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(54) Title: LIGNIN-COATED METAL OXIDE NANOPARTICLES AND USE THEREOF IN COSMETIC COMPOSITIONS

(57) Abstract: While titanium dioxide is commonly used as a sunscreen, concerns persist regarding the catalytic activity of TiO₂ and its generation of reactive oxygen species (ROS). The present invention attempts to address these concerns by providing TiO₂ nanoparticles coated with lignin, yielding a thin-coated metal oxide nanoparticle. The lignin coating is presumed to scavenge ROS before they diffuse away from the particles and interact with the user's body or other components of a cosmetic formulation (such as organic sunscreens). Other metal oxides coated with lignin are also disclosed, as well as methods for preparing lignin coated metal oxide nanoparticles.

LIGNIN ENCAPSULATED NANOPARTICLES AND USES THEREOF

FIELD OF INVENTION

[0001] The present invention relates to nanoparticles, and associated preparative methods. In particular, the present invention relates to nanoparticles coated with lignin, a process to prepare the coated nanoparticles, and uses thereof, including uses in cosmetic formulations and diagnostic applications.

BACKGROUND OF THE INVENTION

[0002] Titanium dioxide (TiO_2) is a common ingredient in many sun protection products, including sunscreens. In fact TiO_2 , along with zinc oxide are the two ingredients that are allowed in the largest concentrations in sunscreens commercialized in North America (up to 25% permitted). This is rather surprising, as the same form of TiO_2 (predominantly anatase) is also used for a wide range of light initiated and free radical mediated processes, including solar cells, self-sterilizing tiles and treatment and purification of polluted waters, due to its high reactivity.

[0003] There have been significant concerns about titanium dioxide safety as a sunscreen ingredient. These concerns reflect the known fact that titanium dioxide generates highly reactive oxygen species (ROS) when exposed to light in the presence of oxygen and humidity.

[0004] Accordingly, new compositions which alleviate some of the concerns relating to TiO_2 adverse effects are highly desirable.

SUMMARY OF THE INVENTION

[0005] It is an object of the invention to provide a thin-coated metal oxide nanoparticle and process for preparing a thin-coated nanoparticle.

[0006] Accordingly, there is provided herein a nanoparticle comprising a metal oxide coated with lignin to form discrete coated particles which have a nanometric size. The metal oxide can in certain embodiments be TiO_2 or ZnO .

[0007] In the above described embodiment, the lignin is cross-linked over a surface of the metal oxide particle. In certain embodiments, the cross-linking is carried out by UVA irradiation.

[0008] In certain embodiments, the coating on the metal oxide particle may be from about 1 to about 10 nm in thickness. In further embodiments, the coating on the metal oxide particle may be from 2 to 5 nm in thickness. In a specific embodiment, the coating on the metal oxide particle is about 3 nm in thickness.

[0009] As described herein, the lignin may be an organo-soluble or water-soluble source of lignin. Moreover, the lignin can be a pure source of lignin, or a source which includes carbohydrates. In certain specific embodiments, the lignin is Kraft lignin, Organosolv lignin, Low Sulfonate Content (LSC) lignin, Sodium lignin, Sodium lignin without sugars or Alkali lignin. Other lignin sources with similar properties may also be used.

[0010] Also provided herein is a process for preparing a lignin coated nanoparticle, comprising

- a. mixing a metal, or a metal oxide nanoparticle precursor with solubilized lignin to form a mixture; and
- b. irradiating the mixture with UVA at a wavelength effective to form particles which have a nanometric size.

[0011] In the described process, the metal oxide may be TiO_2 or ZnO .

[0012] In embodiments of the described process, the lignin is cross-linked over a surface of the metal oxide particle. In certain embodiments the mixture may be irradiated at about 369 nm to carry out the cross-linking. More particularly, the irradiating is carried out for a time effective to produce a coating on the metal oxide particle that is preferably from about 1 to 10 nm in thickness, more typically from 2 to 5 nm in thickness, and in specific embodiments about 3 nm in thickness.

[0013] As mentioned above, the lignin used in the process may be an organo-soluble or water-soluble source of lignin. Moreover, the lignin can be a pure source of lignin, or a source which includes carbohydrates. In certain specific embodiments, the lignin is Kraft lignin, Organosolv lignin, Low Sulfonate Content (LSC) lignin, Sodium lignin, Sodium lignin without sugars or Alkali lignin. Other lignin sources with similar properties may also be used.

[0014] The process can be carried out, in specific embodiments, by combining the metal oxide nanoparticle precursor with an excess of lignin, for example, from about 1:10 to about 1:5 w/w metal oxide:Lignin. In specific non-limiting examples, 1:10 w/w or 1:5 w/w TiO₂:Lignin may be combined in the mixture. In addition, in further embodiments, the irradiating may be carried out for up to 2 hours with stirring to keep the oxide nanoparticle precursor and lignin in suspension. The process may also be carried out in batch or continuously. For example, if a continuous reaction is used for larger scale production, the irradiating may be carried out under continuous flow conditions. For example, continuous flow conditions may be carried out at a flow rate of 1 to 10 mL/sec, or in a specific embodiment, at a flow rate of about 4 mL/sec. In certain embodiments of the continuous flow conditions described herein, it is possible to produce gram scale quantities of lignin-coated metal oxide nanoparticles. Using a small optical width for the UVA irradiation can also increase cross-linking efficiency and reduce reaction times.

[0015] In further embodiments, which may be preferred depending upon user needs, the process may include a further step after irradiation whereby the nanoparticles are separated from the mixture by centrifugation and washed.

[0016] Also provided is a cosmetic composition comprising a nanoparticle as described above, or produced according to a process as described above, and a suitable carrier or excipient. In certain non-limiting embodiments, the cosmetic composition may be a sunscreen or other cosmetic including the nanoparticle defined herein as a sunblock agent.

[0017] For example, the cosmetic composition may be prepared as a topical skin care composition. In such embodiments, the cosmetic composition may be a sunscreen, skin moisturizer, skin cream, body lotion, body spray, mascara, foundation, rouge, face powder,

eyeliner, eyeshadow, nail polish, lipstick, or another personal care composition in which sunblock may be included as an ingredient. In one non-limiting embodiment involving coated TiO₂ nanoparticles, the lignin coating acts as a sacrificial antioxidant preventing the free radical reactions that TiO₂ otherwise initiates. It does so while preserving the sun protection and light scattering properties of TiO₂.

BRIEF DESCRIPTION OF THE DRAWINGS

[0018] These and other features of the invention will become more apparent from the following description in which reference is made to the appended drawings wherein:

FIGURE 1 shows ATR-IR spectra of TiO₂ (black), Kraft lignin (blue) and TiO₂@Kraft (red);

FIGURE 2 shows DR spectra of TiO₂ (black), Kraft lignin (red) and TiO₂@Kraft (blue);

FIGURE 3 shows a TEM image of TiO₂@LSC showing organic shell surrounding the inorganic particle (arrow). Scale bar: 20 nm;

FIGURE 4 shows the percentage of Lignin released (or degraded) upon UVA-UVB irradiation for 2 h. The plot assumes that the absorption coefficient of lignin is constant, that is, insensitive to exposure or release. Data reproducible within $\pm 5\%$;

FIGURE 5 shows the percentage of 2-propanol remaining upon UVA-UVB irradiation in the presence of different Particles. (A) TiO₂ (black), TiO₂@Kraft (blue) and TiO₂@Org (red). (B) TiO₂ (black), TiO₂@LSC (blue), TiO₂@Sodium (red), TiO₂@Sodium without sugars (green), and TiO₂@Alkali (violet);

FIGURE 6 shows the results of avobenzone photodegradation using different amount of particles: A) 0.01 %, B) 0.03 % and C) 0.06 %. Percentage of avobenzone remaining upon UVA-UVB irradiation in the absence (black) and in the presence of TiO₂ (blue), TiO₂@Kraft (red) and TiO₂@Org (green) and TiO₂@LSC (violet);

FIGURE 7 shows kinetic traces of the enzymatic activity of ALP acquired at 405 nm for the dephosphorylation of PNPP. Traces recorded after enzyme pretreatment in absence of

particles (black) and in the presence of TiO₂ (blue), and TiO₂@Kraft (red). Full circle under dark conditions and open circle upon UVA irradiation for 30 min;

FIGURE 8 shows initial rates calculated for the enzymatic activity of ALP under dark and upon UVA irradiation in the absence (black) and in the presence of TiO₂ (blue), TiO₂@Kraft (red) and TiO₂@Org (green) and TiO₂@LSC (violet);

FIGURE 9 shows SPF values measured *in vitro* using Coppertone sunscreen as reference (C = 0.3525) before (black) and after UVA-UVB exposure (blue);

FIGURE 10 shows ATR-IR spectra of TiO₂, lignin and lignin@TiO₂;

FIGURE 11 shows DR spectra of TiO₂, L2@TiO₂, L3@TiO₂, L4@TiO₂;

FIGURE 12 shows emission spectrum obtained after combination of UVA-UVB lamps.

DETAILED DESCRIPTION

[0019] The present inventors have developed a novel approach for scavenging ROS and other species that may be formed before they diffuse away from metal oxide particles, such as TiO₂, and cause damage to either biomolecules or other important sunblock ingredients. This approach is different from the usual modifications of TiO₂ using SiO₂ or Al₂O₃, or from the known attenuation of radical generation upon encapsulation in large pore zeolites, and involves the use of lignin to construct a thin shell around the metal oxide particles.

[0020] Previous work has involved the use of large lignin structures (micrometers and more) in which metal oxide (TiO₂) nanoparticles have been embedded. In such formulations, the nanoparticulate structure of titanium dioxide is lost as it is integrated in a lignin matrix. The nanoparticulate structure is a key characteristic in cosmetic and sunscreen applications as both its incorporation and light scattering properties are directly affected by the size and morphology of the material.

