University of Wollongong

Research Online

Australian Institute for Innovative Materials -
Papers

Australian Institute for Innovative Materials

1-1-2018

Biocompatible Bi(OH)₃nanoparticles with reduced photocatalytic activityas possible ultraviolet **filter** in sunscreens

Kathrin Bogusz

University of Wollongong, kb543@uowmail.edu.au

Dean Cardillo

University of Wollongong, dwc583@uowmail.edu.au

Moeava Tehei

University of Wollongong, moeava@uow.edu.au

Tifenn Boutard

Universite d'Orleans, France

Philip J. Barker

University of Wollongong, pbarker@uow.edu.au

See next page for additional authors

Follow this and additional works at: <https://ro.uow.edu.au/aiimpapers>

Part of the [Engineering Commons](#), and the [Physical Sciences and Mathematics Commons](#)

Research Online is the open access institutional repository for the University of Wollongong. For further information contact the UOW Library: research-pubs@uow.edu.au

Biocompatible Bi(OH)₃nanoparticles with reduced photocatalytic activityas possible ultraviolet filter in sunscreens

Abstract

In this study we investigate readily synthesised Bi(OH)₃nanoparticles as a novel, multifunctional ultraviolet filter for sunscreen. The absorbance of Bi(OH)₃NPs in the ultraviolet region is comparable to that of both ZnO and TiO₂NPs used in commercial sunscreens. In vitro photoprotection results show that the combination of TiO₂/Bi(OH)₃is more efficient than TiO₂/ZnO over the whole UV range, with an increase in sun protection factor of 28%. The emulsions show rheological properties comparable to those of commercial sunscreens. The combination of TiO₂/Bi(OH)₃led to insignificant damage on pre-painted steel panels after exterior exposure for twelve weeks. We also demonstrate that the addition of Bi(OH)₃NPs reduces the degradation of crystal violet by photocatalytically active TiO₂or ZnO NPs under ultraviolet exposure. Finally, assessment of the biocompatibility of Bi(OH)₃with HaCaT keratinocytes and Madin-Darby Canine Kidney (MDCK) cells in vitro is described.

Keywords

biocompatible, bi(oh)₃nanoparticles, reduced, filter, photocatalytic, sunscreens, activityas, possible, ultraviolet

Disciplines

Engineering | Physical Sciences and Mathematics

Publication Details

Bogusz, K., Cardillo, D., Tehei, M., Boutard, T., Barker, P. J., Devers, T., Rosenfeld, A., Dou, S. X., Liu, H. K. & Konstantinov, K. (2018). Biocompatible Bi(OH)₃nanoparticles with reduced photocatalytic activityas possible ultraviolet filter in sunscreens. *Materials Research Bulletin*, 108 130-141.

Authors

Kathrin Bogusz, Dean Cardillo, Moeava Tehei, Tifenn Boutard, Philip J. Barker, Thierry Devers, Anatoly B. Rosenfeld, Shi Xue Dou, Hua-Kun Liu, and Konstantin K. Konstantinov

Biocompatible Bi(OH)₃ nanoparticles with reduced photocatalytic activity as possible ultraviolet filter in sunscreens

Kathrin Bogusz ^{a, b}, Dean Cardillo ^{a, b}, Moeava Tehei ^{b, c, d}, Tifenn Boutard ^e, Philip J. Barker ^c, Thierry Devers ^f, Anatoly Rosenfeld ^{b, d}, Shi X. Dou ^a, Hua K. Liu ^a, Konstantin Konstantinov ^{a, b, *}

^a Institute for Superconducting and Electronic Materials, Australian Institute for Innovative Materials, University of Wollongong, Innovation Campus, Squires Way, North Wollongong NSW 2500, Australia

^b Illawarra Health and Medical Research Institute, University of Wollongong, Northfields Avenue, Wollongong NSW 2522, Australia

^c School of Chemistry, Faculty of Science, Medicine and Health, University of Wollongong, Northfields Avenue, Wollongong NSW 2522, Australia

^d Centre for Medical and Radiation Physics, Faculty of Engineering and Information Science University of Wollongong, Northfields Avenue, Wollongong NSW 2522, Australia

^e ABC Texture, 10 Rue Isaac Newton, 35800 Dinard, France

^f Interface, Confinement, Matériaux et Nanostructures ICMN UMR737, Site IUT de Chartres, France

* Corresponding author. E-mail: konstan@uow.edu.au

Highlights

- Facile synthesis of stable inorganic Bi(OH)₃ nanoparticles.
- High biocompatibility in human skin and dog kidney cells *in vitro*.
- Promising UV filter capable of reducing the photocatalytic activity of TiO₂ and ZnO nanoparticles.

- Incorporation of $\text{Bi}(\text{OH})_3$ in TiO_2 -based sunscreen formulations: significantly improved photoprotection and high photostability in comparison to the classical UV filter TiO_2/ZnO .

Abstract

In this study we investigate readily synthesised $\text{Bi}(\text{OH})_3$ nanoparticles as a novel, multifunctional ultraviolet filter for sunscreen. The absorbance of $\text{Bi}(\text{OH})_3$ NPs in the ultraviolet region is comparable to that of both ZnO and TiO_2 NPs used in commercial sunscreens. *In vitro* photoprotection results show that the combination of $\text{TiO}_2/\text{Bi}(\text{OH})_3$ is more efficient than TiO_2/ZnO over the whole UV range, with an increase in sun protection factor of 28%. The emulsions show rheological properties comparable to those of commercial sunscreens. The combination of $\text{TiO}_2/\text{Bi}(\text{OH})_3$ led to insignificant damage on pre-painted steel panels after exterior exposure for twelve weeks. We also demonstrate that the addition of $\text{Bi}(\text{OH})_3$ NPs reduces the degradation of crystal violet by photocatalytically active TiO_2 or ZnO NPs under ultraviolet exposure. Finally, assessment of the biocompatibility of $\text{Bi}(\text{OH})_3$ with HaCaT keratinocytes and Madin-Darby Canine Kidney (MDCK) cells *in vitro* is described.

Keywords: A. Inorganic compounds, A. Nanostructures, B. Chemical synthesis, B. Optical properties, D. Surface properties

1. Introduction

Excessive exposure to ultra-violet (UV) radiation from the sun can result in photo-aging, immune suppression, genetic damage through the generation of reactive oxygen species (ROS), and sunburn, which can cause the formation of carcinomas and melanoma [1,2]. Both UVA (320 – 400 nm) and UVB (290 – 320 nm), are known to be initiators and promoters of tumour growth. Commonly used sunscreen formulations protect against UV radiation and contain either organic or inorganic additives, or a combination of both. While there are many organic

UV absorbers approved by regulatory bodies, only two inorganic UV filters can be used, namely titanium dioxide (TiO₂) and zinc oxide (ZnO). Traditional sunscreens employing either of these two materials appear white on the skin because the particle size is greater than 100 nm. This white coating acts as a ‘physical blocker’, functioning by scattering and reflecting the incident light. Modern sunscreens employ nanosized materials, typically, nanoparticles (NPs) of ZnO or TiO₂, since, at suitable concentrations, when well dispersed, their solutions and emulsions are transparent in the visible range [3] and are thus invisible after topical application, a factor highly desirable from a cosmetic standpoint. However, when nano particulate (e.g. 10 – 40 nm) they are too small to reflect and scatter efficiently and thus, they function as inorganic UV absorbers relying on their UV absorption spectrum and the Beer-Lambert law for effectiveness. They only become physical blockers when poorly dispersed and agglomerated when, consequentially, they appear visibly white.

It has been shown, however, that these NPs are biologically more reactive than the normal microscopic bulk material, the smaller size allowing them to penetrate skin more readily and reach deeper tissue layers [4]. This penetration is critical, as recent findings reveal that both ZnO and TiO₂ NPs exhibit an intrinsic cyto- and genotoxicity. This intrinsic toxicity is thought to involve the intracellular induction of oxidative stress, leading to a range of metabolic outcomes including glutathione oxidation, lipid peroxidation and changes in phosphorylation and ATP levels, calcium homeostasis and others, ultimately leading to premature cell death. All these processes can be significantly enhanced through photocatalytic activity (PCA) when exposed to UV radiation [5,6]. The photocatalytic reactions are mediated by reactive oxygen species (ROS), such as hydroxyl radicals ($\bullet$ OH), singlet oxygen and superoxide (O₂ $\bullet^-$). PCA can be moderated by a range of methods, and although complete suppression of PCA is difficult [5], sensible guidelines for incorporation of TiO₂ NPs in sunscreen have been provided for formulators to minimise risk.

On the other hand, while the organic additives employed in sunscreens are effective UV absorbers they are often allergenic and many have poor photostability [6]. After topical application, the degradation of organic UV-filters results in a continual reduction of sun protection factor (SPF) upon exposure [7].

Given the drawbacks outlined above, an optimum inorganic NP system for sunscreen formulations therefore, should not exhibit ROS generation either in the presence or absence of UV radiation [8]. Accelerated decomposition of the organic components can potentially be reduced by introducing ROS scavenging properties into the NP system, which must also provide similar UV attenuation to ZnO and TiO₂ NPs. Prospective novel inorganic NPs include pure and doped cerium oxide (CeO₂) [9,10], which are biocompatible, have selective absorbance in the UV region and offer ROS scavenging properties [11,12], and compounds of bismuth, the least toxic of the heavy metals [13]. Moreover bismuth compounds are cost-efficient and already used in medicine and cosmetics [14, 15]. Inorganic bismuth salts are used for the treatment and prevention of gastric and duodenal ulcers [16] and organobismuth compounds have antimicrobial activity, having shown anti-tumour effects in various human cancer cell lines [17,18], while a suspension of (BiO)₂CO₃ and Bi(OH)₃ yields milk of bismuth, which is used as a protective agent in gastrointestinal diseases [19].

