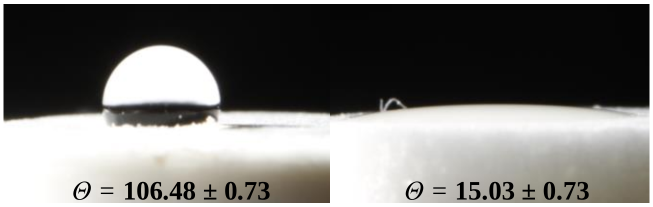

Figure 1: Contact angles of Titanium dioxide in (a) water and (b) liquid paraffin.

By analogy, TiO_2 will stabilize more effectively w/o emulsions, because the contact angle of water greater than 90º and simultaneously, a contact angle of paraffin smaller than 90º (Figure 1).

Particle Size

In this assay it is important to prove that the particles have a size greater than 100 nm [4], in order to avoid regulatory issues, including the needed to perfome cito- and genotoxicity studies [5,6] and to prevent nano-ethical [7] problems.

This premise was thoroughly, as evidenced by the particle size distribution: d(0.5) > 100 nm.

High values of Span were recorded for TiO_2, which can be explained by hydrophobic character of this type and probably associated with an aggregation phenomenon.

Particle size distribution (μm)			
Span	**d (0.1)**	**d (0.5)**	**d (0.9)**
37.36 ± 1.38	0.14 ± 0.01	0.19 ± 0.01	7.12 ± 0.30

Table 2: Particle size distribution of TiO2 (mean ± SD,n=2).

2.2 Characterization Studies of MSS

Emulsion Type

Theoretically the type of emulsion was already defined - w / o, however to assure its veracity further studies concerning the determination of the type of the emulsion were performed.

The emulsions were inspected visually and a drop of emulsion was added to water and to liquid paraffin (oil).

A drop of oil mixed very fast within the emulsion sample, unlike the water drop, showing that oil corresponded to the continuous phase as expected. Finally, the microscopic observation of the emulsion stained with methylene blue (hydrophilic dye) confirmed the presence of a w/o emulsion system (Figure 2).

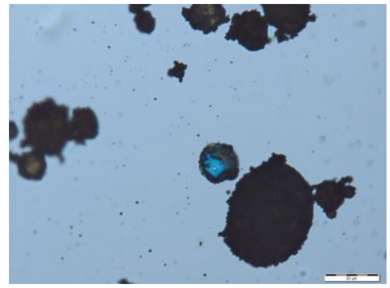

Figure 2: Emulsion dyed with methylene blue visualized by optical microscopy. Scale bar: 20µm.

Droplet Size

In this assay we evaluated the influence of the TiO_2 percentage on Pickering emulsions droplet size. Assuming that by getting smaller droplets it was possible to obtain a more stable emulsion and that an increase in the content of the solid particles leads to a dense coating of the droplet, the excess of TiO_2 will allow forming smaller droplets with a larger surface area.

Based in these results it can be inferred that an increase in the TiO2 will produce an emulsion with smaller droplets (Table 3 and Figure 3).

Similar results have already been published by other authors, such as Li et al. [8], who determined in their experiments that a a higher concentration of solid particles can stabilize a larger total interfacial area, which in turn means that a smaller average droplet size can be achieved.

	Droplet size **distribution (μm)**			
MSS	**Span**	**d (0.1)**	**d (0.5)**	**d (0.9)**
1	0.402	8.365	12.209	22.861
2	0.849	2.161	4.343	7.517
3	1.578	1.273	2.227	4.506
4	2.756	1.006	1.931	6.167

Table 3: Influence of the TiO_2 percentage on Pickering emulsions droplet size – d(0.1), d(0.5), d(0.9) and Span. (mean; n=625).

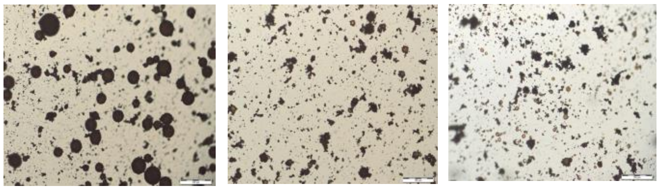

Figure 3: Emulsion samples with different TiO_2 percentage visualized by optical microscopy: left- 7.5%; center- 15% and right- 25%. Scale bar=100 µm.

Efficacy of the Sunscreen Formulations

Theoretical SPF

According to the desired SPF and the limits imposed by the UE, the limit of 25% for TiO_2 could not be exceeded. Thus, it was necessary to combine UV-filters according to its SPF to achieve the maximum protection as possible. Using the BASF simulator and considering the droplet size assay, we tried to establish some formulations that meet the criteria of SPF 50+. Regarding TiO_2 content, we tried not exceed 20% in order to avoid values very close of the maximum allowed concentration and to ensure that this limit wasn't exceeded. Only with 25% of TiO_2 (physical filter) is sufficient to achieve the desired protection factor – 50.

	MSS			
	1	**2**	**3**	**4**
SPF	14	19	30	51.1

Table 4: SPF obtained with different % of TiO_2

Regarding the Pickering Emulsions, they proved to be a good solution, allowing the combination of stabilizing and photoprotective properties of TiO2 with the melatoninergic action. The result obtained was a simple emulsion that offers an excellent compromise between stability, UV protection and cosmeticity and that could allow an easy scale-up.

Figure 4: Topical application of MSS.

3 CONCLUSIONS

The formulation obtained showed good cosmeticity and to be of a low irritant nature. Surprisingly, a TiO_2-based Pickering emulsion containing suitable excipients provided good results in terms of physical stability and high UV protection. Consequently, it offers an excellent compromise between stability, UV protection and cosmeticity.

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