Next Generation Inorganic Nanomaterials for Sunscreening Applications

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Next Generation Inorganic Nanomaterials for Sunscreening Applications

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This thesis is presented as required for the conferral of the degree:

Doctor of Philosophy

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The University of Wollongong
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May 7, 2020
Declaration

I, Alexander Morlando, declare that this thesis is submitted in fulfilment of the requirements for the conferral of the degree Doctor of Philosophy, from the University of Wollongong, is wholly my own work unless otherwise referenced or acknowledged. This document has not been submitted for qualifications at any other academic institution.

__________________________

Alexander Morlando

May 7, 2020
Publications

The following publications resulted directly from this thesis work:


The following publications resulted from direct involvement by the author of this thesis work:


Abstract

The study of nanomaterials is an area of extensive research due to the size and shape dependent properties that arise as a result of confinement to the 1 - 100 nm scale. Materials at this scale exhibit new properties that are neither those of the corresponding bulk or individual molecules making up the material. One reason for this is thought to be due to the fact that, at this scale, many of the atoms making up the material lie at its surface, and so, an interface between the material and its surroundings is formed that it is not observed in the corresponding bulk or individual atoms of the material. This can lead to the generation of new or improved physical, chemical, magnetic and biological properties in nanomaterials compared to their larger scale counterparts. Implementation of nanomaterials, such as nanoparticles, into consumer products have also been shown to have a positive impact on the quality life of the general public. One such example of this is the application of inorganic metal oxide nanoparticles in therapeutic sunscreen products. Sunscreens containing these nanoparticles, namely titanium dioxide (TiO$_2$) and zinc oxide (ZnO), protect the skin from harmful solar ultraviolet (UV) radiation and thus contribute to the prevention of erythema (sunburn), immunosuppression, premature skin ageing and skin cancer. The size reduction of these materials to the nanoscale has been shown to improve their optical UV absorbance properties and increase transparency of formulations containing these nanomaterials in comparison to their microsized or bulk counterparts. However, as a consequence of this nano-phenomenon, the photocatalytic potential of these nanoparticles is also exponentially increased. Like a double-edged sword, absorption of UV radiation by these nanoparticles can also lead to the generation of reactive free radical species, which have the capacity to degrade other organic components in a sunscreen formulation.
The ability for these sunscreen based nanoparticles to generate free radicals is also of concern if they make contact with viable cells within the skin after topical application. Generation of free radical species within cells can result in a state of oxidative stress, a condition that has been implicated in a number of physiological and neurological diseases as well as cancer development. Although a significant number of studies have suggested these particle remain on the surface of the skin, inconsistencies in some results and discrepancies in the sampling methodologies used have still left the scientific community, and the general public, divided on the continued safe use of these nanoparticles. Investigations into alternative inorganic UV filters with complementary properties to those currently used but without the potential toxicological effects has yielded a limited number of candidate materials. More extensive research has focussed on methods for minimizing or removing the free radical generating potential of TiO$_2$ and ZnO and comprise manipulation of the phase composition, particle morphology and surface chemistry. In this thesis work, we investigate different potential coating materials for TiO$_2$ based nanomaterials and assess their suitability based on their impact towards UV light absorption and photocatalytic/phototoxic potential in hopes of improving the safety of sunscreen based inorganic UV filters.

The first work of this thesis investigated the physical, optical and photocatalytic properties of a chitosan/TiO$_2$ nanocomposite material. The nanocomposites were produced via a spray-drying method, in a single step, directly through an aqueous solution for the purpose of reducing the photocatalytic activity of commercially available TiO$_2$ nanoparticles. The photocatalytic activity of the nanocomposite materials were assessed using the organic dye, crystal violet, as the degradation target and irradiating in a photochemical reactor under UV light irradiation. It was found that the photoactivity of the chitosan encapsulated nanoparticles was greatly reduced compared to that of the pristine TiO$_2$ nanoparticles, from 95% degradation after 120 min of irradiation for pristine TiO$_2$ to 40% for the chitosan/TiO$_2$ spray-dried particles. Thus, the work demonstrated the potential for this simple coating process and chitosan material for application as an inactive protective coating for sunblocking applications.
The next body of work explored the deposition of cerium dioxide (CeO$_2$) nanodots onto commercial TiO$_2$ nanoparticles. CeO$_2$ nanoparticles have been demonstrated to display biocompatible properties and antioxidant activity due to redox cycling of the Ce$^{3+}$/Ce$^{4+}$ oxidation states. In this work, CeO$_2$/TiO$_2$ nanocomposites were prepared through a standard precipitation method at atomic concentrations (at%) of Ce relative to Ti of 2.5, 5 and 10 at%, with the aim of reducing the photocatalytic activity of the core TiO$_2$ nanoparticles and improve biocompatibility. The UV absorptive properties of the nanocomposite samples revealed excellent absorbance across the UV region as compared to pristine TiO$_2$ and CeO$_2$. Furthermore, a drastic reduction in the photocatalysed decomposition of crystal violet, when in the presence of the nanocomposite samples, under both UV and solar simulated light was observed compared to the highly photoactive pristine TiO$_2$. An optimal CeO$_2$ nanodot loading, displaying both high UV attenuation and low photocatalytic performance was determined around 5 at% and further in vitro biological testing revealed minimal impact on the cell viability of the human keratinocyte cell line (HaCaT) over a 24 hr period with and without prior exposure to UV irradiation. In contrast, pristine TiO$_2$ nanoparticles induced toxicity to HaCaT cells with prior UV exposure before incubation, particularly at a dosage of 100 mg L$^{-1}$. Thus, the work has demonstrated the effectiveness of CeO$_2$ nanodots in improving biocompatibility and its potential as a coating material for active inorganic UV filters.

The final work explored the synthesis of low photocatalytic rutile TiO$_2$ nanoparticles and the deposition of CeO$_2$ nanodots at their surface. Using a hydrothermal synthesis method, the effects of reaction temperature and nitric acid HNO$_3$ concentration on the crystal phase, composition and morphology were explored to assess the most suitable conditions for reproduction. Optimal reaction conditions for obtaining purely rutile TiO$_2$ nanorods occurred when treating the TiO$_2$ precursor at 150°C for 24 hr in 16 M nitric acid. Here, these rutile nanorods were decorated with CeO$_2$, as a means of producing a material with high UV attenuation and low photocatalytic activity. The nanocomposite sample displayed selective UV absorption whilst also demonstrating a reduction in photocatalytic activity compared to bare rutile TiO$_2$ nanorods of up to 88% and 77% when exposed to
UV and solar simulated light. The results obtained were significant as they would suggest that CeO$_2$/rutile TiO$_2$ could be safely applied as an active inorganic UV filter in sunscreen products.
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<th>Description</th>
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<tr>
<td>6-4PP</td>
<td>(6-4) Pyrimidone</td>
</tr>
<tr>
<td>α-MSH</td>
<td>α-Melanocyte stimulating hormone</td>
</tr>
<tr>
<td>A549</td>
<td>Human alveolar basal epithelial cells</td>
</tr>
<tr>
<td>AG01519</td>
<td>Human foreskin fibroblast cells</td>
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<td>ALS</td>
<td>Amyotrophic lateral sclerosis</td>
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<tr>
<td>AP</td>
<td>Activator protein</td>
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<td>ARPE-19</td>
<td>Human retinal pigment epithelial cells</td>
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<td>ARTG</td>
<td>Australian Register of Therapeutic Goods</td>
</tr>
<tr>
<td>ASR</td>
<td>Age-standardised rate</td>
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<tr>
<td>ASTM</td>
<td>American Society for Testing and Material</td>
</tr>
<tr>
<td>ATP</td>
<td>Adenosine triphosphate</td>
</tr>
<tr>
<td>ATR</td>
<td>Attenuated total reflectance</td>
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<tr>
<td>BALB/c 3T3</td>
<td>Murine embryonic fibroblast cells</td>
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<tr>
<td>BCC</td>
<td>Basal cell carcinoma</td>
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<tr>
<td>BEAS-2B</td>
<td>Human bronchial epithelial cells</td>
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<td>BET</td>
<td>Bruneaur-Emmett-Teller</td>
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<td>BSC</td>
<td>Bio-safety cabinet</td>
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<td>C</td>
<td>Cytosine</td>
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<td>Caco-2</td>
<td>Human intestinal epithelial cells</td>
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<td>cAMP</td>
<td>Cyclic adenosine monophosphate</td>
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<td>CAT</td>
<td>Catalase</td>
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<td>CHL/IU</td>
<td>Chinese hamster lung cells</td>
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<td>CHO</td>
<td>Chinese hamster ovary cells</td>
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<tr>
<td>CPD</td>
<td>Cyclobutane pyrimidine dimers</td>
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<tr>
<td>CV</td>
<td>Crystal violet</td>
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<tr>
<td>DCF</td>
<td>Dichlorofluorescein</td>
</tr>
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<td>DI</td>
<td>Deionized</td>
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DMEM Dulbecco’s modified eagle medium
DMPO 5,5-dimethyl-1-pyrroline N-oxide
DNA Deoxyribonucleic acid
DPBS Dulbecco’s phosphate buffered saline
DSC Differential scanning calorimetry
DTG Differential thermogravimetric
EDS Energy dispersive X-ray spectroscopy
EDTA Ethylenediaminetetraacetic acid
EELS Electron energy loss spectroscopy
EPR Electron paramagnetic resonance
EtOH Ethanol
EU European Union
EWG Environmental Working Group
FDA Food and Drugs Administration
FTIR Fourier transform infrared
FWHM Full-width half maximum
GPX Glutathione peroxidases
GRASE Generally recognized as safe and effective
HAADF High-angle annular dark-field
HaCaT Human keratinocyte cells
HOMO Highest occupied molecular orbital
HT22 Murine hippocampal neuronal cells
IARC International Agency for Research on Cancer
IC$_{50}$ Half maximal inhibitory concentration
IL Interleukin
IN Interferon
IR Infrared
JCPDS Joint Committee for Powder Diffraction Standards
L5178Y Murine lymphoma cells
L929 Murine fibroblast cells
LD$_{50}$ Median lethal dose
LDH Lactate dehydrogenase
LUMO Lowest unoccupied molecular
<table>
<thead>
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<th>Term</th>
<th>Definition</th>
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<tr>
<td>lar orbital</td>
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<tr>
<td>MAPK</td>
<td>Mitogen-activated protein kinases</td>
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<tr>
<td>MC1R</td>
<td>Melanocortin-1 receptor</td>
</tr>
<tr>
<td>MDCK</td>
<td>Madine-Darby canine kidney cells</td>
</tr>
<tr>
<td>MED</td>
<td>Minimum erythemal dose</td>
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<tr>
<td>MH-S</td>
<td>Murine alveolar macrophages</td>
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<td>MITF</td>
<td>Microphthalmia-associated transcription factor</td>
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<td>Matrix metalloproteinases</td>
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<td>Human lung fibroblast cells</td>
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<td>MTS</td>
<td>[3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt]</td>
</tr>
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<td>MTT</td>
<td>3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide</td>
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<td>NAD</td>
<td>Nicotinamide adenine dinucleotide</td>
</tr>
<tr>
<td>NADH</td>
<td>Nicotinamide adenine dinucleotide (reduced)</td>
</tr>
<tr>
<td>NADPH</td>
<td>Nicotinamide adenine dinucleotide phosphate</td>
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<tr>
<td>NER</td>
<td>Nucleotide excision repair</td>
</tr>
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<td>NICNAS</td>
<td>National Industrial Chemicals Notification &amp; Assessment Scheme</td>
</tr>
<tr>
<td>PABA</td>
<td>para-Aminobenzoic acid</td>
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<td>PAF</td>
<td>Platelet-activating factor</td>
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<tr>
<td>PDF</td>
<td>Powder diffraction file</td>
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<tr>
<td>PEG</td>
<td>Polyethylene glycol</td>
</tr>
<tr>
<td>PES</td>
<td>Phenazine ethosulfate</td>
</tr>
<tr>
<td>PLA</td>
<td>Polylactic acid</td>
</tr>
<tr>
<td>PLGA</td>
<td>Poly(lactic-co-glycolic) acid</td>
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<tr>
<td>PMMA</td>
<td>Polymethyl methacrylate</td>
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<td>PRX</td>
<td>Peroxiredoxins</td>
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<td>Murine macrophage cells</td>
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<td>RAW264.7</td>
<td>Murine macrophage cells</td>
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<tr>
<td>RNS</td>
<td>Reactive nitrogen species</td>
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<tr>
<td>ROS</td>
<td>Reactive oxygen species</td>
</tr>
<tr>
<td>SCC</td>
<td>Squamous cell carcinoma</td>
</tr>
<tr>
<td>SCCS</td>
<td>Scientific Committee on Consumer Safety</td>
</tr>
<tr>
<td>SD</td>
<td>Standard deviation</td>
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<tr>
<td>SDS</td>
<td>Sodium dodecyl sulfate</td>
</tr>
<tr>
<td>SE</td>
<td>Secondary electron</td>
</tr>
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<td>SED</td>
<td>Standard erythema dose</td>
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<tr>
<td>Abbreviation</td>
<td>Description</td>
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<tr>
<td>SEM</td>
<td>Scanning electron microscopy</td>
</tr>
<tr>
<td>SeM</td>
<td>Standard error of mean</td>
</tr>
<tr>
<td>SHSY5Y</td>
<td>Human neuroblastoma cells</td>
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<td>SOD</td>
<td>Superoxide dismutase</td>
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<tr>
<td>SPF</td>
<td>Sun protection factor</td>
</tr>
<tr>
<td>SSA</td>
<td>Specific surface area</td>
</tr>
<tr>
<td>T</td>
<td>Thymine</td>
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<tr>
<td>TBT</td>
<td>Titanium butoxide</td>
</tr>
<tr>
<td>TEM</td>
<td>Transmission electron microscopy</td>
</tr>
<tr>
<td>TEWL</td>
<td>Transepidermal water loss</td>
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<td>TGA</td>
<td>Thermogravimetric analysis</td>
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<td>Th1</td>
<td>T-helper type 1 cell</td>
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<td>Th2</td>
<td>T-helper type 2 cell</td>
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<td>THBS</td>
<td>Thrombospondin</td>
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<td>TMCS</td>
<td>Trimethoxycaprylsilane</td>
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<td>TSP</td>
<td>Tumour suppressor protein</td>
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<td>U</td>
<td>Uracil</td>
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<td>Human macrophage cells</td>
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<td>UV</td>
<td>Ultraviolet</td>
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<td>UVAPF</td>
<td>Ultraviolet A protection factor</td>
</tr>
<tr>
<td>UVR</td>
<td>Ultraviolet radiation</td>
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<tr>
<td>UV-Vis</td>
<td>Ultraviolet-visible</td>
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<tr>
<td>WHO</td>
<td>World Health Organisation</td>
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<tr>
<td>WIL2-NS</td>
<td>Human lymphoblastoid cells</td>
</tr>
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<td>XPS</td>
<td>X-ray photoelectron spectroscopy</td>
</tr>
<tr>
<td>XRD</td>
<td>X-ray diffraction</td>
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</table>
Chapter 1

Introduction

1.1 Nanotechnology - New Properties for Old Materials

Nanotechnology is a rather broad term that encompasses a variety of technologies and innovative materials reproduced/manufactured or operating at a scale of 1 to 100 nm in at least one dimension. The concept of nanotechnology and the manipulation of matter at this scale was first brought to light by Richard Feynman in a lecture given in 1959. It was first demonstrated practically by Binnig and Rohrer in 1982 with the development of the scanning transmission microscope and visualization of individual gold atoms. The term 'nanotechnology' itself was not established as a means of describing the manipulation, processing, separation and behaviour of matter at the nanoscale until Taniguchi et al, (1974) used it to describe semiconductor processes occurring at this range. The study of different systems and materials at this scale spans a number of scientific fields including physics, chemistry, biology and materials science, all of which are concerned with the novel properties and behaviours displayed by materials when operating at this scale.