Here, we demonstrate a low temperature synthesis of nanoparticulate bismuth hydroxide Bi(OH)₃ and investigate the UV protective and photocatalytic properties of this material. The SPF was determined in accordance with ISO 24443:2012 for an emulsion containing TiO₂/Bi(OH)₃ and compared to a ‘classical’ emulsion of TiO₂/ZnO. The physical properties of the colorless Bi(OH)₃ NPs alone, and in combination with photocatalytically active TiO₂ and ZnO NPs, were investigated through monitoring of crystal violet degradation under UVA/UVB irradiation and simulated solar irradiation (air mass (AM) 1.5 G, 1 sun equivalent). The

cytotoxicity of the $\text{Bi}(\text{OH})_3$ NPs was determined upon healthy HaCaT keratinocytes and Madin-Darby Canine Kidney (MDCK) cells using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay.

2. Materials and methods

2.1 Materials

All used materials (M) and specimens are summarized in Tables 1, 2, and 3. The $\text{Bi}(\text{OH})_3$ (M1) NPs were the only material that was synthesised in this work. The NPs were synthesised via a modified classical precipitation route [20]. First, 4 g of $\text{Bi}(\text{NO}_3)_3 \cdot 5\text{H}_2\text{O}$ (Sigma-Aldrich) was dissolved in 40 mL of 69% HNO_3 (Merck). Then, 30% NH_4OH (Sigma-Aldrich) was added dropwise until a white precipitate was formed. The precipitate was filtered, washed with deionized (DI) water, resuspended in 150 mL of DI water, and heated under stirring at 80 – 100°C for 5.5 h. The colourless powder was dried in vacuum at 60°C for 48 h.

For the preparation of the homemade sunscreen (HS) emulsions, commercial TiO_2 NPs (Eusolex[®] T-S, Merck KGaA), ZnO NPs (Z-COTE[®] HP1, BASF), and the synthesised $\text{Bi}(\text{OH})_3$ (M1) NPs were used (Table 2). The properties of the homemade emulsions were compared to commercial sunscreen (CS) formulations (Table 3): manufacturer 1 (SPF 50, CS1), manufacturer 1 (SPF 30, CS2), manufacturer 2 (SPF 50, CS3), and manufacturer 3 (SPF 50, CS4).

TiO_2 (Aeroxide[®], P25, M2) and ZnO (≤ 100 nm, M3) NPs with similar morphology and particle size were chosen for all other chemical and biological characterization (Table 1). The nanopowders were obtained from Sigma-Aldrich and used without further purification. For all characterization techniques that involved combinations of TiO_2 and $\text{Bi}(\text{OH})_3$ NPs, or ZnO and $\text{Bi}(\text{OH})_3$ NPs, different concentrations of separately suspended NPs were mixed mechanically.

Table 1: Overview of the materials (M) used.^a

ID	Sample	Trade name/ Supplier	Particle size (nm) and morphology	Crystal phase	Experimental testing
M1	Bi(OH) ₃ NPs	Synthesised in this work	6.04 ± 0.89 (spherical)	-	PCA1, UVF, CT
M2	TiO ₂	Aeroxide [®] , P25	24.08 ± 1.60 (ellipsoidal, cubic)	80% anatase, 20% rutile	PCA1, UVF, CT
M3	ZnO	Sigma Aldrich	44.86 ± 6.38 (spherical, hexagonal, rectangular)	Zincite	PCA1, UVF, CT

^aAbbreviations: PCA1 = photocatalytic activity (degradation of crystal violet), UVF = UV filtering, CT = cytotoxicity.

Table 2: Overview of the homemade sunscreens (HS) used.^a

ID	UV filter	Trade name	Particle size (nm) and morphology	Crystal phase	Experimental testing
HS1	TiO ₂	Eusolex [®] T-S	Rod-like: 20 (width) and 100 (length) [5]	Rutile	PCA2, SPF, rheology
	ZnO	Z-COTE [®] HP1	30 – 200, (rod-like, isometric) [21]	Wurtzite	
HS2	TiO ₂	Eusolex [®] T-S	Rod-like: 20 (width) and 100 (length) [5]	Rutile	PCA2, SPF, rheology
	Bi(OH) ₃	Synthesised in this work (M1)	6.04 ± 0.89 (spherical)	-	

^aAbbreviation: PCA2 = photocatalytic activity (exterior exposure of pre-painted steel panels),
SPF = sun protection factor.

Table 3: Overview of the commercial sunscreens (CS) used.^a

ID	UV filter	Sunscreen	Particle size (nm) and morphology	Crystal phase	Experimental testing
CS1	TiO ₂	Manufacturer 1 (SPF 50)	~ 25 (ellipsoidal, cubic)	80% anatase, 20% rutile	PCA2
CS2	TiO ₂	Manufacturer 1 (SPF 30)	Rod-like: 20 (width) and 100 (length) [5]	Rutile	PCA2
CS3	Organics	Manufacturer 2 (SPF 50)	-		Rheology
CS4	Organics	Manufacturer 3 (SPF 50)	-		Rheology

^aAbbreviation: PCA2 = photocatalytic activity (exterior exposure of pre-painted steel panels).

2.2 Preparation of homemade sunscreen formulations

A water-in-oil emulsion was prepared containing 25 wt.% organic filters and 5 wt.% commercially available TiO₂ NPs (see Table 2). In order to test the performance of commercial ZnO and synthesised Bi(OH)₃ NPs, the emulsions were supplemented with either type of NPs for a final concentration of 3 wt.%, yielding HS1 and HS2.

2.3 Separation of inorganic sunscreen components

To extract the inorganic components from the sunscreen formulations used for the sun exposure tests (manufacturer 1 (CS1), manufacturer 1 (CS2), and the TiO₂-based homemade sunscreen emulsion, 1 – 2 g of sunscreen formulation was placed into a centrifuge tube, as described in the literature [22]. After 30 mL of hexane (Sigma-Aldrich) was added, the mixture was shaken to remove the active organic components, and centrifuged (Eppendorf). The supernatant was

discarded and 30 mL of ethanol (Sigma-Aldrich) was added to the residue to remove the remaining organics. After centrifugation, the supernatant was discarded and 30 mL of DI water was added to the residue to remove the surfactants from the inorganic components. The mixture was shaken, centrifuged, and the remaining inorganic components were washed several times with ethanol. After the inorganic components were dried overnight, the crystalline phase of TiO_2 was determined via XRD.

2.4 Physical characterization

The phase of the synthesized and as-received nanopowders (M1 – M3, CS1, CS2) was characterized using X-ray diffraction (XRD, Enhanced Mini-Materials Analyzer (EMMA) X-Ray Diffractometer, GBC Scientific) with $\text{Cu K}\alpha$ radiation ($\lambda = 1.5418^\circ \text{ \AA}$) at 40 kV and 25 mA. The morphologies and particle sizes of the nanomaterials (M1 – M3) were examined using the JEOL JEM 2010 at the Electron Microscopy Centre at the Australian Institute of Intelligent Materials (AIIM). Transmission electron microscopy (TEM) images were obtained at an accelerating voltage of 200 kV; the samples were prepared on carbon-coated copper grids. The crystal size distribution was surveyed using Image-J software. The Brunauer-Emmett-Teller (BET) specific surface area was measured using a Nova 1000 high speed gas sorption analyser from Quantachrome. The adsorption of N_2 at the temperature of liquid nitrogen was determined, and prior to measuring, the samples were degassed at 60°C for 15 h in vacuum.

2.5 X-ray photoelectron spectroscopy (XPS)

Surface compositions of $\text{Bi}(\text{OH})_3$ (M1), TiO_2 (M2), and ZnO (M3) nanopowders were evaluated using XPS, which was conducted using a SPECS PHOIBOS 100 Analyzer installed in a high-vacuum chamber with the base pressure below 10^{-8} mbar. X-ray excitation was provided by $\text{Al K}\alpha$ radiation with a photon energy of 1486.6 eV at the high voltage of 12 kV and power of 120 W. The XPS binding energy spectra were recorded with the pass energy of

20 eV in the fixed analyser transmission mode. Analysis of the XPS data was carried out using CasaXPS 2.3.15 software.

2.6 UV-visible spectroscopy

Suspensions of Bi(OH)₃ (M1), TiO₂ (M2), and ZnO (M3) NPs, and combinations of the nanomaterials were prepared in DI water at a concentration of 25 µg/mL. In the combinations, each nanomaterial had a concentration of 25 µg/mL, with a total concentration of both nanomaterials of 50 µg/mL. The suspensions were sonicated separately for 2 h using a sonication bath (Branson 3800, Ultrasonics Corp) and then combined. The absorbance was recorded on an UV-3600 spectrophotometer from Shimadzu Corporation (Kyoto, Japan) over the range of 800 to 200 nm by using quartz cuvettes. For a crystalline semiconductor, the optical absorption behaviour near the band edge follows Eq.(1) [23]:

$$(\alpha h\nu)^n = A(h\nu - E_g) \quad (1)$$

where α , h , ν , A , and E_g are the absorption coefficient, Planck constant, light frequency, a constant, and the band gap, respectively. The value of n depends on the type of optical transition (i.e. $n = 2$ for a direct transition and $n = 0.5$ for an indirect transition).

2.7 Photocatalytic Activity

The degradation of crystal violet is used as an indicator to determine hydroxyl radicals generated through photocatalysis. The PCA of Bi(OH)₃ (M1) NPs, and the well-known photocatalysts TiO₂ (M2) and ZnO (M3) under a broad UVA/UVB ultraviolet spectrum was evaluated using a RPR-200 photochemical reactor (Rayonet, Branford, CT, USA), equipped with 300 nm and 350 nm lamps. TiO₂ was chosen as a highly active photocatalyst known to generate •OH radicals upon UV exposure [24]. These radicals react with crystal violet, (tris(4-(dimethylamino)phenyl)methyl) chloride, Sigma Aldrich), resulting in multiple by-products [25] and concomitant loss of colour, monitored by UV-vis spectrometry.