[0021] The present inventors have found that coating metal oxide nanoparticles with a thin shell of lignin (e.g. lignin@TiO₂), maintains the nano-structure needed for effective cosmetic and sunscreen or sunblock applications. Lignin does not necessarily stop the metal oxide from making free radicals, but rather acts as a first line of defense, scavenging the radicals before they escape the shell or vicinity of the nanoparticle, thus preventing the interaction of free radicals with vulnerable biomolecules.

[0022] The lignin coated nanoparticle described herein therefore takes advantage of the free-radical scavenging and antioxidant properties of lignin, which is effectively used as a sacrificial scavenger for the ROS anticipated from TiO₂ and other metal oxides.

[0023] Different kinds of lignins can be attached to the surface of the metal oxide nanoparticles by UVA irradiation. Less than 20% of lignin release has been found (Figure 4) upon irradiation with UVA-UVB light showing good particle stability within the exposure time expected for sunscreens (2 - 4h). Furthermore, studies carried out with one type of particles demonstrate the addition of lignin (LSC) does not affect the SPF values of TiO₂, and does not deteriorate SPF performance of TiO₂ upon UVA-UVB irradiation (Figure 2).

[0024] In addition, the present inventors have tested degradation levels of avobenzone upon UVA-UVB irradiation in the presence of different particles and at different concentrations. The performance of the coated particles (lignin@TiO₂) as avobenzone protectors is shown herein to be equal or greater, depending on the particle concentration, than the pristine TiO₂ NPs (Figure 3). This is especially advantageous since avobenzone is one of the most common sunscreen ingredients, and is widely employed as a UVA protector. However, it suffers from the problem of photodegradation, thus limiting its effectiveness in commercial formulations. These results also suggest that these particles can provide additional protection to other sunscreen ingredients.

[0025] The coated particles (lignin@TiO₂) are also shown, based on experiments using horseradish peroxidase (HRP) as a biological indicator of the bioeffects of TiO₂, that embodiments of the described coated particles can help preserve enzymatic activity when

compared with bare TiO₂ NPs (Figure 7). This indicates that the coated particles described herein can help alleviate certain adverse effects of TiO₂.

[0026] Accordingly, as shown in the following experiments, the coated particles described herein, in certain embodiments, can reduce free radical damage to biomolecules, and work well in conjunction with avobenzone, the most common UVA sunblock, reducing its level of photodegradation (the most common problem with avobenzone).

EXPERIMENTS:

[0027] In order to evaluate the effect of a lignin shell on TiO₂ reactivity and a potential ingredient in sunscreens and cosmetics several types of experiments were performed. First, the inventors prepared TiO₂@Lignin hybrids using various types of lignin and studied their properties, including morphology and stability. Second, given that TiO₂ is a good photocatalyst for the oxidation of alcohols to ketones, the inventors evaluated to what extent the oxidation of isopropanol to acetone is inhibited for lignin-modified TiO₂. This provides a direct measurement of the ability of TiO₂ to catalyze oxidations and is rooted in our knowledge of the catalytic properties of TiO₂. Third, the inventors tested to what extent lignin modifications can reduce the extent of TiO₂-mediated photochange to enzymes. For this purpose, the inventors used the inactivation of Alkaline Phosphatase (ALP) as a test system. Fourth, the inventors examined the possible photoprotection of avobenzone by TiO₂ and lignin modified TiO₂. Avobenzone is a widely used UVA ingredient, largely present in an enol form that photo-degrades readily upon UVA-UVB exposure. Given the ubiquitous use of avobenzone, it was important to establish its compatibility with the new hybrid materials to evaluate to what extent they could help with avobenzone's lack of photostability. The following sections cover the four types of experiments mentioned above.

Materials & Methods

[0028] **Materials:** Lignin alkali low sulfonate content, Lignin alkali, Brij 10, Alkaline phosphatase (ALP) and *p*-nitrophenylphosphate (PNNP) were purchased from Aldrich. Tetrahydrofuran and 2-propanol were purchased from Fischer Scientific and Avobenzone

from Wako. Kraft lignin was purchased from MeadWestVaco and Organosolv lignin (extracted with 1:1 ethanol/water from mixed hardwoods - aspen, maple and birch) was provided by Lignol Energy Corporation; both were a generous gift from Professor T. Baker of Ottawa's Centre for Catalysis Research and Innovation. Lignosulfonate sodium with and without sugars were a gift from Burgo company. TiO₂ P25 was a gift from Evonik Degussa.

[0029] **Instruments:** A High Efficacy 368 nm 11W UV LED Emitter LZ4-00U600 was used to synthesize the particles under irradiation. For all irradiation experiments a Luzchem CCP-4V customized computer-controlled photoreactor, with temperature control was used with 10 UVA lamps and 4 UVB lamps (Figure S4). UV-visible spectroscopy was carried out using a Cary 100 spectrophotometer. The enzymatic assay was performed in a 96 well-plate using a microplate reader SpectraMax M5. NMR spectra were recorded using a Bruker Avance II 300 spectrometer with an appropriate pulse sequence with a spectral width of -0.5 ppm to 12.5 ppm and with the pre-saturation signal centered at 4.706 ppm (proton water signal).

Attenuated Total Reflectance Infrared (ATR-IR) spectra were recorded with a Varian 640 FTIR spectrometer equipped with an ATR accessory in the 500 – 4000 cm⁻¹ range. Diffuse reflectance (DR) spectra were recorded in an Agilent Cary 7000 spectrophotometer equipped with praying mantis (Harrick). The powder X-ray diffraction analysis was carried out at room temperature on Rigaku Ultima IV powder diffractometer in Bragg-Brentano geometry, using Cu K α radiation ($\lambda = 1.5418 \text{ \AA}$). Two theta range of 10° to 100° was covered with 0.02° step width and 1°/min scan speed. The percentage of molar mass of the adsorbed polymer on the surface of TiO₂ was measured by Thermogravimetric analysis (TGA) using a Q5000 IR instrument (TA Instruments; New Castle, DE, USA) under N₂ or air flow (120 mL/min) with a heating rate of 10 °C/min (balance gas with nitrogen 10.0 ml/min; sample gas with nitrogen 25.0 ml/min). The sample TGA data were analyzed by using TA Instruments Universal Analysis 2000 Version 4.5 A. Transmission Electron Microscope (TEM) images were acquired with a Jeol JEM-2100F field emission transmission electron microscope. TEM samples were prepared by drop casting a water suspension of catalysts onto 400 square mesh carbon coated copper grids (Electron Microscopy Sciences).

[0030] **Synthesis of particles.** Briefly, 10 (or 100 mg) of lignin were solubilized in 5 mL of solvent (water or THF, according to the solubility properties of the corresponding lignin) and placed together with 10 mg of TiO₂. The mixture is kept in the dark overnight and then submitted to UVA (368 nm LED) irradiation for 2h under vigorous stirring. The slurry is separated by centrifugation and washed three times. The resulting particles are dried at 100-120 °C for at least 1 h. The particles were characterized by ATR-IR, DR, TEM, and TGA.

[0031] **Photocatalytic oxidation of 2-propanol.** The photoactivity of the NPs was observed using as a reference reaction the photooxidation of 2-propanol to acetone. The reaction was carried out at 35-38°C under combined UVA-UVB irradiation (10 UVA lamps and 4 UVB lamps). Control experiments under dark conditions were also performed (TiO₂, lignin, Lignin@TiO₂) showing no reaction. The conversion of 2-propanol in aqueous solution (5 mM) under stirring was evaluated in presence of TiO₂ and several Lignin@TiO₂ NPs. For this, 1 mL aliquots of particles were used to reach a final concentration of 0.4 mg/mL in 5 mL and the sample was collected each 1 h for 5 h. Each aliquot was centrifuged at 7000 rpm, 20°C, for 10 min and 800 µL of the supernatant was used to record the ¹H NMR spectrum using water suppression sequence with pre-saturation signal centered at 4.706 ppm (proton signal of H₂O) in presence of 3-(trimethylsilyl)-2,2,3,3-tetradeutero propionic acid (sodium salt) (TMSP) in D₂O as external standard to analyze the degradation of 2-propanol over irradiation time using a calibration curve previously done.

[0032] **Enzyme inactivation: TiO₂-mediated photodamage.** Alkaline phosphatase from bovine intestinal mucosa (ALP) (0.02 mg/mL) solution and particles suspension (0.25 mg/mL) were prepared in cold buffer (1.0 M diethanolamine with 0.50 mM magnesium chloride) pH 9.8 at 37 °C. The substrate solution of *p*-nitro phenylphosphate (PNPP) was prepared in water with a concentration of 0.5 mM. The enzyme was submitted to UVA irradiation for 30 min in the absence and in the presence of 50 µg/mL TiO₂ or Lignin@TiO₂ under stirring. Then, the suspensions were centrifuged at 11,000 rpm for 15 min at 0° C. Control reactions under dark conditions were also performed. The enzymatic assay was performed in a 96 well-plate using the following final concentrations: [PNPP] = 25 µM and

[ALP] = 1.5 $\mu\text{g/mL}$. The enzyme activity was followed by monitoring the absorbance changes at 405 nm, where the dephosphorylated product has a maximum absorption.

[0033] ***Compatibility with Avobenzone.*** An Avobenzone aqueous solution (24 μM) was prepared in 1 mM Brij-10 solution ($< 0.04\%$ of 2-propanol). The mixture was sonicated for 3 h and stored in the dark one night. The reaction was carried out using 8 mL of this solution in a quartz test tube placed in a photoreactor equipped with 10 UVA lamps and 4 UVB lamps under stirring. TiO_2 and several Lignin@ TiO_2 NPs were tested using three different Avobenzone/particles ratio: 1/13; 1/41; 1/82 (w/w). Samples (1 mL) were collected at 1h intervals for 4h and centrifuged at 7000 rpm, 20°C, 10 min. Each aliquot was analyzed by UV spectroscopy recording absorbance at 362 nm.

