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## Lignosulfonate is an efficient SPF booster: Application to eco-friendly sunscreen formulations

Faustine Lorquin<sup>a,b</sup>, Jean Lorquin<sup>b,\*</sup>, Magalie Claeys-Bruno<sup>a</sup>, Marion Rollet<sup>c</sup>, Maxime Robin<sup>a</sup>, Carole Di Giorgio<sup>a</sup>, Philippe Piccerelle<sup>a</sup>

<sup>a</sup> Aix-Marseille Université, Mediterranean Institute of Marine and Terrestrial Biodiversity and Ecology (IMBE), 27 Boulevard Jean Moulin, 13385, Marseille Cedex 5, France

<sup>b</sup> Aix-Marseille Université, Mediterranean Institute of Oceanology (MIO), 163 Avenue de Luminy, 13288, Marseille Cedex 9, France

<sup>c</sup> Aix-Marseille Université, Institut de Chimie Radicalaire (ICR), 52 Avenue Escadrille Normandie Niemen, 13397, Marseille Cedex 20, France

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### ABSTRACT

Sunscreen oil-in-water emulsions containing few ingredients and two EU-authorized organic filters had been developed in an eco-friendly approach. Based on their photostability, spectroscopic features, and the lack of data on toxicity, BEMT (UVA/B range; bis-ethylhexyloxyphenol methoxyphenyl triazine) and DHHB (UVA; diethylamino hydroxybenzoylhexyl benzoate) were selected and incorporated at minimal concentrations to reduce the risk of impact on human health and coastal marine ecosystems. Despite the inconclusive results previously reported, the use of the w-soluble and largely available Na-lignosulfonate (LiS) had been reconsidered with success. Since BEMT and DHHB alone or in combination were not able to higher the sun protection factor (SPF) value at 50, results showed that it becomes possible by supplementing with LiS at 5% (w/w), ensuring stability, anti-radical property, and a non-toxicity of the sun emulsion. After defining the range doses for the three components, minimizing concentrations was achieved by experimental design studies using a response surface methodology in which SPF values before and after irradiation has been considered. Consequently, an SPF<sub>30</sub> and SPF<sub>50</sub> emulsions containing only 9 and 12% total filter respectively and 5% LiS each had been developed. This high boosting effect led to discussions on how LiS interacts, suggesting the involvement of J aggregation, the formation of LiS micelles that would partly encapsulate the o-soluble filters, and the mode of adsorption at the solid-liquid interface of the poly(methyl-methacrylate) plate or the skin.

### 1. Introduction

To prevent the damaging effects of sun exposures, organic filters have been developed usually as aromatic compounds that absorb UV radiations and release lower energy rays. However, most of the time these synthetic absorbers may have negative effects on human health and the environment. Indeed, (1) UV absorption may activate organic filters that consequently interact with cutaneous molecules, cause dermatitis or photosensitivity reactions (Dromgoole and Maibach, 1990), exhibit neurotoxic effects (Ruszkiewicz et al., 2017), pro-carcinogenic activities (Kerdivel et al., 2013), and generate ROS which are potential mutagens when applied topically to the UV-exposed skin (Hanson et al., 2006). Consequently, the terms ‘endocrine disruptor’ and ‘allergenic substance’ appeared a few years ago for most of the synthetic sunscreens (Schlumpf et al., 2001; Singh et al., 2011;

Birkhäuser, 2016). (2) Most of them are not stable under UV radiation, e.g. the widely used homosalate (HMS, UVB) and avobenzone (BMDM, UVA) (see Supplementary material 2), which are photodegraded into free radicals (Allen et al., 1996; MacManus-Spencer et al., 2011; Miura et al., 2012). If their stabilization could be assessed by immobilization in a sol-gel matrix or most widely by the inclusion of triplet-triplet quenchers (Chodorowsky-Kimmes, 2004; Paris et al., 2009), extra ingredients are needed. (3) Due to their continuous and persistent usage, some organic UV filters became potential threats to the ecological environment. Higher sunscreen concentrations were found in rivers, lakes, seawaters, taps, groundwaters, and swimming pools where they may generate chlorine by-products (Ramos et al., 2015). In coastal seawaters, they are endocrine disorders for mussels and fishes by mimicking estrogens and cause alterations of fecundity in aquatic animals (Kunz and Fent, 2006; Fent et al., 2008; Nakata et al., 2012). Notably,

\* Corresponding author.

E-mail address: [jean.lorquin@mio.osupytheas.fr](mailto:jean.lorquin@mio.osupytheas.fr) (J. Lorquin).

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sunscreens had been reported to cause complete bleaching of some representative coral species from the Pacific, Atlantic, and the Indian Ocean, at extremely low concentrations, by inducing the lytic viral cycle in symbiotic zooxanthellae with latent infections (Danovaro et al., 2008). As another illustration, benzophenone-3 was reported as a genotoxicant, transforming planulae and cells of the hard coral *Stylophora pistillata* to a deformed, sessile condition (Downs et al., 2016).

Nowadays all over the world, industries are trying to reduce the number of ingredients in their cosmetic preparations, especially in sunscreen preparations. Replacing synthetic filters with natural, sustainable, and non-toxic molecules had also been considered. Natural polyphenols not only are potential skin protectors with high antioxidant abilities, but they also absorb at UV light wavelengths, exhibit SPF boosting properties (Stevanato et al., 2014; de Alencar Filho et al., 2016), repair DNA (Nichols and Katiyar, 2010), and rarely give sensitization effects probably because they are components of the diet. For instance, apigenin, caffeic acid, and resveratrol were found to exhibit interesting sun protection factors (SPF) of 28.8, 28.0, and 19.2 respectively, at only 7% (w/v) concentration (Stevanato et al., 2014), as well as other known flavonoids (de Alencar Filho et al., 2016). However, they have poor photostability and their sunscreen performance would weaken after UV irradiation (Chaabana et al., 2017). From a legal point of view, as regards current legislation imposed in each country, cosmetic manufacturers must formulate solar filters that belong to a defined and restricted list. Article 14 from the EU-Regulation number 1223/2009, states that “cosmetic preparations cannot contain UV filters other than those belonging to the annex VI”, a list of 28 filters comprising two minerals. For instance, karanja oil from seeds of *Pongamia pinnata* was very attractive for manufacturers but is not an authorized UV filter, and if it is used, companies must prove that the SPF is the result of other authorized sunscreens. The only strategy thus remains in finding an efficient natural booster able to raise the SPF to a high value from low authorized filter concentrations.

SPF boosters were merely emollients and polymers that efficiently dissolved UV organic absorbers mainly crystalline. For a few years, this view has changed and could no longer be limited to emollient actions or a contribution of extra UV-absorbers added. Recently, lignin, the second most abundant component in plants and discharged as byproducts, was found to significantly enhance the sunscreen performance of commercial creams supplemented with octyl methoxycinnamate (OMC, UVB filter) and avobenzone (BMDM, UVA) (Qian et al., 2016, 2017). These two commonly used filters while photo-unstable can be stabilized by combining them with triplet quenchers such as octocrylene (OCR) or triazine derivatives (Chatelain and Gabard, 2001; Damiani et al., 2006; Paris et al., 2009). Moreover, OMC has demonstrated an endocrine disruptor (Klammer et al., 2005; Heneweer et al., 2005; Seidlová-Wuttke et al., 2006). The case of BMDM is more delicate since, considered safe on MCF-7 cells (Schlumpf et al., 2001, 2004), it has recently been described as an endocrine disruptor on other more adapted cell lines (Klopčič et al., 2017). Qian et al. (2016) also showed that non-treated hydrophobic lignin such as organosolv (OLV) lignin had better sunscreen performance than hydrophilic counterparts Na-lignosulfonate (LiS) and alkali (ALK) lignin. The addition of 1, 5, and 10% (w/w) of OLV increased the SPF value of the emulsion to 31.5, 55.7, and 91.6 respectively, since LiS at the same doses enhanced the SPF to 34.6, and decreased after at 22.0 and 20.1, respectively.

In this work, we have developed sunscreen formulations containing few ingredients in an eco-friendly approach. Based on photostability and safe properties, two EU-authorized organic filters BEMT and DHHB have been selected then incorporated in the simplified oil-in-water emulsion. To enhance the SPF value and minimize the filter content, the sustainable and largely available Na-lignosulfonate has been re-considered. By experimental design studies using response surface methodology, the concentration of the three active components has been minimized for the highest UV protection, allowing the expansion of a more environmentally friendly sun lotion line.

## 2. Materials and methods

### 2.1. Chemicals and lignin providing

Chemicals were of the highest grade available and purchased from Sigma (Saint-Quentin-Fallavier, France). All solvents were of HPLC-mass grade and supplied by Biosolve (Dieuze, France), SEC (size exclusion chromatography) standards by Agilent Technologies (Les Ulis, France). Media for human keratinocytes and mouse fibroblasts cultures were from Dutscher (France). For preparing formulations, common chemicals such as Na<sub>2</sub>-EDTA, glycerin, and xanthan gum were from Fagron (Nazareth, Belgium), Tribehenin PEG-20 esters, and caprylic-capric triglycerides (Labrafac CC) from Gattefossé (Saint-Priest, France). The other surfactants tested were Simulgel 600, Montanov L, Simulgreen 18-2 (from Seppic, La Garenne Colombes, France), and Emullium Melifera (Gattefossé, France). For the UV filters, bis-ethylhexyloxyphenol methoxyphenyl triazine (BEMT, or Tinosorb S), diethylamino hydroxybenzoylhexyl benzoate (DHHB, Uvinul APlus), ethylhexyl triazine (EHT, Uvinul T150) were provided by BASF Personal Care and Nutrition (Monheim, Germany). The others such as ethylhexyl salicylate (EHS, Eusolex OS), ethylhexyl methoxycinnamate (OMC, Eusolex 2292), butylmethoxydibenzoylmethane (BMDM, Eusolex 9020), octocrylene (Eusolex OCR), and homosalate (HMS, Eusolex HMS), were supplied by Merck KGaA (Darmstadt, Germany). Pure cream used was a Nivea refreshingly soft moisturizing cream (200 mL) bought from large-area drug markets (France). All lignins were in the form of fine powder previously dried at 70 °C for 24 h before use. Sodium lignosulfonate lignin (LiS, CAS 8061-51-6) was extracted from needle-leaved trees in Japan through sodium sulfite treatment and was purchased from TCI (product number L0098, see Supplementary material 1) as a dark orange powder. Organosolv lignin (OLV, brown-black color) from wheat straw was a gift of Compagnie Industrielle de la Matière Végétale (CIMV, laboratory of Toulouse, France), and Kraft lignin (brown) from softwood pine a gift of Fibre Excellence Co. (plant of St Gaudens, France). Alkaline lignin (ALK, CAS 8068-05-1, product number 471003, brown) was purchased from Sigma.

### 2.2. Formulation procedure

The composition is shown in Table 1. The preparation consisted of

**Table 1**

Ingredients used in the formulation (see 2.2. for preparation). A, aqueous phase; B, oil phase. The citrate buffer was an appropriate mixture of citric acid (1x H<sub>2</sub>O) solution adjusted at pH 6.0 with a 3Na-citrate (2x H<sub>2</sub>O) solution, each at 100 mM in milliQ-H<sub>2</sub>O.