The development of nanomaterials is an area of extensive research due to the size and shape dependent properties that arise as a result of the spatial confinement at the nanoscale. Nanomaterials typically display new properties that are neither those of the corresponding bulk or individual molecules making up the material. One reason for this is thought to be due to the fact that, at this scale, many of the atoms making up the material lie at the surface, and so, an new interface between the material and its environment is
formed unlike that observed for the corresponding bulk or individual atoms. Another way of putting this is to consider the example of a bag of sugar made up of very small crystals and another bag of sugar cubes, much larger in size than that of the small crystals. When each bag is poured into their own cups of water, it would be observed that the smaller sugar crystals dissolve at a faster rate than that of the large sugar cubes. This is a result of the increased amount of exposed surface area of the smaller sugar crystals as compared to the sugar cubes, leading to an increase in the chemical dissolution. The same size dependent properties are observed in nanomaterials because, as with the example outlined, the surface area to volume ratio of nanomaterials is vastly higher than that of their corresponding bulk. Optical properties are also affected by these size dependent properties. This can best be observed when comparing the appearance of bulk gold and gold nanoparticles. At the macro-scale, we observe gold to be, well, gold in colour, which we assign to being due to particular electronic transition between valence atomic orbitals, resulting in absorption of specific visible light wavelengths and it’s subsequent appearance. The electrons in gold nanoparticles however are inhibited in there movement due to the effects of quantum confinement, an effect observed at the nanoscale. This confinement of electrons in gold nanoparticles leads to a phenomena known as plasmonic resonance, a collective oscillation of the surface atoms of the gold nanoparticles when exposed to specific electromagnetic frequencies. The oscillation of these confined electrons occurs at specified frequencies which, in the case of gold, happens to correspond to wavelengths in the red light region of the electromagnetic region. (Figure 1.1).

Figure 1.1: Macroscopic and nanoscopic appearance of gold (Au). TEM micrograph of gold nanoparticles reproduced from Raliya et al, (2017).
Advances in our understanding of nanomaterials and the development of devices and instruments to manipulate materials at this scale has led to the incorporation of nanomaterials in numerous commercial products. Silver nanoparticles may be incorporated in band-aids and bandages owing to their antimicrobial activity. Metal oxide nanoparticles are used in commercial sunscreen products as active UV filtering ingredients. Nanstructured anode/cathode materials based upon silicon, carbon and metal chalcogenides are used in lithium ion batteries due their high surface area and high electron transport rates. Graphene, a two dimensional array of carbon atoms, and graphene-based nanocomposite materials have been incorporated into two of the highest selling vehicles produced by the Ford Motor Company due to improvements in heat transfer, noise reduction and strength imparted. Development of new nano-fields combining pharmaceutical and biomedical sciences have also paved the way for the development of novel nanomedicines including novel drugs and imaging agents that show improvements in targeting, efficacy and bioavailability as compared to traditional medicines. Superparamagnetic iron oxide ($\text{Fe}_3\text{O}_4$) have been investigated for targeted drug delivery by manipulation of their magnetic properties. Polymeric nanoparticles composed of L-glutamic acid, L-alanine, L-lysine and L-tyrosine are used as an immunomodulator in the treatment of multiple sclerosis. Nanoparticles composed of self-assembled liposomes have also been used as drug-carriers for the delivery of specific drugs to target locations.

However, this commercialisation and increased production of nanomaterials has also raised concerns over the potential human health and environmental risks posed by such materials. The release of nanomaterials into the environment may occur from direct sources such as production facilities, waste water treatment plants or landfills or indirect sources such as wash-off of cosmetics or other products containing nanomaterials. Much like the accumulation of heavy metals and radioisotopes, persistent nanomaterials may be bioaccumulated in flora and fauna and carried up through the food-chain. Exposure of organisms to high levels of nanomaterials has also been demonstrated to have an impact on health and regular functionality. Internalisation may occur through accidental digestion or inhalation, whilst permeation through the skin may also occur through
lipid channels between cells in the stratum corneum or through hair follicles. Various *in vitro* and *in vivo* studies have shown that exposure to nanomaterials can result in cellular internalisation as well as cytotoxic/genotoxic effects, occasionally mediated through the production of free radical species within the cell.\textsuperscript{14–16} As such, there is an urgent need to ensure new and current nanomaterials, and their unique properties, are understood and well characterized. This will enable better understanding of the toxicological effects these materials may have to both humans and the environment and will enable minimization or removal of any potential harm that could imparted by such new materials.

### 1.2 Ultraviolet (UV) Radiation - Australia at the Forefront

UV radiation is a constituent of the electromagnetic spectrum, spanning the wavelength range of 10 - 400 nm. Of all the solar electromagnetic radiation reaching the earth’s atmosphere, approximately 9% corresponds to wavelengths in the UV region, although this can vary across the seasons of a year and by geographical location.\textsuperscript{17} The UV region can also be further subdivided based upon the differing biological effects associated with different UV band ranges. As such, the UV electromagnetic wavelength regions of most biological importance comprise of the UVC region (100 - 290 nm), UVB region (290 - 320 nm), UV AII (320 - 340 nm) and UVAI (340 - 400 nm) regions.\textsuperscript{17,18} The composition of UV radiation incident on the earth’s surface also varies as a result of atmospheric processes, such as absorption by stratospheric ozone, leading to total absorption of wavelengths in the UVC region. Of the terrestrial UV radiation present, approximately 6% corresponds to UVB radiation and the remaining 94% to UVA radiation.

Living in Australia, people are exposed to some of the highest intensities of solar UV radiation experienced across the globe. The reason for this is due to a combination of factors including the geographical location of the continent, earth’s position and orientation relative to the sun during summer periods and the higher level of air quality in the southern hemisphere as compared to the northern hemisphere. These factors contribute
to the roughly 15% higher UV irradiance of Australia compared to other countries in the Northern hemisphere (Table 1.1).\textsuperscript{19,20}

<table>
<thead>
<tr>
<th>Latitude (\textdegree{N})</th>
<th>Location</th>
<th>Yearly UVR (SED*)</th>
<th>Population</th>
</tr>
</thead>
<tbody>
<tr>
<td>19.5</td>
<td>Mauna Loa, USA</td>
<td>17,700</td>
<td>376</td>
</tr>
<tr>
<td>26.0</td>
<td>Naha, Japan</td>
<td>10,172</td>
<td>319,435</td>
</tr>
<tr>
<td>41.6</td>
<td>Barcelona, Spain</td>
<td>8,200</td>
<td>1,609,000</td>
</tr>
<tr>
<td>47.3</td>
<td>Garmisch, Germany</td>
<td>5,494</td>
<td>26,178</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Latitude (\textdegree{S})</th>
<th>Location</th>
<th>Yearly UVR (SED*)</th>
<th>Population</th>
</tr>
</thead>
<tbody>
<tr>
<td>12.4</td>
<td>Darwin, Australia</td>
<td>16,750</td>
<td>132,045</td>
</tr>
<tr>
<td>31.9</td>
<td>Perth, Australia</td>
<td>12,234</td>
<td>1,980,000</td>
</tr>
<tr>
<td>33.9</td>
<td>Sydney, Australia</td>
<td>9,502</td>
<td>4,640,000</td>
</tr>
<tr>
<td>34.9</td>
<td>Adelaide, Australia</td>
<td>10,500</td>
<td>1,310,000</td>
</tr>
</tbody>
</table>

Table 1.1: Comparison of yearly total incident UVR as SEDs\* between Australian cities and northern hemisphere cities. Data produced from Gies, (2003).\textsuperscript{19} \*Standard Erythema Dose (SED) - 1 SED is equivalent to an erythemal radiant exposure of 100 Jm\textsuperscript{-2}.

The standardized indicator for UV radiation intensity is notated as the UV index, a scale adopted by the World Health Organisation (WHO) in 1994\textsuperscript{21} and introduced in Australia in 1996.\textsuperscript{20} The UV index is a measure of the intensity of UV radiation incident on the earth’s surface during clear-sky conditions and is an indicator of potential skin damage. A scale ranging from 0 upwards to 11 and beyond is typically used as a means of not only indicating the intensity of incident UV radiation, but also to provide an idea of the level of protection required at that particular value. It also serves to highlight the extent to which damaging effects can occur, with higher values suggesting greater potential for skin and eye damage and lower exposure times for such effects to occur.\textsuperscript{22} Day-to-day UV indices are calculated from the maximum biological effective solar UV radiation (\textit{UVR}_{\text{eff}}), measured over a period of approximately 30 min. \textit{UVR}_{\text{eff}} is obtained from the summation of weighted contributions of the erythemal (sunburn) effect of incident UV wavelengths in
the range of 280 - 400 nm, as expressed by the following equation:

\[ UVR_{eff} = \sum_{280\text{nm}}^{400\text{nm}} E_\lambda S_\lambda \triangle \lambda \]  

(1.1)

where \(E_\lambda\) is the solar spectral irradiance (W m\(^{-2}\) nm\(^{-1}\) or standard sun), \(S_\lambda\) the erythemal spectral effectiveness (Figure A.1) and \(\triangle \lambda\) the bandwidth (nm) of the measured intervals.

Figure 1.2 highlights the global UV index spread during the winter and summer months of 2015/2016, from which it is clear that, not only Australia, but many regions around the world are exposed to very high levels of UV radiation and, for Australia in particular, is a leading factor in the substantial diagnosis of skin cancers each year. In fact, statistics from the WHO attribute approximately 50-90\% of malignant melanomas and non-malignant basal cell carcinomas, as well as 50-70\% of non-malignant squamous cell carcinomas in light-skinned populations due to sun exposure and incident UV radiation.\(^{23}\) In addition to this, studies of the Australian workforce have shown that outdoor workers, on average, experience greater exposure to UV radiation as compared to outdoor workers in overseas countries including Canada and the United Kingdom.\(^ {24}\) This also accounts for the higher skin cancer rates observed in Australia with its largely light-skinned population and the country having the highest incidence of these types of cancers in the world.\(^ {25}\)

\[ \begin{array}{c}
\text{Erythematic UV Index} \\
KIMR/ESA \end{array} \]

\[ \begin{array}{c}
\text{Clear-sky} \\
1\text{July 2015} \\
1\text{January 2016} \end{array} \]

\[ \begin{array}{c}
0 \quad 2 \quad 4 \quad 6 \quad 8 \quad 10 \quad 12 \quad 14 \quad 16 \quad 18 \\
0 \quad 2 \quad 4 \quad 6 \quad 8 \quad 10 \quad 12 \quad 14 \quad 16 \quad 18 \\
\end{array} \]

**Figure 1.2:** Global UV index recorded in the middle of the Australian winter (left) and summer (right) months during 2015-16. The scales shown represent the variation of UV index, with higher values representing higher UV intensities. Figure reproduced from TEMIS, (2016).\(^ {26}\)

In addition to the carcinogenic potential of UV radiation, high levels of exposure have
also been shown to induce a range of skin-related conditions including erythema, immunosuppression and premature skin ageing.\textsuperscript{27,28} The cause behind these conditions is thought to, in part, be attributed to the generation of free radical species, such as reactive oxygen species (ROS), within viable cells. ROS and other reactive species are a regular by-product of the cell cycle and metabolism and thus natural antioxidant pathways exist within cells to cope with these species.\textsuperscript{29,30} In addition, certain free radical species are important in various cellular function and play a role in intracellular signalling and the immune response to foreign bodies.\textsuperscript{31,32} However, an excess of free radicals or overloading of the inherent cellular mechanisms for dealing with free radicals can result in a state of oxidative stress within afflicted cells. This can lead to oxidative damage of important cellular features such as organelles, the cell membrane and even deoxyribonucleic acid (DNA), accounting for the link between UV exposure and the development of skin cancers.\textsuperscript{33} A number of strategies have been implemented to increase public awareness of the harmful effects of UV radiation and to encourage the general public to avoid or limit sun exposure during times of high UV intensities. However, societal norms have limited the effectiveness of such warnings and so, more effective measures for defence against UV rays have been developed and integrated into the routine of consumers over the last 50 years through the development and commercialisation of sunscreen products.