Results and Discussion

Synthesis and characterization

[0034] Different kinds of lignin were used for the synthesis of the new material ranging from water soluble lignin to lignins that can only be solubilized in organic solvents (Table 1). Briefly, 10 mg of lignin are solubilized in 5 mL of solvent (water or tetrahydrofuran –THF-, according to the solubility properties of the corresponding lignin) and placed together with 10 mg of TiO_2 . The mixture is kept under dark overnight and then submitted to UVA (368 nm LED) irradiation for 2h under vigorous stirring. The slurry is separated by centrifugation and washed with water (or THF, respectively) three times. The resulting particles are dried at 100-120 °C for at least 1 h. The particles were characterized by Attenuated Total Reflectance Infrared (ATR-IR), Diffuse Reflectance (DR), Transmission Electron Microscopy (TEM), and Termogravimetry analysis (TGA).

[0035] The particles can thus be synthesized under very mild conditions taking advantage of the photocatalytic activity of TiO_2 . Upon UVA irradiation a mixture of a lignin solution (organic or aqueous solution depending on the type of lignin used) in the presence of TiO_2 , lignin can be cross-linked over the particle surface (due to light-induced ROS generation) and lead to lignin-coated TiO_2 nanoparticles within 1-2 hours.

[0036] Figures 1 and 2 show the functionalization of TiO₂ using Kraft lignin. Similar results were found for the other types of lignin used (Figures 10 and 11). According to the IR spectrum of TiO₂, the band at 3400 cm⁻¹ is due to the OH stretching, while at 1630 cm⁻¹ OH bending vibrations can be observed. Between 1000 and 400 cm⁻¹, the broad band is related to the Ti-O-Ti stretching bonds. On the other side, the characteristic peaks of lignin are at 2900 cm⁻¹ for sp³ C-H stretching, 1600 cm⁻¹ for C=O stretching and below 1500 cm⁻¹ for the aromatic rings bending. In general, all particles clearly show signals corresponding to both TiO₂ and lignin. The diffuse reflectance (DR) spectra in Figure 2 show that the particles can slightly extend the absorption of TiO₂ to the visible light region (typically below 400 nm) due to the presence of lignin. Note the thin lignin coating makes these compositions cosmetically acceptable not only in terms of light absorption and scattering but also in terms of the visible color and appearance. In fact, lignin@TiO₂ NPs show a very light tint suitable for skincare formulations. Additionally, as the particles are insoluble in water, they are effectively waterproof.

[0037] Figure 3 is a HR-TEM image suggesting organic shell surrounds the TiO₂ particles, and more important, that the particles retain their nanometric size (≤ 50 nm). Finally, Table 2 shows the amount of lignin found on each particle using TGA. Of note, the organo-soluble lignins generate particles with higher loadings, presumably due to the presence of more conjugated structures in those types of lignin that can interact better with the free radicals generated by TiO₂.

Table 1. Different types of lignin used for TiO₂ encapsulation

Name	Type of Lignin	Solubility
L ₁	Kraft Lignin	Organo-soluble
L ₂	Organosolv Lignin	
L ₃	Low Sulfonate Content (LSC)	
L ₄	Sodium Lignin	Water-soluble
L ₅	Sodium Lignin without sugars	
L ₆	Alkali Lignin	

Table 2. Weight percentage of lignin in each particle found after thermogravimetric analysis (TGA).

Particle	Lignin (wt %) ^a	Shell thickness (nm) [*]
TiO ₂ @Kraft	43	9.5
	13 ^b	3.6
TiO ₂ @Org	18	4.7
	8 ^b	2.3
TiO ₂ @LSC	9	2.6
	5 ^b	1.5
TiO ₂ @Sodium	8	2.3
TiO ₂ @Sodium no sugars	6	1.8
TiO ₂ @Alkali	3	0.9

^a Synthesized using 100 mg of lignin unless otherwise indicated. ^b Synthesized using 10 mg of lignin. *Shell thickness calculated with a density of 3.8 g mL⁻¹ for anatase (10 mg) and assuming a 50 nm particle size.

Stability test

[0038] The stability of the particles in an aqueous solution upon UVA-UVB irradiation was monitored by UV spectroscopy, following the absorption at the wavelength of maximum absorption of the corresponding lignin. Thus, the absorbance due to leached or degraded lignin can be measured in the supernatant of the mixture after 2 h of irradiation.

[0039] Dried NPs (2.5 mg) were re-suspended in aqueous solutions (10 mL) and their stability was analysed at 35-40°C under UVA/UVB irradiation. At specific times, an aliquot of 1 mL of each sample was taken and centrifuged at 7000 rpm, 20°C for 10 min. The supernatant was analyzed by UV spectroscopy to evaluate the possible presence of small compounds originated from leaching and/or degradation of lignin. Control experiments in dark conditions show same tendency within the experimental error. As can be seen in Figure 4, the particles show great stability under UVA-UVB exposure (see also Figure 12). As it was expected, the % of Kraft and organosolv lignin (organo-soluble lignins L₁ and L₂) released was lower compared with water-soluble lignin.

Photocatalytic oxidation of 2-propanol

[0040] The photoactivity of NPs was observed using as reference reaction the photooxidation of 2-Propanol to acetone. The reaction was carried out at 35-38°C under UVA-UVB irradiation. Control experiments under dark conditions were also run. The conversion of 2-propanol in aqueous solution (5 mM) under stirring was evaluated in presence of TiO₂ and several TiO₂@lignin NPs. For this, 2 mg of particles were used and the sample was collected each 1 h for 5 h. Each aliquot was centrifuged at 7000 rpm, 20°C, for 10 min and 800 µL of the supernatant was used to record the ¹H NMR spectrum using water suppression sequence in presence of 3-(trimethylsilyl)-2,2,3,3-tetradeutero propionic acid (sodium salt) (TMSP) in D₂O as external standard to analyze the degradation of 2-propanol over irradiation time. Figure 5 shows the photocatalytic activities exhibited by the different Lignin@TiO₂ composites compared to the pristine TiO₂. Notice that 2-propanol is totally consumed after 3 h of irradiation in the presence of TiO₂ but different percentages of alcohol still remain when treated with lignin-modified TiO₂. Thus, while the strategy used to synthesize the lignin@TiO₂ is based on the photocatalytic activity of TiO₂, the new composites exhibit the capacity to inhibit free radical reactions. Particles showing the worst photocatalytic activity are indeed the ones chosen as potential sunblock active ingredients. From Figure 5, TiO₂@Kraft, TiO₂@Org and TiO₂@LSC were selected for further examination, although L4@TiO₂ (Sodium Lignin) also shows excellent performance.

Photodegradation of Avobenzone

[0041] In order to determine the compatibility of these new particles with other sunscreen ingredients, the photoprotection of avobenzone was tested. Avobenzone is a widely used UVA protector, largely present in an enol form that photodegrades readily upon UVA-UVB exposure, through a mechanism involving a photo-induced enol-keto transformation. Other sunblock agents can stabilize avobenzone, either by competitive light absorption (or scattering) or by quenching its excited states. Given the ubiquitous use of avobenzone, it is important to establish its compatibility with the new hybrid materials to evaluate to what

extent they could be involved in the process of photodegradation or photoprotection of avobenzone.

[0042] The avobenzone aqueous solution (24 μ M) was prepared in 1 mM Brij-10 solution (< 0.04% of 2-propanol). The mixture was sonicated for 3h and stored in dark overnight. The reaction was carried out using 8 mL of this solution in a quartz test tube placed in a photoreactor equipped with 10 UVA lamps and 4 UVB lamps under stirring. TiO₂ and several TiO₂@lignin NPs were tested using three different particle concentrations: 0.01; 0.03; and 0.06 wt%. The sample (1 mL) was collected each 1h for 4h and centrifuged at 7000 rpm, 20°C, 10 min. Each aliquot was analyzed by UV spectroscopy recording absorbance at 362 nm. Figure 6 shows the photodegradation of avobenzone at two different times (2 and 4 h of UVA-UVB irradiation) and using different amount of particles. Lower particles concentration TiO₂ can act as a photoprotector (graphs A and B), although when the TiO₂ particle concentration is increased this ability is lost. In contrast, the new particles retain the photoprotection ability even at high TiO₂ concentrations. These results clearly show that new particles not only preserve the photoprotection properties that TiO₂ provides to avobenzone (graph A) but also prevent the photodegradation of avobenzone when the amount of TiO₂ added generates high concentration of ROS (graph C). This opens the opportunity to increase the amount of TiO₂ particles in formulations preserving the integrity of other organic active ingredients.

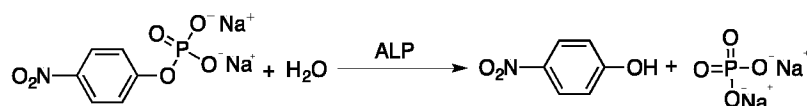
Alkaline phosphatase assay

[0043] Alkaline phosphatase from bovine intestinal mucosa (ALP) (0.02 mg/mL) solution and particles suspension (0.25 mg/mL) were prepared in cold buffer (1.0 M Diethanolamine with 0.50 mM Magnesium Chloride) pH 9.8 at 37°C. The substrate solution of p-nitro phenylphosphate (PNPP) was prepared in water with a concentration of 0.5 mM. The enzymatic activity of ALP was performed after enzyme was pre-treated with different particles and under different conditions. Briefly, the enzyme was submitted to UVA irradiation during 30 min in the absence and in the presence of TiO₂ and TiO₂@lignin NPs

under stirring. Then, the suspensions were centrifuged at 11,000 rpm for 15 min at 0° C. Control reactions under dark conditions were also carried out.

[0044] ALP assay was performed in a 96 well-plate with ([PNPP] = 25 μM; [ALP] = 1.5 μg/mL) and the enzymatic activity was followed by the absorbance changes at 405 nm, where the dephosphorylated product has an absorption maximum. TiO₂ and several TiO₂@lignin NPs were tested using 50 μg/mL.