Phase	Ingredient (INCI name)	Supplier	Function	% (w/w)
A	H <sub>2</sub> O milliQ	–	–	qsp
A	Na-citrate buffer 0.1 M, pH 6.0 (in H <sub>2</sub> O)	Sigma	excipient	60
A	EDTA	Fagron	ion chelator	0.1
A	Glycerin	Fagron	humectant	3.0
A	Xanthan gum	Fagron	texturing agent	0.2
A	Lignosulfonic acid sodium salt	TCI Europe	SPF booster	1–5
B	Tribehenin PEG-20 esters (Emullium 22)	Gattefossé	emulsifier	4.0
B	Caprylic-capric triglycerides	Gattefossé	emollient	7.0
B	Bis-ethylhexyloxyphenol methoxyphenyl triazine (BEMT, or Tinosorb S)	BASF	sunblocker UVA/B	see text
B	Diethylamino hydroxybenzoyl hexyl benzoate (DHHB, or Uvinul A Plus)	BASF	sunblocker UVA	see text
<b>Total</b>				<b>100</b>

two oil-in-water (o/w) phases with a reduced number of ingredients and the o-phase contained two organic filters. The w-phase was prepared by dissolving solid Na<sub>2</sub>-EDTA in the adequate volume of Na-citrate buffer 100 mM at pH 6.0, with a high-speed agitator VMI-Rayneri Turbotest V2004 (Montaigu, France) at 300 rpm for 2 min, in which Naliginosulfonate was added until complete dissolution by gently sprinkling to avoid foam formation. Then glycerin (liquid) and xanthan gum (fine powder) were added slowly by heating at 85 °C and the agitation was maintained for 45 min. The o-phase was prepared by successively mixing under moderate magnetic agitation at 85 °C, Tribehénin PEG-20 esters (liquid), caprylic-capric triglyceride (liquid), and one or two organic UV filters until complete dissolution, depending on the assays. For SPF<sub>30</sub> and SPF<sub>50</sub> preparation, BEMT and DHHB both as powder were added in the o-phase. In the final emulsion stage, the o-phase was added to the w-phase and the mixture homogenized with an Ultra-Turax IKA digital T25 (Staufen, Germany) equipped with a 1 cm stator diameter, at 10,000 rpm for 5 min at 85 °C. The resulting emulsion was agitated again but this time with a Heidolph Hei-Torque 200 blade-stirrer (Schwabach, Germany) at 250 rpm for at least 20 min until the decrease of the temperature at 30–40 °C. Finally, 100 g of sunscreen preparation were stored in hermetically closed 150 mL flasks in the dark at room temperature.

### 2.3. In vitro SPF and UVA-PF determination, UV-visible spectrophotometry

To evaluate SPF and UVA-PF (UVA protection factor), the emulsion was deposited on the roughness face of 50 × 50 mm square poly(methyl-methacrylate) (PMMA, from Europlast, Aubervilliers, France) plates with defined physical properties, *i.e.* transparent to UV radiations, 6 μm roughness to simulate the texture of human skin, and 3 mm thickness. The amount of sunscreen was ~30 mg per plate (1.3 mg cm<sup>-2</sup>) which were precisely weighed and deposited in nine small well-distributed piles, and then evenly spread over the entire surface for 30 s with the finger (Fageon et al., 2009), until a homogeneous distribution over the entire surface was achieved. Then the plated samples were left for 15 min in the dark at room temperature to ensure a leveling of the formula and resulting in a weight of the plate of 15 ± 5 mg. Three UV transmission spectra (from 290 to 400 nm, in steps of 1 nm) were recorded for each side of the square plate, with a spectrophotometer Shimadzu UV-2600 equipped with an integrating sphere accessory to collect and measure the scattered light from the PMMA plate. According to Diffey and Robson (1989), SPF and UVA-PF data were acquired by the SPF-Calculator software (from Shimadzu). Data were averages of five determinations, *i.e.* five PMMA plates for 3 determinations (3 sides) per plate, acceptable covariance must be ≤ 17%. For SPF prediction, the BASF sunscreen simulator ([www.sunscreensimulator.basf.com](http://www.sunscreensimulator.basf.com)) was configured with a value of 1.5 mg cm<sup>-2</sup> in the software settings.

BEMT and DHHB separately and mixed were dissolved in tetrahydrofuran (THF) and analyzed by UV-visible spectrophotometry from 290 to 400 nm in quartz cuvettes with a Cary 60 device (Agilent Technologies). The stock solutions of BEMT and DHHB in THF were at 85 and 35 mg mL<sup>-1</sup>, respectively, and diluted 4000x each before scanning. The mixture miming the composition of an SPF<sub>50</sub> emulsion contained 85 mg BEMT and 35 mg DHHB in one mL THF and was diluted and analyzed in the same manner.

## 2.4. Experimental design and mathematical modeling

### 2.4.1. Experimental domain

To determine the minimal filter concentrations in presence of LiS with fixed and desired SPF values, an experimental design using the Azurad software (a company of Marseille), has been conducted. The experimental responses Y<sub>i</sub> were the SPF values taken before (Y<sub>1</sub>) and after irradiation (Y<sub>2</sub>). The study considered three factors (X<sub>i</sub>, all in w/w) defining an experimental domain, within the following experimental

range imposed: X<sub>1</sub>, percentage of BEMT (5–10%); X<sub>2</sub>, percentage of DHHB (0–5%); X<sub>3</sub>, percentage of LiS (1–5%). The domain of interest was represented by a cubic form, in which the three dimensions correspond to the concentrations of the three factors.

### 2.4.2. Mathematical modeling and choice of the experimental design

To achieve the objectives, an infinite number of experiments in all points of the domain of interest would be necessary. To help us collect the same information with fewer experiments, an empirical mathematical model was used. This chosen tool is a postulated second-degree polynomial model that considers possible curvatures in the domain. After validation, the chosen model will represent the phenomenon throughout the experimental domain; it was written as follows:

$$Y_i = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{33} X_3^2 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3$$

The coefficients β<sub>i</sub> were estimated by using the experimental results obtained for the runs carefully chosen. For that, a central composite design was used (Myers and Montgomery, 1995; Sarabia and Ortiz, 2009), with 14 experiments (1–14), and a three-repeated experiment (number 15, *i.e.* 15-1 to 15-3) in the center of the domain (summarized in Table 4). The model coefficients β<sub>i</sub> were calculated from the 17 experiments using multi-linear regression for the two responses (Y<sub>1</sub>, Y<sub>2</sub>). Further, to validate the previous model, four test-points (runs 16 to 19) chosen in the domain were added (Claeys-Bruno et al., 2006).

### 2.4.3. Desirability

The desired final response SPF before (Y<sub>1</sub>) and after irradiation (Y<sub>2</sub>) had been chosen for elaborating two emulsions (SPF<sub>30</sub> and an SPF<sub>50</sub>) adapted to the market. Desirability function (D), a multicriterion optimization tool has further been investigated for developing formulations containing minimal active ingredients concentrations with the desired SPF value. This tool allowed finding the best compromise between the two responses: at any point of the domain, predicted response values have been transformed into a D function representing the degree of satisfaction. To maximize the SPF values before and after irradiation, two curves of the D function was drawn: the d<sub>1</sub> and d<sub>2</sub> functions have been respectively set at 0% when the responses are unacceptable (SPF<sub>1</sub> < 30, and SPF<sub>2</sub> < 50), and at 100% when the values are equal or more than 30 and 50 (desired values). The overall D is calculated within the experimental domain by D = (d<sub>1</sub> × d<sub>2</sub>)<sup>1/2</sup>, with D = 0% for undesirable values of the response (Y<sub>1</sub> or/and Y<sub>2</sub>), D = 100% for desirable values, and 0 < D < 100% for acceptable values of the response *i*. When an undesirable value is obtained for, at least one response (Y<sub>1</sub> < 30 or Y<sub>2</sub> < 50), the overall desirable value D is 0% and no compromise has been found. On the contrary, when each requirement gets completely satisfied, the overall D value is 100%. Finally, when 0 < D < 100%, an acceptable compromise between the different responses has been found.

## 2.5. Toxicological assays and biological activities

Because LiS lignin cannot be dissolved together with the two sunscreens in the same solvent, toxicological properties were first evaluated from the SPF<sub>50</sub> and SPF<sub>30</sub> emulsions developed in this work. LiS was prepared alone at 5 and 10 mg mL<sup>-1</sup> stock solutions in milliQ-H<sub>2</sub>O for determination of the antioxidant activity and cytotoxicity-phototoxicity assays, respectively.

### 2.5.1. Cytotoxicity and phototoxicity

The viability of human epidermal keratinocytes neonatal cells was expressed by the vital dye Neutral Red (NR) technique, as already described (Lorquin et al., 2021), except that the medium was replaced by 200 μL of complete medium containing the sample, *i.e.* 8 concentrations at 0–1000 μg mL<sup>-1</sup> diluted in HBSS (Hank's Balanced Salt Solution, from Dutscher). The concentration of the pigment causing a 50%

Table 2

SPF boost effect and photo-stability studies with the developed formulation containing one organic filter and lignosulfonate (LiS), before and after radiation.

Filter <sup>a</sup>	UV region	Pure state	LiS (%)	pH	Viscosity (mPa.s)	Before irradiation				After irradiation				Irr. effect (± %) <sup>b</sup>
						SPF	Boost (%)	UVA-PF	Boost (%)	SPF	Boost (%)	UVA-PF	Boost (%)	
BEMT	UVA/B	solid	0	5.8	5599	13.35 ± 1.75	–	8.19 ± 0.85	–	13.33 ± 2.04	–	7.75 ± 0.83	–	NS <sup>c</sup>
			5	5.8	17316	20.51 ± 3.01	53.6	10.44 ± 0.99	27.5	19.93 ± 2.66	–	9.99 ± 0.89	28.9	NS
			10	5.9	19916	19.95 ± 2.66	49.4	10.02 ± 0.88	22.3	21.62 ± 1.79	–	10.58 ± 0.50	36.5	NS
DHBB	UVA	solid	0	5.9	12997	3.71 ± 0.26	–	11.55 ± 0.63	–	3.70 ± 0.36	–	11.44 ± 1.00	–	NS
			5	5.8	32513	4.62 ± 0.67	24.5	11.60 ± 1.31	0.4	4.12 ± 0.41	–	9.84 ± 1.13	<0	NS
			10	5.9	39592	8.72 ± 1.11	135.0	17.2 ± 2.44	48.9	8.27 ± 1.02	–	17.58 ± 2.41	53.7	NS
EHS	UVB	liquid	0	5.7	7758	2.53 ± 0.05	–	0.86 ± 0.01	–	1.76 ± 0.14	–	0.84 ± 0.01	–	–30.4
			5	5.8	27794	3.79 ± 0.13	49.8	1.08 ± 0.01	25.6	1.01 ± 0.06	–	1.1 0.01	20.2	–73.3
			10	5.8	28714	5.64 ± 0.23	122.9	1.36 ± 0.02	58.1	4.14 ± 0.39	–	1.31 ± 0.02	55.9	–26.6
OMC	UVB	liquid	0	5.9	17276	8.06 ± 0.71	–	0.96 ± 0.00	–	6.45 ± 0.31	–	1.00 ± 0.01	–	–19.9
			5	5.9	18036	12.12 ± 0.74	50.3	1.21 ± 0.03	26.0	9.81 ± 0.74	–	1.22 ± 0.02	22.0	–19.0
			10	5.8	21395	12.78 ± 1.12	58.5	1.44 ± 0.03	50.0	11.16 ± 1.34	–	1.46 ± 0.03	46.0	–12.7
BMDM	UVA	solid	0	5.7	21755	4.26 ± 0.28	–	8.95 ± 0.85	–	2.86 ± 0.19	–	4.62 ± 0.57	–	–32.8
			5	5.8	10998	7.34 ± 0.19	72.3	12.75 ± 0.55	42.4	5.18 ± 0.25	–	8.10 ± 0.62	75.3	–36.4
			10	5.8	14317	10.68 ± 0.83	150.7	17.25 ± 1.53	92.7	6.46 ± 0.66	–	6.57 ± 1.21	42.2	–61.9
OCR	UVB	liquid	0	5.9	11358	5.75 ± 0.39	–	1.25 ± 0.03	–	5.42 ± 0.46	–	1.22 ± 0.03	–	NS
			5	5.9	31313	8.51 ± 0.77	48.0	1.53 ± 0.02	22.4	7.71 ± 0.59	–	1.46 ± 0.03	19.6	NS
			10	5.9	31913	11.40 ± 1.66	98.2	1.87 ± 0.08	49.2	11.31 ± 1.51	–	1.80 ± 0.06	47.5	NS
HMS	UVB	liquid	0	5.8	3439	2.55 ± 0.07	–	0.83 ± 0.01	–	2.00 ± 0.13	–	0.82 ± 0.01	–	–21.5
			5	5.9	13917	3.40 ± 0.23	33.3	1.04 ± 0.01	25.3	2.73 ± 0.08	–	1.00 ± 0.01	21.9	–19.7
			10	5.7	19356	4.85 ± 0.15	90.2	1.30 ± 0.02	56.6	4.07 ± 0.16	–	1.24 ± 0.02	51.2	–16.0
EHT	UVB	solid	0	5.8	4479	6.12 ± 0.07	–	0.87 ± 0.01	–	6.45 ± 0.16	–	0.87 ± 0.01	–	+5.4
			5	5.9	8878	8.59 ± 0.29	40.3	1.15 ± 0.03	32.1	9.33 ± 0.43	–	1.21 ± 0.03	39.0	+8.6
			10	5.8	11679	11.45 ± 1.32	87.0	1.39 ± 0.06	59.7	11.24 ± 1.39	–	1.34 ± 0.06	54.0	NS

