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Use of *Agave tequilana*-lignin and zinc oxide nanoparticles for skin photoprotection

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ABSTRACT

The use of sunscreens is essential for preventing skin damage and the potential appearance of skin cancer in humans. Inorganic active components such as zinc oxide (ZnO) have been used commonly in sunscreens due to their ability to block UVA radiation. This ultraviolet (UV) protection might be enhanced to cover the UVB and UVC bands when combined with other components such as titanium dioxide (TiO_2). In this work we evaluate the photoprotection properties of organic nanoparticles made from lignin in combination with ZnO nanoparticles as active ingredients for sunscreens. Lignin nanoparticles were synthesized from *Agave tequilana* lignin. Two different pulping methods were used for dissolving lignin from agave bagasse. ZnO nanoparticles were synthesized by the precipitation method. All nanoparticles were characterized by SEM, UV–Vis and FT-IR spectroscopy. Nanoparticles were mixed with a neutral vehicle in different concentrations and in-vitro sun protection factor (SPF) values were calculated. Different sizes of spherical lignin nanoparticles were obtained from the spent liquors of two different pulping methods. ZnO nanoparticles resulted with a flake shape. The mixture of all components gave SPF values in a range between 4 and 13. Lignin nanoparticles showed absorption in the UVB and UVC regions which can enhance the SPF value of sunscreens composed only of zinc oxide nanoparticles. Lignin nanoparticles have the added advantage of being of organic nature and its brown color can be used to match the skin tone of the person using it.

1. Introduction

Daily exposure to sunlight makes the use of a UV radiation screen essential. UV rays can damage the skin by sunburn [1], premature aging [2], photo-allergies [3], and can ultimately produce skin cancer [4]. UV radiation ranges from 200–400 nm and is classified into UVC (100–280 nm), UVB (280–315 nm) and UVA (315–400 nm). UVA has been subdivided into UVAI and UVAII (315–340 nm and 340–400 nm, respectively) [5]. The whole range of UV radiation is, up to some extent, harmful to the skin. However, UVC radiation may be neglected because it is absorbed by the ozone layer. On the other hand, over 95% of the UV radiation reaching the Earth's surface is UVA and only 5% is UVB radiation, the latter being the main cause of damage to the skin due to its greater amount of energy. This is why sunscreens are usually limited to the care and protection against UVB.

There are two types of active ingredients in sunscreens that provide protection against UV radiation. They are classified as chemical or organic, and physical or inorganic [5]. Chemical ingredients absorb UV radiation and convert it into heat; other pathways for excited state decay are radiative decay (fluorescence), isomerization (i.e. octinoxate) and photodegradation (i.e. avobenzone) [5–6]. On the other hand, physical or inorganic active ingredients create a layer on the skin that screens solar radiation by scattering and/or reflecting UV radiation. Common physical shields are ZnO and TiO_2 (titanium dioxide) [7]. Regardless of the type of active ingredient that sunscreens contain in any case, the bottom line is to protect and help to prevent skin damage from UV radiation. The ability to avoid erythema (sunburn) by a sunscreen is indicated by the SPF, which is a measure of how much more sun exposure it takes to undergo sunburn. Erythema is mainly caused by UVB radiation; however, it is necessary to cover the UVA and visible regions because they also damage the skin [8]. This fact has spurred research on inorganic components, such as TiO_2 and ZnO, which reflect or scatter UV radiation, besides absorbing, because they also provide protection against light in the visible range.

There are natural organic substances that absorb UV radiation. A clear example of this type of substance is lignin, which is an aromatic polymer from vascularized plants, whose absorbance region is exactly in the UVC and UVB regions [9]. Lignin is a highly complex biopolymer considered the second biggest source of carbon in the planet after cellulose. It performs vital functions for the structural strength of terrestrial plants, the conduction of water and the defense against pathogens. Despite these roles, the study of lignin has been limited by its own nature. The fact that the macromolecular pattern can vary from species to

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species has made it almost impossible to generate a detailed model of lignin [10–11]. The composition of lignin varies naturally among species, tissues within a given plant, and as a function of plant development. Lignin is an abundantly available, low-cost waste product from pulp industries and bio-refineries that hydrolyze cell wall polysaccharides from renewable resources (such as woody biomass, corn stover and wheat straw) and convert the monosaccharides into fuels and chemicals [12]. The chemical and technological uses of lignin modification products are enormous, beginning with the great amounts of raw material, available from the paper industries that usually dispose or burn them as a secondary energy source. Lignin has great energy content because of the presence of aromatic rings. Also, the carbon skeleton presents a wide range of substitution and additional reaction sites. These products have also great compatibility with basic chemicals like sulfur, sodium and nitrogen, have good absorbent, adhesion and ion exchange properties, and are a direct source of various kinds of phenolic and aromatic compounds [13]. This biopolymer is an ideal candidate for use as an active ingredient in a sunscreen against UV radiation, and its combination with ZnO will help provide better coverage against the full spectrum of UV radiation from the sun and in the visible range. The aim of this paper is to use nanoparticles of lignin (ligninNPs) and ZnO (ZnONPs) as active ingredients in a sunscreen formulation targeted to provide adequate protection against a broad UV spectral range.

2. Materials and Methods

2.1. Chemicals

Neutral body cream (Lubriderm® daily moisture lotion cream, normal to dry skin) was used as a vehicle for sunscreen preparation.