[0045] The following equation shows the de-phosphorylation reaction used to determine the enzymatic activity, simply by monitoring the formation of *p*-nitrophenol using UV-vis spectroscopy.



[0046] The kinetic traces (Figure 7) acquired at 405 nm for the formation of *p*-nitrophenol are a reflection of the activity of the enzyme. Each curve is then fitted with the expression:

$$A_{405\text{ nm}} = a + bt + ct^2$$

where A is the absorbance and *t* the time. The coefficients a, b and c are fitting parameters.

The derivative of this expression with respect to *t* is given by:

$$\frac{dA}{dt} = b + ct$$

which at *t* = 0 corresponds to b. That is, the first coefficient (b) of the quadratic fit is the calculated initial slope. These slopes have been used as a measure of the enzymatic activity.

[0047] Figure 8 shows the initial rates calculated for the enzymatic activity of ALP after treatment with TiO₂ and TiO₂@lignin particles. As can be noticed, TiO₂ can decrease the enzymatic activity simply by contact (dark conditions). This inhibition is increased under UVA exposure (Light conditions). Coating the TiO₂ nanoparticles with any kind of lignin

prevents the enzyme inactivation even under light conditions. These results indicate clearly that the UVA irradiation does not affect the enzyme and, more important, lignin@TiO₂ NPs are innocuous for the enzymatic activity under dark conditions. The TiO₂-mediated photodamage under UVA irradiation is highly reduced in presence of lignin and the L₁@TiO₂ (Kraft Lignin) composite shows no enzyme inactivation. Clearly the changes that prevent alcohol photooxidation also inhibit enzyme inactivation.

Sun Protection Factor (SPF) determination *in-vitro*

[0048] 0.75 mg/cm² of TiO₂ and TiO₂@Lignin NPs suspension in glycerol (5%; 10%) were placed on a quartz slide (9.75 cm²) covered by 3M Transpore Nexcare tape. The emulsion was weighed on the slide and evenly distributed on the surface. The sample is left to dry under air during 20 min. Five different plates for each sample were analyzed. The light transmittance of each sample was measured between 290 to 400 nm before and after exposure to UVA-UVB light for 2h. The same protocol was followed using Coppertone sunscreen with SPF value informed equal to 30.

[0049] The SPF value was calculated in according to COLIPA standard protocol by the following equation:

$$SPF_{in-vitro} = \frac{\int_{\lambda=290\text{ nm}}^{\lambda=400\text{ nm}} E(\lambda) * I(\lambda) * d\lambda}{\int_{\lambda=290\text{ nm}}^{\lambda=400\text{ nm}} E(\lambda) * I(\lambda) * 10^{-A_0(\lambda) * C} * d\lambda}$$

where E(λ) is the erythema action spectrum, I(λ) the solar spectral irradiance, d(λ) the spectral transmittance of the sample, C a coefficient of adjustment and A₀(λ) correspond to the mean monochromatic absorbance measured per plate of the test product layer before UV exposure.

Conclusions

[0050] Regardless of its great light absorption and scattering properties, there are some health concerns about the use of TiO₂ because of its intrinsic photocatalytic properties. Thus, TiO₂

can generate ROS in the presence of water upon UVA irradiation. The *in vitro* studies reported here demonstrate that TiO₂ particles can be modified in order to decrease their photocatalytic activity, while retaining the absorption and scattering properties desirable for sunscreens and cosmetic uses. Thus, the potential risks from TiO₂-mediated free radical generation are curtailed by shielding the particles with a good antioxidant. Here the inventors have used a non-toxic, biocompatible shell made by lignin that neutralizes the free radicals by scavenging them with neutral antioxidants before they exit the new TiO₂-lignin composites, preserving the scattering and the UV absorption characteristics. For this purpose, it was demonstrated that this stable lignin@TiO₂ composite plays an important role reducing the photocatalytic activity of TiO₂ in a chemical and enzymatic reaction, improving the photoprotection of the other ingredients even when they are present at high concentrations. As such, the particles described here, showing a nanometric size and a very light color, are promising candidates as ingredients in skincare formulations, especially for sunscreens, given that they are non-toxic and waterproof.. Additionally, this approach regarding the use of a nontoxic and extremely versatile material, mainly a by-product of the paper industry, also contribute to the development of environmentally-friendly processes for the cosmetic industry. While illustrated here with lignin, it is clear that the same strategy could be implemented with other polymeric or polymerizable antioxidants.

[0051] One or more currently preferred embodiments have been described by way of example. It will be apparent to persons skilled in the art that a number of variations and modifications can be made without departing from the scope of the invention as defined in the claims.

[0052] The terminology used herein was chosen to best explain the principles of the embodiments, the practical application or technical improvement over technologies found in the marketplace, or to enable others of ordinary skill in the art to understand the embodiments disclosed herein.

[0053] All documents cited in this application are herein incorporated by reference in their entirety.

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- (1) Jacobs, J. F.; van de Poel, I.; Osseweijer, P., "*Sunscreens with Titanium Dioxide (TiO₂) Nano-Particles: A Societal Experiment*", Nanoethics 2010, 4, 103-113.
- (2) Morsella, M.; Giammatteo, M.; Arrizza, L.; Tonucci, L.; Bressan, M.; d'Alessandro, N., "*Lignin coating to quench photocatalytic activity of titanium dioxide nanoparticles for potential skin care applications*", RSC Advances 2015, 5, 57453-57461.
- (3) Hancock-Chen, T.; Scaiano, J. C., "*Enzyme Inactivation by TiO₂ Photosensitization with UVA*", J. Photochem. Photobiol. B: Biol. 2000, 57, 193-196.
- (4) Ricci, A.; Chrétien, M. N.; Scaiano, J. C., "*TiO₂-promoted mineralization of organic sunscreens in water suspension and sodium dodecyl sulfate micelles*", Photochem. Photobiol. Sci. 2003, 2, 487-492.
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- (16) WO 2013/124459
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- (19) US 8,431,143
- (20) US 2005/0129634
- (21) US 2015/0166836
- (22) WO 2014/164418
- (23) AU 2008/366085
- (24) US 2015/0225531
- (25) EP 1 438 361

WHAT IS CLAIMED IS:

1. A nanoparticle comprising a metal oxide particle coated with lignin to form coated particles which have a nanometric size.
2. The nanoparticle of claim 1, wherein the metal oxide is TiO_2 or ZnO .
3. The nanoparticle of claim 1, wherein the lignin is cross-linked over a surface of the metal oxide particle.
4. The nanoparticle of claim 3, wherein the lignin is cross-linked by UVA irradiation.
5. The nanoparticle of claim 1, wherein the coating on the metal oxide particle is from about 1 to 10 nm in thickness.
6. The nanoparticle of claim 1, wherein the coating on the metal oxide particle is from 2 to 5 nm in thickness.
7. The nanoparticle of claim 1, wherein the coating on the metal oxide particle is about 3 nm in thickness.
8. The nanoparticle of claim 1, wherein the lignin is Kraft lignin, Organosolv lignin, Low Sulfonate Content (LSC) lignin, Sodium lignin, Sodium lignin without sugars or Alkali lignin.
9. The nanoparticle of claim 1, wherein the lignin is a pure source of lignin or is a source which includes carbohydrates.
10. A process for preparing a lignin coated nanoparticle, comprising
 - a. mixing a metal oxide nanoparticle precursor with solubilized lignin to form a mixture; and
 - b. irradiating the mixture with UVA at a wavelength effective to coat the metal oxide nanoparticle precursor and form particles which have a nanometric size.

11. The process of claim 10, wherein the metal oxide is TiO_2 or ZnO .
12. The process of claim 10, wherein the lignin is cross-linked over a surface of the metal oxide particle.
13. The process of claim 10, wherein the mixture is irradiated at about 369 nm.
- 5 14. The process of claim 10, wherein the irradiating is carried out for a time effective to produce a coating on the metal oxide particle from about 1 to 10 nm in thickness.
15. The process of claim 10, wherein the irradiating is carried out for a time effective to produce a coating on the metal oxide particle from 2 to 5 nm in thickness.
- 10 16. The process of claim 10, wherein the irradiating is carried out for a time effective to produce a coating on the metal oxide particle of about 3 nm in thickness.
17. The process of claim 10, wherein the lignin is Kraft lignin, Organosolv lignin, Low Sulfonate Content (LSC) lignin, Sodium lignin, Sodium lignin without sugars or Alkali lignin.
- 15 18. The process of claim 10, wherein the lignin is a pure source of lignin or is a source which includes carbohydrates.
19. The process of claim 10, wherein the metal oxide nanoparticle precursor and lignin are combined with an excess of lignin by weight in the mixture.
20. The process of claim 10, wherein the irradiating is carried out for up to 2 hours with stirring to keep the oxide nanoparticle precursor and lignin in suspension.
- 20 21. The process of claim 10, wherein the irradiating is carried out in batch or continuously.
22. The process of claim 21, wherein the continuous irradiating is carried out under continuous flow conditions.

23. The process of claim 22, wherein the continuous irradiating is carried out under continuous flow conditions at a flow rate of 1 to 10 mL/sec.

24. The process of claim 23, wherein the continuous irradiating is carried out under continuous flow conditions at a flow rate of about 4 mL/sec.

5 25. The process of claim 10, wherein after irradiation the nanoparticles are separated from the mixture by centrifugation and washed.

26. A cosmetic composition comprising as a sunblock agent a nanoparticle as defined in any one of claims 1 to 9, or produced according to a process of any one of claims 10 to 21, and a suitable carrier or excipient.

10 27. The cosmetic composition according to claim 26, which is a topical skin care composition.

28. The cosmetic composition according to claim 26, which is a sunscreen, skin moisturizer, skin cream, body lotion, body spray, mascara, foundation, rouge, face powder, eyeliner, eyeshadow, nail polish, or lipstick.