<sup>a</sup> All filters were here at 5%, LiS at 0, 5, and 10% (w/w), and each SPF value was determined from 5 PMMA plates (see methods).

<sup>b</sup> Irradiation effects were determined from the SPF values corresponding to the UV region covered by the filter. Filters tested were BEMT (or Tinosorb S), bis-ethylhexyloxyphenol methoxyphenyl triazine; EHS, ethylhexyl salicylate; OMC, ethylhexyl methoxycinnamate; BMDM, butyl methoxydibenzoylmethane; DHBB, diethylamino hydroxybenzoyl hexylbenzoate; OCR, octocrylene; HMS, homosalate; EHT, ethylhexyl triazone. The covariance was acceptable (<17%, according to EU legislation).

<sup>c</sup> NS, non-significative value. All formulations were stable after the centrifugation method at 48 h. In all cases,  $\lambda_c > 370$  nm, and the UVA/UVB ratio  $\geq 0.33$ .

release of NR as compared to the control culture ( $IC_{50}$ , in  $\mu\text{g}\cdot\text{mL}^{-1}$ ), was calculated using the Phototox v2.0 software (Zebet, Germany). Phototoxicity was evaluated by the in vitro and normalized 3T3 NRU assay (OECD number 432) except that the medium was replaced by 100  $\mu\text{L}$  of HBSS containing 8 sample concentrations, 0–1000  $\mu\text{g}\cdot\text{mL}^{-1}$  diluted in HBSS. The Photo-Irritation-Factor (PIF) defined by the ration  $IC_{50}(-\text{Irr})/IC_{50}(+\text{Irr})$  was expressed to finalize the results, a test substance exhibiting a PIF <2 predicts no phototoxicity,  $2 < \text{PIF} < 5$  a probable, and PIF >5 a phototoxicity.

### 2.5.2. LiS DPPH-antioxidant activity

The assay consisted of mixing 0.6 mL of 350  $\mu\text{M}$  DPPH (1,1-diphenyl-2-picrylhydrazyl; in MeOH) with 0.3 mL of aqueous LiS lignin samples at

0–500  $\mu\text{g}\cdot\text{mL}^{-1}$  final concentration, and measurements were performed as already described (Lorquin et al., 2021). Trolox, ascorbic acid, and propyl gallate (3,4,5-trihydroxybenzoic acid propyl ester) were used as positive controls, the radical scavenging activity was expressed as the mean (triplicate)  $\pm$  SD of the  $EC_{50}$  value (in  $\mu\text{g}\cdot\text{mL}^{-1}$ ) determined by the Prism 7.0 software (Lorquin et al., 2021).

### 2.6. Irradiation and photostability assays

Photostability studies were performed on the emulsion-containing PMMA plates (see section 2.3) or on the formulations previously spread on a Petri dish which was placed and irradiated in a Suntest CPS<sup>+</sup> solar simulator (from Atlas, Moussy-Le-Neuf, France) equipped with a

**Table 3**

SPF and UVA-PF values with the developed formula, before and after irradiation, with combinations of BEMT and DHHB at different concentrations (maximal EU authorized), and in presence of amounts of LiS (1 or 5%, w/w). The covariance was acceptable (<17%, according to EU legislation). In all cases, the critical wavelength  $\lambda_c > 370$  nm, and the UVA/UVB ratio  $\geq 0.33$ .

Composition	pH	Before irradiation		After irradiation	
		SPF	UVA-PF	SPF	UVA-PF
<b>Control</b>					
- BEMT, - DHHB, - LiS	6.0	0.86 ± 0.03	0.84 ± 0.01	0.81 ± 0.01	0.85 ± 0.02
- BEMT, - DHHB, + LiS 5%)	6.0	1.44 ± 0.04	1.07 ± 0.01	1.27 ± 0.03	1.04 ± 0.01
<b>Max. concentrations studied</b>					
BEMT 10%	5.9	18.48 ± 2.25	11.67 ± 2.57	14.82 ± 2.35	8.53 ± 1.90
BEMT 10%, LiS 5%	5.8	<b>57.46</b> ± 2.15	<b>24.69</b> ± 3.08	<b>44.45</b> ± 3.01	<b>16.50</b> ± 2.41
DHHB 5% <sup>b</sup>	5.9	3.71 ± 0.12	11.55 ± 0.05	3.70 ± 0.36	11.44 ± 0.20
DHHB 5%, LiS 5% <sup>b</sup>	5.8	4.62 ± 0.08	11.60 ± 0.22	4.12 ± 0.13	9.84 ± 0.29
BEMT 10%, DHHB 5%	5.9	27.22 ± 2.13	23.33 ± 1.10	26.51 ± 2.88	21.91 ± 1.43
BEMT 10%, DHHB 5%, LiS 1%	5.9	<b>54.23</b> ± 3.82	<b>38.56</b> ± 3.03	<b>73.08</b> ± 4.07	<b>44.56</b> ± 4.55
BEMT 10%, DHHB 5%, LiS 5%	5.7	<b>78.96</b> ± 4.48	<b>52.96</b> ± 3.79	<b>106.0</b> ± 5.01	<b>61.69</b> ± 4.97
<b>Applications (see Exp. design)</b>					
BEMT 7%	5.8	16.37 ± 1.74	9.54 ± 0.64	14.29 ± 2.67	8.81 ± 1.01
BEMT 7%, LiS 5%	5.8	29.51 ± 1.43	15.30 ± 0.34	28.73 ± 3.96	13.11 ± 1.53
DHHB 2%	5.9	1.70 ± 0.09	3.78 ± 0.22	1.61 ± 0.10	3.66 ± 0.30
DHHB 2%, LiS 5%	5.8	2.77 ± 0.14	4.88 ± 0.34	2.52 ± 0.14	4.52 ± 0.32
BEMT 7%, DHHB 2%	5.9	20.01 ± 2.04	13.90 ± 0.84	19.46 ± 1.84	14.09 ± 1.02
BEMT 7%, DHHB 2%, LiS 5%	5.7	<b>34.13</b> ± <b>5.04</b>	<b>22.50</b> ± <b>2.51</b>	<b>34.78</b> ± <b>5.99</b>	<b>22.60</b> ± <b>3.75</b>
BEMT 8.5%	5.9	20.31 ± 4.23	12.93 ± 1.80	18.20 ± 3.04	11.91 ± 0.98
BEMT 8.5%, LiS 5%	5.8	39.05 ± 3.62	20.05 ± 3.07	34.05 ± 4.21	22.68 ± 4.3
DHHB 3.5%	5.9	2.47 ± 0.18	6.36 ± 0.51	2.37 ± 0.19	6.20 ± 0.63
DHHB 3.5%, LiS 5%	5.8	3.64 ± 0.21	6.95 ± 0.50	3.34 ± 0.20	6.74 ± 0.48
BEMT 8.5%, DHHB 3.5%	5.8	25.01 ± 4.34	16.12 ± 2.66	24.62 ± 2.86	17.34 ± 1.83
BEMT 8.5%, DHHB 3.5%, LiS 5%	5.7	<b>50.26</b> ± <b>4.67</b>	<b>31.34</b> ± <b>2.22</b>	<b>52.84</b> ± <b>7.61</b>	<b>30.09</b> ± <b>3.09</b>

UVC cut-off glass filter restricting transmission of light below 290 nm, and another near IR-blocking filter. The xenon lamp energy was fixed at 550 W m<sup>-2</sup> for 30 min (99 J cm<sup>-2</sup> UVA-visible irradiation), the incident dose composed of 8.9 J cm<sup>-2</sup> UVA and 90.1 J cm<sup>-2</sup> visible-light. In the simulator, the specimen table was maintained at 4 °C by a fluid circulating underneath and the air chamber at 25 °C, ensuring a constant ambient temperature of the samples during irradiation. The photostability of LiS was evaluated on 4 mL glass-closed tubes (external diameter 15 mm) containing 3 mL of LiS solution at 0.05 and 0.1 mg mL<sup>-1</sup> in milliQ-H<sub>2</sub>O. In the simulator, tubes placed horizontally were irradiated respecting the ICH Q1B guidelines (European Medicines Agency). The strong irradiance was 550 W m<sup>-2</sup> for 1 h (i.e. 198 J cm<sup>-2</sup> UVA-visible irradiation). Changes in the lignin structure were monitored by UV-visible spectroscopy from 200 to 700 nm and size exclusion chromatography (SEC, see below), comparatively to non-irradiated samples.

## 2.7. Extraction and HPLC control of the sunscreens in emulsions

One g of cream in a 15 mL Falcon tube was extracted by adding 10 mL of a cocktail of hexane-AcOEt-iPrOH (1:1:1, v/v/v), and the mixture vigorously agitated for 5 min with a vortex. After centrifugation (6500 g, 20 min), the yellow supernatant was removed, evaporated to dryness *in vacuo* with a rotavapor, the residue dissolved in 60 mL THF, and 5 µL analyzed by RP-HPLC. HPLC separations were carried out with a Waters system composed of a 1525 binary pump, a 2996-diode array detector, a Rheodyne 7725i manual injector, and files acquired with the Empower software. The column was a Cortecs C<sub>18</sub> column (4.6 × 75 mm, 2.6 µm pore size, from Waters) protected by a guard cartridge, and eluted by an isocratic solvent of acetonitrile-THF-H<sub>2</sub>O (40:40:20, v/v), at 50 °C (oven temperature) and a flow rate of 1.0 mL min<sup>-1</sup> for 30 min. DHHB and BEMT were visualized at 330 nm, identified by their respective retention time (rt) and UV-visible spectra (Shaht, 2010), compared to those of a standard mixture of 10 mg of each filter in 50 mL THF (0.2 mg mL<sup>-1</sup> each) injected in the same conditions. To quantify, standard curves were

**Table 4**

Parameters of experimental runs with the responses obtained before and after irradiation. All covariance was acceptable (<17%, according to EU legislation). In all cases, the critical wavelength  $\lambda_c > 370$  nm, and the UVA/UVB ratio  $\geq 0.33$ .