Suspensions of Bi(OH)₃ (M1), TiO₂ (M2), and ZnO (M3) NPs, and combinations of the nanomaterials were prepared in DI water and sonicated for 2 h using a sonication bath (Branson 3800, Ultrasonics Corp). In the combinations of TiO₂ + Bi(OH)₃ and ZnO + Bi(OH)₃, the concentration of TiO₂ and ZnO was maintained at 5 mg/L, while the amount of the Bi(OH)₃ suspension added varied between 1 – 5 mg/L. Crystal violet dye with an initial concentration of 12 µmol/L was added to the nanomaterial suspensions and the nanoparticle and crystal violet dispersions were equilibrated by stirring in the dark for 60 minutes. In a typical run, an aliquot was taken prior to irradiation, centrifuged to remove the photocatalyst and the absorbance at 590 nm was measured. The aliquot was resuspended and returned to the reaction mixture. Upon exposure to UV light, aliquots were withdrawn every 5 min for a total of 30 min, and the absorbances were measured. For the sun simulation experiments, a halogen lamp (50 watt power) was used, and the degradation of crystal violet was investigated under illumination of AM 1.5 G one sun (100 mW/cm²). The irradiation time ranged from 0 – 6 h, and the absorbance was measured in intervals of 30 min.

The kinetics of the dye degradation was investigated using a first-order reaction. The apparent rate constant k was calculated following Eq. (2) [26]:

$$c = c_0 e^{-kt} \quad (2)$$

where c is the concentration as the reaction progresses, c_0 is the initial concentration, and t is the time.

2.8 Sun exposure of sunscreen formulations

Another cheap and reliable method for evaluating potential photocatalytic sunscreen activity has been described by Barker and Branch [22] and a similar protocol was employed here. Flat panels (250mm x 150mm) of pre-painted steel product were obtained from a production run of a coil-coating paintline in Port Kembla NSW.

Test formulations were applied to these panels using a #10 drawdown bar, which gives a uniform applied wet-film thickness of $\approx 13\mu\text{m}$. Typically, 3g of the emulsion was placed halfway along the length of the test panel and was drawn down, thus covering half the panel, the top half of the panel remained uncovered and was used as a negative control for comparison. The panels were then mounted lengthwise on an open, fence-type, exterior exposure rack facing north at 45° , at a latitude of $\sim 34.5^\circ\text{S}$ (Wollongong, NSW) for a total of twelve weeks. The exposure time of twelve weeks was chosen since significant differentiation after this particular time can be expected if photocatalytic components are present in the formulation under test [22]. Changes in the surface roughness were observed over time using scanning electron microscopy (SEM). A $4.5\text{ cm} \times 2.0\text{ cm}$ area was cut from each panel after twelve weeks exposure and cleaned of debris under warm running water with a fine hair paint brush. The test strip was then coated with a thin layer of platinum, and surface roughness investigated using a JEOL JSM-7001F scanning electron microscope. SEM images were obtained at an accelerating voltage of 5 kV.

The steel panels were painted with five different sunscreen formulations: three homemade sunscreen emulsions (base emulsion ('all organics'), and base emulsion supplemented with TiO_2/ZnO (HS1) and $\text{TiO}_2/\text{Bi}(\text{OH})_3$ (HS2) NPs) and two different commercially available sunscreen formulations (CS1, CS2), which have inorganic ingredients (i.e. TiO_2 NPs), were tested.

2.9 Rheological properties

Rheological properties were determined for the TiO_2/ZnO (HS1) and $\text{TiO}_2/\text{Bi}(\text{OH})_3$ (HS2) homemade sunscreen emulsions, and compared to two sunscreen formulations commercially available in Australia: manufacturer 2 (SPF 50, CS3) is a viscous cream dispensed from a bottle, while manufacturer 3 (SPF 50, CS4) is applied through a spray dispenser. These two

commercial products were chosen in order to compare the viscosity and the ease of topical application of the **homemade sunscreen emulsions (HS1) and (HS2)** to those of commercially available sunscreen formulations. For a typical measurement, a small amount of emulsion was placed on the base plate of a Physica MCR 301 (Anton Paar, Graz, Austria) rheometer, and the viscosity was measured at room temperature with a test gap of 0.3 mm over a range of rotational shear rates, which increased from 0.01 to 1000 s⁻¹. The viscosity was recorded every 5 seconds for a total of 50 measurement points. The obtained rheograms were analysed using the Ostwald Law and the flow index n was calculated using Eq. (6) [27]:

$$\tau = Kr^n \quad (6)$$

where τ is the shear stress, r is the shear rate, and K is the consistency index. The flow index and the consistency index can be calculated by plotting the log of shear stress vs. the log of shear rate, which are given by the slope and antilog of the y-intercept, respectively (Eq. (7)):

$$\log(\tau) = \log(K) + n\log(r) \quad (7)$$

2.10 Photoprotection efficiency

In-vitro SPF and UVA protection factor (PF) measurements of the **homemade sunscreen emulsions** were performed using a Labsphere UV2000S in accordance with ISO 24443:2012, in order to evaluate the photoprotection efficiency in the UVA and UVB range. 30 mg of **homemade sunscreen emulsion (HS1, HS2)** was placed on the surface of a standard poly(methyl methacrylate) (PMMA) plate (Europlast, Aubervilliers, France) and spread homogeneously across its surface by finger. After spreading, 15 +/- 0.5 mg of emulsion remained on the PPMA plate, which was then left to dry under ambient conditions. Four plates were prepared for each emulsion and the transmission was measured between 290 and 400 nm at six different locations on the PMMA plate. The SPF was calculated using Eq. (3) [28]:

$$SPF = \frac{\int_{290}^{400} E\lambda.S\lambda.d\lambda}{\int_{290}^{400} E\lambda.S\lambda.T\lambda.d\lambda} \quad (3)$$

where $E\lambda$ is the erythemal spectral effectiveness, $S\lambda$ is the solar spectral irradiance, and $T\lambda$ is the spectral transmittance of the emulsion. The UVA-PF was also calculated from the obtained measurements, according to Eq. (4) [28]:

$$\text{UVA-PF} = \frac{\int_{320}^{400} E\lambda \cdot S\lambda \cdot d\lambda}{\int_{320}^{400} E\lambda \cdot S\lambda \cdot d\lambda \cdot T\lambda} \quad (4)$$

The critical wavelength is typically used to evaluate the efficiency of protection in the UVA region and is determined by using the first value of wavelength for which the following ratio, R , in Eq. (5) is ≥ 0.9 [29]:

$$R = \frac{\int_{290}^{\lambda} A\lambda \cdot d\lambda}{\int_{290}^{400} A\lambda \cdot d\lambda} \quad (5)$$

where $A\lambda$ is the absorbance.

2.11 Cell culture

Cellular experiments were carried out with HaCaT cells, which are transformed keratinocytes derived from histologically normal adult human skin and epithelial MDCK cells, derived from a normal female adult Cocker Spaniel. The cells were purchased from the European Collection of Cell Cultures (ECACC). Dulbecco's Modified Eagle Medium (DMEM), phosphate buffered saline (PBS), fetal bovine serum (FBS), penicillin, streptomycin, and trypsin ethylenediaminetetracetic acid (trypsin–EDTA) were purchased from Life Technologies. Cell cultures were grown and maintained in a T75 cm² tissue culture flask (Falcon) containing DMEM with L-glutamine, which was supplemented with 10% (v/v) FBS and 1% (v/v) penicillin/streptomycin at 37°C and 5% (v/v) CO₂.

2.12 Cell viability assay

The viability of HaCaT and MDCK cells was assessed by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay as described by Mosmann [30], with a slight modification. The MTT assay is a quantitative calorimetric method based on the reduction of

water-soluble yellow tetrazolium salt to water-insoluble purple formazan, which takes place in the mitochondria of viable cells. The amount of formazan produced is directly proportional to the number of viable cells and can therefore be used to determine cell viability.

The cells were seeded in a 96-well plate for the controls (growth curve) and the NP exposure, and incubated at 37°C with 5% (v/v) CO₂. Suspensions of Bi(OH)₃ (M1), TiO₂ (M2), and ZnO (M3) NPs were prepared in PBS (without Ca²⁺ and Mg²⁺) at a concentration of 1 mg/mL, and sonicated for 2 h using a sonication bath (Branson 3800, Ultrasonics Corp). The NP suspensions were diluted in fresh medium and were then added to the treatment wells at concentrations of 0, 5, 10, 25, 50, 100, 250, and 500 µg/mL and incubated for 24 h. At the end of the exposure, the culture medium was removed from each well and replaced with 100 µL of new medium and 10 µL of MTT solution (0.5 mg/mL in PBS). The cell culture plate was incubated for 4 h at 37°C with 5% (v/v) CO₂ until a purple-coloured formazan product was formed. All but 25 µL of the test solution was removed, and the formazan crystals were dissolved in 100 µL of dimethyl sulfoxide (DMSO). After incubation at 37°C for 10 min, the well plate was centrifuged at 1200 rpm for 5 min to settle the remaining NPs and avoid additional absorbance, which would interfere with the reading for the reduction of MTT. Then, 100 µL of supernatant was transferred to a new well of a 96-well plate, and the absorbance was measured at a wavelength of 540 nm with a microplate reader (SpectraMax 384 Plus (Molecular Devices)). The cell viability was expressed as percentage of absorbance relative to the control (without NP incubation), and experiments were performed independently in triplicate. The MTT dye and DMSO were obtained from Sigma Aldrich.