15 29. A sunscreen comprising as a sunblock agent a nanoparticle as defined in any one of claims 1 to 9, or produced according to a process of any one of claims 10 to 21, and a suitable carrier or excipient.

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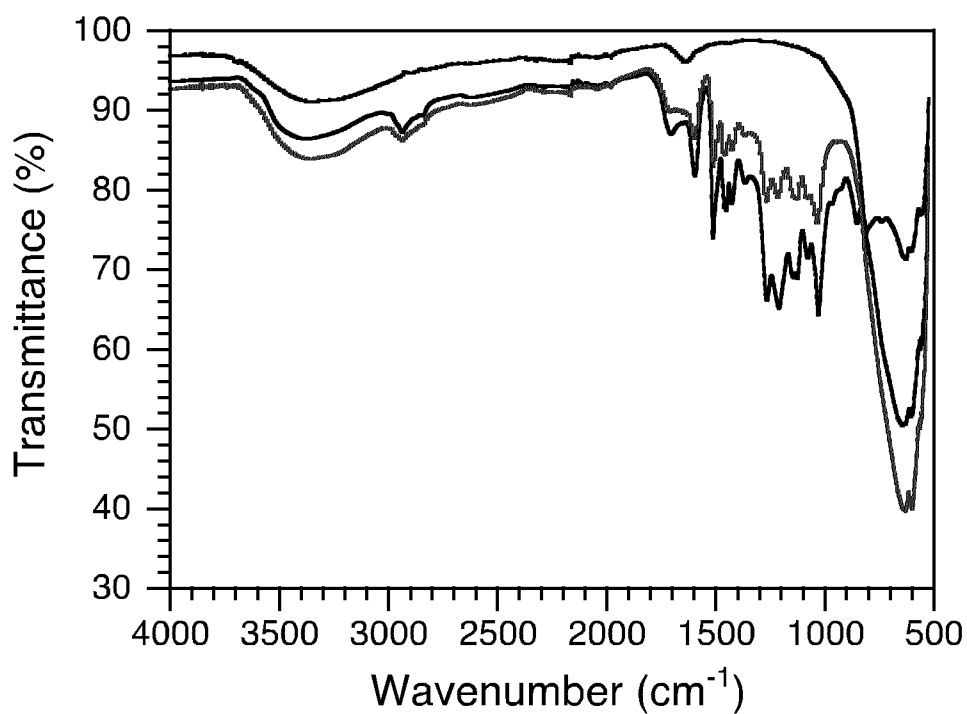


Figure 1

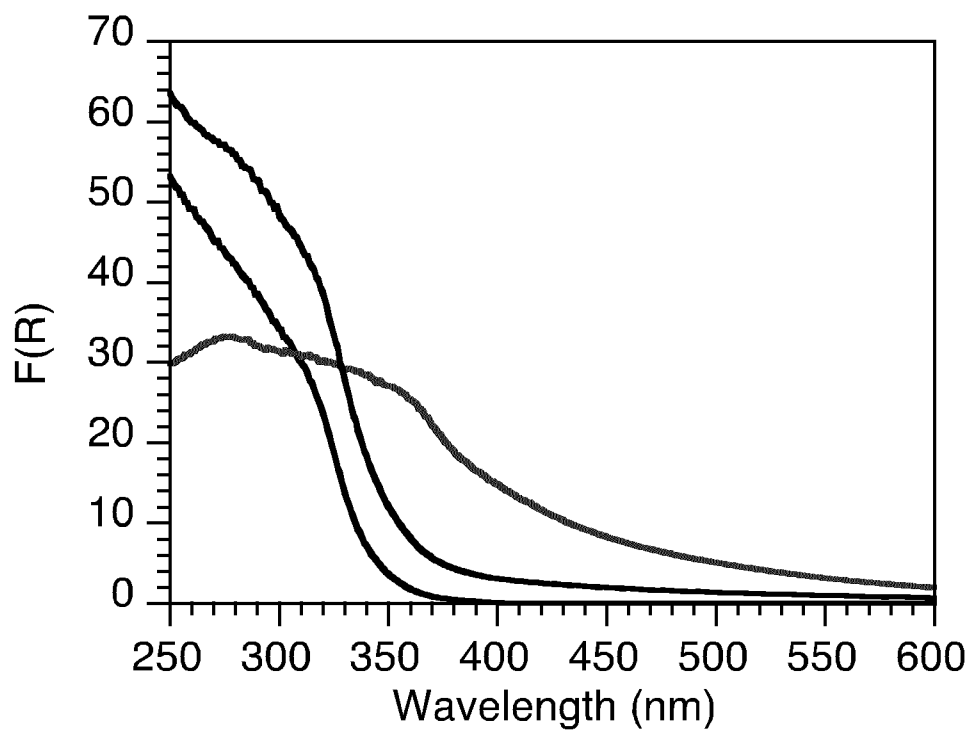


Figure 2

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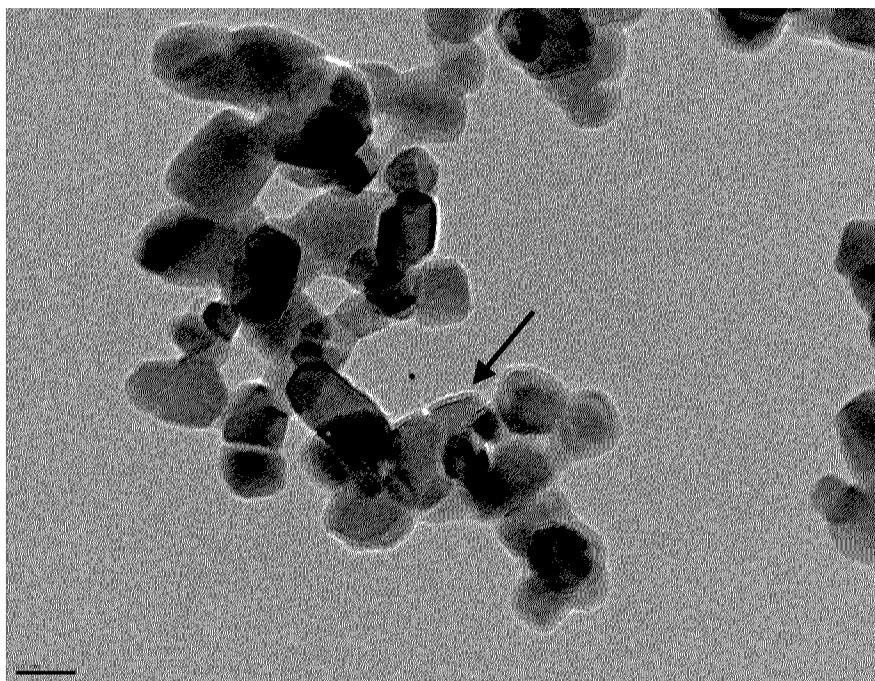