Exp n°	<sup>a</sup> X <sub>1</sub> [TinS]	<sup>a</sup> X <sub>2</sub> [DHHB]	Total filter	<sup>a</sup> X <sub>3</sub> [LiS]	Viscosity (mPa.s)	pH	Before irradiation			After irradiation		
							Y <sub>1</sub> SPF	UVA-PF	<sup>b</sup> CoV	Y <sub>2</sub> SPF	UVA-PF	<sup>b</sup> CoV
1	5	0	5	1	1180	5.9	11.36	7.3	0.072	12.85	7.64	0.068
2	10	0	10	1	1420	6.0	38.13	20.01	0.055	27.3	14.1	0.153
3	5	5	10	1	2230	6.0	20.21	19.92	0.092	21.3	20.06	0.131
4	10	5	15	1	3749	5.9	54.23	38.56	0.139	73.08	44.56	0.033
5	5	0	5	5	3369	5.8	15.97	9.06	0.090	16.01	8.61	0.101
6	10	0	10	5	3729	5.8	53.57	25.99	0.074	27.99	14.1	0.109
7	5	5	10	5	7378	5.8	26.84	22.82	0.065	29.02	23.8	0.036
8	10	5	15	5	8598	5.8	78.96	52.96	0.115	106.05	61.69	0.096
9	5	2.5	7.5	3	2529	5.8	20.57	14.08	0.145	20.23	13.84	0.107
10	10	2.5	12.5	3	4139	5.8	38.02	26.81	0.138	47.26	29.1	0.091
11	7.5	0	7.5	3	1330	5.8	19.06	11.35	0.063	15.8	9.28	0.032
12	7.5	5	12.5	3	2290	5.9	31.19	26.25	0.093	37.17	28.8	0.144
13	7.5	2.5	10	1	2020	6.0	30.6	22.09	0.090	34.89	23.13	0.095
14	7.5	2.5	10	5	3409	5.8	42.78	25.22	0.062	41.17	22.62	0.038
15-1	7.5	2.5	10	3	1920	5.9	45.28	25.83	0.132	45.2	24.1	0.138
15-2	7.5	2.5	10	3	2519	5.9	48.47	27.7	0.051	47.08	25.91	0.114
15-3	7.5	2.5	10	3	2859	5.7	40.1	24.79	0.030	43.07	24.56	0.022
16	6.5	1.9	8.4	2.7	2669	5.8	31.57	19.15	0.104	29.51	17.51	0.081
17	8.5	1.9	10.4	2.7	3779	5.9	40.71	24.19	0.063	38.55	22.43	0.152
18	7.5	3.7	11.2	2.7	3349	5.9	34.6	25.22	0.087	48.52	30.4	0.151
19	7.5	2.5	10	4	3559	5.8	41.46	26.18	0.062	42.07	24.56	0.058

constructed with the diluted 2, 5, 10, 20x standard mixture from which 10  $\mu$ L was injected. Concentrations of DHHB and BEMT in the formula were determined using five extractions and three HPLC injections per extraction.

## 2.8. LiS physicochemical determination

The size of the LiS was assessed by size exclusion chromatography (SEC) performed with two detectors online at 40 °C (UV<sub>270 nm</sub> and RI) and using two MCX columns from PSS (Perfect Separation Solution, Germany), as already described (Lorquin et al., 2021). About 2.5 mg dried LiS was dissolved in 1 mL of NaOH 0.1 N containing 2–3  $\mu$ L ethylene glycol (flow marker), from which 20  $\mu$ L were injected. Data were acquired by the software to display  $M_w$  (molecular weight),  $M_n$  (number average molecular weight),  $M_p$  (molecular weight of the highest peak), and  $\text{Đ}$  (dispersity,  $M_w/M_n$ ). The SEC system was calibrated by Na-polystyrene sulfonate standards ( $M_p$  1260–340 kDa) and a typical equation is shown in Fig. 1SB. Elemental analysis (C, H, N, S) of LiS was performed according to Lorquin et al. (2021).

## 2.9. Emulsion control

### 2.9.1. Stability

It was carried out by the backscattering (BS) and light transmission (T) technique using a TurbiScan Lab device (from Formulacion, Toulouse, France) which evaluated the particle size variation (floculation or coalescence) and particle migration (creaming or sedimentation) according to Mengual et al. (1999). The 20 mL o/w emulsion freshly prepared was placed into a cylindrical sample cell and scanned every 1 h over 7 days at 40 °C (total scans 168). The Turbiscan stability index (TSI) was calculated from raw T and BS signals recorded in the instruments. It provided a single value to rapidly assess and rank formulation stability independently on occurring phenomena, and was calculated by the formula  $[\sum(x_i - x_{BS})^2 / n - 1]^{1/2}$ , where n is the number of scans,  $x_i$  the average backscattering per min of measurement, and  $x_{BS}$  the average  $x_i$  (Li et al., 2020). A high TSI value (>1) indicates a less stable emulsion sample. All measurements were made in duplicate. The emulsion stability was also controlled visually after centrifugation (3000 g, 30 min), at 48 h.

### 2.9.2. Viscosity

It was determined at 12 rpm velocity with a Brookfield DV-II-pro viscometer (Middleboro, USA) equipped with a spindle LV S63 model. Viscosity was expressed in mPa.s taking the criterion that a percentage value greater than 20% validated the result.

### 2.9.3. Microscopy

The micrographs of the o/w emulsions were captured on a Nikon Eclipse E600 optical microscope fitted with a digital camera Nikon DS Fi3. The emulsion samples were directly deposited on the glass slide and observed at a 100x resolution. Particle size and homogeneity were determined with the software.

## 3. Results

In presence of OMC and BMDM (total filters 11.5% w/w) and Naliginosulfonate (LiS), they indicated a moderate SPF enhancement and formulations demulsified over time. In our laboratory and the same manner as the literature (Qian et al., 2015, 2016), the first experiments performed from a Nivea common cream blended with OMC, BMDM, and a LiS provided by another supplier (see Methods), gave similar SPF values. On the other hand, to find an alternative to the use of soda in the formulations, solubilization of OLV lignin (wheat straw), kraft lignin (softwood pine trees), and ALK lignin (Sigma) was tried in a multitude of safe and non-safe personal care products including mixtures of  $\gamma$ -valerolactone-H<sub>2</sub>O (Lê et al., 2016), unfortunately without success. In the same way, fractionation of these lignins with acetone (Sadeghifar et al., 2016; Domínguez-Robles et al., 2018) did not lead to a soluble extract, redirecting the strategy toward a formula containing LiS and despite the reported demulsifying effect. We have thus undertaken to elaborate a stable, simple, and eco-design formulation containing LiS by conventional formulation techniques.

### 3.1. Characterization, antiradical properties, and toxicity of LiS

LiS has the advantage to be highly soluble in water, cheaper, easily available than the hydrophilic OLV, kraft, and ALK lignin. It is highly produced for uses in several fields, as an anionic surfactant in many application areas, as concrete water reducers, dispersant for water-coal-slurry, corrosion, as scale inhibitor, pesticide dispersant, binders, and mainly for cement industries. However, the used LiS has been partially

characterized by the manufacturer (see Supplementary material 1).

### 3.1.1. Molecular weight and chemical formula

The LiS was shown by SEC to have a molecular weight ( $M_w$ ) of  $7700 \text{ g mol}^{-1}$  which designed a mean of masses eluted in the peak, and a large dispersity  $\mathcal{D}$  ( $M_w/M_n$ ) of 49.5 (Fig. 1SA, Supplementary material 1). Identical values were obtained whatever the detector used (RI or UV). The size of LiS was generally reported to range from 5 to 50 kDa with a smaller  $\mathcal{D}$  (6–8) (Kai et al., 2016; Aro and Fatehi, 2017). In our laboratory, previous studies have shown that if the lignin is not fully solubilized, the pigment forms high  $M_w$  agglomerates ( $\sim 40\text{--}50 \text{ kDa}$ ) which could bias the results by elution on aqueous or organic SEC supports. Solubilize and elute LiS in NaOH 0.1 N was therefore essential. On the other hand, element analyses of LiS led to the chemical formula  $\text{C}_{244}\text{H}_{369}\text{O}_{254}\text{N}_5\text{S}_{10}$  that corresponds to  $M_w$  of  $7751 \text{ g mol}^{-1}$ , thus very close to the value obtained by SEC. This weak difference would be due to some ions complexed with the polymer as already reported (Aro and Fatehi, 2017).

### 3.1.2. Photostability studies

Photostability was determined on LiS at two concentrations ( $0.1$  and  $0.05 \text{ mg mL}^{-1}$ ) in aqueous solutions submitted to strong radiation three times higher than the authorized minimal dose (see Methods). They showed that the UV-visible spectrum of the LiS and its  $M_w$  evaluated by SEC have not changed after irradiation, compared to the non-irradiated solution (data not shown). This indicated that LiS is a high photostable and suitable ingredient for use in sun emulsions.

### 3.1.3. DPPH-antioxidant activity

LiS was studied along with known antiradical agents. Fig. 1 indicates that LiS has a good antioxidant activity ( $\text{EC}_{50}$   $135.2 \mu\text{g mL}^{-1}$ ), however less important than the standards ascorbic acid ( $29.0 \mu\text{g mL}^{-1}$ ), Trolox ( $12.0 \mu\text{g mL}^{-1}$ ), propyl gallate (PG,  $4.2 \mu\text{g mL}^{-1}$ ) (Lorquin et al., 2021), better than  $\alpha$ -tocopherol ( $224 \mu\text{g mL}^{-1}$ ; Castro et al., 2006) commonly used in cosmetology, and much better than the synthetic butylated hydroxytoluene (BHT,  $\text{EC}_{50} \mu\text{g mL}^{-1}$ ; Yao and Qi, 2016). Note that PG induced DNA damages (Matsuda et al., 2016), and BHT was reported cytotoxic (Ito et al., 1986). We have thus demonstrated that LiS could diminish the action of ROS, remembering that under irradiation, keratinocytes are a source of ROS that may affect neighboring skin cells such as melanocytes, and influence the process of melanogenesis or contribute to the progression of vitiliginous lesions.