3. Results and Discussion

3.1. Morphology and surface composition of Bi(OH)₃ (M1), TiO₂ (M2), and ZnO (M3) NPs

Fig. 1a displays the XRD patterns of the synthesised Bi(OH)₃ (M1) NPs and the as-received TiO₂ (M2) and ZnO (M3) nanopowders. The XRD pattern of the Bi(OH)₃ NPs shows reflections which are identified as single phase but are broadened due to the very small particle size [31]. Since no JCPDS file can be assigned to the Bi(OH)₃, the XRD pattern is not indexed. The XRD pattern of the as-received ZnO NPs shows the single zincite structure (JCPDS 00-036-1451), whereas the TiO₂ NPs are composed of mixed phases of anatase (JCPDS 01-075-2552) and rutile (JCPDS 01-075-1753).

Fig. 1b shows TEM images of all the nanomaterials. The Bi(OH)₃ (M1) NPs are mostly agglomerates ~100 nm in size, with an average single particle size of 6.04 ± 0.89 nm, and round and ellipsoidal shapes (left images). The TiO₂ (M2) NPs are mostly spherical, ellipsoidal, and cubic in shape, with similar particle sizes of 24.08 ± 1.60 nm (centre right image). The particle size distribution is shown in Fig. 1c. The ZnO (M3) NPs are composed of particles with various morphologies, such as ellipsoidal, cubic, spherical, and hexagonal, with an average particle size of 44.86 ± 6.38 nm (right image). There is also a significant amount of rectangular plate-like particles with an average length of 92.99 ± 20.97 nm and width of 44.52 ± 5.50 nm, making the ZnO NPs the largest in comparison to the Bi(OH)₃ and TiO₂ NPs. Moreover, since the particle sizes vary greatly, the associated error is consequently much higher. For the particle size distribution of the ZnO NPs, the cross-section of the rectangular-shaped particles was used to calculate an equivalent spherical diameter.

The BET specific surface area of all materials was determined to be 47.8 ± 0.5 m²/g, 21.8 ± 0.2 m²/g, and 6.4 ± 0.1 m²/g for the TiO₂ (M2), Bi(OH)₃ (M1), and ZnO (M3) NPs, respectively.

Fig. 1. Physical characterization of $\text{Bi}(\text{OH})_3$ (M1), TiO_2 (M2), and ZnO (M3) NPs. (a) XRD patterns of $\text{Bi}(\text{OH})_3$ NPs (M1, top), ZnO (M3, centre), and TiO_2 (M2, bottom) NPs. (b) TEM images and (c) particle size distribution of $\text{Bi}(\text{OH})_3$ (M1) NPs, TiO_2 (M2), and ZnO (M3) NPs. (d) High-resolution XPS spectra of the O 1s region of M1, M2 and M3.

In order to investigate if the surface composition deviates from the bulk composition, XPS analysis was performed. Fig. 1d displays both the high-resolution XPS spectra of the O 1s peak and the deconvolution results for the Bi(OH)₃ (M1), TiO₂ (M2), and ZnO (M3) NPs. The survey spectra of all nanomaterials are shown in Fig. S1 of the Supporting Information (SI). The O 1s peak for all tested nanomaterials is resolved into multiple peaks with different intensities relative to each other. The O 1s peaks for Bi(OH)₃ at binding energies of 530.43 ± 0.04 eV and 532.81 ± 0.13 eV can be assigned to O²⁻ ions in Bi–O bonds [32] and hydroxide species (Bi–OH) adsorbed on the NP surfaces [32,33], respectively. Similarly to Bi(OH)₃, the O 1s peak for ZnO at 530.36 ± 0.25 eV represents O²⁻ ions in Zn–O bonds, and the peak at 531.95 ± 0.10 eV can be assigned to hydroxide and chemisorbed oxygen species (Zn–OH) [34]. The O 1s spectrum of TiO₂ displays peaks at 528.96 ± 0.08 eV associated with lattice oxygen in Ti–O and at 530.90 ± 0.11 eV, which is ascribed to hydroxyl groups (Ti–OH) on the surface, while the peak at 531.95 ± 0.16 eV corresponds to chemisorbed water (Ti–OH₂) [35].

3.2 UV-visible study of Bi(OH)₃ (M1), TiO₂ (M2), and ZnO (M3) NPs

The UV-visible absorption spectra of Bi(OH)₃ (M1), TiO₂ (M2), and ZnO (M3) NPs are shown in Fig. 2. The absorption spectra were all obtained at the same concentration (25 µg/mL) and indicate a higher and more selective absorbance of TiO₂ NPs within the UV region compared to ZnO and Bi(OH)₃ NPs. A comparison of the latter two shows a significant increase in the absorbance maximum for the Bi(OH)₃ NPs, which is similar to that of TiO₂ in lower wavelengths < 300 nm, while the ZnO NPs show a reduced and broader absorbance throughout the whole UV range. Therefore, TiO₂ NPs are considered UVB and UVA2 (320 – 340 nm) blocker, while ZnO NPs provide protection in the UVB and UVA1 (340 – 400 nm) area [36]. According to these categories, Bi(OH)₃ NPs would – similarly to TiO₂ – be a suitable blocker of UVB radiation, and thus, a potential candidate as active sunscreen ingredient.

This observation is reflected in the increase of the optical direct band gap of the $\text{Bi}(\text{OH})_3$ NPs with 4.06 ± 0.08 eV, compared to TiO_2 NPs with 3.39 ± 0.07 eV and ZnO NPs with 3.08 ± 0.05 eV. There is no literature available in regard to the measurement or calculation of the band gap of $\text{Bi}(\text{OH})_3$, and our results suggest the presence of a direct band gap at 4.06 ± 0.08 eV. With its wide band gap, $\text{Bi}(\text{OH})_3$ NPs have a high optical transparency in the visible light range, making it potentially viable as a cosmetic material. The band gaps were calculated using a Tauc plot (Fig. S2, SI, Eq. (1)), and are in agreement with the literature, where the band gap for ZnO ranges from 3.1 to 3.3 eV [37]. The band gap for TiO_2 ranges from approximately 3.0 eV (rutile), to 3.13 eV (brookite) and 3.2 eV (anatase) [38].

Fig. 2. UV-visible absorption spectra of $\text{Bi}(\text{OH})_3$ (M1), TiO_2 (M2), and ZnO (M3) NPs at 25 $\mu\text{g/mL}$ in deionised (DI) water. The concentration of the combined materials (M2 + M1; M3 + M1) is 25 $\mu\text{g/mL}$ each, implying a total concentration of 50 $\mu\text{g/mL}$.

The combination of materials, in particular TiO_2 and $\text{Bi}(\text{OH})_3$, and ZnO and $\text{Bi}(\text{OH})_3$ with equal concentrations (each material = 25 $\mu\text{g/mL}$) results in absorption spectra that show the characteristics of both materials. For instance, the combination of ZnO and $\text{Bi}(\text{OH})_3$ NPs clearly shows the peak maximum at approximately 375 nm, which corresponds to ZnO , and a linear increase in absorbance starting at approximately 300 nm, which is ascribed to $\text{Bi}(\text{OH})_3$.

3.3 Photocatalytic activity of $\text{Bi}(\text{OH})_3$ (M1), TiO_2 (M2), and ZnO (M3) NPs under UV irradiation

Intuitively, the presence of photocatalytically active anatase TiO_2 would not be expected in sunscreen formulations for topical application since TiO_2 , in particular the anatase phase, is known to be a highly active photocatalyst, which generates $\bullet\text{OH}$ radicals upon exposure to UV light. Several recent studies, however, have confirmed the presence of a highly photoactive P25 phase in commercial formulations [24,39,40].

Under Ultraviolet Exposure (300 nm and 350 nm)

The photoactivity of $\text{Bi}(\text{OH})_3$ (M1), TiO_2 (M2), and ZnO (M3) NPs was evaluated under UV irradiation using lamps with emission at 300 nm (UVB) and 350 nm (UVA), and is shown in Fig. 3a. The exposure of a crystal violet solution with TiO_2 (5 mg/L) leads to significant degradation, with only 10% of the initial absorbance present after irradiation with UV light for 30 minutes (Fig. 3a). Similarly, the addition of ZnO NPs, which are also known for their PCA, also resulted in degradation albeit at a reduced rate.

In contrast, the exposure to $\text{Bi}(\text{OH})_3$ NPs at the same concentration leads to little dye degradation over a similar period, meaning that the NPs do not generate many $\bullet\text{OH}$ radicals upon exposure to UV light. Moreover, the small PCA of the $\text{Bi}(\text{OH})_3$ NPs is most likely not related to the reduced number of active sites for the photodegradation reaction in comparison to TiO_2 , since the surface area of the ZnO NPs is even smaller, with the NPs displaying greater photodegradation than the $\text{Bi}(\text{OH})_3$ NPs. The large band gap of > 4 eV for $\text{Bi}(\text{OH})_3$ makes the excitation of electrons from the valence band to the conduction band less likely.

Fig. 3. Photoactivity of Bi(OH)₃ (M1), TiO₂ (M2), and ZnO (M3) NPs. (a) Relative decrease in absorbance of crystal violet solutions containing only the dye, M3 (5 mg/L), M2 (5 mg/L), and M1 NPs with concentrations ranging from 1 to 5 mg/L, and combinations of M2 (5 mg/L) + M1 (1 – 5 mg/L) and M3 (5 mg/L) + M1 (5 mg/L) under ultraviolet light exposure (300 nm and 350 nm). (b) Relative decrease in absorbance of crystal violet solutions containing M2 (5 mg/L), M3 (5 mg/L), and M1 (5 mg/L) NPs under exposure of AM 1.5 G one sun (100 mW/cm²). The data represent the mean of three independently prepared samples, which were measured separately.

When added to suspensions of TiO₂ or ZnO prior to irradiation, the Bi(OH)₃ NPs cause a significant decrease in crystal violet degradation and apparent rate constant k (see Eq. (2)), as shown in Table 4. The plots used to find the apparent rate constant were calculated with help of the Langmuir–Hinshelwood model [41] and are shown in Fig. S3a of the SI. More importantly, the dye degradation is reduced close to that of Bi(OH)₃ alone indicating almost complete reduction of the PCA of TiO₂.