Figure 3

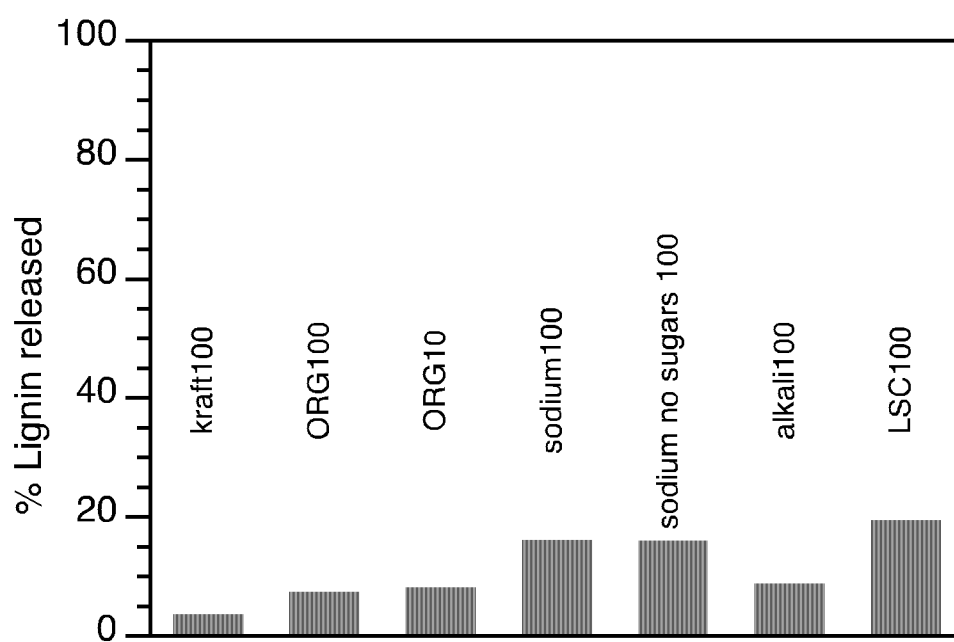


Figure 4

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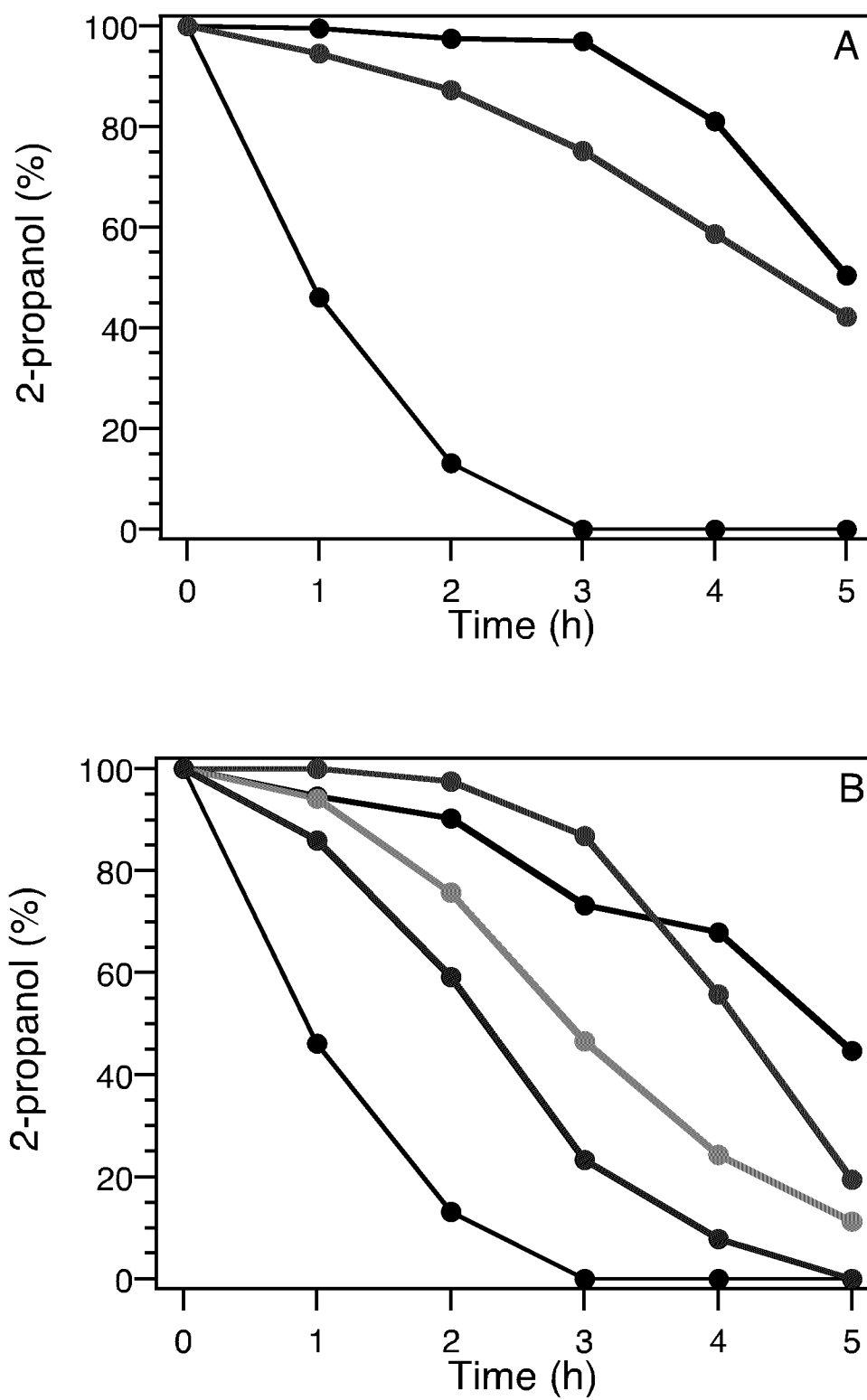


Figure 5

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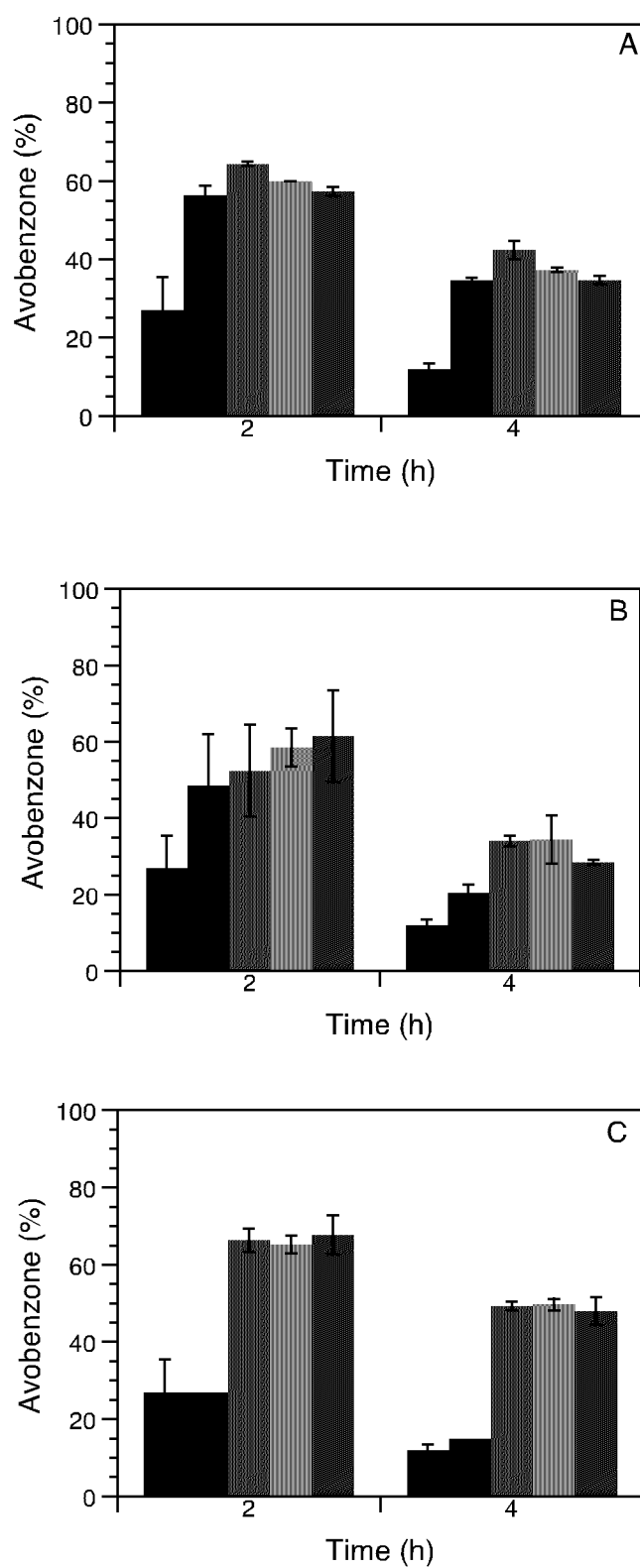


Figure 6

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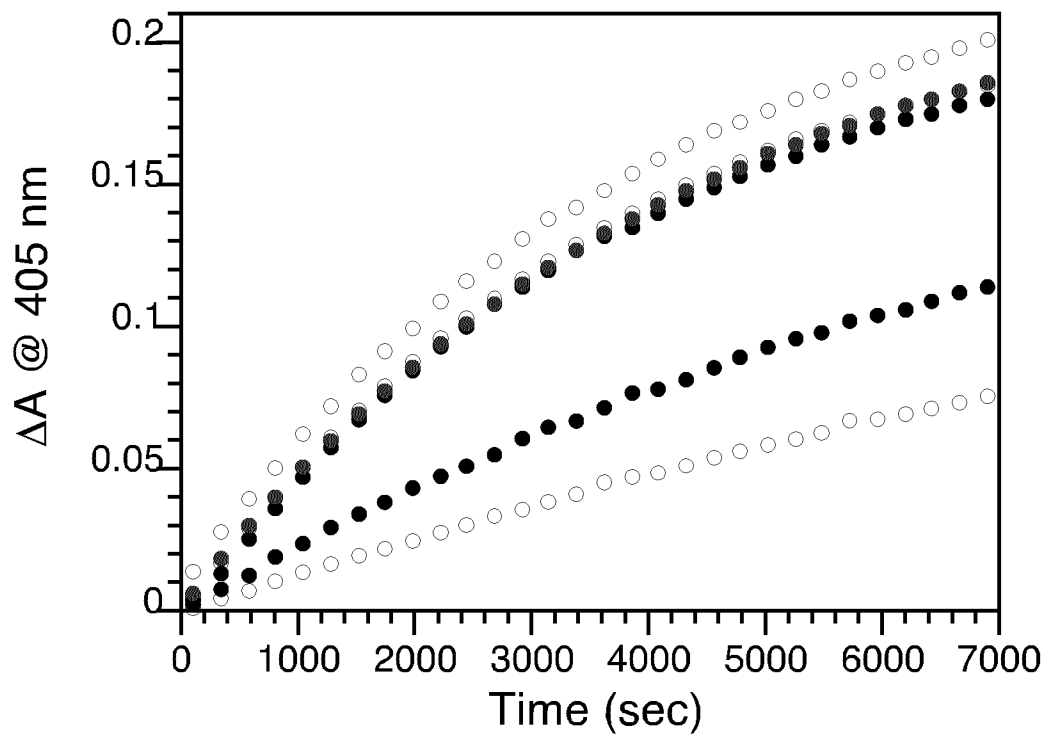