1.3 Sunscreens and Nanomaterials

The use of products or minerals containing UV blocking or filtering ingredients is no modern invention, with evidence suggesting the use of clay products by Ancient Egyptians containing UV absorbing iron oxides dating as far back as 3100 BC.\textsuperscript{34} However, mainstream commercialisation and patenting of specific sunscreen formulations and did not occur until the 1920s.\textsuperscript{35} Even at this stage, a shift in societal behaviour during the later half of the 20\textsuperscript{th} century was leading to all time high levels of UV exposure. In addition, an increasing amount of evidence was mounting in highlighting the link between UV radiation and skin cancers which, combined with the increasing rates of melanoma diagnoses, was of particular concern to human populations residing in countries exposed
1.3 Sunscreens and Nanomaterials

to high levels of UV, such as Australia. A health campaign promoted in Australia during the 1980s, colloquially known as the Slip! Slop! Slap! SunSmart Campaign, helped in educating and encouraging the general public to use sunscreen products during outdoor activities. According to the Australian Cancer Council, the popularisation of the campaign has helped play a key role in shaping the sun protection attitudes and behaviour of people in the years since the campaign was run.36

Despite increased public awareness of UV radiation and the need for sunscreen products, the incidence of skin cancers are still on the rise. A part of this can be attributed to recent consumer concerns over certain sunscreen features developed over the past 20 years and a lack of certainty in the safe use of these products. Recent analysis from the Cancer Council’s National Sun Protection Survey have revealed worrisome statistics about the Australian public’s perception of sunscreen products.37 According to the survey, 45% of adults could not agree with whether sunscreens could be used safely on a daily basis, whilst 20% of adults believed regular use could lead to Vitamin D deficiencies and 17% of adults believing the ingredients present in sunscreens were bad for health if regularly used. Publication of news articles in recent times pertaining to the potential bleaching effects of sunscreens on corals, absorption of certain sunscreen ingredients and concerns surrounding the use of nanoparticles in sunscreens have also propagated the uncertainty in such products.38–40 However, misinterpretation of experimental evidence by online groups lacking specific background knowledge in the field41 has also contributed to the spread of misinformation and is likely also a contributing factor in the survey results obtained by the Cancer Council.

The ingredients comprising a sunscreen formulation serve a range of purposes and vary from emulsification agents, preservatives, antioxidants and the 'active' ingredients that provide specific protection from incident UV radiation. These active ingredients are typically classified as organic or inorganic UV filters and are regulated in Australia by the Therapeutic Goods Administration (TGA).42 The TGA are responsible for ensuring sunscreen manufacturers comply to regulation guidelines pertaining to the UV protective ability of these active ingredients and their safety, and govern the list of approved UV
1.3 Sunscreens and Nanomaterials

filters, classified as therapeutics, that may be used in sunscreen formulations. A number of health and environmental concerns surrounding the use of organic UV filters in sunscreen products have arisen since their initial inception into the commercial market in the 1940s, however, they are not the main focus of this thesis work (although a further look at organic UV filters will be given in Chapter 2). The two TGA approved inorganic UV filtering compounds are materials based upon titanium dioxide (TiO$_2$) and zinc oxide (ZnO). These materials were initially introduced into commercial formulations in the form of particles, generally in the micrometer range. Owing to a difference in the physical properties of these compounds, as compared to organic UV filters, sunscreen formulations containing these particles typically appeared opaque when applied and left an unappealing whiteness to the skin even after rubbing in. However, with advances in manufacturing methods and the fruition of nanotechnology, modern sunscreen formulations containing these two materials have been tailored to improve transparency whilst also affording increased protection from incident UV radiation.\(^{43}\) The cause for this advancement has been brought about by the size reduction of these inorganic particles to below 100 nm, thus forming nanoparticles. With this size reduction, less visible light is scattered, meaning formulation aesthetics can be improved (Figure 1.3). In addition, the effects of size quantization become more prominent, leading to improved UV absorption by these nanoparticles as compared to their microparticle counterparts.\(^7\)

![Figure 1.3: Calculated UV attenuation curves for spherical particles, demonstrating light scattering effects as a function of particle size. Figure reproduced from Schilling et al, (2010).\(^{44}\)](image)
of this size reduction, has also brought concerns over their potential harm to consumers when incorporated in commercial goods, such as sunscreens. As a result of the drastic increase in the surface area to volume ratio of nanoparticles compared to their bulk equivalents, increases in chemical, photochemical and photocatalytic reactivity occur.\textsuperscript{45} TiO$_2$ nanoparticles in particular have been extensively investigated in photocatalysis applications due to its well known photocatalytic properties and propensity to generate various free radicals, including ROS.\textsuperscript{46} Furthermore, concerns over the potential for these nanoparticles to penetrate the skin barrier when topically applied has been a topic of much discussion since their inception into commercial sunscreens. This has been a significant cause of concern due to mounting evidence demonstrating the cytotoxic, genotoxic and phototoxic potential of TiO$_2$ and ZnO nanoparticles towards various human cell lines and animal models.\textsuperscript{47–52} Many of these studies have also linked the toxicity induced to the increased generation of free radical species by these nanoparticles, resulting in states of oxidative stress. Moreover, a study performed in 2008 revealed that many sunscreen products containing TiO$_2$ nanoparticles were in a compositional form similar to that of commercial-grade TiO$_2$ nanoparticles sold specifically for photocatalysis applications.\textsuperscript{53} In this study, it was found that the sunscreen-based TiO$_2$ nanoparticles displayed photocatalytic activities on par with the commercial-grade photocatalytic TiO$_2$ powder, prematurely ageing and degrading coatings on steel roofing panels through a free radical mediated process. Increased consumer awareness of these nanoparticles in sunscreen products and surmounting scientific evidence of their potential toxicological effects paved the way for a review of literature by the Australian TGA, firstly in 2013 and later updated in 2016.\textsuperscript{54} The primary focus of this review was on the potential for these sunscreen nanomaterials to penetrate the skin and reach viable cells and considered both \textit{in vitro} and \textit{in vivo} studies. It was in their opinion that the ‘weight of evidence’ suggested these nanoparticles cannot reach viable cells when applied topically to skin and that they largely remain atop the stratum corneum, the outermost layer of superficial skin. As such, it was inferred that they do not pose any significant threat to consumers using nanoparticle-containing sunscreens. Despite the conclusions drawn by the TGA, irregularities in testing proto-
cols and inconsistencies in skin models used for a various number of studies cited have still left the scientific community and consumers divided on the matter. The review also highlighted the need for additional long-term case studies involving the continuous topical application of nanoparticulate sunscreens to, not only healthy human skin, but also abraded and pre-damaged skin models to better account for long-term health effects and to consider the implications of a reduced skin barrier to external entities.

Another important parameter needed to be considered for the continued safe use of nanoparticulate inorganic UV filters is their photocatalytic activity. Both TiO$_2$ and ZnO nanoparticles have been studied for use in various photocatalysis applications including dyesensitized solar cells, water purification and splitting and self-cleaning glasses.$^{46, 55, 56}$ The underlining principle for these nanomaterials and their application is their ability to react with chemically adsorbed molecules through interaction of photoexcited charge species generated within the material upon UV exposure. Under certain conditions, such as within mammalian cells, this can lead to the generation of harmful ROS such as the hydroxyl and superoxide radicals and can contribute to states of oxidative stress in viable cells.$^{57}$ In addition, generation of such free radical species can impact the efficacy of sunscreen formulations by degrading organic based active ingredients, thus reducing the protection afforded when applied.$^{58}$ Sunscreen manufacturers are aware of this photocatalysis property and typically modify these inorganic UV filters by applying inert surface coatings to the nanoparticles and include antioxidant compounds in formulations to minimize and limit the impact of photogenerated free radicals. Such strategies however may bring about further issues, for instance, the addition of antioxidant compounds, which are typically organic in nature, may enhance the propensity for the formulation to induce inflammatory and allergenic reactions in sensitive skin. As for the coating strategies, the use of coatants such as aluminium and silicon based oxides and hydroxides have been shown to aid in reducing the photocatalytic activity of TiO$_2$ and ZnO.$^{59}$ However, it has also been demonstrated that excessive coating can impair the UV absorptive ability of the core nanoparticle material, thus limiting the overall efficiency of the UV protection afforded and increasing the need for greater nanoparticle loadings in sunscreens to
achieve and maintain a high level of UV attenuation (Figure 1.4). \(^{60}\)

**Figure 1.4:** Diminished UV absorption of a range of TiO\(_2\)/SiO\(_2\) and TiO\(_2\)/SiO\(_2\)/APTES nanocomposite particles. APTES refers to 3-aminopropyltriethoxysilane. Figure reproduced from Bai et al., (2017). \(^{60}\)

Novel UV filtering nanomaterials have been explored throughout the 21st century, with various alternatives to TiO\(_2\) and ZnO displaying prominent UV absorptive properties rivaling those of the currently approved inorganic UV filters. These include such doped and undoped variants of cerium oxides (CeO\(_2\)), iron oxides (Fe\(_2\)O\(_3\)), tin oxides (SnO\(_2\)) as well as biocompatible polymeric nanoparticles and organic/inorganic hybrid nanomaterials. \(^{61-65}\) A major drawback with developing new UV filtering ingredients, in particular inorganic based filters, is the extensive level of physical, chemical and biological characterisation required to be submitted to regulating bodies before approval can be given. \(^{66}\) This can be a timely and costly process, so manufacturers prefer to work with currently approved UV filters. This could be adjusting loading concentrations or testing certain combinations of different filters to achieve high levels of UV protection. Additionally for inorganic UV filters, manufacturers are given some limited free range to manipulate the physical properties of these nanomaterials. In the case of inorganic TiO\(_2\) UV filtering
nanoparticles, the TGA have recently adopted guidelines outlined by the European Union (EU) Scientific Committee on Consumer Safety (SCCS) based upon recommendations made in an earlier report.\textsuperscript{67,68} One of the critical components stipulated by these guidelines for TiO\textsubscript{2} nanomaterials is to ensure that they do not have photocatalytic activity or, at most, up to 10\% photocatalytic activity compared to a corresponding non-coated or non-doped reference material. Thus the possibility for exploring different coating materials and methods for applying these materials is relatively open, provided the resultant composite can adhere to the guidelines outlined.

1.4 Research Objectives and Thesis Outline

Both TiO\textsubscript{2} and ZnO nanomaterials are used in consumer products such as sunscreens, however, concerns have been raised over the safety of these materials due to a combination of their nanometric scale, photocatalytic properties and the subsequent toxicological effects that may result. Thus, one focus of this thesis was to explore pathways for reducing the photocatalytic activity of such nanomaterials, in particular TiO\textsubscript{2} nanoparticles, or consider alternative materials that may display similar, if not, improved sunscreen relevant properties compared to current inorganic UV filters. Finally, after assessing the most ideal methodology for inhibiting photocatalysis, based upon a literature review, new nanocomposite variants based upon TiO\textsubscript{2} nanoparticles will be developed and assessed for applicability in sunscreen products. The individual aims of this research thesis include:

(a) To develop and optimize a methodology for synthesizing TiO\textsubscript{2} nanoparticles of specific physical and chemical characteristics suitable for UV filtration.

(b) To investigate and prepare a polymer/TiO\textsubscript{2} nanocomposite material and to assess the suitability of the encapsulation process used as a means of inhibiting the photocatalytic activity of the core metal oxide nanoparticles whilst still maintaining adequate levels of UV protection.

(c) To develop a metal oxide/TiO\textsubscript{2} nanocomposite material with deposition of poten-
tially free radical scavenging nanoparticles in the form of CeO$_2$ and to assess the effects of these particles of the UV absorptive and photocatalytic properties of the composite material, under both UV and solar-simulated light irradiance.

(d) To combine TiO$_2$ nanoparticles that display ideal physical and chemical properties for use as an inorganic UV filter with free radical scavenging CeO$_2$ nanoparticles and to assess the changes in UV protection afforded and photocatalytic activity exerted.

(e) To assess the cytotoxic and phototoxic potential of TiO$_2$ and TiO$_2$-based nanomaterials towards a selected human skin epithelial cell line.

The research conducted over the course of this PhD thesis and the content of this thesis is split into several chapters as described below:

**Chapter 1** Provides a general introduction into nanotechnology, nanomaterials and their application in commercial products. In addition, an overview of UV radiation and its geographical incidence is provided. The connection between UV radiation, commercial sunscreen products and nanotechnology is given. Finally, the key motivations and goals of this thesis work are given.

**Chapter 2** A detailed review of current and past literature pertaining to the effects of UV radiation and the role of ROS in human health complications is given. Furthermore, an overview of sunscreen products, their regulation in Australia and the role and function of active ingredients in these products is outlined. A thorough analysis of the potential dangers of inorganic metal oxide nanoparticles present in sunscreen products is also given and current methodologies for minimizing consumer concern in relation to these particles is shown. An introduction to alternative inorganic UV filtering ingredients is also given, however, the main focus of thesis work is on the modification of currently approved TiO$_2$ based nanoparticles.
Chapter 3 This encompasses the physical, chemical and biological methods employed to synthesize and characterise the various nanomaterials studied in this thesis work. A brief outline of the characterisation techniques used to investigate various physical, chemical and biological properties of the nanomaterials prepared, including particle size, morphology, elemental composition, crystal phase composition, optical properties, photocatalytic properties and cytotoxic properties, is given followed by a procedural outline of the experiments performed.

Chapter 4 Presents a study focussed on the development and characterisation of a nanocomposite material based upon the encapsulation of TiO$_2$ nanoparticles by a natural polymer, chitosan. The study highlights the effectiveness of the encapsulation process in terms of mitigating the photocatalytic properties of the core TiO$_2$ nanoparticles as well as its effect on the optical properties of the resultant material. The applicability of the encapsulation process as an alternative to current commercial coating methods of sunscreen based TiO$_2$ is assessed.

Chapter 5 Focusses on the compatibility of potentially free-radical scavenging CeO$_2$ nanoparticles and commercial TiO$_2$ nanoparticles bound together through a chemical precipitation method. The effect of CeO$_2$ loading on the optical and photocatalytic properties of the core TiO$_2$ nanoparticles under both UV and solar-simulated light irradiance were assessed. In addition, the biological effects of the nanocomposite material, as compared to the pristine components, were assessed through cytotoxic and phototoxic assays performed using human keratinocyte (HaCaT) cells.