2.2. Methods

2.2.1. Isolation of Agave Lignin

Lignin was obtained from *A. tequilana* Weber bagasse by soda and organosolv pulping. Soda pulping was carried out at a liquor to solids ratio 10:1 (NaOH 20 wt.% solution) at 195 °C, 1 h. In order to precipitate lignin, the obtained black liquor was treated by bubbling CO_2 at 85 °C until reaching pH 8. Lignin was separated then by filtration in a Gooch filter (porosity 10–15 μm) and extensively washed. This lignin was further purified by dissolving it again in NaOH 5 wt.% (1:10 lignin: NaOH solution) and thereafter precipitated with sulfuric acid (0.3 N) until reaching pH 2, filtered, washed with hot water (60 °C) and vacuum dried for further use.

Organosolv pulping was performed at a liquid to solids ratio 10:1 with ethanol (48 vol.%), and 3 wt.% acetic acid as catalyst at 175 °C for 2.5 h. Organosolv lignin was obtained by evaporation of ethanol in a rotaevaporator (Büchi B-490) and purified in the same manner as the soda lignin (vide supra).

2.2.2. Synthesis of LigninNPs and ZnONPs

LigninNPs were synthesized based on the method described else were [14]. Briefly: a lignin slurry 17 wt.% was magnetically stirred (600 rpm) in a beaker for 1 h, and then 3.67 wt.% NaOH (based on lignin content) was added. After 2 h, ammonium hydroxide was added (active ammonium [NH_4], 7.90 wt.%) and the mixture was subjected to high intensity mixing with an Ultra-turrax (IKA, T10) at 24,000 rpm for 5 min, then active formaldehyde was added (vide infra) and the temperature was raised to 85 °C and left 2 h under magnetic stirring at 600 rpm for crosslinking and nanoparticle formation in suspension. Six types of ligninNPs were prepared, three for each type of lignin source, using 18 and 27 wt.% of active formaldehyde (CH_2O) and the lignin samples in absence of formaldehyde as blanks. These ligninNPs were labeled as: L1 (without formaldehyde or blank), L2 (18 wt.% CH_2O) and L3 (27 wt.% CH_2O) for each type of lignin source, i.e. organosolv or soda.

The ZnONPs used in this work were synthesized by a direct precipitation method using zinc nitrate and KOH as precursors. Aqueous solutions of 0.2 M zinc nitrate hexahydrate and 0.4 M KOH was prepared with deionized water. The KOH solution was slowly added into the zinc nitrate solution at room temperature under vigorous stirring, which resulted in the formation of a white suspension. The white product was centrifuged at 5000 rpm for 20 min and washed three times with distilled water, and with absolute alcohol at last. The obtained product was calcined at 500 °C in air for 3 h.

2.3. Scanning Electron Microscopy Analysis

Morphological characterization of ZnONPs and ligninNPs was performed by scanning electron microscopy (SEM) in a FEI Inspect F50 system. Both NPs were analyzed directly from the powder placed on the carbon tape of the microscope. Lignin and ZnONPs size distribution was analyzed using Image J 1.45 software [15]. Particle size was calculated by measuring 200 particles in random field of view.

2.4. UV–Vis Spectroscopy Analysis

Absorbance spectra of ZnONPs, bulk lignin and ligninNPs suspended in diluted NaOH (1.25 mM) were recorded using a UV–Vis Redtide-Ocean Optics spectrophotometer, with 1 cm pathway cell. The baseline during the experiment was made using only DI water as reference.

2.5. FT-IR Spectroscopy Analysis

FT-IR spectra of lignins (both bulk and ligninNPs) were obtained with a Spectrum GX spectrometer (Perkin-Elmer), in the 4000–550 cm^{-1} range with a resolution of 4 cm^{-1}. Bulk lignin and ligninNPs were analyzed directly from the powder.

2.6. Sunscreen Preparation

It is important that the preparation can be spread on the skin without irritation problems, allergies, or any other side effects; therefore, Lubriderm® daily moisture lotion cream was chosen as a vehicle because it is neutral, without UV protection and no adverse side effects. In order to measure the transmission spectra and to obtain the SPF the mixture has to be perfectly quantized, and the proportions of active components need to be similar to those of commercial sunscreens (1–20 wt.%). Sunscreens were prepared by adding 5.0 wt.% ZnONPs and varying the amount of ligninNPs in percentages of 5.0, 10.0 and 15.0% by weight of cream. Sunscreens containing only ZnONPs and only ligninNPs in the range 5–15 wt.% were also prepared. Homogeneous dispersions were accomplished by using a high intensity mixer with an ultrasonic tip (Ultra-Turrax). The amounts of active components indicated above were mixed with the Ultra-turrax starting at 8000 rpm for 5 min, and then at 10,750 rpm for 5 more minutes and then at 13,500 rpm for 10 min.

2.7. SPF Values Measurement

SPFs were measured by homogeneously depositing each sample (2 mg/cm^2, standard amount recommended by dermatologists) on quartz substrates (1 cm × 1 cm). A gloved (latex) finger (*digitus secundus*) was used to achieve as uniform a thickness as possible with a circular light rubbing motion for about 10 s; this technique gives a high reproducibility and re-use compared to other methods for in vitro test [16]. Transmission measurements were then carried out. Each substrate was placed on a Redtide-Ocean Optics spectrophotometer and the percent transmission was measured in the 200 to 800 nm range. All measurements were made at ambient temperature under the same conditions (21 °C at 50% relative humidity). SPFs were obtained from transmission measurements results using Eq. (1) [16]. The