Since TiO₂ is the more active photocatalyst, this reduction in activity was studied further by varying the concentration of mechanically added Bi(OH)₃ NPs. The reduction in PCA is greater for the addition of lower concentrations of Bi(OH)₃ NPs. This effect is most likely not only related to the effects of absorption and scattering from the additional particles, as a substantial

decrease in the degradation of crystal violet can also be observed for the lowest concentration of Bi(OH)₃ NPs used (1 mg/L).

Table 4: Photoactivity of Bi(OH)₃ (M1), TiO₂ (M2), and ZnO (M3) NPs. Apparent rate constant k for the degradation of crystal violet solutions containing M2 (5 mg/L), M3 (5 mg/L), and M1 NPs, in concentrations ranging from 1 to 5 mg/L, and combinations of M2 (5 mg/L) + M1 (1 – 5 mg/L) and M3 (5 mg/L) + M1 (5 mg/L) under ultraviolet light exposure (300 nm and 350 nm) and AM 1.5 G one sun (100 mW/cm²). The errors indicated are the standard deviation obtained through the linear regression.

Sample	$k \times 10^{-2}$ (min ⁻¹) UV irradiation	$k \times 10^{-2}$ (min ⁻¹) sunlight irradiation
Dye only	0.05 ± 0.01	0.47 ± 0.10
TiO ₂ (5 mg/L)	7.08 ± 0.22	46.67 ± 0.99
ZnO (5 mg/L)	1.45 ± 0.07	18.91 ± 2.23
Bi(OH) ₃ (5 mg/L)	0.89 ± 0.02	4.58 ± 0.12
ZnO (5 mg/L) + Bi(OH) ₃ (5 mg/L)	0.52 ± 0.02	-
TiO ₂ (5 mg/L) + Bi(OH) ₃ (5 mg/L)	0.94 ± 0.03	-
Bi(OH) ₃ (2.5 mg/L)	0.36 ± 0.02	-
TiO ₂ (5 mg/L) + Bi(OH) ₃ (2.5 mg/L)	0.57 ± 0.03	-
Bi(OH) ₃ (1 mg/L)	0.13 ± 0.01	-
TiO ₂ (5 mg/L) + Bi(OH) ₃ (1 mg/L)	0.47 ± 0.02	-

CeO₂, for instance, has the ability to ‘scavenge’ free radicals due to the presence of a large number of surface defects on the surfaces of CeO₂ NPs for small particle sizes, which can lead to oxygen deficiencies and reversible oxidation state changes in the cation [42,43]. This mechanism enables scavenging of excess free radical species, and consequently, the PCA of photocatalysts is reduced [44]. It is therefore likely that the reduced degradation of crystal violet is related to the scavenging of •OH radicals generated from the photocatalytically active TiO₂ and/or the reduction of active surface sites on the TiO₂ surface through the adsorption of Bi(OH)₃ NPs. Very low concentrations of Bi(OH)₃ NPs are enough to reduce the PCA of TiO₂ significantly: by adding a suspension of Bi(OH)₃ NPs that resulted in a concentration of 1 mg/L to a suspension of TiO₂ at a concentration of 5 mg/L prior to irradiation, the degradation of crystal violet decreased by more than 90%. This ultimately means that in the final suspension the ratio Bi(OH)₃:TiO₂ is 1:5.

There are no reports on the scavenging effects of $\text{Bi}(\text{OH})_3$ NPs, but the very small diameter of the NPs suggests that the effect might be related to the surface properties of the material. The $\text{Bi}(\text{OH})_3$ NPs display a very small particle size with low crystallinity, resulting in the presence of more surface defects, which, in turn, can act as ROS scavengers.

Under Ultraviolet Exposure (350 nm)

In order to exclude the possibility that $\text{Bi}(\text{OH})_3$ NPs absorb the radiation in the UV range and thus reduce the effective UV radiation for the TiO_2 and ZnO NPs, PCA tests were performed with a modified UV range. As shown via UV-visible spectroscopy, the band gap of $\text{Bi}(\text{OH})_3$ NPs lies at 305 nm (4.06 eV), and for TiO_2 and ZnO at 366 nm (3.39 eV) and 403 nm (3.08 eV), respectively. By limiting the UV radiation only to lamps with an emission at 350 nm (UVA), the absorbance of UV radiation by $\text{Bi}(\text{OH})_3$ NPs can be minimized. At the same time, the energy is sufficient to induce photocatalytic effects in TiO_2 and ZnO NPs.

The degradation of crystal violet, including the apparent rate constant, is shown in Fig. S4 of the SI, which gives results for ZnO (M3), TiO_2 (M2), and $\text{Bi}(\text{OH})_3$ (M1) NPs at a concentration of 5 mg/L. The photodegradation properties of crystal violet, for all nanomaterials under light irradiation at 350 nm are summarized in Table S1 of the SI. The performance of the ZnO NPs remains comparable to a full irradiation with both 350 (UVA) and 300 (UVB) nm lamps, most probably due to the absorbance in the broader wavelength range, with the absorbance maximum reached at approximately 375 nm, while TiO_2 shows a clear absorbance peak at wavelengths < 300 nm.

The addition of $\text{Bi}(\text{OH})_3$ NPs to the crystal violet solution results in very low dye degradation with an apparent rate constant $k = 0.08 \pm 0.01 \text{ min}^{-1}$, which is very similar to the control sample of only crystal violet with $k = 0.02 \pm 0.01 \text{ min}^{-1}$. It is therefore likely that the irradiation at 350 nm does not lead to any significant absorbance by $\text{Bi}(\text{OH})_3$, and that the decreased PCA of

TiO₂ NPs can be assigned to either a mechanism related to the active scavenging of photogenerated intermediates, or, more likely, the decrease in active surface area through the physical adsorption of Bi(OH)₃ NPs. Consequently, the addition of a suspension of Bi(OH)₃ (5 mg/L) to a suspension of ZnO (5 mg/L) appeared to completely inhibit crystal violet degradation, while addition of Bi(OH)₃ to TiO₂ (5 mg/L) reduced the PCA by 70%.

3.4 Photocatalytic activity of Bi(OH)₃ (M1), TiO₂ (M2), and ZnO (M3) NPs under simulated solar irradiation

In order to test the PCA under conditions that are more realistic for sunscreen applications, suspensions of Bi(OH)₃ (M1), TiO₂ (M2), and ZnO (M3) NPs in presence of crystal violet were exposed to AM 1.5 G one sun (100 mW/cm²) for a total time of 6 h. The degradation curves and apparent rate constant curves are displayed in Fig. 3b and Fig. S3b of the SI, respectively, and highlight the degradation of crystal violet when exposed to TiO₂ and ZnO NPs. Although there is some visible decrease in dye degradation for the treatment with Bi(OH)₃ NPs, these NPs show a much lower PCA compared to TiO₂ and ZnO NPs, especially in the early hours of irradiation (0 – 2 h) (Table 4).

3.5 Sun exposure of commercial sunscreens (CS1, CS2) and of homemade sunscreens (HS1, HS2)

One major drawback of modern sunscreen formulations is the aggressive degradation of third party applications, such as surface coatings on wood and steel, which is mediated by photocatalytic reactions [22,45]. The photocatalytic mechanism includes the formation of radicals if oxygen, water, and light are present.

In order to test the reduced PCA of the Bi(OH)₃ NPs in comparison to the photocatalytically more active ZnO NPs, both homemade sunscreen emulsions (with additional TiO₂ NPs: HS1, HS2) were applied on dark blue pre-painted steel sheets. In addition, two commercially

available sunscreen formulations were tested: manufacturer 1 (SPF 50, CS1) and manufacturer 1 (SPF 30, CS2), which also have organic compounds in addition to TiO₂ NPs as active ingredients.

After an exterior exposure of twelve weeks, SEM images of the panels were collected to investigate the surface morphology (Fig. 4). The untreated control test panel (top row, left image) maintained a smooth surface after the exposure, with the surface coating still intact. The panels treated with the commercial sunscreen formulations, however, showed signs of photocatalytic degradation: while the treatment with CS2 (top row, centre image) results in minor damage to the panel's surface, the treatment with CS1 (top row, right image) clearly leads to the degradation of the surface coating of the steel panels. It has been shown that the photocatalytically active particles will first destroy the sunscreen matrix, followed by a decomposition of the coating [22].

Similarly, the steel panel treated with the homemade TiO₂/ZnO emulsion (HS1, bottom row, centre image) displays a visible surface roughness, although it is lower in comparison to a treatment with CS1.

In contrast, panels coated with the homemade base emulsion prepared in this work ('all organics'; bottom row, left image) and the TiO₂/Bi(OH)₃ emulsion (HS2, bottom row, right image) showed no visible degradation of the panel surface, underlining the lack of PCA of the TiO₂/Bi(OH)₃ emulsion in comparison to the TiO₂/ZnO emulsion. This conclusion is also supported by the fact that the only difference in the emulsions is the replacement of ZnO NPs with Bi(OH)₃ NPs, while keeping all other ingredients the same.

Fig. 4. Sun exposure of commercial sunscreens and of homemade sunscreens. SEM images of the pre-painted steel panels without, and panels treated with, commercial sunscreen formulations (manufacturer 1 (CS1, CS2)), the homemade base emulsion, and homemade sunscreens containing TiO_2/ZnO (HS1) and $\text{TiO}_2/\text{Bi}(\text{OH})_3$ (HS2) after exposure to the Australian sun at a latitude of $\sim 34.5^\circ\text{S}$ (Wollongong, NSW) for twelve weeks.