### 3.1.4. Toxicity

The EFSA (European Food Safety Authority) through the FEEDAP organization (group on additives and products used in animal feed)

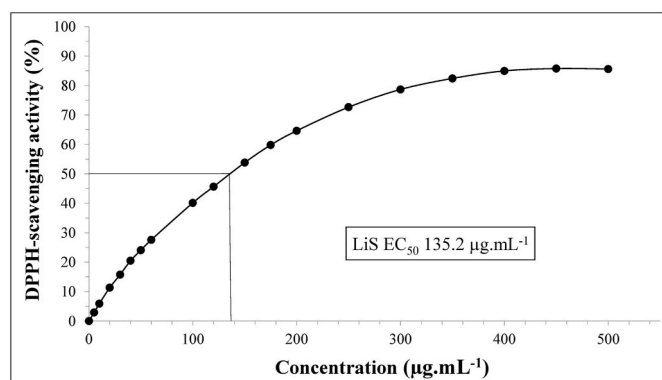


Fig. 1. DPPH-antioxidant activity (in %) of the used Na-Lignosulfonate (LiS). The mean of the triplicate tubes led to standard deviations less than 5%, which were not represented. Conditions: DPPH solution at  $350 \mu\text{M}$ , wavelength  $517 \text{ nm}$ .

concluded that LiS is not an irritant to the skin and the eyes, nor a skin sensitizer (Andersson and Göransson, 1980; EFSA-opinion, 2015;). Studies have also noted the low toxicity of lignosulfonates in marine life, which further increases the potential for their safe use and friendly for marine ecosystems (Neff et al., 1981). Lignin is degraded by a variety of soil bacteria and fungi. LiS is a polymer different from lignin that does not naturally occur in the environment, and therefore in the absence of additional information, an environmental risk assessment is necessary. Cytotoxicity and phototoxicity of LiS were evaluated on keratinocytes, the most abundant cells of the epithelial layer of the skin used as a part of the 3D skin model for the assessment of the toxic hazard of cosmetic ingredients. Confluent keratinocyte cells were incubated for 24 h in the presence of different concentrations of LiS, and the cell metabolic activity was quantified, showing no reduction of this activity as compared to the non-treated cells, thus formally postulating the absence of toxic effect on skin cell metabolic activity for LiS until  $1000 \mu\text{g mL}^{-1}$ . Furthermore, using the normalized OECD protocol commonly used for cosmetology product evaluation, LiS was not phototoxic ( $\text{PIF} < 2$ ).

### 3.2. A short and eco-design formulation containing Na-lignosulfonate

A formulation containing few ingredients had been developed (Table 1) and was composed of glycerin as a humectant, xanthan gum as thickener, caprylic-capric triglycerides as a softener, which represent the natural (or alternative) part, in addition to Tribehenin PEG-20 esters as the synthetic emulsifier. This latter brings SPF boosting to sun cares due to its optimal dispersion effect (Gattefossé technical sheets). Especially in this procedure, a crucial difference brought was the pH stabilization ensured by a Na-citrate buffer at a suitable ionic force of  $60 \text{ mM}$  (in final). Indeed, the addition of strong negative charges provided by LiS considerably destabilized the pH, gave SPF variations, even by replacing the Tribehenin emulsifier with other surfactants specifically resistant to highly ionic media (electrolytes), such as Simulgel 600, Montanov L, Simulgreen 18-2, and Emullium Melifera. At least, special care must be paid to the temperature during solubilization of the filters at  $85 \text{ }^\circ\text{C}$ , as well as the several agitation times and velocities to be strictly applied to ensure full success, i.e. a stable and optimal SPF-exhibiting formula with great reproducibility.

To validate the formulation, experiments were conducted in presence of OMC (7.5%), BMDM (4.0%), and increasing amounts of LiS (0, 1, 3, 5, 10%), with and without post-irradiation. Results in Table 2S were quite different from Qian et al. (2016) and showed that SPF increased non-linearly along with LiS amounts, reaching 53.1 (LiS 3%) and 69.0 (10%). Compared to the results of Qian et al. (2016) which reported a constant SPF of 20–22 between 5 and 10% LiS, a break at 5% lignin (SPF 40.8) was observed in our experiments, a behavior that has no plausible explanation at moment. Importantly, 30-min irradiation led to a drastic SPF decrease of 48.3% (without) and 34.7% (with 10% LiS), and 50.5% (without), and 49.7% (with 10% LiS) for UVA-PF (Table 2S). In this formula, OMC at 4% did not prevent the photo-instability of BMDM which is known to generally decomposes by irradiation in absence of quenchers, commonly octocrylene (OCR), OMC, and BEMT (Chatelain and Gabard, 2001; Damiani et al., 2006; Nash and Tanner, 2014). Photodegradation of BMDM was attributed to a Norrish I reaction that cleaves the diketone moiety (Damiani et al., 2006; Paris et al., 2009). To conclude this part, the formulation developed here with OMC, BMDM, and LiS, gave high SPF values, and were different from those reported by Qian et al. (2016). However, the negative effect of irradiation constitutes an impediment to improvement and development. Replace OMC and BMDM with non-photodegradable and non-toxic UV filters became necessary.

### 3.3. Selection of two safe-described sunscreens

#### 3.3.1. Selection by sorting

The strategy had been directed toward a broad-spectrum two-in-one



organic filter and covering UVA and UVB as wide as possible. Among the 26 UV-authorized organic sunscreens from EU statements (annex VI of 2009, revised in 2015 and 2017) and data summarized in Table 1S (Supplementary material 2), the selection was made by conjunction of spectroscopic data, impact on human health comprising mutagenic, photo-induced damaged effects, endocrine disruptor, allergenic properties, and on ecosystems mainly expressed by estrogenic disorders on living species. Especially, the molar extinction coefficient ( $\epsilon$ ) had been regarded as a crucial parameter because could promote the use of lower concentrations. The UVA/B authorized filters are six in number and include the benzophenone series (-3, -4, -5), DTS, MBBT (Tinosorb M), and BEMT (Tinosorb S). Benzophenone-3 is an endocrine-androgenic disruptor notably against coral (Heweweer et al., 2005; Downs et al., 2016) with allergenic properties (Warshaw et al., 2013), and was discarded. Benzophenone-4, -5, and DTS (Drometrizole trisiloxane) do not cause risk but they have a low  $\epsilon$  and a narrow UV area (Table 1S), they were also discarded. BEMT and MBBT have the best spectroscopic data, a great photostability (Chatelain and Gabard, 2001; Damiani, 2007), and neither of these two agents possess intrinsic in vitro estrogenic/antiestrogenic or androgenic/antiandrogenic activity (Ashby et al., 2001). BEMT has a higher  $\epsilon$  (Table 1S) and the advantage to exert a strong and better anti-inflammatory activity with 76% inhibition of edema than MBBT (56%) (Couteau et al., 2012). Therefore, BEMT was selected.

The EU list contains only four strict UVA filters, such as BMDM, DHHB, and two ionic compounds TDSA (terephthalylidene dicamphor sulfonic acid) and PDTA (phenyl dibenzimidazole tetrasulfonate, see Table 1S). The photodegradable and unsafe BMDM was discarded (Allen et al., 1996; Heweweer et al., 2005; Paris et al., 2009; Klopčić et al., 2017). DHHB has a high  $\epsilon$  and up to now, no described effect on human cells nor environmental impact. Indeed, it was not toxic, non-mutagenic, not clastogenic, and not phototoxic in vitro. DHHB can be degraded by UV/H<sub>2</sub>O<sub>2</sub> in aqueous media, but at high peroxide level and over 254 nm (Gong et al., 2015). Comparatively, TDSA ( $\lambda_{\max}$  345 nm,  $\epsilon$  750%/cm) and PDTA ( $\lambda_{\max}$  335 nm,  $\epsilon$  770%/cm) seemed interesting UVA absorbers. TDSA protects genomic DNA (Fourtanier et al., 2006) but is not photostable; PDTA was signaled to provoke strong anaphylactic reactions (Lange-Asschenfeldt et al., 2004). Finally, DHHB ( $\lambda_{\max}$  354 nm,  $\epsilon$  925%/cm) was preferable for its better spectroscopic data, covering a larger UVA zone, safe properties, and photostability.

### 3.3.2. Confirmation by experiments

The two selected BEMT and DHHB were assayed separately at 5% concentration in the formula (Table 1), in presence of 5 and 10% LiS, before and after irradiation, relatively to the control (without lignin). BMDM (UVA) and other commonly used UVB absorbers such as EHS (weak  $\epsilon$ ), OMC (estrogenic and carcinogenic compound), OCR (low  $\epsilon$ , estrogenic), HMS (weak  $\epsilon$ , potential endocrine disruptor), and EHT (high  $\epsilon$ , low allergic risk) (Table 1S), were assayed in parallel. Results in Table 2 indicate for the whole filters a significant SPF increase along with the amount of LiS, whether before and after irradiation. Best effects were observed with BEMT, EHS, OMC, and OCR. After irradiation, EHS, OMC, and HMS exhibited a loss of SPF, BMDM a loss of UVA-PF, whereas DHHB at 5% confirmed its photo-stability. However, UVA-PF of DHHB enhanced only beyond 5% LiS (Table 2). Besides, for each filter studied here, the viscosity increased along with the LiS content, making the emulsions consistent comparatively to the milkier emulsions performed in absence of lignin.

### 3.4. Solubility and UV-visible spectroscopy of BEMT and DHHB

To evaluate their spectral properties, BEMT and DHHB were assayed for their solubility at 1, 2, 5, and 10% w/w (10–100 mg mL<sup>-1</sup>) in various solvents such as ethanol, ethanol-H<sub>2</sub>O (1:1), dimethyl sulfoxide, 1,4-dioxane, methanol, tetrahydrofuran (THF), propanol-2, acetonitrile (ACN), and acetone (Ac<sub>2</sub>O). At these all concentrations, BEMT was

soluble in 1,4-dioxane, THF, and Ac<sub>2</sub>O only, and insoluble in all others. DHHB is soluble in all the solvents tested, except in the mixture ethanol-H<sub>2</sub>O (low solubility). To characterize the spectral complementarity and covering in the UV-visible range, BEMT and DHHB in THF were studied separately and mixed at concentrations deduced from the next study (see below Desirability), i.e. BEMT 8.5% and DHHB 3.5% (w/w). Spectra in Fig. 2S (Supplementary material 1) indicated that, at these concentrations, the addition of DHHB slightly complements the UVA protection and provokes an increase in the absorbance of the mix all over the UVB and UVA range (290–400 nm).

### 3.5. Stability of the two filters during the process

BEMT and DHHB are solid in the pure state, have a melting point of 80 °C and 54 °C respectively, both decompose at temperature >300 °C (Chemical Book search engine), hence they should not be affected by the temperature of the process at 85 °C. To confirm this, by an organic extraction of two emulsions containing known quantities of these two filters (BEMT 8.5%, DHHB 3.5%, LiS 5%), we demonstrated by RP-HPLC analysis and calibration with the two corresponding standard curves, a recovery of 98.7% and 99.2% (n = 5 experiments, p < 0.05) for BEMT and DHHB, respectively (not shown). In absence of LiS in the formula, equivalent values were also been obtained. We concluded that both filters were not degraded during the process whether the presence or absence of LiS. To simultaneously estimate the BEMT and DHHB content in emulsions, the procedure developed here and using THF for solubilizing and in the mobile phase (HPLC eluent), will be useful to control the quality of future formulations containing these two filters.

### 3.6. Concentration range selected for experimental design studies

To select the range concentration of BEMT, DHHB, and LiS, combinations of filters used at their maximum authorized concentrations were tested. From the SPF data compiled in Table 3, conclusions were the following.