Figure 7

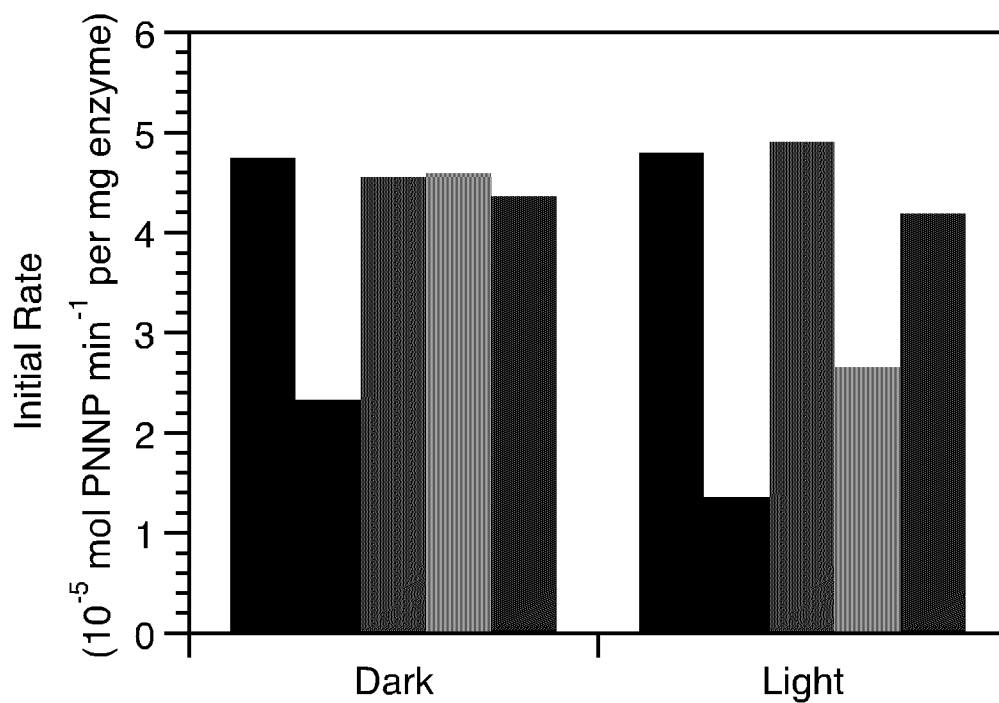


Figure 8

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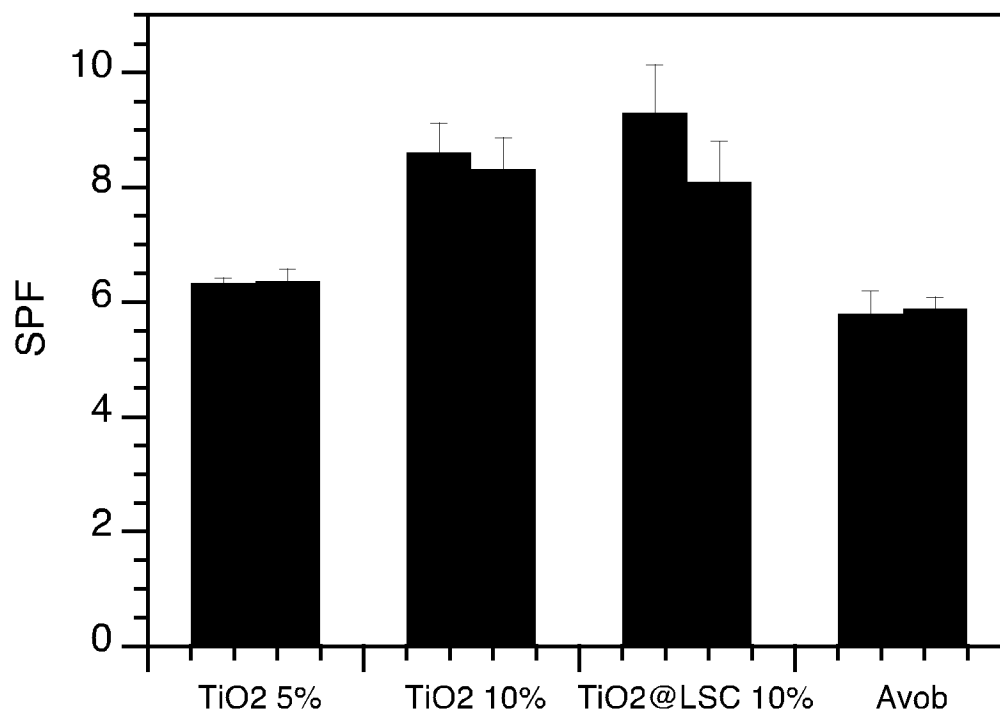


Figure 9

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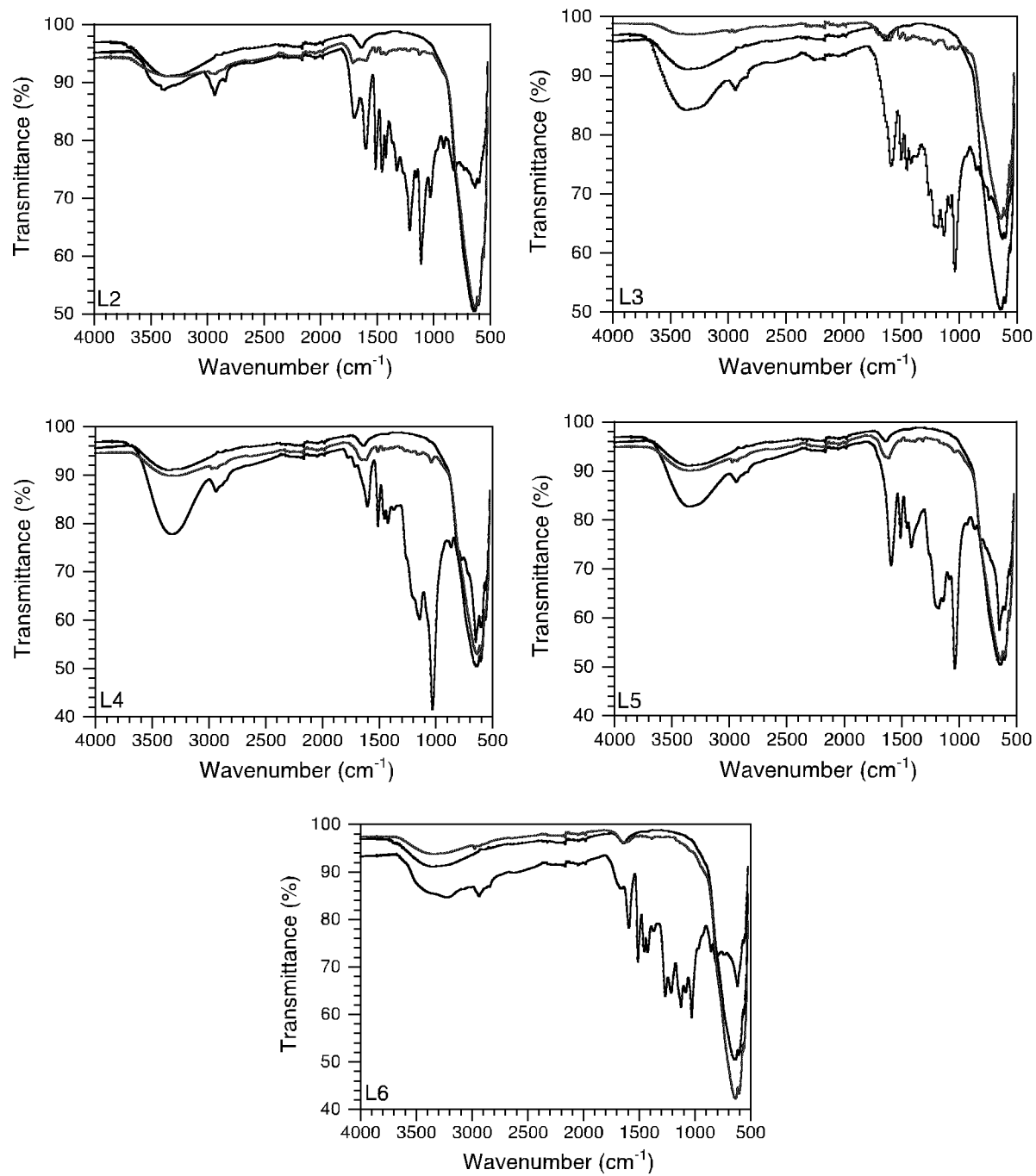


Figure 10

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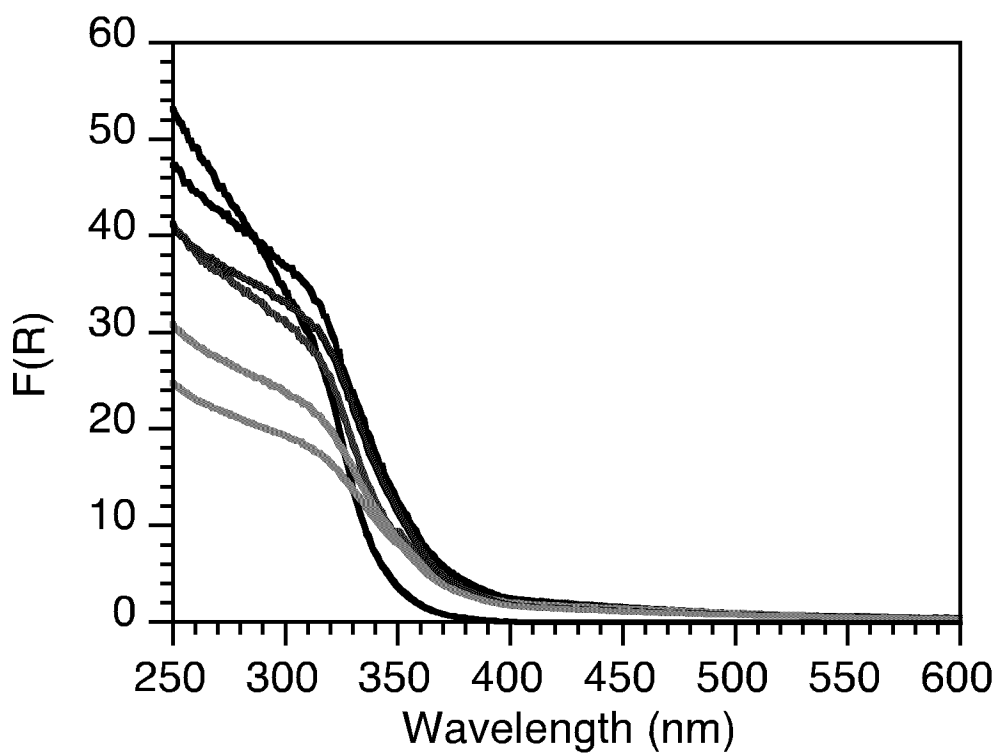