Chapter 6 An in-depth study on the development of TiO$_2$ nanoparticles and CeO$_2$/TiO$_2$ nanocomposites focussed on addressing specific criteria pertaining to certain materials parameters for sunscreen based TiO$_2$ is given. The study covers the initial optimization of synthesis parameters in producing TiO$_2$ nanoparticles of the rutile crystal phase. Followed by this, a comparative investigation of the optical and photocatalytic properties of
the rutile TiO$_2$, a CeO$_2$/TiO$_2$ nanocomposite prepared using the rutile TiO$_2$ and commercial TiO$_2$ nanoparticles is presented.

Chapter 7 Summarizes the outcomes of this thesis work and addresses the future work needed to be undertaken.
Chapter 2

Literature Review

2.1 UV and its Effects on Humans

Terrestrial solar light is a major source of incident UV radiation, particularly in the wavelength region of 290 - 400 nm which comprise the biologically relevant UVB and UVA wavelength bands. Exposure to UV radiation has long been linked to the generation of harmful cancers such as malignant melanoma. It has also lead to the development of consumer products designed to provide protection from these high energy wavelengths, as well as the promotion of health awareness campaigns to further aid in educating the general public and increase awareness of the risks of UV exposure. Small doses of UV radiation are still necessary for humans, particularly for the synthesis of vitamin D. Absorption of UVB radiation (around 300 nm) has been shown to stimulate the production of vitamin D firstly through the conversion of 7-dehydrocholesterol to previtamin D followed by isomerisation to vitamin D3 by the kidneys and liver. Additional reported benefits of UV exposure include treatment and prevention of certain skin and non-skin related diseases, such as atopic dermatitis, rickets and psoriasis, as well as increasing cutaneous melanin count, providing a very minimal amount of natural sun protection. However, more often than not, people are subjected to periods of exposure to terrestrial UV far exceeding what is required, thus leading to a variety of photo-induced skin related health issues and diseases. The major concern associated with UV exposure is its carcinogenic effect, however, a range of additional side effects are implicated such as im-
2.1 UV and its Effects on Humans

munosuppression, erythema (sunburn) and premature skin ageing. This section will give an overview of free radicals and ROS and will include an outline of their role in regular cellular metabolic processes as well as various diseases. In addition, an outline of the deleterious effects of UV exposure on biological tissues will be given as well as an outline of the mechanisms involved in these effects.

2.1.1 Free-radicals and the Human Body

A major factor in health-related issues associated with UV exposure to the body is the production of free radicals. Free radicals are molecular species containing one or more unpaired electrons in an atomic orbital. This means that, generally, free radicals are highly unstable, reactive and are capable of donating or accepting an electron, thus acting as both an oxidising or reducing agent. The abstraction of an electron from biomolecules results in the start of a series of chain reactions which, if left unchecked, can cause cellular damage. Some of the most important free radical species in biological systems are those derived from oxygen and include the following generated through oxygen or indirectly from oxygen through catalysis by a transition metal:

\[ \text{O}_2 + e^- \rightarrow \text{O}_2^\cdot^- \]  
(2.1)

\[ \text{O}_2 + 2e^- + 2\text{H}^+ \rightarrow \text{H}_2\text{O}_2 \]  
(2.2)

\[ 2\text{O}_2^\cdot^- + 2\text{H}^+ \rightarrow \text{H}_2\text{O}_2 + \text{O}_2 \]  
(2.3)

\[ \text{O}_2^\cdot^- + \text{H}_2\text{O}_2 \rightarrow \text{OH}^\cdot + \text{OH}^- + \text{O}_2 \]  
(2.4)

\[ \text{H}_2\text{O}_2 + \text{Fe}^{2+} \rightarrow \text{OH}^\cdot + \text{OH}^- + \text{Fe}^{3+} \]  
(2.5)

Of these three main ROS molecules, superoxide (\( \text{O}_2^\cdot^- \)), hydrogen peroxide (\( \text{H}_2\text{O}_2 \)) and hydroxyl radical (\( \text{OH}^\cdot \)), the hydroxyl radical is considered the most reactive and damag-
2.1 UV and its Effects on Humans

O$_2^•−$ has been shown to be mainly reductive in nature and is significant primarily as a source of hydrogen peroxide. Whilst H$_2$O$_2$ is an oxidising agent, in the absence of a metal catalyst it, as well as O$_2^•−$, are considered by some to be harmless when the body is under homeostatic conditions and can be scavenged efficiently by antioxidant enzymes present in cells such as superoxide dismutase (SOD). Table 2.1 highlights the various ROS, and reactive nitrogen (RNS), molecules that may be produced during cell metabolism including species that, as with H$_2$O$_2$, are classified as non-radical but can lead to the production of free radicals in living organisms.

Free radicals in biological systems and cells are important and are deliberately produced by certain cellular entities to play a role in a number of cellular functions including cellular electron signalling, mitogenesis and redox regulation. They are also heavily implicated in a number of physiological conditions and diseases when present at elevated levels, resulting in a state of oxidative stress. Oxidative stress can lead to damaging of the cellular membrane, proteins and even DNA which can contribute to, not only the ageing process, but other diseases including neurodegenerative, arthritic and cardiovascular diseases.

<table>
<thead>
<tr>
<th>Free Radical</th>
<th>Symbol</th>
<th>Half-life</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>ROS</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Radicals</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Superoxide</td>
<td>O$_2^•−$</td>
<td>10$^{-6}$ s</td>
</tr>
<tr>
<td>Hydroxyl</td>
<td>OH$^•$</td>
<td>10$^{-10}$ s</td>
</tr>
<tr>
<td>Alkoxy</td>
<td>RO$^•$</td>
<td>10$^{-6}$ s</td>
</tr>
<tr>
<td>Peroxyl</td>
<td>ROO$^•$</td>
<td>17 s</td>
</tr>
<tr>
<td>Non-radicals</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hydrogen peroxide</td>
<td>H$_2$O$_2$</td>
<td>Stable</td>
</tr>
</tbody>
</table>

Table 2.1: Biologically relevant ROS and RNS produced during cellular metabolism. Table reproduced from Phaniendra *et al.*, (2015). $^a$ Half-life dependent on the environmental medium. Half life units are in seconds (s) and minutes (min).
### Sources of Important ROS in Biological Systems

Free radicals are produced by cellular entities as part of the normal metabolic progression of cells and in response to certain external stimuli. The production of free radicals in biological systems generally arises through a chain-type reaction and can be self-propagating (Equations 2.1 - 2.5).

One of the most common free radicals generated is the ROS, $O_2^{•−}$. The main source of $O_2^{•−}$ is as an accidental by-product of the mitochondrial electron transport chain.
2.1 UV and its Effects on Humans

In this process, electrons from reduced nicotinamide adenine dinucleotide (NADH) are passed through a series of enzymatic electron donors and acceptors to convert molecular oxygen into water. This transfer of electrons creates a proton gradient across the membrane of the mitochondria and enables the production of adenosine triphosphate (ATP). The production of $O_2^{•−}$ occurs due to direct leakage of a single electron from the transport chain that reduces oxygen into a ROS (approximately 1 - 2 % incidence rate).$^{86,87}$ $O_2^{•−}$ also forms during autoxidation of haemoglobin, a process that can occur at physiological pH due to the higher redox potential of oxygen compared to iron, and is enhanced when in a state of hypoxia (oxygen deficiency).$^{88,89}$ If not adequately dismutated, $O_2^{•−}$ can serve as the starting point for other free radicals or cellular damaging species. For instance, under oxidative stress or certain pathological conditions, the intensification of haemoglobin autoxidation enables nitric oxide to react with $O_2^{•−}$ to produce ONOO$, a powerful oxidant that can initiate a cascade of ROS generation, leading to protein and DNA damage.$^{90,91}$

It has also been established that $O_2^{•−}$ is generated through the activation of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase by phagocytic immune cells when in the process of consuming and breaking down microbes.$^{92}$

Although not a free radical in itself, $H_2O_2$ is important as it acts as a generator for both radical and non-radical species. It is also permeable to cell membranes and can be significantly biologically damaging to cells, mainly acting as a precursor to harmful radicals such as OH$. It has long been known that $H_2O_2$ is produced as a by-product of oxygen metabolism, whereby, oxygen consumed by mitochondria is first converted to $O_2^{•−}$, then $H_2O_2$. Another major source of $H_2O_2$ is through autoxidation or redox cycling of various xenobiotics, as well as physiological compounds such as heme and flavoproteins.$^{94,95}$ This autoxidation process of flavoproteins also contributes to the production of $O_2^{•−}$. The reason for this due to the nature of the electron transfer within the flavoprotein and its various redox moieties. Thus, in the initial electron transfer step, if oxygen is present, a free electron can hop to it and form $O_2^{•−}$. At this stage, $O_2^{•−}$ may escape the newly formed flavosemiquinone and propagate further production of $O_2^{•−}$ molecules or, it can undergo spin inversion and form a peroxy adduct with flavoprotein, leading to the
eventual cleavage and release of $\text{H}_2\text{O}_2$.\textsuperscript{96,97}

The neutral $\text{OH}^\bullet$ radical is a highly reactive radical known to cause oxidative damage to both organic and inorganic biomolecules varying from proteins, lipids and even DNA.\textsuperscript{98,99} The primary mode of generation for $\text{OH}^\bullet$ is through the Fenton reaction (Equation 2.5) of $\text{H}_2\text{O}_2$, catalysed by metal ions such as $\text{Fe}^{2+}$ and $\text{Cu}^+$ bound in proteins such as ferritin and ceruloplasmin. The propagation of $\text{OH}^\bullet$ can also be further increased when cells are under oxidative stress, whereby, elevated levels of $\text{O}_2^\bullet^-$ enable the release of free metal ions from complexed proteins, allowing for more efficient catalysis of $\text{H}_2\text{O}_2$. Mitochondria are thought to be the prime region for $\text{OH}^\bullet$ production within cells due to the close proximity of precursor and catalyst molecules within the mitochondrial matrix.\textsuperscript{99} Thus under mitochondrial oxidative stress conditions, $\text{OH}^\bullet$ production is favoured and driven by the reduction of $\text{Fe}^{3+}$ by $\text{O}_2^\bullet^-$, leading to substantial cellular damage.

External stimuli also contribute to the generation of ROS (and RNS) in multicellular organisms. Ionizing radiation, such as X-ray and $\gamma$ radiation can cause extensive cellular damage due to the production of ROS. Although sufficient in energy to directly excite biomolecules, $\text{H}_2\text{O}$ being the major constituent of cells leads to the generation of ROS and an indirect mechanism for radiation damage.\textsuperscript{100} Irradiation of water with such high energy radiation can result in one of two events occurring. The water may be ionized to produce a free electron and charged water molecule, which can both interact with other water molecules or break down further to produce free radical species such as $\text{OH}^\bullet$ or the hydrogen radical, $\text{H}^\bullet$. Alternatively, irradiated water may undergo a process known as ly‐sis, in which the molecule is immediately broken into free radical components consisting of the $\text{OH}^\bullet$ and $\text{H}^\bullet$ species. Cosmic rays are a major source of such ionizing radiation, however, much of it is absorbed and scattered in the earth’s upper atmosphere before reaching the earth’s surface. Background radiation that is experienced at the surface of the planet is estimated to induce oxidative free radical damage on orders of magnitude less than that of the natural processes of aerobic cells thanks largely in part to the shielding of such cosmic rays.\textsuperscript{101,102}
2.1 UV and its Effects on Humans

Role of Important ROS in Biological Systems

At moderate to low concentrations, ROS can play a role in various physiological functions including cell signalling, the immune response, mitogenesis and redox regulation.\textsuperscript{81,82,103} H\textsubscript{2}O\textsubscript{2} is produced in all aerobic organisms as a by-product of normal cellular processes but it can also be produced in response to various stimuli including cytokines and growth factors.\textsuperscript{104–106} It can contribute to various biological signalling pathways such as stimulation of cell growth, differentiation and apoptosis.\textsuperscript{107–110} The response to H\textsubscript{2}O\textsubscript{2} can vary between different types of cells and its concentration. For instance, in mammalian cells the expression of different p53-regulated genes is reflected in the different levels of H\textsubscript{2}O\textsubscript{2} present within the cell. At low H\textsubscript{2}O\textsubscript{2} levels, antioxidants are produced so as mitigate further ROS production and prevent oxidative damage whilst at high levels of H\textsubscript{2}O\textsubscript{2}, pro-oxidants are produced to enhance oxidative damage and induce apoptosis.\textsuperscript{111} H\textsubscript{2}O\textsubscript{2} is also involved in the functioning of various transcription factor kinase and phosphatase type proteins. An example of this is the oxidation of the bacterial transcriptional activator, OxyR. Selective oxidation of the cysteine residues by H\textsubscript{2}O\textsubscript{2} of the protein enables the transcription of antioxidant genes, which aid in promoting cell growth and survival in response to elevated ROS levels.\textsuperscript{112} H\textsubscript{2}O\textsubscript{2} has also been shown to be involved in the activation of human T-cells and B-cells. It acts primarily as a redox modifier that enables oxidation of cysteine residues in important signalling molecules involved in the activation of these immune cells.\textsuperscript{113,114}

As mentioned prior, O\textsubscript{2}•− also plays a role in the immune response, particularly towards microbial pathogens. Electron transfer from membrane-bound NADPH oxidase proteins on phagocytic immune cells to molecular oxygen results in the generation of O\textsubscript{2}•−. O\textsubscript{2}•− then serves as the starting point for the generation of other ROS which can also aid in the immune response, provided the generation rate is tightly regulated (too much ROS may cause damage to surrounding tissues).\textsuperscript{115} These subsequent ROS include ONOO\textsuperscript{−} (through reaction with NO) and HOCl (through reaction with H\textsubscript{2}O\textsubscript{2} and Cl\textsuperscript{−} catalysed by myeloperoxidase), as well as non-ROS H\textsubscript{2}O\textsubscript{2} (through dismutation with SOD) which further contribute to ROS generation.\textsuperscript{116,117} Extracellular release of these ROS enable oxida-
tive degradation of incident bacterial and fungal pathogens. The secretion of $O_2^{•−}$ inside of phagolysosome formed during phagocytosis is also of importance as it aids in initiating the release of proteases that allow for the degradation of ingested pathogens.\textsuperscript{115,118}