- (i) When BEMT 10% was used alone, the initial SPF at 18.5 (simulator at 18.2) showed a loss of 19.8% after irradiation (14.8), thus contrasted with the previous photo-stability recorded for this absorber at 5% (Table 2). Hence, BEMT 10% used alone should be excluded. The addition of 5% LiS boosted the initial SPF to 57.5, while the irradiation led to an SPF of 44.4 that corresponds to a more important loss of 22.6%.
- (ii) Association of BEMT 10% and DHHB 5% (total filter 15%) was a benefit for the photo-stabilization of BEMT and the boost effect; indeed, LiS from 1 to 5% efficiently raised the SPF at 54.2 (UVA-PF 38.5) and 79 (UVA-PF 52.9), respectively, while irradiation provoked this time a high and positive enhancement reaching 73 (UVA-PF 44.5) and 106 (61.7). Such an effect had already been observed with Nivea emulsions supplemented with OMC, BMDM, OLV (or ALK lignin), but not with LiS (Qian et al., 2016). On the other hand, it has been observed that a 10% LiS concentration has no incidence on the stability of emulsions containing only BEMT (Table 2) but rapidly led to unstable creams when the two filters BEMT and DHHB were combined (not shown).
- (iii) By the empiric BASF simulator software, BEMT (5–10%) and DHHB (1–5%, w/w) doses were further combined to predict the SPF value. A total of 30 mass combinations were entered, varying from 6 (SPF 9.7, UVA-PF 8.5) to 15% (SPF 22.2, UVA-PF 21.9) total filter content, all respected a critical wavelength  $\lambda_c > 370$  nm and a UVA/UVB ratio  $\geq 0.33$ . Finally, the predicted data were found of the same order of magnitude as those of our results (see Table 3, SPF before irradiation, -LiS) and confirmed that LiS is essential to higher the SPF number over 30.

Thus, these primarily experiments suggested that (a) the percentage

ranges chosen for BEMT (5–10%) and DHHB (0–5%) are satisfactory, (b) the total percentage of filter does not have to exceed 10–12%, and (c) the booster LiS does not have to exceed 5%.

### 3.7. Experimental design model for desired SPF

#### 3.7.1. Mathematical modeling

An empirical mathematical model was postulated to calculate the SPF values in function of sunscreen (BEMT, DHHB) and lignin (LiS) concentrations in their limiting ranges defined before. Responses such as UVA-PF, UVA/UVB ratio, and critical wavelength ( $\lambda_c$ ) were not considered because their values were systematically found in the appropriate range. By the Azurad software (see Methods), Table 4 shows the defined experimental runs and the related results corresponding to the two responses, SPF before ( $Y_1$ ) and after ( $Y_2$ ) irradiation.

In the first step, the model was validated using the four test points (Table 4, lines 16–19) for which experimental values have been further compared to the previously calculated ones. Data showed a non-significant difference with significance values largely  $>5\%$  (Tables 3S and 4S), indicating that the model and the experience values were close. Then these four test points were integrated for the calculation of the coefficients as follows:

$$Y_1 = 36.8302 + 16.6559X_1 + 7.0715X_2 + 6.3348X_3 - 1.0601X_1^2 - 5.8489X_2^2 + 5.9651X_3^2 + 2.7625X_1X_2 + 3.6454X_1X_3 + 1.4684X_2X_3, \text{ and}$$

$$Y_2 = 38.7482 + 18.1092X_1 + 16.8665X_2 + 4.9907X_3 + 0.5232X_1^2 - 6.2665X_2^2 + 4.8152X_3^2 + 12.8322X_1X_2 + 2.8720X_1X_3 + 4.5634X_2X_3$$

At this stage, with a higher number of experiments and therefore a greater degree of freedom, ANOVA became possible. As illustrated in

Tables 5S and 6S, the regression mean squares (458.266 for  $Y_1$ , 912.386 for  $Y_2$ ) and the residual mean squares (60.446 for  $Y_1$ , 55.712 for  $Y_2$ ) allowed the Fisher ratios for assessing the linearity of the regression. In both cases, the regression was good: 86.1% and 93.0% for  $Y_1$  and  $Y_2$ , respectively (not shown). That indicated that the variation of the response was due to the variation of the parameters and not only due to the experimental error. This second-order predictive model for desired SPF values was accepted and can now be applied to predict the SPF values in the whole domain of interest.

#### 3.7.2. Interpretation of response surfaces

SPF responses were represented in graphic form by two-dimensional sectional planes from the three-dimensional cube. The 18 projection planes, 9 in each case ( $Y_1$  and  $Y_2$ ), corresponded to the responses of the two external faces and the middle of the cube. This middle depends on the range concentration defined previously and was fixed by the pitch of 2.5 for BEMT and DHHB, 2 for LiS. Responses illustrated in Fig. 2A (for  $Y_1$ ) and 2B ( $Y_2$ ) with the associated color code, clearly showed that the three factors studied, BEMT, DHHB, and LiS have an important effect on the SPF before ( $Y_1$ ) and after irradiation ( $Y_2$ ). Experimental design allows us to confirm the expected effects of LiS with the best knowledge of its influence over the domain and the range of robustness of the method. As a first conclusion, before ( $Y_1$ ) and after irradiation ( $Y_2$ ), the more the concentration of each factor increase, the more the SPF will be high. In each case,  $Y_1$  and  $Y_2$  responses evolve in the same way but are different. At low filter concentrations, for instance, BEMT 5%, DHHB 0%, and whatever the lignin concentration (1–5%),  $Y_1$  cannot reach 50. In contrast, after irradiation ( $Y_2$ ), a 50 SPF value can be obtained in the same concentration conditions. That confirmed the impact of LiS on the response after irradiation (Table 3). At high filter concentrations, for

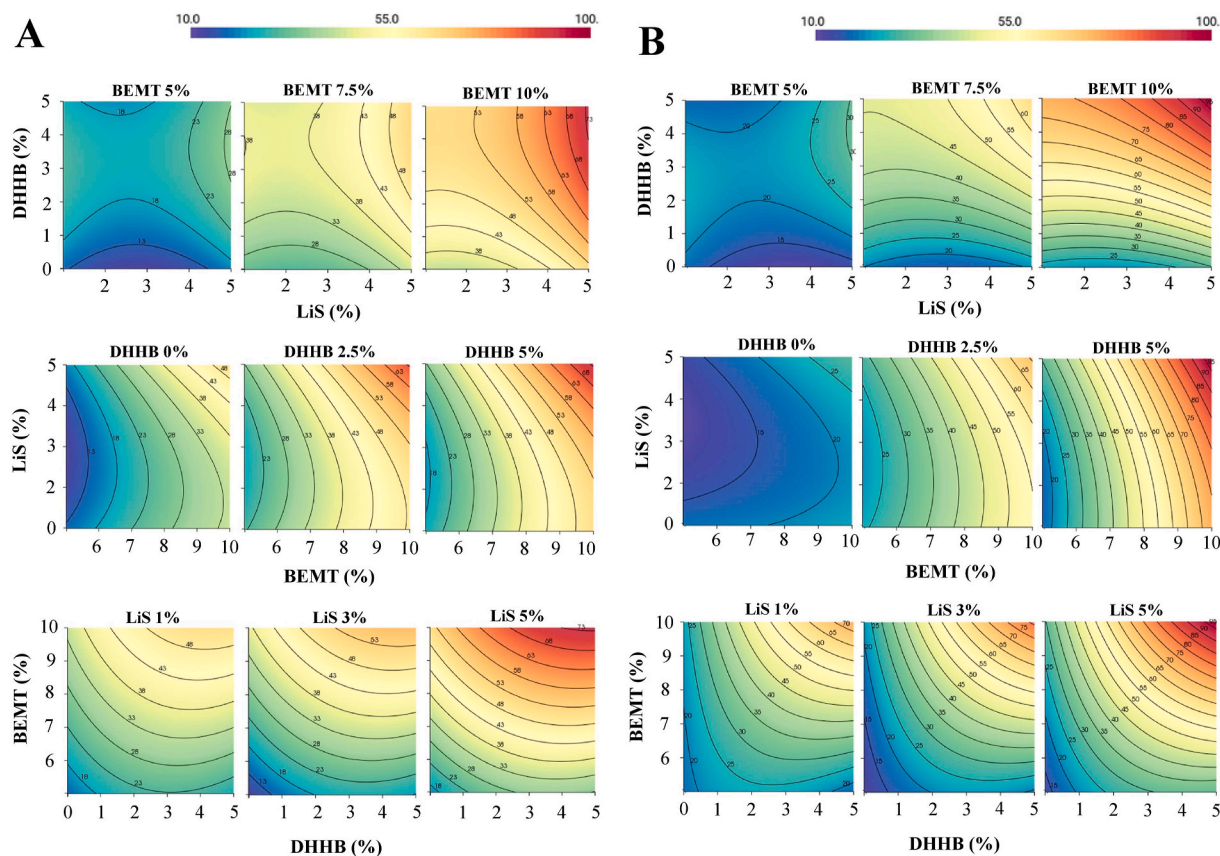


Fig. 2. Iso-response curves corresponding to the two-dimensional results of the sectional plane from the three-dimensional cube, before (A,  $Y_1$  response) and after (B,  $Y_2$ ) irradiation. The nine illustrations for each condition ( $Y_1$  or  $Y_2$ ) represented the two external faces and the middle responses (section) of the cube which were given at a fixed pitch of BEMT, DHHB, and LiS.

instance, BEMT 10% and DHHB 5%, the responses in Fig. 2 confirmed the results of Table 3. Consequently, to use lower BEMT-DHHB concentration, a value of 5% LiS is necessary for all SPF-desired emulsions.

### 3.7.3. Desirability

This final part aims to define an experimental zone of acceptable compromises in which all responses are satisfactory. For that, the D function (global desirability) is the “compromise function” first calculated from “partial desirability functions”. Then, it will be possible, everywhere in the domain, to get 3D spatial representation (response surfaces from a cube) of the studied factors in the same manner as previously. At this stage and from all results (Table 3, and previous response surfaces), a value of 5% for LiS was the most preferable for reducing as well as possible the filter concentrations in the emulsion.

Further, for marketing and related to the demand, two emulsions SPF<sub>30</sub> and SPF<sub>50</sub>, both evaluated before and after irradiation, were taken for applying the model and the D function. The D surfaces were illustrated in Fig. 3A and B. In the blue and blue-green zones, a point in Fig. 3A was selected, and another in Fig. 3B. The SPF<sub>30</sub> composition deduced was BEMT 7.0%, DHHB 2.0%, LiS 5% (total filter 9%); for the SPF<sub>50</sub>, the deduced composition was BEMT 8.5%, DHHB 3.5%, LiS 5% (total filter 12%). To validate these theoretical compositions, the two emulsions were developed according to the formulation process (Table 1), the SPF was evaluated by the integrated-sphere spectrophotometer and compared to the emulsions prepared without LiS.

### 3.7.4. Control and validation of the model

A formulation containing 8.5% BEMT, 3.5% DHHB (total filter 12%), and 5% LiS, was found to exhibit an SPF of 50.2 (UVA-PF 31.3) before, and 52.8 (30.1) after irradiation. In the same manner, a formulation with 7.0% BEMT, 2.0% DHHB (total filter 9%), and 5% LiS has an SPF of 34.1 (UVA-PF 22.5) before, and 34.7 (22.6) after irradiation (summarized in Table 3). They were defined SPF<sub>50</sub> and SPF<sub>30</sub> emulsions, respectively, and validated the postulated model and the previous experimental design studies.