Figure 11

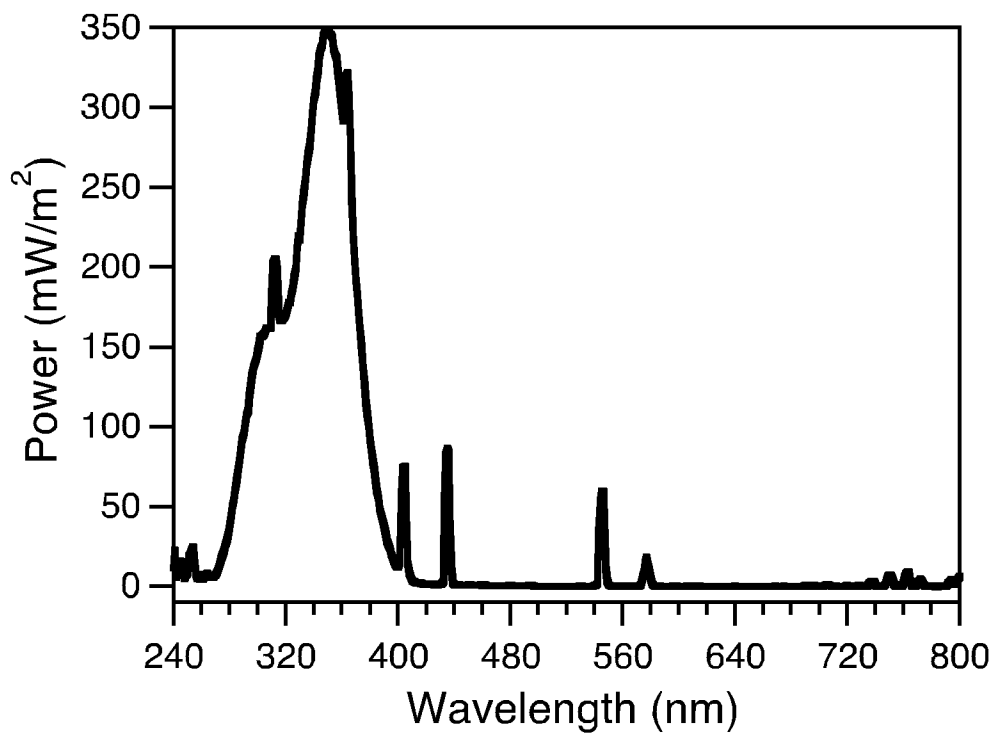


Figure 12

INTERNATIONAL SEARCH REPORT

International application No.

PCT/CA2017/050613

A. CLASSIFICATION OF SUBJECT MATTER

IPC (2006.01): **A61K 8/84** , **A61K 8/11** , **A61K 8/29** , **A61Q 17/04** , **B82Y 30/00** , **B82Y 40/00**

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC (2006.01): **A61K 8** , **A61Q 17**, **B82Y 30**, **B82Y 40**

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic database(s) consulted during the international search (name of database(s) and, where practicable, search terms used)

Databases: Canadian Patent Database, Orbit (FAMPAT), Google, Scopus

Keywords: lignin, lignins, titanium, zinc, cellulose

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P, X	MORSELLA, <i>et al.</i> , ACS OMEGA, Volume 1, pages 464-469, 23 September 2016 (23-09-2016). See entire document. --	1-29
P, X	WO 2017/093184 A1 (HAMBARDZUMYAN) 8 June 2017 (08-06-2017) See abstract; page 4, lines 4-27; page 11, lines 4-31; and the examples. --	1-29
P, X	CN 106633967 A (YANG, <i>et al.</i>) 10 May 2017 (10-05-2017) See abstract. --	1-29
Y	MORSELLA, <i>et al.</i> , RSC ADVANCES, Volume 5, pages 57453-57461, 2015. See abstract; "Synthesis of TiO ₂ -lignin clusters" on page 57455; "Characterization" on page 57457; "Shielding activity" on pages 57459-57460; and "Conclusions" on page 57460. --	1-29

☒ Further documents are listed in the continuation of Box C.☒ See patent family annex.

* "A" "E" "L" "O" "P"	Special categories of cited documents: document defining the general state of the art which is not considered to be of particular relevance earlier application or patent but published on or after the international filing date document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) document referring to an oral disclosure, use, exhibition or other means document published prior to the international filing date but later than the priority date claimed	"T" "X" "Y" "&"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art document member of the same patent family
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Date of the actual completion of the international search
8 September 2017 (08-09-2017)Date of mailing of the international search report
12 September 2017 (12-09-2017)Name and mailing address of the ISA/CA
Canadian Intellectual Property Office
Place du Portage I, C114 - 1st Floor, Box PCT
50 Victoria Street
Gatineau, Quebec K1A 0C9
Facsimile No.: 819-953-2476

Authorized officer

Philip O. Brown, Ph.D. (819) 639-8596

INTERNATIONAL SEARCH REPORT

International application No.

PCT/CA2017/050613

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 2010/093573 A2 (SENGPUTA) 19 August 2010 (19-08-2010) See abstract; paragraphs 3, 6, 9, 10, 13, 17, 18, and 24. --	1-29
Y	CA 2681153 A1 (KARPOV, <i>et al.</i>) 2 October 2008 (02-10-2008) See abstract; page 1, lines 6-11 and 35-38; page 4, lines 5-41; and page 10, lines 27-31. --	1-29
A	WO 2005048970 A1 (HASSELWANDER, <i>et al.</i>) 2 June 2005 (02-06-2005) See abstract. ----	1-29

INTERNATIONAL SEARCH REPORT

International application No.

PCT/CA2017/050613**Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of the first sheet)**

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claim Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☒ Claim Nos.: 1 and 3-29
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

The above claims do not clearly and concisely define the subject matter for which protection is sought, and thus fail to comply with Article 6 of the PCT. By the same token, the subject matter encompassed by said claims has not been clearly and completely disclosed in the description, such that the application does not comply with Article 5 of the PCT. See extra sheet for further details.

3. ☐ Claim Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claim Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

Continuation of Box II:

Claims 1, 3-10, and 12-29 do not clearly define the matter for which protection is sought, and thus fail to comply with Article 6 of the PCT. The failure to clearly define the nature of the "metal oxide" to be used causes ambiguity. Metal oxides are commonly understood to comprise oxygenated forms of a vast array of elements. Said compounds vary wildly in terms of structure and properties, such that it is not clear what materials are actually encompassed by the claims. Furthermore, the application only provides adequate disclosure within the meaning of Article 5 of the PCT as to the utility of coated nanoparticles of TiO₂, such that a search beyond said scope would not be meaningful. Consequently, the search has only been established for the parts of the application which appear to be clear and supported, namely nanoparticles of TiO₂ (and the related ZnO) coated with lignin.

Claims 3, 4, and 9 do not clearly define the matter for which protection is sought, and thus fail to comply with Article 6 of the PCT. The inclusion of references relating to broad morphological or chemical parameters (*i.e.*, "the lignin is cross-linked over a surface of the metal oxide particle"), the means by which the nanoparticle is prepared (*i.e.*, "the lignin is cross-linked by UVA irradiation"), or the presence of materials beyond those forming part of the nanoparticle (*i.e.*, "the lignin is a pure source of lignin or is a source which includes carbohydrates (*sic*)") causes confusion. Said references attempt to limit the nanoparticles in terms of qualitative properties or the means by which they are produced. With respect to the qualitative properties, it is not clear what specific structural features are encompassed by such references. In the case of the references to aspects of how the nanoparticles are produced, it is not clear what structural limitations arise from such processes. The resulting claims are so unclear in scope that no search has been conducted in relation to them.

Claims 10-25 do not clearly define the matter for which protection is sought, and thus fail to comply with Article 6 of the PCT. The failure to clearly and explicitly define the specific steps and parameters required to obtain the desired lignin-coated nanoparticles causes confusion. Independent claim 10 relates to a process involving mixing a "metal oxide nanoparticle precursor" with "solubilized lignin", and then irradiating the resulting mixture with UVA at a wavelength "effective" to coat the precursor particles to yield nanoscale coated particles. It is not clear what constitutes an appropriate "precursor" or "solubilized lignin". More significantly, what appears to be the key step of the process is defined in terms of a desired result, rather than an explicit definition of the conditions required to yield the desired materials. The various dependent claims do not rectify these defects, as they are drafted such that they do not interact with each other (as well as introduce further functional limitations).

Furthermore, the disclosure relating to the processes is so incomplete with regards to the meaning of Article 5 such that said claims appear to lack support with regards to the meaning of Article 6. As a result, a search of the full breadth of these claims is not possible. Instead, said claims have been searched on the basis of the processes actually disclosed in the application (namely, those embodied by the exemplary method disclosed in paragraph 30 of the description).

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.

PCT/CA2017/050613

Patent Document Cited in Search Report	Publication Date	Patent Family Member(s)	Publication Date
WO2017093184A1	08 June 2017 (08-06-2017)	FR3044239A1	02 June 2017 (02-06-2017)
CN106633967A	10 May 2017 (10-05-2017)	None	
WO2010093573A2	19 August 2010 (19-08-2010)	WO2010093573A3 CA2750198A1 EP2396085A2 MX2011006954A US2010202985A1	22 December 2011 (22-12-2011) 19 August 2010 (19-08-2010) 21 December 2011 (21-12-2011) 03 August 2011 (03-08-2011) 12 August 2010 (12-08-2010)
CA2681153A1	02 October 2008 (02-10-2008)	AU2008231831A1 BRPI0809159A2 BRPI0809159B1 CN101641077A CN101641077B EP2129360A1 JP2010521411A JP5393652B2 KR20090125194A KR101455887B1 US2010119829A1 WO2008116790A1	02 October 2008 (02-10-2008) 16 September 2014 (16-09-2014) 07 June 2016 (07-06-2016) 03 February 2010 (03-02-2010) 16 January 2013 (16-01-2013) 09 December 2009 (09-12-2009) 24 June 2010 (24-06-2010) 22 January 2014 (22-01-2014) 03 December 2009 (03-12-2009) 03 November 2014 (03-11-2014) 13 May 2010 (13-05-2010) 02 October 2008 (02-10-2008)
WO2005048970A1	02 June 2005 (02-06-2005)	DE10354380A1	16 June 2005 (16-06-2005)