**Regulation of Important ROS in Biological Systems**

Homeostasis of the intracellular free radical system is essential for proper cell functionality and survival. As such, cells are equipped with extensive antioxidant defences for regulating intracellular free radical levels. Examples of enzymatic antioxidant entities include SOD, peroxiredoxins (PRX), glutathione peroxidases (GPX) and catalase (CAT).\textsuperscript{29,119} Humans contain three variants of SOD, each with different metal-centres. These include copper/zinc (Cu/Zn)-SOD, located generally in the cytoplasm and extracellular space of cells and manganese (Mn)-SOD, generally occurring in the mitochondria.\textsuperscript{120} Some enzymatic antioxidants simply convert specific ROS from one form to another, as is the case with SOD’s and their role in the conversion of $O_2^{•−}$ to $H_2O_2$.\textsuperscript{121} As such, combinations of antioxidant enzymes work together to minimize the concentration of free ROS in cells and to maintain appropriate levels needed for proper cell functionality. Thus, $H_2O_2$ is subsequently removed from cells by CAT and/or GPX peroxidases by converting it to $H_2O$ and $O_2$.\textsuperscript{122} Antioxidant enzyme activity can also be regulated by modification of the protein post-synthesis. In this manner, concentration gradients of ROS can be established in selective/appropriate locations throughout the body to contribute towards biological signalling in response to certain cellular stimuli. An example of this is the action of PRX in removing peroxides and peroxynitrates. Reduction of these species requires an initial disulfide reduction of the antioxidant by thioredoxin before scavenging may occur.\textsuperscript{123}

Non-enzyme antioxidants include compounds such as vitamin C (ascorbic acid), vitamin E (which encompasses a variety of lipophilic molecules such as $α$-, $β$- and $γ$-tocopherol), uric acid and glutathione. Non-enzyme antioxidants tend to be indiscriminate in their activity, whereas, enzymatic antioxidants generally act specifically towards a particular free radical species.\textsuperscript{124} Furthermore, antioxidant vitamins cannot be produced by the
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Vitamins act as free-radical ‘chain-breakers’ as they generally cannot scavenge radicals such as OH• but instead work in close proximity to the cell membrane to mitigate lipid peroxidation. For instance, α-tocopherol is an efficient lipid peroxyl radical (LOO•) scavenger that intercepts and terminates lipid peroxidation chain reactions induced by ROS such as OH•. Uric acid is a potent antioxidant compound and the most abundant aqueous antioxidant found in human plasma. Although not a direct scavenger of O2•−, it can scavenge, carbon-centred radicals, peroxyl radicals (ROO•−) and peroxynitrate (ONOO−) in hydrophilic environments. When in the presence of ascorbic acid, it has also been shown to be important in preventing the uncoupling of nitric oxide synthases that help modulate blood pressure and regulate smooth muscle relaxation and vasodilation through the production of nitric oxide (NO).

Detrimental Effects of Important ROS in Biological Systems

Although the presence of ROS species is important in maintaining regular cellular functionality, when at appropriate concentrations, an excess of these species can induce a state of oxidative stress. This imbalance occurs when the rate of generation of ROS (or free-radicals in general) in a cell is outweighed by its capacity to remove them. In absence of adequate antioxidant defences, excess ROS can lead to oxidative damage of important cellular features including the membrane, organelles, lipids, proteins, the nucleus and DNA. These elevated levels of ROS in cells have been implicated in a variety of physiological and neurological diseases due to their deleterious effects.

As a result of the potential oxidative damage that may occur to DNA in cells, ROS induced oxidative stress has been suggested to be a cause for certain cancers. Oxidative damage to DNA can result in strand breaks, base pair lesions, DNA cross-linking and rearrangement of base pairs which in turn can lead to transcription errors, abnormal cell growth and the activation of oncogenes. Metastasis of cancer cells have also been suggested to be aided by ROS as they can regulate and activate relevant signalling pathways and transcription activities. For instance, certain mitogen-activated protein kinases (MAPK),
which can regulate cell growth, differentiation, mitosis and apoptosis, have been shown to be activated through oxidative processes by ROS without the need for accompanying ligands. Elevated levels of ROS and a state of oxidative stress have also been implicated in neurological diseases, including Alzheimer’s disease. Oxidative stress in the cells composing brain tissue is of major concern due to the abundance of lipids susceptible to oxidative damage and the lack of means for binding free metal ions which can catalyse ROS production, as compared to other tissues. Experimental evidence has also shown that the production of β-amyloid, a toxic peptide found at elevated levels in patients with Alzheimer’s disease, is reliant on oxidative action by ROS. Without an efficient antioxidant system, mitochondrial dysfunction in cells can result in the excessive release of ROS, oxidative stress and β-amyloid formation, contributing to the ageing process and neuron degeneration in diseases such as Alzheimer’s. Other neurological diseases for which ROS play a role include Parkinson’s disease, amyotrophic lateral sclerosis (ALS) and multiple sclerosis. There is also evidence to suggest that oxidative stress, and thus ROS, play an important role in the development of cardiovascular diseases such as hypertension, atherosclerosis and heart failure, kidney diseases such as renal failure and uremia as well as rheumatoid arthritis.

2.1.2 UV-induced Human Health Conditions

Extensive UV exposure has traditionally been associated with erythema (sunburn), but a number of physiological issues may arise in addition to this. These UV induced conditions are influenced by, not only the dosage of UV, but also the absorbing chromophore. ROS and the generation of ROS also play a significant role in these UV-induced health conditions, which include immunosupression, premature skin ageing and skin cancer.

Immunosuppression

Langerhan cells in the skin help regulate the immune response to skin-related diseases by communicating with both T and non-T cell lymphocytes. In combination with cytokine releasing keratinocytes and lymph nodes, the collective system is termed 'skin-associated lymphoid tissues'. Exposure to UV radiation, leading to subsequent DNA
damage (as DNA is an inherent chromophore for a broad range of UV wavelengths), has been suggested to induce immunosuppression by affecting these skin-associated lymphoid tissues at the sites of irradiation.\textsuperscript{142} The regular response of Langerhan cells to skin-associated diseases results in the secretion of cytokines interleukin (IL)-12 and IL-4. IL-12 promotes the differentiation of naive T-cells into T-helper type 1 (Th1) cells, which inhibit the production of T-helper 2 (Th2) cells and up-regulates IL-12 and interferon-\(\gamma\) (IN-\(\gamma\)) production.\textsuperscript{143,144} IN-\(\gamma\) further aids in regulating the immune response by down-regulating Th2 cell activity and activating macrophages. IL-4 operates to modulate and suppress the immune response towards foreign entities by promoting Th2 differentiation. Th2 cells in turn produce a variety of cytokines which suppress macrophage activity and activate a type of white blood cell called eosinophils.\textsuperscript{71} The combined activation of these factors leads to a down-regulation of Th1 cells and overall suppression of the Th1 cell mediated immune response.\textsuperscript{145} \textit{In vitro} and \textit{in vivo} investigations have shown that UV exposure can disrupt the immune response upon irradiation by impacting the ratio and activity of Th1 and Th2 cells.\textsuperscript{28,146,147} Simon \textit{et al}, (1990) showed functional inactivation of Th1 cells in C3H/HeN mice exposed to UVB (200 J/m\(^2\)/day) radiation by showing significant decreases in the production of IN-\(\gamma\) and IL-2 cytokines between irradiated/non-irradiated mice, whilst also showing minimal changes in Th2 relevant cytokines.\textsuperscript{28} Also through a mouse model, Elnazar \textit{et al}, (2015) demonstrated a suppression of IL-12 for specimens exposed to UVB and overall shifts in Th1/Th2 cell responses.\textsuperscript{148} Nishigori \textit{et al}, (1996) also showed suppression of T-cell mediated immune responses \textit{in vitro} using murine keratinocytes after exposure to UV radiation.\textsuperscript{149} It was suggested that unrepaired DNA damage caused by the irradiation process lead to the production of cytokines that down-regulate the immune response. UV-mediated immunosuppression has also been implicated as an indirect cause of skin cancer, with evidence of higher risks of incidence associated with patients undergoing immunosuppressive therapies.\textsuperscript{150,151} Photoperoxidation of polyunsaturated phospholipids in keratinocytes has also been implicated in UV-induced immunosuppression. The increased levels of ROS due to UVA exposure in these cells results in the production of platelet-activating factor (PAF)-like ligands that play a
role in the suppression of the immune system which, when produced in combination with UV exposure, help promote metastasis and tumor growth.\textsuperscript{152–154}

**Premature Skin Ageing**

Skin ageing is a natural-occurring process that can be influenced and accelerated by a number of factors which include genetics, hormonal changes, metabolic processes, time and environmental factors.\textsuperscript{155} Substantial experimental evidence has shown that premature skin ageing, or photoageing, is linked and strongly caused by cumulative exposure to terrestrial solar radiation.\textsuperscript{27} Collagen and elastin are the major components of the extracellular matrix which aid in binding tissues and providing structural and biochemical support for surrounding cells, particularly in the skin. Secretion of type-I procollagen (precursor compound to collagen) into the dermal extracellular tissue occurs in health skin where it undergoes a process called fibrillogenesis. In this process, the procollagen structure is rearranged to associate with other extracellular matrix proteins and to form collagen bundles, which give the skin its strength and elasticity.\textsuperscript{156} Studies have shown that specific exposure to UV radiation can induce damage to these bundles and other skin connective tissues, resulting in a loss of skin elasticity.\textsuperscript{155} The biological cause of this damage is believed to be linked to the photochemical generation of ROS, resulting in activation of certain cellular signalling pathways and the activation of certain endoproteinases.

UV absorbing chromophores endogenous to the human body include the NADH/NADPH cofactor, *trans*-urocanic acid and tryptophan. Energy transfer from these entities to molecular oxygen produces $\text{O}_2^{•−}$, which may be dismutated to $\text{H}_2\text{O}_2$ by SOD and subsequently be converted to $\text{OH}^{•}$ if in the presence of $\text{Fe}^{3+}$ or $\text{Cu}^{+}$.\textsuperscript{157} The increased production of $\text{O}_2^{•−}$ can also amplify MAPK signalling pathways primarily through the activator protein (AP)-1 effector.\textsuperscript{155} AP-1 is a transcription factor that regulates genes governing cellular growth and differentiation as well as regulate the activity of matrix metalloproteinases (MMP). These MMP’s are a group of endoproteases that, collectively, can degrade all manner of extracellular proteins and are generally produced by cells in an inactive form (zymogen). Upon UV exposure and the increased production of AP-1, MMP’s
become up-regulated and contribute to premature skin ageing through activation of MMP-1, MMP-3 and MMP-9 which collectively can degrade type I, II, IV fibrillar collagens and collagen fragments. The activation of these MMP’s has been shown to occur in vivo in human skin exposed to UV light and is consistent with the collagen breakdown observed after irradiation.\textsuperscript{158} Furthermore, the UV mediated activation of AP-1 further contributes to skin-ageing by inhibiting the production of new collagen by down-regulating the genes that encode for type I procollagen, thus furthering UV-induced skin damage.

The extent of UV-induced skin ageing and skin damage has been investigated in a few studies. A study of a Queensland population with individuals aged 20 to 55 years found that 72\% of young men and 47\% of women (aged between 20-29 years) displayed skin characteristics of moderate to severely photoaged skin.\textsuperscript{159} Another study demonstrated that Australian adults are much more susceptible to photoageing than European adults, owing to the higher intensity of incident UV radiation present in the subtropics.\textsuperscript{160, 161} This difference in UV incidence based on geographical location has been further demonstrated in a study by Fritschi \textit{et al}, (1995), whereby, 33\% of schoolchildren between the ages of 13 and 15 years in Scotland displayed signs of mild skin damage as compared to the 40-70\% rate of incidence for Queensland children.\textsuperscript{162}

\textbf{Skin Cancer}

Exposure to UV radiation has long been linked to the formation of non-melanoma and melanoma skin cancers, which are the most common forms of carcinomas that occur. A more detailed overview of the mechanisms behind UV-induced carcinogenesis is given in Section 2.1.3, but a brief introduction to the different skin cancers that may be induced by UV radiation is given here.

Squamous cell carcinoma (SCC) and basal cell carcinoma (BCC) are typically the most common forms of non-melanoma skin cancers and are highly prevalent in Australia. Both SCC and BCC may originate from stem or progenitor cells found in the stratum basale of the epidermis, the outer most layer of skin, whilst BCC can additionally arise from the bulge region of hair follicles.\textsuperscript{163, 164} SCC frequently occurs due to chronic UV exposure
and in people with already UV damaged skin. It is also usually preceded by other inflammatory skin responses and diseases such as Bowen’s disease and actinic keratosis.\textsuperscript{163} BCC on the other hand is associated with infrequent exposure to high UV intensities and generally does not produce signs of precancerous lesions as with SCC.\textsuperscript{165} BCC also accounts for approximately 80\% of non-melanoma skin cancer cases with SCC making up the rest.\textsuperscript{166} Although still potentially fatal (688 recorded deaths in Australia during 2018\textsuperscript{167}), these forms of cancer are considered much more treatable and manageable than the malignant melanoma counterpart.

According to the Australian Institute of Health and Welfare, 14,778 new cases reported as a malignant neoplasm of the skin (melanoma) were accounted for in Australia during 2018, of which, 1,684 cases were fatal.\textsuperscript{167} Melanoma accounted for approximately 10\% of all new cancer cases in Australia that year and approximately 3\% of all fatalities resulting from cancers (Figure 2.1). Malignant melanoma arises from epidermal melanocytes (Figure 2.3) and, although the exact processes for melanoma development are not clear, it has been suggested to occur due to mutations in the p16 thrombospondin (THBS) gene, which allows for the uncontrolled growth and proliferation of mutated, cancerous melanocytes.\textsuperscript{168}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure21.png}
\caption{(left) Incidence and mortality rates for Australians towards melanoma through the years 1982 - 2018. 2019 - 2021 are projected estimates. (right) Comparison of the number of incidences and mortalities associated with the most common cancers in Australia during 2018. Data obtained from the Australian Institute of Health and Welfare, Australian Government.\textsuperscript{167} *ASR corresponds to the age-standardised rates per 100,000 people.}
\end{figure}
The incidence of skin cancers are also inherently linked to a person's phenotype and susceptibility to UV damage. Light skinned individuals with freckles, light coloured eyes and an inability to tan are at greater risk of skin cancer incidence.\textsuperscript{169} Men are also much more likely to develop BCC or SCC cancers than women, which may be attributable to increased exposure to UV radiation during outdoor leisure activities.\textsuperscript{166} Inherited diseases are also linked with increased prevalence of skin cancers. Xeroderma pigmentosum (colloquially known as vampire syndrome) is an inheritable genetic disease that affects the ability for skin cells to repair UV damaged DNA. As a result, individuals with this condition are highly susceptibility to all forms of skin cancers brought about by UV exposure.