## 3.8. Characterization of the SPF<sub>30/50</sub> emulsions

### 3.8.1. Toxicity

Since it was not possible to evaluate the toxicity of BEMT and DHHB in THF or oils in reason to the insolubility of the samples in the keratinocytes culture medium, the SPF<sub>50</sub> emulsion that contained LiS 5% w/w diluted in the HSSB solution (see Methods) was controlled. With the

same protocol as for LiS toxicity evaluation, the emulsion did not show any cyto- and phototoxicity until 1000 µg mL<sup>-1</sup>, giving insurance for direct applications and marketing with both emulsions.

### 3.8.2. Stability

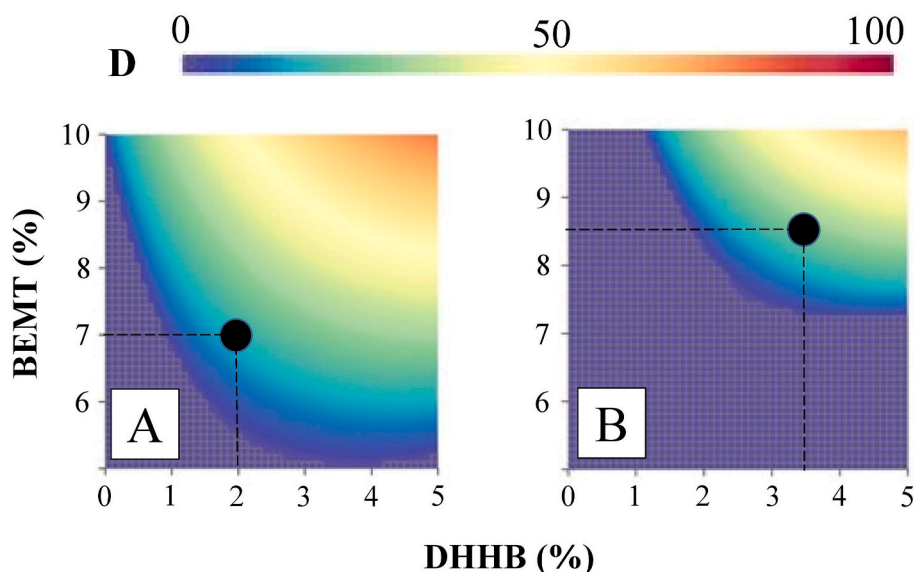
SPF<sub>30</sub> and SPF<sub>50</sub> emulsions have shown high stability by centrifugation (3000 g, 30 min) at 48 h. For the long-term, stability of the two emulsions was controlled with the Turbiscan apparatus at 40 °C using the manufacturer parameters. In this case, the transmission (T) value for opaque emulsions was invalid (<0.2%), hence only the variation of backscattering (ΔBS) was taken. Since for the two samples SPF<sub>30</sub> and SPF<sub>50</sub> emulsions, ΔBS was weak (~1%) (Fig. 3SA and 3SB) and the calculated TSI <1 all along the 7 days (Fig. 3SC, Supplementary material 1), it was concluded that the two emulsions are stable over time, predicting the absence of destabilization mechanism, i.e. creaming, sedimentation, flocculation, coalescence, etc.

### 3.8.3. Physical characterization

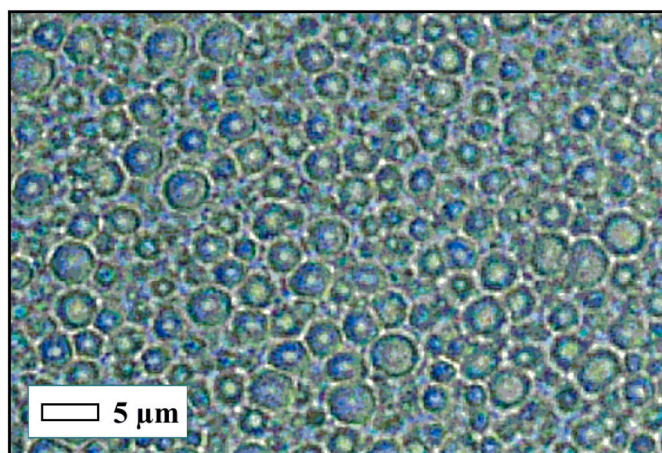
Viscosity measured for both emulsions was similar, with a value of 19036 mPa s for the SPF<sub>30</sub> and 19456 mPa s for the SPF<sub>50</sub> preparation, indicating that LiS at 5% brought a sufficient consistency of the emulsion for correct spreading on the skin. The pH at 5.8–6.0 (skin pH) was constant during the process due to the citrate buffer. Furthermore, viscosity and pH were at the same value after 48 h and over time. Emulsions SPF<sub>30</sub> and SPF<sub>50</sub>, all at 5% LiS, comparatively to emulsions that did not contain LiS (4 samples in total), were also observed by optical microscopy. All emulsions showed quite homogeneous droplets with 2.2–3.8 µm particle size measured with the software, as illustrated in Fig. 4. In all emulsions, we observed no change in droplet size or organization.

## 4. Discussions

In an ecological and public health approach, reduce organic filter concentrations meanwhile maintaining high the protection required to find an effective SPF booster among plant kingdom, lower the concentration of the selected sunscreens, and formulate with ingredients limited in number and natural as far as possible. Results on lignin as SPF booster have been reported for the first time from nonconventional preparations in which commercial pure creams were directly blended by various lignin and two organic sunscreens, OMC and BMDM (total filters 11.5% w/w), with vigorous magnetic stirring (Qian et al., 2015, 2016). However, in presence of these filters and Na-lignosulfonate (LiS), a moderate SPF enhancement was obtained and formulations demulsified



**Fig. 3.** Two-dimensional desirability surfaces resulting from the Azurad software. Here, LiS was fixed at 5% (w/w), (A) designed the SPF<sub>30</sub> zone before and after irradiation; (B) the SPF<sub>50</sub> zone before and after irradiation. The violet zone corresponds to the non-satisfactory area, the other colors (blue to red) the satisfactory zone of the desired response, blue and blue-green zones correspond to the minimal filter concentrations, and a point has been selected. The SPF<sub>30</sub> (total filter 9%) and SPF<sub>50</sub> (12%) composition were deduced (see text).



**Fig. 4.** Micrograph of the SPF<sub>30</sub> o/w emulsion deposited on a glass slide and captured at a 100x resolution. The preparation contained 5% LiS, 7% BEMT and 2% DHHB. A similar micrograph and particle repartition were obtained with the SPF<sub>50</sub> emulsion.

over time. On the other hand, due to their side effects on human health and impact on sea ecosystems, the selection of non-toxic organic UV filters that are stable and protect a large UV range at low concentrations remained a difficult challenge. Beforehand, an extensive sorting based on their UV-visible performance, photostability, and the absence of toxic effects or further toxicological studies, made it possible to select two other organic sun blockers, BEMT (or Tinosorb S, UVA/B range protection) and DHHB (UVA). Photostability is rarely indicated on sunscreens, and yet it should be the most important characteristic of an effective sunscreen since the light-induced decomposition not only reduces its photoprotective power but also can promote phototoxic or photoallergic contact dermatitis (Herzog et al., 2009). There is a need for a standardized method to measure photostability that should be marked on the sunscreen product. In this study, we have applied moderate light radiation of  $99 \text{ J cm}^{-2}$  to the emulsions. The two selected UV filters were safe; however, no data exist on their microbial degradation and accumulation in ecosystems in the long term. By comparing concentrations used in most commercial formulas, we had taken the commitment not to exceed 12% of total filters in our formulations for achieving high SPF.

The first challenge was to develop a formulation with few compounds in number. Such formula validated here for use in UV protection contains nine ingredients including five natural compounds among which the assimilated caprylic-capric triglycerides, two synthetic organic filters, and one plant byproduct as the SPF booster. This oil-in-water emulsion has low lipid content (max. 22.5% w/w) (Table 1), BEMT and DHHB are lipophilic molecules, BEMT has a high molecular weight of  $628 \text{ g mol}^{-1}$ , 397 for DHHB, thus decreasing the risk of these two filters to permeate the stratum corneum barrier and reach the epidermis and dermis (Durand et al., 2009). Recently, following finite dose Franz cell studies with porcine skin over 12 h, there was no skin permeation of BEMT and DHHB (Haque et al., 2016). Considering the higher permeability of porcine skin compared to human skin, and the higher dose used, these experiments also suggested no effective transdermal permeation of the compounds will occur in normal human skin.

Since BEMT alone or in combination with DHHB were not able to bring the SPF value to 50 when used in the limited 12% total filter concentration, the main challenge was to efficiently boost the SPF by addition of raw, water-soluble, and untreated Na-lignosulfonate (LiS) lignin. In contrast to other lignin, LiS considerably facilitated the process and gave stability to the emulsion in the maximum limit of 5% use. Finally, by defining the concentration range of the three active ingredients, the success of this work was due to experimental design studies based on a cubic mathematical model constructed from only 19

experiments and leading to validated equations. Consequently, a SPF<sub>50</sub> formulation containing 8.5% BEMT, 3.5% DHHB (total filter 12%) and 5% LiS was found to exhibit a SPF of 50.2 (31.3 UVA-PFA) before irradiation, and 52.95 (30.1) after. One of the main questions was to understand the role of DHHB in this tripartite association. From the UV-spectra of the filters in THF, DHHB brings a complementary UVA overlap to the absorption of BEMT (Fig. 2S) and enhanced the absorbance of BEMT from 290 to 400 nm. Although DHHB alone (until 5%) was not SPF boosted by LiS when incorporated in the short-developed formula, in contrast, it generated a high SPF enhancement when associated with BEMT and LiS together, whether before and after irradiation (Tables 3 and 4). These results also showed that DHHB photostabilizes BEMT which, if left alone, degrades slightly under irradiation (Table 3).

Whatever the second-degree mathematical model was able to propose a line of sunscreens ranging from SPF 15 to 50 with total filter content  $\leq$  of 12%, the SPF having no realistic signification beyond 50 (Wilson et al., 2012). Finally, the SPF<sub>30</sub> and SPF<sub>50</sub> emulsions made here are stable in time as determined by the two techniques (centrifugation, Turbiscan), have a pH like that of the skin, a viscosity typical of sunscreen emulsions, and as expected, are not toxic on keratinocyte cells, nor phototoxic. Advantageously, the emulsion emits a very pleasant smell of wood due to LiS, this lignin would bring an antiradical property to the emulsion as suggested by the DPPH assay ( $\text{EC}_{50} 135.2 \mu\text{g mL}^{-1}$ ), and probably an antimicrobial role (Dong et al., 2011; Jha and Kumar, 2018). As an argument, there was no contamination of the closed emulsions after several months. Whatever, extensive antibacterial and antifungal assays are required on LiS and the emulsion-containing LiS. On the other hand, since the anti-inflammatory activity of BEMT and DHHB has been reported in vivo (Couteau et al., 2012), activities by the pro-inflammatory LPS-induced assay on mouse keratinocytes failed in reason to the insolubility of these filters prepared in a non-aqueous organic solvent, in the culture medium, even after high dilution.