These results support the findings that were obtained from the photocatalytic degradation of crystal violet. Compared with TiO_2 (M2) and ZnO (M3), $\text{Bi}(\text{OH})_3$ (M1) NPs showed the lowest PCA and more importantly, $\text{Bi}(\text{OH})_3$ NPs were able to reduce the PCA of TiO_2 NPs. Moreover, it was previously shown that the formation of defects is associated with the PCA of particular components of the sunscreens and that the photocatalytic degradation accelerates the weathering of the coating by 100 fold [22].

The difference in photostability of the tested commercial sunscreens is related to the phase of the TiO_2 NPs present in the formulations. The separation of the sunscreen ingredients and the XRD analysis of the inorganic components identified either the rutile phase or a combination of rutile and anatase TiO_2 [22,39,46]. In the present case, sunscreen CS1 contains an anatase

and rutile composite phase similar to M2, while sunscreen CS2 contains a single rutile TiO₂ phase as shown in Fig. S5 of the SI.

Generally, anatase TiO₂ is photocatalytically more active than rutile TiO₂ [46,47], but the mixed phase with 85% anatase and 15% rutile is particularly active [48]. The homemade TiO₂/ZnO (HS1) and TiO₂/Bi(OH)₃ (HS2) sunscreens have Eusolex[®] T-S as ingredient, which is an inorganic UV filter composed of rutile type TiO₂ and thus, less photocatalytically active.

3.6 Rheological properties of commercial sunscreens (CS3, CS4) and of homemade sunscreens (HS1, HS2)

An important factor that needs consideration when formulating sunscreen products is their uniformity, which is related to rheology. The dependence of the viscosity on the shear rate is shown in Fig. 5a and was measured for the homemade sunscreens of TiO₂/ZnO (HS1) and TiO₂/Bi(OH)₃ (HS2), as well as for two commercially available sunscreen products. In order to classify the spreadability of the homemade emulsions, two sunscreen products with different viscosity were chosen.

The commercial sunscreen, which is dispensed directly from the bottle (manufacturer 2, CS3), showed the highest viscosity, while the sunscreen applied through a spray dispenser (manufacturer 3, CS4) displayed the lowest viscosity. Both homemade sunscreen emulsions of TiO₂/ZnO and TiO₂/Bi(OH)₃ exhibit a viscosity in between the two commercially available sunscreen products, which emphasizes the adequate spreadability of the homemade emulsions and the associated eligibility for the use in sunscreen formulations. More importantly, both homemade emulsions demonstrate comparable rheological properties, exhibiting greater viscosity for lower shear rates.

While both commercial sunscreens show a linear relationship between the viscosity and the shear rate, the homemade sunscreens show non-linearity for higher shear rates. The increase in

viscosity is associated with the presence of agglomerates of NPs, since the concentration of ceramic NPs is 8 wt.%. This effect is less pronounced for the **homemade** emulsion of $\text{TiO}_2/\text{Bi}(\text{OH})_3$, which underscores the greater homogeneity in comparison to the ‘classical’ emulsion of TiO_2/ZnO .

Fig. 5. Rheological properties of commercial sunscreens and of homemade sunscreens. (a) Viscosity measurements and (b) rheograms of two commercially available sunscreens (CS3, CS4) and the homemade sunscreens containing TiO_2/ZnO (HS1) and $\text{TiO}_2/\text{Bi}(\text{OH})_3$ (HS2).

The relationship between the shear rate and the shear stress is shown in Fig. 5b. All tested **sunscreen** formulations exhibited nonlinear behaviour and consequently can be referred to as non-Newtonian fluids [49]. The flow index n classifies the deviation of a system from Newtonian behaviour, which is found for $n = 1$ (see Eq. (6)) [50]. Pseudoplasticity or shear thinning is typically indicated by $n < 1$, whereas shear thickening is present for values of $n > 1$ [51].

As displayed in Table 5, the **sunscreen** formulations showed a flow index ranging from 0.23 to 0.68, indicating pseudoplastic behaviour for all tested **sunscreens**. The more viscous sunscreen CS3 displayed the lowest flow index, which is in agreement with the generally lower flow rate of thicker bases. The advantage of a pseudoplastic fluid lies in its shear thinning properties, allowing for it to be easily spread on the skin, while simultaneously preventing the applied film

from running, as its viscosity instantly increases when no shear is applied [52]. In contrast, a Newtonian fluid runs quickly once applied to the skin, consequently reducing the protective effect of the applied film [53].

It is important to note that the flow index of the **homemade TiO₂/Bi(OH)₃ (HS2) sunscreen** is very similar to that of the commercially available sunscreen **CS4**, whereas the ‘classical’ **homemade sunscreen** of TiO₂/ZnO (**HS1**) exhibits a significantly greater flow index closer to those of Newtonian fluids.

The consistency index K of the formulations (see Eq. (6)) was calculated to range between 29 for **commercial** sunscreen **CS4** and 3175 for the more viscous **commercial** sunscreen **CS3**. These values are in agreement with commonly reported consistency indexes [51]. Generally, a high consistency index is associated with low spreadability, which is in agreement with the obtained results. More importantly, the **homemade TiO₂/Bi(OH)₃ (HS2) sunscreen** showed a consistency index significantly smaller than that of **commercial** sunscreen **CS3**, which underscores the improved spreadability properties of the TiO₂/Bi(OH)₃ **sunscreen**.

It was further shown that the viscosities of seven tested sunscreen formulations had a correlation with their SPF values: an increased SPF value always coincided with a higher viscosity [51]. These findings are in agreement with the obtained results for the two **homemade sunscreens**: the TiO₂/Bi(OH)₃ (**HS2**) **sunscreen** with a larger SPF value also shows greater viscosity in comparison to the ‘classical’ **sunscreen** with TiO₂/ZnO (**HS1**).

Table 5. Rheological properties of commercial sunscreens and of homemade sunscreens. Flow index (n) and consistency index (K) of two commercially available sunscreens (**CS3**, **CS4**) and the **homemade sunscreens** of TiO₂/ZnO (**HS1**) and TiO₂/Bi(OH)₃ (**HS2**). The errors indicated are the standard deviation obtained through the linear regression.

Sample	n	K
Commercial sunscreen CS3	0.238 ± 0.003	3175.411 ± 63.955
Commercial sunscreen CS4	0.455 ± 0.001	29.000 ± 1.682
TiO ₂ /ZnO emulsion	0.679 ± 0.016	62.765 ± 6.475

TiO ₂ /Bi(OH) ₃ emulsion	0.481 ± 0.023	180.082 ± 27.271
--	---------------	------------------

3.7 Photoprotection of homemade sunscreens (HS1, HS2)

The UV absorbance of the ‘classical’ sunscreen made of TiO₂/ZnO (HS1), and of the sunscreen made of TiO₂/Bi(OH)₃ (HS2) was determined *in vitro* over the entire UV spectrum (290 – 400 nm) using substrate spectrophotometry.

According to the recommendation of the European Commission, the ‘classical’ sunscreen with a measured SPF of 39.02 ± 1.26 can be classified in the category of ‘high protection’ [54]. In addition, this sunscreen shows the characteristics of a broad spectrum sunscreen which can give photoprotection against longer UV wavelengths, since the critical wavelength of 372.88 ± 0.07 nm is higher than 370 nm. The UVA-PF is 14.99 ± 0.39 .

The sunscreen made of TiO₂ and Bi(OH)₃ is significantly more efficient in the UVA and UVB range, with a SPF of 49.93 ± 1.66 , which represents an increase of 28% compared to the ‘classical’ sunscreen. Furthermore, the TiO₂/Bi(OH)₃ sunscreen shows a UVA-PF of 16.57 ± 0.38 , which is equivalent to an increase of 11% in comparison to the TiO₂/ZnO sunscreen. The UVA-PF is 33.2% of the total SPF for the TiO₂/Bi(OH)₃ sunscreen and therefore falls within the recommendations of the United States Food and Drug Administration (FDA) [29].

The obtained SPF is significantly larger than previously reported SPF values, such as for CeO₂ NPs. For instance, it was shown that a sunscreen of TiO₂ and calcium-doped CeO₂ NPs resulted in a maximum SPF of approximately 40 with a UVA-PF of approximately 11.5 [55].

The critical wavelength of the TiO₂/Bi(OH)₃ sunscreen was determined to be 372.01 ± 0.08 nm, resulting in broad-spectrum protection according to the FDA [29]. Since the critical wavelength only depends on the shape and width of the absorbance spectrum, and not on the

amplitude or the thickness of **sunscreen** application, the protection in the high-wavelength UVA region of both tested **sunscreens** is underlined.

An *in-vitro* study of 59 commercially available sunscreen products with UVA-filters, such as TiO₂ and ZnO, showed that only 10% of the tested sunscreens had a critical wavelength ≥ 370 nm [49]. This study demonstrates that a simple addition of long wavelength UVA active ingredients does not ensure true broad-spectrum protection, and thus underscores the importance of the obtained results for the **homemade sunscreen** of TiO₂/Bi(OH)₃.

3.8 Cytotoxicity of Bi(OH)₃ (M1), TiO₂ (M2), and ZnO (M3) NPs in healthy HaCaT and MDCK cells

In order to be considered a potential material in sunscreen formulations, the Bi(OH)₃ NPs need to be biocompatible. The cytotoxicity of **Bi(OH)₃ (M1), TiO₂ (M2), and ZnO (M3)** NPs was determined using the MTT assay, which is often employed to evaluate the effects of anticancer drugs [56].

Mitochondria are crucial for the maintenance of cellular function via aerobic adenosine triphosphate production and are therefore targets for toxic injury by numerous types of compounds [57]. Since the reduction of the tetrazolium salt occurs only in functional mitochondria, a decrease in MTT dye reduction indicates mitochondrial damage.

Non-cancerous human skin cells (HaCaT) and **dog** kidney cells (MDCK) were chosen, which were exposed to the NPs for 24 h at concentrations ranging from 5 – 500 $\mu\text{g/mL}$ (Fig. 6). While HaCaT keratinocytes are typically used to examine potential adverse effects of NPs in sunscreens [58,**59**], MDCK cells are typically used to determine biocompatibility of nanomaterials since kidney cells represent a possible detoxification route [**60,61**].