\subsection*{2.1.3 Human Skin Exposure to UV Radiation and DNA Damage}

Extreme UV radiation (10 nm \( \leq \lambda \leq \) 120 nm) is sometimes classified as a type of ionizing radiation, capable of stripping atoms and molecules from biological tissues and altering the course of chemical reactions in the body.\textsuperscript{170} Terrestrial UV radiation, typically in the wavelength range of 290 - 400 nm, is also capable of inducing biological changes and mutations. The natural source of UV radiation provided by sunlight means people are exposed to UV on a daily basis. The harmful effects associated with UV radiation are strongly dependent on the length of exposure, the susceptibility of individuals and the wavelengths of the incident UV radiation. Many organisms, humans included, contain UV-absorbing pigments to act as a first line of defence, however this type of radiation is still capable of penetrating through superficial tissue and reaching DNA.\textsuperscript{171–173}

The major factors involved in the carcinogenic effect of UV radiation include generation of mutations in key proto-oncogenes and tumour suppressor genes which help regulate apoptosis, DNA repair and cell division/arrest.\textsuperscript{71,174} The mechanisms behind the carcinogenic effects of extended UV exposure vary between UVA and UVB wavelengths and are inherently linked to their fundamental photon energies and permeation capabilities.
Human Skin Response to UV Exposure

Upon exposure to UV radiation, the human body aims to protect the cells found in the hypodermis, the layer just below the dermis, through the stimulation of melanocytes in a process termed melanogenesis. Melanocytes are a type of cell found in the stratum basale of the epidermis and comprise between 1 - 2% of epidermal cells. The primary function of melanocytes is to produce the pigment melanin, a natural absorber of UV radiation (Figure 2.3 (top-right)) and also the major determinant of hair, skin and eye colour. Different types of melanin exist in the form of the brown/black eumelanin and the red/yellow pheomelanin and are produced and stored in melanosomes, organelles found in melanocytes. Upon UV exposure, particularly UVB radiation, stimulation of the p53 gene occurs as a result of DNA damage which in turn stimulates the production of p53 tumour suppressor protein (TSP). This protein is important in the prevention of carcinomas as it helps activate DNA repair mechanisms whilst also stimulating other transcription factors that can mitigate the spread of damaged/altered DNA through a delay in cell cycle or induction of apoptosis. p53 TSP is also important in the production of melanin from melanocytes. It stimulates cleavage of proopiomelanocortin, a precursor compound produced in the pituitary but can also be found in melanocytes and keratinocytes. Cleavage
of this precursor results in the synthesis and secretion of α-melanocyte stimulating hormone (α-MSH) which acts upon melanocortin receptors, the most important of which is the melanocortin-1 receptor (MC1R).\textsuperscript{178,179} Notably, variances in the MC1R gene is often associated with increased risk of SCC, BCC and melanoma skin cancers as mutation of this gene has been consistently found in people with these diseases.\textsuperscript{180}

**Figure 2.3:** (left) The molecular and biological steps involved in response to UV exposure. (top-right) Absorption spectra of eumelanin (dashed line) and pheomelanin (solid line) along with (bottom-right) corresponding chemical structures. Figures reproduced from Garibyan et al, (2010)\textsuperscript{71} and Tran et al, (2006),\textsuperscript{181} respectively.

Activation of extracellular MC1R leads to elevated levels of cyclic adenosine monophosphate (cAMP), an important intracellular secondary messenger that increases transcription of microphthalmia-associated transcription factor (MITF) in melanocytes.\textsuperscript{71} From this, initiation of melanin synthesis from tyrosine occurs with the subsequent pigments being stored in melanosomes. Melanosomes containing melanin are exported to keratinocytes via pseudopodia, temporary projections of the melanocyte cell membrane that may be engulfed by adjacent keratinocytes. Differentiation in skin pigmentation arises due to differences in the number, size, composition and distribution of these melanosomes in keratinocytes,\textsuperscript{176} not the melanocyte number. These melanosomes are then positioned over the nuclei in keratinocytes to aid in UV protection (nuclear ‘capping’) and prevent further nucleic DNA damage. The increased activity of these melanocytes upon UV exposure, both UVA and UVB, and increase in pigmentation is actually a delayed tanning
response. The immediate pigment darkening response occurs within seconds upon UV exposure and results from the redistribution of melanin moieties already present in the skin. This is then followed by the increased activity of melanocytes and the production of melanosomes, resulting in delayed tanning. Thus in response to UV exposure, the ideal biological result involves repair of any damaged DNA before DNA synthesis and mitosis may occur or controlled cell death limiting the spread of mutated genes. In addition, an increase in melanin levels in keratinocytes to further mitigate cellular and nucleic damage.

**Direct Carcinogenesis from UVB Exposure**

UVB exposure exceeding a certain threshold dosage induces a cascade of cellular mediator responses such as the release of cytokines and vasoactive/neuroactive mediators. The release of these mediators results in an inflammatory response observed in skin known as erythema or, more commonly termed, 'sunburn'. Thus, UVB exposure is often associated as being the wavelength band responsible for sunburn (Figure 2.4). Although present in lower abundance as compared to UVA radiation, UVB radiation is also most commonly associated with photocarcinogenesis and can instigate this response at much lower doses as compared to UVA radiation. This is owing to the fact that DNA, as well as RNA, are natural chromophores of UVB radiation, with maximum absorbance centering around 260 nm. The primary route for photo-induced damage occurs through the absorption of UVB by pyrimidine derived nucleobases, which comprise a component of the nucleotides making up DNA. These nitrogenous bases, namely thymine (T) and cytosine (C), undergo photochemical reactions upon UVB excitation to form a series of photoproduct adducts between adjacent pyrimidine sites. One class of photoproduct produced are the cis/trans cyclobutane pyrimidine dimers (CPD) formed through the [2+2] cyclo-addition of adjacent pyrimidine bases, and typically occur between thymine residues (TT). These cyclic products introduce conformational, replication and transcription issues in DNA and have the potential for mutagenesis but are often repaired through natural cellular repair mechanisms. Light absorption at wavelengths greater than 300 nm by photoreactivating enzymes help facilitate the reversal of
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this process, restoring the DNA to its normal conformation. There is also significant evidence suggesting these CPD’s, particularly those formed in keratinocytes and Langerhan cells, can have immunomodulatory effects, such as immunosuppression, in addition to carcinogenicity. CPD’s may also be formed between CT and CC residues but occur less frequently than those formed between TT. However, these CPD’s are considered to be highly mutagenic as a result of the presence of the cytosine base, which causes CPD’s formed to be highly unstable and undergo deamination to form uracil (U). The presence of these uracil containing CPD’s causes further issues during DNA transcription and replication and impede the action of enzymatic DNA polymerase from repairing the damaged strand. Subsequent templating and replication of these damaged strands leads to mutation of the daughter DNA molecules produced. Thus, mutations through this dimerisation route consist primarily of C→T and CC→TT transitions and are, in fact, found in 90% of SCC cases and mutations of the p53 TSP gene.

Another form of lesion produced by UVB irradiation that may occur is the pyrimidine (6-4) pyrimidone photoproduct (6-4PP). In this instance, a single covalent bond is formed between adjacent residues at the C6 and C4 carbon positions of each base ring (Figure 2.5). It has been estimated that the relative amounts of CPD formed upon UVB/UVA exposure compared to 6-4PP is 3:1, hence the higher mutagenicity and carcinogenicity associated with CPD’s in mammalian cells. It is also believed 6-4PP’s are corrected (excised from the genome) more efficiently than CPD’s, leading to fewer muta-

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**Figure 2.4:** UV effectiveness spectra highlighting wavelengths responsible for erythema (sunburn), ROS generation and immuno-suppression. Figure reproduced from Osterwalder et al, (2013).
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It is also known that 6-4PP can inter-convert to their Dewar valence isomers upon UV excitation at wavelengths around 325 nm. These Dewar isomers distort DNA and induce DNA bending, similar to that of their parent 6-4PP, but to a lesser extent. They have also been suggested to be less mutagenic than their 6-4PP counterparts but are capable of inducing a broader range of mutations. As with the 6-4PP lesions, Dewar isomers are believed to be efficiently repaired through the nucleotide excision repair (NER) pathway due to more easy recognition by repair proteins sensitive to significant structural changes in DNA, in contrast to CPD lesions. However, mutations due to UV exposure in the p53 TSP genes that govern these repair pathways, such as NER, can inhibit the recovery of DNA and dysregulate apoptosis. Thus, uncontrolled cell growth of cancerous cells may occur, an effect which is seen in the mitosis of affected keratinocytes and the growth of skin cancers.

**Figure 2.5:** Chemical structure of the main photoproducts formed by UVB-induced photoreaction of thymine (T) residues in DNA. Figure reproduced from Cadet et al, (2005).

### Indirect Carcinogenesis from UVA Exposure

For a long time, the harmful and carcinogenic effects of UV radiation were primarily attributed to the UVB wavelength range. However, it is now known that UVA radiation can also damage DNA, as well as RNA, indirectly through the production of ROS. The depth of penetration of UVA radiation is also greater than that of UVB, in that, the so-called ‘fingerprint’ mutations for UVA are found predominantly in the stratum basale of the epidermis, whilst those for UVB are found mainly in the stratum granulosum. This means that ROS generated by UVA radiation are in closer proximity to a wider variety of cell types, lipids and extracellular components, thus having the potential to exert greater
oxidative damage to the body than UVB radiation.

Photosensitization of DNA by UVA occurs through indirect oxidative damage. DNA is a poor chromophore for UVA radiation, however, UVA may trigger the generation of ROS, including $O_2^{•−}$ and $OH^{•}$, through intermediate photosensitizers. Some of the important photosensitizer compounds present in human skin include porphyrins (uroporphyrins, coproporphyrins and protoporphyrin IX), melanin and melanin precursors, B$_6$ vitamers (pyridoxal), vitamin K, \textit{trans}-urocanic acid and tryptophan.$^{211}$ Absorption of UVA radiation by these photosensitizers results in elevation in the electronic energy state of the absorbing molecule to an excited singlet state.$^{211}$ Following excitation, the excited molecule may relax back to ground state, through irradiative emission of the absorbed energy or through heat dissipation, or undergo intersystem crossing and transition to a reactive triplet energy state. In this triplet state, the excited molecule can again relax back to the ground state through light emission or partake in photochemical reactions to transfer the excess absorbed energy to surrounding molecules. In this manner, damage to DNA bases can occur directly from the photosensitizer (type I photosensitization) or ROS may be formed through interaction of the photosensitizer with molecular oxygen (type II photosensitization). In type II photosensitization, $^1O_2$ is formed by direct energy transfer from an excited triplet state chromophore to a ground level triplet state oxygen molecule. $O_2^{•−}$ may subsequently be formed by electron injection from another excited chromophore, which also results in the formation of a radical cation of the photosensitizer. With $O_2^{•−}$ present, additional ROS such as lipid peroxides may be formed, as well as $H_2O_2$ following enzymatic dismutation, thus elevating the levels of ROS present in cells.$^{212}$ In addition, if the photosensitizers are positioned in relatively close proximity to DNA, oxidative DNA damage may occur upon UVA exposure.$^{211}$
Generation of these ROS has been demonstrated to enable indirect UVA-induced DNA damage by causing single strand breaks and DNA cross-linking as well as oxidative damage to pyrimidines and purines in mammalian cells. 213, 214 The most common DNA lesions produced by UVA mediated ROS damage is 8-oxoguanine, the photoproduct of oxidized guanine residues, and TT site CPD’s. 215, 216 The formation of TT, CT and TC CPD’s have been detected in mammalian cells, including human skin cells, exposed to UVA radiation but predominantly occur at TT sites, similar to UVB induced CPD’s. 216 The rate of incidence of these CPD’s is also significantly lower than that induced by UVB or UVC irradiance. 6-4PP’s have not been detected in humans exposed to UVA radiation but it has been shown that UVA radiation may photoisomerize 6-4PP’s formed by UVB to Dewar isomers. 216, 217 Thus, the main biomarkers for indirect UVA-induced DNA damage are the generation of 8-oxoguanine, TT CPD’s and Dewar isomers.

2.2 Protection from UV Radiation: Sunscreens

The biologically harmful effects of UV radiation bring to light the need for adequate methodologies for protection. Calculating the protective effect of melanin in even the
most dark-skinned individuals through minimal erythemal dosage has shown only 10 - 15 fold increases compared to an absence of melanin, suggesting relatively low levels of protection.\textsuperscript{218} The most efficient means of protection is non-exposure, however, outdoor leisure and social activities have become a societal norm, rendering such a measure infeasible. The next appropriate measure is minimisation of exposure and wearing of appropriate attire but, again, societal pressures, whether due to the latest fashion trends or leisure activities generally correlates to high levels of skin exposure on a daily basis. This, along with the increased levels in ambient UV radiation due to changes in stratospheric ozone levels, coincides with the increase in melanoma incidence observed over the years. As such, cosmetic and therapeutic products have been developed to aid in combating the deleterious effects of UV radiation and to combat the incidence of skin cancers. These products, termed, sunscreens, contain ingredients capable of preventing the transmittance of terrestrial UV from reaching the skin. In this Section, an overview of the historical developments and regulation of sunscreen products and ingredients is described. Furthermore an explanation of the protective effect provided by these products and the types of ingredients used is given.