If these findings are encouraging, the main difficulties remained the explanation of the boosting effect observed and how LiS interacts with sunscreens. LiS is insoluble in organic solvents, and unfortunately, we did not find a matrix able to solubilize BEMT or DHHB together with LiS. This was the major impediment for molecular interaction studies with these three components, for instance, to consider differential scanning micro-calorimetry (DSC) experiments. Interestingly and as previously observed for OLV lignin (Qian et al., 2016), special attention must be paid to the association of BEMT 10%, DHHB 5% (total 15%) with only LiS 1% that raised the SPF at 54 before and 73 after irradiation (Table 3), a phenomenon that needs to be exploited later. As the main starting point, J aggregation which is one type of  $\pi$ - $\pi^*$  stacking, had been suggested between lignin and OMC (Qian et al., 2016), and led to larger conjugated structures with a tilt angle of the dipole moment of the chromophores less than  $54.7^\circ$ , as demonstrated with alkali lignin in presence of iodine (Deng et al., 2011). Finally, the interaction was proved by the decreasing energy for the  $\pi$ - $\pi^*$  transition that became lower (Qian et al., 2016). The terms “ $\pi$ -stacking” or “ $\pi$ - $\pi$  interactions” do not accurately describe the forces that drive the association between organic sunscreens and lignin. In reason to the electrostatic considerations relating to polarized  $\pi$  systems, these terms thus should no longer be used (Hunter and Sanders, 1990; Martinez and Iverson, 2012). Direct electrostatic interactions between polarized atoms of substituents as well as solvation-desolvation effects must also be considered and even dominate in many circumstances (Martinez and Iverson, 2012), however, they are difficult to occur in complex mixtures such as cosmetic emulsions.

The other essential point concerns the LiS structure. By using dynamic light scattering (DLS), Qiu et al., 2010 measured the critical aggregation concentration (CAC) of Na-LiS in water-solution at a value of  $0.38 \text{ g.L}^{-1}$ , revealing the state as well as dynamics of the formation of the LiS micelles. LiS was the state of individual molecules at a concentration less than the CAC, whereas the individual LiS molecules started to aggregate above, and micelles formed and grew with increasing LiS

concentration. With a 5% LiS concentration (this work), a spherical hollow vesicular structure could be proposed as a model of the aggregated micelles of LiS in the emulsion, these micelles might encapsulate only a part of the total sunscreens. On the other hand, when LiS is adsorbed on PMMA plates, a skin-mimicking system, the aggregation and the conformation of LiS changes with pH and the relationship between the solution behaviors and the adsorption characteristics of LiS (Qian et al., 2011). Indeed, the adsorption thickness and surface morphology depend on the aggregation state and molecular conformation of LiS, and at pH around 6, the carboxyl groups might be ionized, and the phenolic hydroxyl groups would not. Thus, the performance of lignosulfonate in boosting the SPF would also depend on its adsorption characteristics at the solid-liquid interface, and its molecular weight. Extensive experiments on this last point might still diminish the sunscreen concentration in such an emulsion.

## 5. Conclusions

In this work, Na-lignosulfonates have rightly been re-considered; ideal associations with two selected and safe-described filters were thanks to an experimental design strategy. We have shown that a line of SPF<sub>15</sub> to SPF<sub>50</sub> emulsions on request, which are photostable and non-cytotoxic, contain few ingredients with only 5% LiS and minimal concentrations of organic filters, was feasible to decrease the risks of impact on the sea environment and human health. Would this correspond to an eco-concept? Either way, it is a good approach. The ideal solar filter does not exist because generally microbial degradation has not been elucidated nor its accumulation in ecosystems. The use of a high SPF sunscreen does not necessarily allow indefinite sunbathing without significant damage to the skin. For these reasons and according to this work, we still might be able to reduce the concentrations of BEMT and DHHB, for instance by adjusting the adsorption parameters at the solid-liquid interface and optimize the micellar sunscreen-containing structure. As another starting argument, the SPF value of sunscreen containing only 2% organic filters has been boosted by 10% middle size OLV lignin colloidal spheres, from 10.7 to 47.7. In this study, the SPF values determined in vitro reflect the reality, however, the evaluation of SPF by the in vivo method is mandatory for marketing (packaging), and the legislation, this final evaluation will be overestimated and due to the anti-inflammatory activity of BEMT and DHHB.

## Author contributions

F. Lorquin designed, executed all experiments, analyzed the data, and wrote the manuscript. J. Lorquin initiated the lignin study, supervised the experiments, analyzed the data, and reviewed the manuscript. M. Claeys-Bruno helped in the Azurad software operation and corrected the relevant part. M. Robin developed the extraction-identification methodologies. M. Rollet determined the molecular weight. C. Di G. (with F. Lorquin) managed the toxicological aspects. P.P supervised this work, the financial aspects, reviewed the manuscript.

## Declaration of competing interest

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

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.scp.2021.100539>.

## References

- Allen, J.M., Gossett, C.J., Allen, S.K., 1996. Photochemical formation of singlet molecular oxygen in illuminated aqueous solution of several commercially available sunscreen active ingredients. *Chem. Res. Toxicol.* 9, 605–609. <https://pubs.acs.org/doi/10.1021/tx950197m>.
- Andersson, R., Göransson, K., 1980. Contact allergy to calcium lignosulphonate. *Contact Dermatitis* 6, 354–355. <https://doi.org/10.1111/j.1600-0536.1980.tb04968.x>.
- Aro, T., Fatehi, P., 2017. Production and application of lignosulfonates and sulfonated lignin. *ChemSusChem* 10, 1861–1877. <https://doi.org/10.1002/cssc.201700008>.
- Ashby, J., Tinwell, H., Plautz, J., Twomey, K., Lefevre, P.A., 2001. Lack of binding to isolated estrogen or androgen receptors, and inactivity in the immature rat uterotrophic assay, of the ultraviolet sunscreen filters Tinosorb M-active and Tinosorb S. *Regul. Toxicol. Pharmacol.* 34, 287–291. <https://doi.org/10.1006/rtp.2001.1511>.
- Birkhäuser, M., 2016. UV-Filter und Endokrinium. *Gynäk. Endokrinol.* 14, 165–173. <https://doi.org/10.1007/s10304-016-0086-y>.
- Castro, I., Rogero, M.M., Junqueira, R.M., Carrapeiro, M.M., 2006. Free radical scavenger and antioxidant capacity correlation of  $\alpha$ -tocopherol and Trolox measured by three in vitro methodologies. *Int. J. Food Sci. Nutr.* 57, 75–82. <https://doi.org/10.1080/09637480600656199>.
- Chaabana, H., Ioannou, I., Paris, C., Charbonnel, C., Ghoula, M., 2017. The photostability of flavanones, flavonols and flavones and evolution of their antioxidant activity. *J. Photochem. Photobiol. Chem.* 336, 131–139. <https://doi.org/10.1016/j.jphotochem.2016.12.027>.
- Chatelain, E., Gabard, B., 2001. Photostabilization of butyl methoxydibenzoylmethane (avobenzone) and ethylhexyl methoxycinnamate by bis-ethylhexyloxyphenol methoxyphenyl triazine (Tinosorb S), a new UV broadband filter. *Photochem. Photobiol.* 74, 401–406. [https://doi.org/10.1562/0031-8655\(2001\)074<401:POBMAA>2.0.CO;2](https://doi.org/10.1562/0031-8655(2001)074<401:POBMAA>2.0.CO;2).
- Chodorowsky-Kimmes, S., 2004. Photostabilized dibenzoylmethane sunscreens immobilized within sol/gel matrices. *Patent US 2004/0057918A1*. <https://www.freepatentsonline.com/y2004/0057918.html>.
- Claeys-Bruno, M., Vandenabeele-Trambouze, O., Sergent, M., Geffard, M., Bodet, D., Dobrijevic, M., Commeyras, A., Phan Tan Luu, R., 2006. Methodological approaches for histamine quantification using derivatization by chloroethylnitrosourea and ELISA measurement. Part I: optimisation of derivated histamine detection with coated-plates using optimal design. *Chemometr. Intell. Lab. Syst.* 80, 176–185. <https://doi.org/10.1016/j.chemolab.2005.06.006>.
- Couteau, C., Chauvet, C., Papis, E., Coiffard, L., 2012. UV filters, ingredients with a recognized anti-inflammatory effect. *PLoS One* 7 (12), e46187. <https://doi.org/10.1371/journal.pone.0046187>.
- Damiani, E., Rosati, L., Castagna, R., Carloni, P., Greci, L., 2006. Changes in UV absorbance and hence in protective against lipid peroxidation of organic sunscreens after UVA-irradiation. *J. Photochem. Photobiol., B* 82, 204–213. <https://doi.org/10.1016/j.jphotobiol.2005.03.011>.
- Damiani, E., Baschong, W., Greci, L., 2007. UV-Filter combinations under UV-A exposure: concomitant quantification of overall spectral stability and molecular integrity. *J. Photochem. Photobiol., B* 87, 95–104. <https://doi.org/10.1016/j.jphotobiol.2007.03.003>.
- Danovaro, R., Bongiorno, L., Corinaldesi, C., Giovannelli, D., Damiani, E., Astolfi, P., Greci, L., Pusceddu, A., 2008. Sunscreens cause coral bleaching by promoting viral infections. *Environ. Health Perspect.* 116, 441–447. <https://doi.org/10.1289/ehp.10966>.
- de Alencar Filho, J.M.T., Sampaio, P.A., Valença Pereira, E.C., de Oliveira Júnior, R.G., Silva, F.S., da Silva Almeida, J.R.G., Araújo Rolim, L., Pereira Nunes, X., da Cruz Araújo, E.C., 2016. Flavonoids as photoprotective agents: a systematic review. *J. Med. Plants Res.* 10, 848–864. <https://doi.org/10.5897/JMPR2016.6273>.
- Deng, Y.H., Feng, X.J., Zhou, M.S., Qian, Y., Yu, H.F., Qiu, X.Q., 2011. Investigation of aggregation and assembly of alkali lignin using iodine as a probe. *Biomacromolecules* 12, 1116–1125. <https://doi.org/10.1021/bm101449b>.
- Diffey, B.L., Robson, J., 1989. A new substrate to measure sunscreen protection factors throughout the ultraviolet spectrum. *J. Soc. Cosmet. Chem.* 40, 127–133. <https://libary.sconline.org/v040n03/1>.
- Domínguez-Robles, J., Tamminen, T., Liittä, T., Soledad Peresin, M., Rodríguez, A., Jääskeläinen, A.-S., 2018. Aqueous acetone fractionation of kraft, organosolv and soda lignins. *Int. J. Biol. Macromol.* 106, 979–987. <https://doi.org/10.1016/j.jbiomac.2017.08.102>.



- Stevanato, R., Bertelle, M., Fabris, S., 2014. Photoprotective characteristics of natural antioxidant polyphenols. *Regul. Toxicol. Pharmacol.* 69, 71–77. <https://doi.org/10.1016/j.yrtph.2014.02.014>.
- Warshaw, E.M., Wang, M.Z., Maibach, H.I., Belsito, D.V., Zug, K.A., Taylor, J.S., Mathias, T., Sasseville, D., Zirwas, M.J., Fowler, J.F., DeKoven, J.G., Fransway, A.F., DeLeo, V.A., Marks, J.G., Pratt, M.D., Storrs, F.J., 2013. Patch test reactions associated with sunscreen products and the importance of testing to an expanded series: retrospective analysis of North American Contact Dermatitis Group data, 2001 to 2010. *Dermatitis* 24, 176–182. <https://doi.org/10.1097/der.0b013e3182983845>.
- Wilson, B.D., Moon, S., Armstrong, F., 2012. Comprehensive review of ultraviolet radiation and the current status on sunscreens. *J. Clin. Aesthet. Dermatol.* 5, 18–23.
- Yao, Z.Y., Qi, J.H., 2016. Comparison of antioxidant activities of melanin fractions from chestnut shell. *Molecules* 21, 487. <https://doi.org/10.3390/molecules21040487>, 487.