In general, a reduction in cell viability was observed in a dose-dependent manner for all the tested nanomaterials, which was more distinct in MDCK cells. The results indicate a significant decrease in mitochondrial activity for the ZnO NPs, with a cell viability of 48.7% and 0.5% in HaCaT and MDCK cells, respectively, at the highest tested concentration of 500 $\mu\text{g/mL}$. The TiO_2 NPs demonstrate only moderate cytotoxicity with a cell viability ranging between 61.5 – 71.2% at the same concentration. Similar findings regarding the cytotoxicity of ZnO and TiO_2 NPs in HaCaT cells are described in the literature [58].

The $\text{Bi}(\text{OH})_3$ NPs exhibited only a minor change in the reduction of MTT dye for all the tested concentrations in both cell lines, indicating that the mitochondrial function was not altered much at these doses. At the highest tested dose of 500 $\mu\text{g/mL}$ the NPs showed a cell viability of 83.5% and 82.5% in HaCaT and MDCK cells, respectively. The high biocompatibility of $\text{Bi}(\text{OH})_3$ NPs compared to the controls points to their suitability for use in sunscreens as opposed to ZnO NPs.

Fig. 6. Effects of $\text{Bi}(\text{OH})_3$ (M1), TiO_2 (M2), and ZnO (M3) NPs on the mitochondrial function in non-cancerous human skin (HaCaT) cells and dog kidney (MDCK) cells. The cells were treated with the NPs at concentrations of 0 (control), 5, 10, 25, 50, 100, 250, and 500 $\mu\text{g/mL}$ for 24 h. At the end of exposure, the mitochondrial function was determined using the MTT reduction assay, as described in Section 2. The data are presented as the mean of three independently prepared experiments.

4. Conclusions

The free radical generating properties of the TiO_2 and ZnO NPs currently used in some commercial sunscreen products could have the potential to cause adverse health effects, either through the direct interaction of these radicals with human tissue, or through the accelerated decomposition of other organic compounds within the formulation. These reactions not only have the potential to decrease the SPF of the formulation more rapidly, but also yield products with unknown effects on the human body.

In this study we investigated the potential of colourless $\text{Bi}(\text{OH})_3$ NPs that were synthesised through a facile, low-temperature route as an active ingredient in sunscreen formulations. The $\text{Bi}(\text{OH})_3$ NPs showed low PCA throughout the whole UV-visible spectrum, and more importantly, they were able to reduce the PCA of TiO_2 and ZnO NPs. We further showed that sunscreen formulations containing $\text{TiO}_2/\text{Bi}(\text{OH})_3$ have better photostability than commercially available sunscreen formulations, while displaying excellent rheological properties.

With its comparable absorbance to ZnO and TiO_2 in the UV region, and its significantly increased SPF and UVA-PF, $\text{Bi}(\text{OH})_3$ NPs offer a novel and cost-effective approach to improve the UV blocking properties of commercial sunscreen formulations and act as an antioxidant stabilizer for the organic components of the product, reducing the potential harm of these products to the health of the consumer.

Moreover, the $\text{Bi}(\text{OH})_3$ NPs exhibited high biocompatibility in healthy HaCaT and MDCK cells, while introduction of ZnO NPs resulted in a significant decrease in cell viability over the exposure time of 24 h at the same tested concentrations.

Acknowledgements

This work was supported by the Australian Research Council (ARC DP160102627) and the 2017 University of Wollongong Global Challenges Seed Grant “New generation sunscreens

optimized for Australian extreme exposure conditions and reliable in vitro test protocols for SPF and critical wavelength”. Furthermore, the authors acknowledge the use of the facilities within the Electron Microscopy Centre at the University of Wollongong. We would also like to thank Dr. D. Shi at the University of Wollongong for conducting the XPS measurements and Dr. T. Silver for help with editing the manuscript.

Appendix A. Supplementary data

Supplementary information associated with this article can be found, in the online version, at xxx.

References

- [1] J. D'Orazio, S. Jarrett, A. Amaro-Ortiz, T. Scott, UV radiation and the skin, *Int. J. Mol. Sci.* 14 (2013) 12222 – 12248.
- [2] F. L. Meyskens, P. Farmer, J. P. Fruehauf, Redox regulation in human melanocytes and melanoma, *Pigment Cell Melanoma Res.* 14 (2001) 148 – 154.
- [3] A. P. Popov, J. R. Lademann, A. V. Priezzhev, R. Myllylä, Effect of size of TiO₂ nanoparticles embedded into stratum corneum on ultraviolet-A and ultraviolet-B sun-blocking properties of the skin, *J. Biomed. Opt.* 10 (2005) 064037.
- [4] J. Wu, W. Liu, C. Xue, S. Zhou, F. Lan, L. Bi, H. Xu, X. Yang, F. D. Zeng, Toxicity and penetration of TiO₂ nanoparticles in hairless mice and porcine skin after subchronic dermal exposure, *Toxicol. Lett.* 191 (2009) 1 – 8.
- [5] T. G. Smijs, S. Pavel, Titanium dioxide and zinc oxide nanoparticles in sunscreens: focus on their safety and effectiveness, *Nanotechnol. Sci. Appl.* 4 (2011) 95 – 112.
- [6] L. Gaspar, P. M. Campos, Rheological behaviour and the SPF of sunscreens, *Int. J. Pharm.* 250 (2003) 35 – 44.

- [7] N. Serpone, A. Salinaro, A. V. Emeline, S. Horikoshi, H. Hidaka, J. Zhao, An in vitro systematic spectroscopic examination of the photostabilities of a random set of commercial sunscreen lotions and their chemical UVB/UVA active agents, *Photochem. Photobiol. Sci.* 1 (2002) 970 – 981.
- [8] J. F. Reeves, S. J. Davies, N. J. Dodd, A. N. Jha, Hydroxyl radicals (OH) are associated with titanium dioxide (TiO₂) nanoparticle-induced cytotoxicity and oxidative DNA damage in fish cells, *Mutat. Res.* 640 (2008) 113 – 122.
- [9] N. Zholobak, V. Ivanov, A. Shcherbakov, A. Shaporev, O. Polezhaeva, A. Y. Baranchikov, N. Y. Spivak, Y. D. Tretyakov, UV-shielding property, photocatalytic activity and phototoxicity of ceria colloid solutions, *J. Photochem. Photobiol.* 102 (2011) 32 – 38.
- [10] L. Truffault, M. T. Ta, T. Devers, K. Konstantinov, V. Harel, C. Simmonard, C. Andreazza, I. P. Nevirkovets, A. Pineau, O. Veron, Application of nanostructured Ca doped CeO₂ for ultraviolet filtration, *Mater. Res. Bull.* 45 (2010) 527 – 535.
- [11] D. Schubert, R. Dargusch, J. Raitano, S. W. Chan, Cerium and yttrium oxide nanoparticles are neuroprotective, *Biochem. Biophys. Res. Commun.* 342 (2006) 86 – 91.
- [12] J. Colon, L. Herrera, J. Smith, S. Patil, C. Komanski, P. Kupelian, S. Seal, D. W. Jenkins, C. H. Baker, Protection from radiation-induced pneumonitis using cerium oxide nanoparticles, *Nanomedicine* 5 (2009) 225 – 231.
- [13] V. Rodilla, A. T. Miles, W. Jenner, G. M. Hawksworth, Exposure of cultured human proximal tubular cells to cadmium, mercury, zinc and bismuth: toxicity and metallothionein induction, *Chem. Biol. Interact.* 115 (1998) 71 – 83.
- [14] G. G. Briand, N. Burford, Bismuth compounds and preparations with biological or medicinal relevance, *Chem. Rev.* 99 (1999) 2601 – 2658.

- [15] B. Bradley, M. Singleton, A. Po, Bismuth toxicity – a reassessment, *J. Clin. Pharm. Ther.* 14 (1989) 423 – 441.
- [16] L. Tillman, F. Drake, J. Dixon, J. Wood, Safety of bismuth in the treatment of gastrointestinal diseases, *Aliment. Pharmacol. Ther.* 10 (1996) 459 – 467.
- [17] K. Iuchi, Y. Hatano, T. Yagura, Heterocyclic organobismuth (III) induces apoptosis of human promyelocytic leukemic cells through activation of caspases and mitochondrial perturbation, *Biochem. Pharmacol.* 76 (2008) 974 – 986.
- [18] K. A. Smith, G. B. Deacon, W. R. Jackson, E. R. T. Tiekink, S. Rainone, L. K. Webster, Preparation and anti-tumour activity of some arylbismuth (III) oxine complexes, *Met. Based Drugs* 5 (1998) 295 – 304.
- [19] J. Klaveness, A. Berg, T. Almen, K. Golman, M. Droege, S. B. Yu, United States Patent, US 5817289 A, 1998.
- [20] K. Bogusz, M. Tehei, C. Stewart, M. McDonald, D. Cardillo, M. Lerch, S. Corde, A. Rosenfeld, H. K. Liu, K. Konstantinov, Synthesis of potential theranostic system consisting of methotrexate-immobilized (3-aminopropyl)trimethoxysilane coated α - Bi_2O_3 nanoparticles for cancer treatment, *RSC Adv.* 4 (2014) 24412 – 24419.
- [21] European Commission, Health and Consumers, Scientific Committees, Questions on zinc oxide, https://ec.europa.eu/health/scientific_committees/opinions_layman/zinc-oxide/es/1-3/3.htm (assessed 10 May 2018).
- [22] P. J. Barker, A. Branch, The interaction of modern sunscreen formulations with surface coatings, *Prog. Org. Coat.* 62 (2008) 313 – 320.
- [23] J. Tauc, *Amorphous and liquid semiconductors*, Springer, Boston, MA, 1974.
- [24] S. Senthilkumaar, K. Porkodi, Heterogeneous photocatalytic decomposition of crystal violet in UV-illuminated sol-gel derived nanocrystalline TiO_2 suspensions, *J. Colloid Interface Sci.* 288 (2005) 184 – 189.