\subsection{2.2.1 Historical Developments}

The application of ingredients and formulations used specifically for protecting the skin dates as far back as the Ancient Egyptian period (3100 BC - 330 BC).\textsuperscript{34} The discovery of preserved papyri and paintings in tombs have revealed the identity of these ingredients which include various oils and mineral clays frequently applied to the skin to maintain a fair complexion and minimize skin damage. A number of these ingredients even include compounds that are used in modern cosmetic products such as red ochre (iron oxide) and henna oil (lawsone).\textsuperscript{219,220} The first developed sunscreen product released for commercial purchase was in the United States in 1928 and consisted of a formulation with two active UV filtering ingredients, benzyl salicylate and benzyl cinnamate.\textsuperscript{221} Further advances in sunscreen technology and ingredients led to the development of red petrolatum during World War II, which contained a mixture of both organic compounds and inorganic
2.2 Protection from UV Radiation: Sunscreens

Particles capable of protecting against UV.\textsuperscript{35,219} During the 1940s, the first patented UV filter, specifically \textit{para}-aminobenzoic acid (PABA), was registered, whilst patenting and commercialisation of formulations containing the inorganic compounds, TiO\textsubscript{2} and ZnO, did not occur until the late 1980s and early 1990s.\textsuperscript{222} With the continued development of new sunscreen actives and an increased understanding of photobiology, a need for a standardized method for assessing the effectiveness of these filters was required. This led to the eventual introduction of the sun protection factor (SPF) rating, still used today, in indicating to consumers the level of protection afforded against UV (or more specifically UVB) radiation by the given formulation.\textsuperscript{223,224}

2.2.2 Regulation of Sunscreen Products in Australia

Before commercialization, a sunscreen product goes through a rigorous review process which requires adherence to specific product and ingredient guidelines. In Australia, ingredients listed in sunscreen products are regulated by the Therapeutic Goods Administration (TGA) and require registration in the Australian Register of Therapeutic Goods (ARTG).\textsuperscript{42} These include ingredients present only in therapeutic sunscreens, not cosmetic sunscreens. Therapeutic sunscreens refer to all primary sunscreen products designed for UV protection with SPF ratings 4 or greater and secondary sunscreens such as insect repellants and moisturisers with SPF values of 4 and 15, respectively. Cosmetic sunscreens on the other hand refer to cosmetic products that contain ingredients with UV protective capabilities but are not marketed specifically for UV protection.\textsuperscript{66} Such cosmetic sunscreens are instead regulated by the National Industrial Chemicals Notification & Assessment Scheme (NICNAS) and the associated Cosmetics Standard and NICNAS Cosmetics Guidelines. The major focus of the TGA standards for sunscreen ingredients is on their safety. Prior to registration of a new UV filtering ingredient, various \textit{in vitro} and \textit{in vivo} toxicological information must be provided that adequately demonstrates that no or limited toxicological potential is exerted by the ingredient. This includes data pertaining to acute toxicity, local tolerance, allergenicity, genotoxicity, reproductive toxicity and carcinogenicity. The guidelines used for registering such new ingredients in Australia
have been adopted from EU ‘non-clinical’ guidelines by the TGA, despite the differences in classification of primary sunscreens (therapeutic in Australia as opposed to cosmetic in EU). This gives certain ingredients and manufacturers leeway in the data that is required for approval, provided the absence of said data is justified. For example, a lack of long term *in vivo* carcinogenicity data for a potential new ingredient may be allowed provided it can be shown the ingredient displays a lack of *in vivo* dermal absorption or low persistence in the skin.\(^66\) Data pertaining to the actual UV protective abilities of the filter must also be available in the form of its UV spectral characteristics and specific level of UVB and UVA protection.

In addition to TGA guidelines, therapeutic sunscreen products must abide by the Australian/New Zealand *Standard AS/NZS 2604:2012 Sunscreen products - Evaluation and classification*.\(^225\) The main purpose of this standard is to provide sunscreen manufacturers specific guidance on the measurement of the SPF and broad spectrum protection afforded by their products. The specific methodologies employed for determining these quantities refer to the International Standards:

- ISO 24443 Determination of sunscreen UVA photoprotection *in vitro*
- ISO 24444 Cosmetics - Sun protection test methods - *In vivo* determination of the sun protection factor (SPF)

For simplicity, all mentioning of sunscreens, sunscreen products and the ingredients used here onwards refers to therapeutic sunscreens unless stated otherwise.

### 2.2.3 Sun Protection Factor (SPF) and UVA Protection Ratings

A number of ingredients constitute the composition of a sunscreen formulation, contributing to formulation factors such as emulsion stability, viscosity and shelf-life. The specific ingredients included to protect users from UV radiation are listed as the ‘active’ ingredients of a particular formulation. These ‘active’ ingredients protect the skin through modes of absorption, reflection and/or scattering of incident UV radiation. The level of protection provided by these products is quoted as the sun protection factor (SPF), which
is defined as the minimal erythemal dose (MED) of UV radiation required to produce sunburn as a ratio of protected to unprotected skin, thus, is primarily an indicator of UVB radiation protection. Different sunscreen formulations and quantities of active ingredients lead to different SPF ratings. Theoretically, a sunscreen product labelled with SPF 30 implies that, with proper application, the user may remain exposed for thirty times as long as without protection before an observable sunburn is seen (Figure 2.7). In practice however, it is often the case that users do not apply the recommended amount of sunscreen (2 mg cm$^{-2}$). Both \textit{in vitro} and \textit{in vivo} experiments have shown that reducing the amount of sunscreen formulation applied by half can decrease the SPF effectiveness of the formulation by up to half that labelled. As such, the SPF shouldn’t be considered as an absolute measure of sun protection over a period of time and a justification for prolonged sun exposure but instead as a means of ranking the relative protection of different formulations.

![Figure 2.7](image)

\textbf{Figure 2.7}: (\textit{left}) Sunburning (MED) dose for a person susceptible to the dose within 10 minutes in absence of sunscreen and the affects of different SPF value sunscreens on this timeframe. (\textit{right}) Bar graph representation of the endpoints shown in (\textit{left}) for different SPF values. Figure reproduced from the Australian/New Zealand Standard for Sunscreen products, (2012).

With the increased understanding of the carcinogenic effects of UVA exposure, sunscreen products may also display a rating or statement indicating its protection across this wavelength region. In Australia, sunscreen products may be labelled as ‘broad’ spectrum protecting, provided they meet the legislative requirements. Ingredients listed with an SPF of 30 or higher are required to provide broad spectrum protection (Table 2.2), whilst those with an SPF less than 30 are not, as according to the TGA. However, the AS/NZS
2.2 Protection from UV Radiation: Sunscreens

AS/NZS 2604:2012 standard stipulates that sunscreen products with SPF 4+ must have broad spectrum protection. The specification of being broad spectrum requires that the product or ingredient meets two criteria:

- That the UVA protection factor (UVAPF) is equal to or greater than one-third the labelled SPF
- That the critical wavelength is equal to or greater than 370 nm.

Interestingly, the methodology for determining the UVAPF outlined in this Australian standard refers to the international standard, ISO24443:2012, which is an in vitro method, contrary to the in vivo method used for SPF. As such, instead of human substrates and evaluation of pigmentation darkening, the method uses roughened substrates and measures the transmittance of UV through a thin layer of an applied formulation. The critical wavelength is defined as the wavelength at which 90% of the cumulative area under the total absorbance curve between 290 and 400 nm occurs. Setting the limitation for broad spectrum protection to products with critical wavelengths equal to or greater than 370 nm infers that equal to or greater UVA protection is afforded by the product as compared to UVB.

Table 2.2: Different SPF categories and classifications and the labelling permitted for such sunscreen formulations according to the AS/NZS 2604:2012 standard.

<table>
<thead>
<tr>
<th>SPF</th>
<th>Labelled SPF</th>
<th>Category description</th>
<th>Broad spectrum labelling</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Primary</td>
</tr>
<tr>
<td>1-3</td>
<td>Not allowed</td>
<td>Not allowed</td>
<td>Not allowed</td>
</tr>
<tr>
<td>4-14</td>
<td>4, 6, 8, 10</td>
<td>Low</td>
<td>Compulsory</td>
</tr>
<tr>
<td>15-29</td>
<td>15, 20, 25</td>
<td>Medium or moderate</td>
<td>Compulsory</td>
</tr>
<tr>
<td>30-59</td>
<td>30, 40, 50</td>
<td>High</td>
<td>Compulsory</td>
</tr>
<tr>
<td>60 or higher</td>
<td>50+</td>
<td>Very high</td>
<td>Compulsory</td>
</tr>
</tbody>
</table>
### 2.2.4 Organic and Inorganic UV Filters

The active UV filtering ingredients in sunscreen products may be broadly categorized as organic or inorganic. Table B.1 lists UV filters approved by the TGA for use in Australia and their maximum loading amount. Initially, the mechanism of protection through this classification system was generalized such that organic filters protected through means of UV absorption whilst inorganic filters protected through processes of scattering and reflection. However, advances in UV filtering materials have led to the development of novel organic and inorganic materials capable of providing protection opposite to that of the classical means or even a combination of the two.

**Organic Filters**

The main mode of protection from UV radiation by organic UV filters is through absorption. These chemical filters generally consist of organic compounds belonging to one of several groups shown in Figure 2.8 and can be subdivided as either UVA (benzophenone, anthranilates and dibenzoylmethanes) or UVB (PABA derivatives, salicylates, cinnamates and camphor derivatives) absorbers. Absorption of particular wavelengths by organic molecules can occur when the incident photon energy is sufficient to excite an electron from its highest occupied molecular orbital (HOMO) to the lowest unoccupied molecular orbital (LUMO). Relaxation from the excited state may occur through a number of pathways. For instance, in the singlet state, de-excitation may occur through non-radiative vibrational relaxation, internal conversion or radiative relaxation through emission of a photon in a process known as fluorescence. Intersystem crossing may also occur, in which the excited electron's spin is no longer paired with the ground state, leading to the formation of a triplet state. De-excitation of this triplet state can similarly occur through internal conversion and also through the emission of a photon which, in this instance, is termed phosphorescence due to the longer lived state relative to fluorescence.
2.2 Protection from UV Radiation: Sunscreens

This absorption and de-excitation mechanism is the basis for how, classically, organic UV filters provide protection from incident UV when applied to the skin, although dissipation of the absorbed energy by re-emission of a photon is not ideal for sunscreen actives. Another relaxation pathway available to both singlet and triplet state excitations is through photochemical reaction and is one of the major disadvantages associated with organic UV filters, as it can result in a loss of UV filtering functionality and lead to the formation of unwanted by-products and accidental photochemical reactions. One important example of this instability is with avobenzone (butyl methoxydibenzoylmethane), a commercially important UVA filter with an absorption maximum at 357 nm. Exposure to UV can cause fragmentation of the avobenzone molecule into free radical species which, in turn, can cause further damage to other active UV filtering ingredients and a loss of protection when topically applied. In addition to the stability issues associated with organic UV
filters, substantial concern has been raised over the penetration of organic filters into epidermal cells and their absorption into fatty tissues.\textsuperscript{64,230,231} The detection of metabolites produced from these filters in urine and breast milk samples after topical application has also been a topic of much debate due to the potential consequences of their metabolism, such as endocrine disruption and estrogen mimetic activity.\textsuperscript{232–234} One countermeasure being researched is the development of so-called Dalton-500 molecules. These compounds are designed to possess very high molecular weights, so as to minimise dermal permeation, and possess multiple chromophoric moieties, enabling broad-spectrum UV protection.\textsuperscript{235} Encapsulation of organic UV filters is another protective pathway being explored using biocompatible polymers. Polylactic acid (PLA) is one such example, having already been established in drug delivery and has been shown to improve the photostability of organic UV filters such as octinoxate, avobenzone and octocrylene.\textsuperscript{236,237} Poly(methyl methacrylate) (PMMA), chitosan, ethyl cellulose and a variety of co-block polymers such as poly(lactic-co-glycolic acid) (PLGA) have also been investigated for improving the photostability of organic UV filters and preventing/minimising their permeation when applied.\textsuperscript{238–241}

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{avobenzone_fragmentation}
\caption{Fragmentation of avobenzone upon UV exposure, leading to a loss UV absorptive functionality and production of two reactive species.}
\end{figure}

Another concern often associated with organic UV filters is their propensity to induce allergic and photoallergic reactions when topically applied. A number of specific filters have been reported to cause such adverse effects, usually some time after their introduction into the sunscreen market, and has led to the complete removal of some filters by governing health organisations in various countries.\textsuperscript{242}
Inorganic Filters

The mineral compounds, TiO$_2$ and ZnO, are the only certified inorganic UV filters listed in the ARTG and find use in a range of additional cosmetic products such as foundations and eye shadows.$^{243}$ Initially introduced as microfine particles (primary particle size $>$0.2-0.5 $\mu$m), these active ingredients provided protection by means of scattering and reflecting incident UV radiation. However, due to the size of such particles, scattering of wavelengths across the visible light region (400 - 700 nm) lead to formulations appearing opaque and often left a white-residue even when rubbed in.$^{244}$ To improve the cosmetic aesthetics of such mineral containing formulations, modern formulations now often contain ultrafine variants of these UV filters (primary particle size $<$100 nm), leading to enhanced absorption across the UVB and UVA wavelengths regions and increased transparency in the visible light wavelength band (Figure 2.10).

![Figure 2.10: UV-Vis absorption properties of microfine (200 - 500 nm) (x) and ultrafine (<100 nm) (y) particles of (left) TiO$_2$ and (right) ZnO. Figure reproduced from Dransfield, (2000).$^{43}$](image)

TiO$_2$ is the main oxide of titanium and can occur naturally as either the anatase or rutile polymorphs or as brookite if at high pressure. Aside from its application in sunscreen products, TiO$_2$ has also been utilized in commercial applications such as self-cleaning surfaces, food additives, anti-bacterial agents and as a pigment in paints, paper textiles and inks.$^{245-249}$ TiO$_2$, in its various crystal forms, has also been researched extensively for use in supercapacitors, battery materials, water purification and splitting, sensors and photocatalysis owing to its unique electronic structure and versatile functional perfor-
Of the three main crystal forms, the rutile phase is considered to be the most thermodynamically stable in the bulk, whilst the anatase phase is metastable and brookite considered unstable. At the nanoscale, the thermostability is flipped, in that, the anatase phase becomes the predominately more thermodynamically stable phase. Although each crystal form comprises of TiO$_6$ octahedra, their orientation in space is what differentiates them. The anatase and rutile crystal phases both belong to the tetragonal crystal system whilst brookite belongs to the orthorhombic crystal system. Furthermore, these TiO$_6$ octahedra are slightly distorted from regular octahedral coordination. There is further still a difference in this distortion between these crystal phases which leads to differences in certain physical and chemical properties. For instance, due to the slightly more compact structure of the rutile phase as compared to anatase, it has a higher refractive index, density and greater chemical stability than anatase.