- [25] R. E. Palma-Goyes, F. L. Guzmán-Duque, G. Peñuela, I. González, J. L. Nava, R. A. Torres-Palma, Electrochemical degradation of crystal violet with BDD electrodes: effect of electrochemical parameters and identification of organic by-products, *Chemosphere* 81 (2010) 26 – 32.
- [26] A. Cornish-Bowden, *Fundamentals of enzyme kinetics*, Weinheim, Germany, Wiley-Blackwell, 2012.
- [27] H. Andersson, J. Aarseth, N. Braud, B. Dandapat, Flow of a power-law fluid film on an unsteady stretching surface, *J. Nonnewton. Fluid Mech.* 62 (1996) 1 – 8.
- [28] B. Diffey, J. Robson, A new substrate to measure sunscreen protection factors throughout the ultraviolet spectrum, *J. Soc. Cosmet. Chem.* 40 (1989) 127 – 133.
- [29] United States Department of Health and Human Services Food and Drug Administration Center for Drug Evaluation and Research, Guidance for industry labeling and effectiveness testing: sunscreen drug products for over-the-counter human use – small entity compliance guide, <https://www.fda.gov/drugs/guidancecomplianceregulatoryinformation/guidances/ucm330694.htm> (accessed 01 January 2018).
- [30] T. Mosmann, Rapid calorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays, *J. Immunol. Methods* 65 (1983) 55 – 63.
- [31] M. A. Hobosyan, S. A. Yolchinyan, K. S. Martirosyan, A novel nano-energetic system based on bismuth hydroxide, *RSC Adv.* 6 (2016) 66564 – 66570.
- [32] V. S. Dharmadhikari, S. Sainkar, S. Badrinarayan, A. Goswami, Characterisation of thin films of bismuth oxide by X-ray photoelectron spectroscopy, *J. Electron. Spectrosc. Relat. Phenom.* 25 (1982) 181 – 189.

- [33] R. Al-Gaashani, S. Radiman, A. R. Daud, N. Tabet, Y. Al-Douri, XPS and optical studies of different morphologies of ZnO nanostructures prepared by microwave methods, *Ceram. Int.* 39 (2013) 2283 – 2292.
- [34] N. Gogurla, A. K. Sinha, S. Santra, S. Manna, S. K. Ray, Multifunctional Au-ZnO plasmonic nanostructures for enhanced UV photodetector and room temperature NO sensing devices, *Sci. Rep.* 4 (2014) 6483.
- [35] F. L. Toma, G. Bertrand, S. Begin, C. Meunier, O. Barres, D. Klein, C. Coddet, Microstructure and environmental functionalities of TiO₂-supported photocatalysts by suspension plasma spraying, *Appl. Catal. B* 68 (2006) 74 – 84.
- [36] D. R. Sambandan, D. Ratner, Sunscreens: an overview and update, *J. Am. Acad. Dermatol.* 64 (2011) 748 – 758.
- [37] V. Srikant, D. R. Clarke, On the optical band gap of zinc oxide, *J. Appl. Phys.* 83 (1998) 5447 – 5451.
- [38] D. Reyes-Coronado, G. Rodríguez-Gattorno, M. Espinosa-Pesqueira, C. Cab, R. D. de Coss, G. Oskam, Phase-pure TiO₂ nanoparticles: anatase, brookite and rutile, *Nanotechnology* 19 (2008) 145605.
- [39] L. Tiano, T. Armeni, E. Venditti, G. Barucca, L. Mincarelli, E. Damiani, Modified TiO₂ particles differentially affect human skin fibroblasts exposed to UVA light, *Free Radic. Biol. Med.* 49 (2010) 408 – 415.
- [40] A. Rampaul, I. P. Parkin, P. Cramer, Damaging and protective properties of inorganic components of sunscreens applied to cultured human skin cells, *J. Photochem. Photobiol. A* 191 (2007) 138 – 148.
- [41] R. Djellabi, M. Ghorab, Solar photocatalytic decolourization of crystal violet using supported TiO₂: effect of some parameters and comparative efficiency, *Desalin. Water Treat.* 53 (2015) 3649 – 3655.

- [42] E. G. Heckert, A. S. Karakoti, S. Seal, W. T. Self, The role of cerium redox state in the SOD mimetic activity of nanoceria, *Biomaterials* 29 (2008) 2705 – 2709.
- [43] I. Celardo, M. de Nicola, C. Mandoli, J. Z. Pedersen, E. Traversa, L. Ghibelli, Ce^{3+} ions determine redox-dependent anti-apoptotic effect of cerium oxide nanoparticles, *ACS Nano* 5 (2011) 4537 – 4549.
- [44] D. Cardillo, M. Weiss, M. Tehei, T. Devers, A. Rosenfeld, K. Konstantinov, Multifunctional $\text{Fe}_2\text{O}_3/\text{CeO}_2$ nanocomposites for free radical scavenging ultraviolet protection, *RSC Adv.* 6 (2016) 65397 – 65402.
- [45] P. J. Barker, Modern sunscreens make a lasting impression on surface coatings, *Journal of Surface Coatings Australia* (ISSN 2206-4818) 53:3 (2016) 10 – 17.
- [46] Australian Government, NMI Nanometrology, XRD phase analysis of TiO_2 sunscreens, <https://1bps6437gg8c169i0y1drtgz-wpengine.netdna-ssl.com/wp-content/uploads/2017/legacy/NMI-report-XRD-anatase.pdf> (accessed 01 January 2018).
- [47] K. M. Tyner, A. M. Wokovich, W. H. Doub, L. F. Buhse, L. P. Sung, S. S. Watson, N. Sadrieh, Comparing methods for detecting and characterizing metal oxide nanoparticles in unmodified commercial sunscreens, *Nanomedicine* 4 (2009) 145 – 159.
- [48] D. C. Hurum, A. G. Agrios, K. A. Gray, Explaining the enhanced photocatalytic activity of Degussa P25 mixed-phase TiO_2 using EPR, *J. Phys. Chem. B* 107 (2003) 4545 – 4549.
- [49] B. L. Diffey, P. R. Tanner, P. J. Matts, J. F. Nash, In vitro assessment of the broad-spectrum ultraviolet protection of sunscreen products, *J. Am. Acad. Dermatol.* 43 (2000) 1024 – 1035.
- [50] A. Metzner, R. Otto, Agitation of non-Newtonian fluids, *AIChE J.* 3 (1957) 3 – 10.

- [51] D. Nesseem, Formulation of sunscreens with enhancement sun protection factor response based on solid lipid nanoparticles, *Int. J. Cosmet. Sci.* 33 (2011) 70 – 79.
- [52] M. Pader, *Dentifrice rheology, Cosmetic Science and Technology Series* (1993) 247 – 247.
- [53] G. Dahms, Einfluss der Thixotropie auf die Lichtschutzwirkung von Sonnenschutzemulsionen, *Parfümerie und Kosmetik* 75 (1994) 675 – 679.
- [54] European Commission, Commission Recommendation of 22 September 2006 on the efficacy of sunscreen products and the claims made relating thereto (notified under document number C(2006) 4089), http://eur-lex.europa.eu/legal-content/EN/ALL/?uri=uriserv:OJ.L_.2006.265.01.0039.01.ENG (accessed 01 January 2018).
- [55] L. Truffault, B. Winton, B. Choquet, C. Andreazza, C. Simmonard, T. Devers, K. Konstantinov, C. Couteau, L. J. Coiffard, Cerium oxide based particles as possible alternative to ZnO in sunscreens: effect of the synthesis method on the photoprotection results, *Mater. Lett.* 68 (2012) 357 – 360.
- [56] M. J. Akhtar, M. Ahamed, S. Kumar, M. Khan, J. Ahmad, S. A. Alrokayan, Zinc oxide nanoparticles selectively induce apoptosis in human cancer cells through reactive oxygen species, *Int. J. Nanomed.* 7 (2012) 845 – 857.
- [57] S. Hussain, K. Hess, J. Gearhart, K. Geiss, J. Schlager, In vitro toxicity of nanoparticles in BRL 3A rat liver cells, *Toxicol. In Vitro* 19 (2005) 975 – 983.
- [58] F. Rancan, B. Nazemi, S. Rautenberg, M. Ryll, S. Hadam, Q. Gao, S. Hackbarth, S. Haag, C. Graf, E. Rühl, Ultraviolet radiation and nanoparticle induced intracellular free radicals generation measured in human keratinocytes by electron paramagnetic resonance spectroscopy, *Skin Res. Technol.* 20 (2014) 182 – 193.

- [59] K. Bogusz, M. Tehei, M. Lerch, S. X. Dou, H. K. Liu, K. Konstantinov, $\text{TiO}_2/(\text{BiO})_2\text{CO}_3$ nanocomposites for ultraviolet filtration with reduced photocatalytic activity, *J. Mater. Chem. C* 6 (2018) 5639 – 5650.
- [60] S. Xuan, F. Wang, J. M. Lai, K. W. Sham, Y. X. J. Wang, S. F. Lee, J. C. Yu, C. H. Cheng, K. C. F. Leung, Synthesis of biocompatible, mesoporous Fe_3O_4 nano/microspheres with large surface area for magnetic resonance imaging and therapeutic applications, *ACS Appl. Mater. Interfaces* 3 (2011) 237 – 244.
- [61] A. Mewada, S. Pandey, S. Shinde, N. Mishra, G. Oza, M. Thakur, M. Sharon, M. Sharon, Green synthesis of biocompatible carbon dots using aqueous extract of *Trapa bispinosa* peel, *Mater. Sci. Eng. C* 33 (2013) 2914 – 2917.