TiO$_2$ found in sunscreen formulations will often consist of the rutile crystal phase and, to a lesser extent, a mixture of the anatase and rutile phases. TiO$_2$ nanoparticles used in sunscreen products primarily act as UVB absorbers. In Australia, the maximum allowed amount of TiO$_2$ applicable in formulations is 25 \% (w/w), as according to the

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**Figure 2.11:** The crystal structures for the different polymorphs of TiO$_2$ including the (top-left) anatase, (top-right) rutile, (bottom-left) brookite and (bottom-right) TiO$_2$(B) forms. Figure reproduced from Ma et al, (2014).
TGA, and the crystal phase composition is not as well regulated despite the significantly different properties that surmount. Furthermore, sunscreen manufacturers in Australia are not required to display labels on their products stating whether the product contains nanoparticulate TiO$_2$ (or ZnO). Other global governing institutions do regulate the crystal composition of sunscreen based TiO$_2$. For instance, the SCCS of the EU strictly regulates the composition of TiO$_2$, in particular nano-TiO$_2$, as well as the composition of any surface coatings that are applied. Under EU regulation, manufacturers are also required to label cosmetic products containing nanoparticles and must provide details regarding the names of the chemicals involved, their size, physicochemical properties and toxicity.\textsuperscript{258,259} The key parameters outlined by the SCCS for the safe use of nanoparticulate TiO$_2$ in sunscreen formulations include:\textsuperscript{67}

- having a TiO$_2$ purity of $\geq 99\%$ or lesser purity if the impurities have be demonstrated for safe use in cosmetic formulations
- being composed of the rutile phase with up to a maximum of 5\% anatase allowed
- having a mean article size between 30 to 100 nm, demonstrated through different particle measurement methodologies eg transmission electron microscopy and dynamic light scattering
- having an aspect ratio from 1.0 and up to 4.5 such that the particles are primarily spherical in morphology with some elongation allowed
- being coated with a photostable and formulation stable coating material
- not having photocatalytic activity or up to 10\% of the activity of a non-coated or non-doped reference material

Coating materials used for both TiO$_2$ and ZnO nanoparticles are often based upon aluminium and silicon stearates, oxides and hydroxides and are utilized for addressing suspension stability issues and the inherent photocatalytic activity of these semiconductor materials. It is important to note also that these guidelines are for TiO$_2$ nanoparticles used in sunscreen creams, not sprayable products. Further discussion of the photocatalytic
activity and coating materials of inorganic UV filters is given in Section 2.4.

Figure 2.12: SEM and TEM images of commercial sunscreens containing the inorganic UV filters, TiO$_2$ and ZnO. a) and b) corresponds to TiO$_2$ nanoparticles whilst d) and e) are of ZnO. c) is an example of a blank sample and f) a mixture of both TiO$_2$ and ZnO. Figure reproduced from Lewicka et al, (2011).260

ZnO, as with TiO$_2$, also finds use in a wide variety of applications outside of its use as an inorganic UV filter. Owing to its light absorption properties and photocatalytic activity, it shares a number of applications with TiO$_2$ such as in hydrogen production, sensor devices and battery materials. It has also been studied for use in various optoelectronic and laser technology devices.261–264 The main crystal forms of ZnO are wurtzite, zinc-blende and rocksalt.262 At room temperature, the thermodynamically stable phase is the wurtzite phase whilst zinc-blende can be stabilized through specialized growth substrates and rocksalt obtained at relatively high pressures. The main ZnO crystal phase employed in sunscreen products is the wurtzite phase and is primarily a UVA absorber. Due to the limitations in the wavelength ranges covered, quite often both ZnO and TiO$_2$ will be used in combination with each or other organic UV filters so as to provide broad spectrum coverage.

There are no current loading limits for the use of ZnO nanoparticles in sunscreen creams in Australia however the SCCS places a load limit of 25 % (w/w) for European manufacturers. As with TiO$_2$ nanoparticles, ZnO nanoparticles are also prohibited for application in spray-based cosmetic products owing to the toxic effects they may exert through inhalation.
2.2 Protection from UV Radiation: Sunscreens

2.2.5 Health Related Issues Associated with Organic UV Filters

In an ideal world, sunscreen products should include ingredients that do not cause irritations to the skin when applied, be able to prevent UV radiation from reaching the skin without diminishing effectiveness over time and not be harmful to internal organs if accidentally consumed orally. However, as touched on previously, the reality is that many sunscreen ingredients, primarily organic UV filters, are readily absorbed through the skin and can be found quantifiably in blood, urine and even breast milk samples. In the United States, when sunscreen products were first beginning to garner mainstream commercial recognition during the 1970s, many ingredients previously used in specialized situations were immediately approved for commercial use by the Food and Drug Administration (FDA) without a review of the potential hazards. Only as recently as February 2019, have the FDA re-reviewed all currently approved UV filters, resulting in the discovery of at least 12 active ingredients that did not meet the necessary safety requirements. These ingredients include organic UV filters that have been used in sunscreen products since the very beginning of commercial sunscreen products such as oxybenzone, octinoxate, octocrylene and avobenzone. The only two filters that met the FDA safety requirements and were considered to be generally recognized as safe and effective or GRASE were TiO\textsubscript{2} and ZnO.

The TGA is also currently in a state of reviewing the Australian regulatory guidelines for sunscreens (2020). Whether this will result in a review of individual ingredients is unknown, however, past sunscreen compliance reviews, adhering to the AS/NZS 2604:2012 standard and the Therapeutic Goods Act 1989 have been performed as recently as 2018. Of the listed sunscreen products tested, no major compliance deficiencies were found in relation to the safety and efficacy of the formulation. Only minor issues pertaining to the labelling and advertisement of a third of the sunscreens were identified whilst a third of sunscreens reviewed were removed from the ARTG due to compliance with outdated standards that allowed elevated levels of the preservatives methylisothiazolinone and methylchloroisothiazolinone. No specific issues with the active ingredients, specifically organic UV filters, were found.
2.2 Protection from UV Radiation: Sunscreens

Regardless of the regulation of active ingredients in Australia, an American non-profit organisation dedicated to maintaining the environment and improving human health, the Environmental Working Group (EWG), developed a hazard rating scheme for various organic UV filters based upon their potential for dermal permeation, allergenicity, endocrine disruption and other causes for concern. A list of these ingredients, their hazard scores and health related concerns is shown in Table B.2.

**Skin Permeation**

Frequent application and reapplication of sunscreen formulations and the detection of organic UV filters and UV filter metabolites in urine and breast milk samples suggests systematic absorption of these active ingredients through the skin. This is a cause of concern owing to the potential for these ingredients to impact endocrine function and impair reproductive development. The ability for these ingredients to penetrate through the skin is strongly dependent on their chemical structure which governs the molecular weight and lipophilicity of the molecule in question. The type of formulation applied and presence of certain other ingredients can also influence the cutaneous penetration of these active UV filtering ingredients. Both *in vitro* and *in vivo* studies have been performed to assess the permeation of organic UV filters through the skin and their potential to reach viable skin layers. Jiang *et al*, (1999) investigated the absorption of various commercial sunscreen emulsions containing UV filters such as octyl methoxycinnamate, oxybenzone, titanium dioxide, octylsalicylate, octocrylene and butyl methoxy dibenzoylmethane through human skin epidermis using a Franz diffusion cell. It was found that all the filters tested permeated into the epidermis but only oxybenzone was found in the receptor fluid 8 hr after application. A further study performed by Hayden *et al*, (2005) found that the sunscreensing agents avobenzone, oxybenzone, octinoxate, octocrylene and padimate O, were present in the stratum corneum and viable epidermis of a skin model 24 hr after exposure to the sunscreensing agents when applied in a mineral oil. However, subsequent cytotoxic investigations of these tested organic UV filters with human epidermal keratinocytes suggested that the concentrations absorbed through the skin would be insufficient to induce any significant toxicity to viable skin cells. *In vivo* percuta-
neous permeation studies of oxybenzone have shown the ingredient to have skin penetrative abilities after being detected in urine samples from human and animal subjects up to 48 hr after application.\textsuperscript{271,272} Another study investigating the penetration of various organic UV filters, including octyl methoxycinnamate, 4-methyl benzylidene camphor and oxybenzone, in human volunteers also found detectable levels of the ingredients in urine samples.\textsuperscript{273} Recent advances in the encapsulation of UV filtering ingredients have enabled the development of micro- and nano-carrier systems capable of minimizing the permeation of these compounds through the skin.\textsuperscript{274,275} The use of zeolitic frameworks and polymeric delivery systems have been shown to aid in minimizing skin penetration of organic UV filters and to maintain topical retention of the applied sunscreens but whether the application of such advanced preparation methods can be transferred industrially is unclear.\textsuperscript{64,276,277}

**Endocrine Disruption**

Endocrine disrupting substances refer to chemicals capable of blocking, mimicking or changing the behaviour of hormones produced by the endocrine system.\textsuperscript{278} Some the TGA approved organic UV filters that have been implicated as endocrine disrupting chemicals include oxybenzone, sulisobenzone, 4-methyl benzylidene camphor and octyl methoxycinnamate.\textsuperscript{279}

Oxybenzone is an organic UVB filter that has been used in sunscreen products since the 1980s, however, studies have shown the filter to be capable of having estrogenic effects. Estrogens are steroid-type compounds that are important regulators of physiological development in many vertebrates and are associated with regulation of immune function, mineral homeostasis and reproduction in both sexes.\textsuperscript{280–282} The type of effect estrogen disruptive compounds can have on these functions can vary according to the chemical structure of the disruptor as well as the presence of other co-regulators or transcription factors around the estrogen receptor. Oxybenzone, and other benzophenone type UV filters used outside of Australia, have been shown to have estrogenic effects and are capable of inducing developmental and reproductive toxicity \textit{in vitro}.\textsuperscript{283,284} \textit{In vivo} studies using
2.2 Protection from UV Radiation: Sunscreens

rat and fish based subjects have also highlighted the estrogenic activity of oxybenzone and sulisobenzone and the resultant toxicity induced.\textsuperscript{234,285} Oxybenzone has also been shown to display anti-androgenic activity in an \textit{in vitro} study using a bone tissue cell line, U2-OS, abundant in androgen receptors and enabling more selective and sensitive measurement of the interaction occurring.\textsuperscript{286} There is also \textit{in vivo} evidence to suggest hydroxylated benzophenones, such as sulisobenzone, may exert anti-androgen activity in rat and fish larvae subjects.\textsuperscript{284,285}

Camphor derivatives used as UVB filters, such as 4-methyl benzylidene camphor are also causes of concern not only due to their potential for estrogenic activity, but their propensity for absorption through the skin as a result of their highly lipophilic nature.\textsuperscript{287–289} Exposure to 4-methyl benzylidene camphor and another UV filter, 3-benzylidene camphor, were also found to impact prostate gland growth and induce delayed puberty in rats subjected to daily 7 mg kg\textsuperscript{−1} doses of either chemical for 90 days, displaying activity similar to that of other estrogen mimetic compounds.\textsuperscript{287,290} Octyl methoxycinnamate has also been shown to disrupt estrogen and androgen activity.\textsuperscript{291,292} Pre-natal administration of octyl methoxycinnamate to pregnant rat subjects resulted in decreased sperm counts in male offspring eight months later correlating to decreased testes mass, whilst dosed dams showed marked decreases in thyroxine T4 levels.\textsuperscript{293} Both results suggest the administered UV filter can impact reproductive and neurological development if systematically exposed.

Allergenicity

Contact dermatitis is a skin condition brought about by exposure to an allergen. This results in the occurrence of red, itchy rashes on the skin and can be uncomfortable if left unchecked. Organic UV filters and, in particular organic UVA filters have been shown to induce allergic reactions.\textsuperscript{294} In addition, organic UV filters may undergo molecular changes in their chemical structure after UV exposure. These photoproducts can also act as allergens and induce allergic reactions to the skin.\textsuperscript{295} The incidence of allergic and photoallergic reactions is relatively low and is most prominent in people with a hist-
2.2 Protection from UV Radiation: Sunscreens

tory of photosensitivity. In a large-scale study performed in the UK, 1155 patients were tested for contact allergy and photoallergy towards suncreening active ingredients, which included current TGA approved organic UV filters such as butyl methoxy dibenzoylmethane, isomethyl methoxycinnamate, octocrylene, octyl methoxycinnamate, octyl triazone, oxybenzone and sulisobenzone. Of the 1155 patients tested, 130 (11.3%) exhibited either contact or photoallergic reactions, with the most common photoallergen being oxybenzone.

Although not as serious as other side effects of organic UV filter use, the immune response induced by some organic UV filters was seen as sufficient cause for manufacturers to incorporate inorganic UV filters in new formulations since, even at high loadings, allergenic responses do not occur.

Photostability

Organic UV filters should ideally be able to absorb incident UV radiation and dissipate the absorbed energy through photophysical and photochemical pathways that do not result in the formation of ROS or harmful reactive photoproducts. However, some organic sunscreen agents have been shown to undergo photoisomerization and photofragmentation reactions after UV exposure. This can lead to a loss of UV protection, as the fragmented species are generally less UV absorbing than the parent species, and can result in the generation of harmful ROS and other reactive fragments that can cause further damage to formulation ingredients. Combined with skin permeation and internalization, the photo-instability of certain organic UV filters is a cause of concern owing to the oxidative or free radical mediated damage they may induce. Serpone et al, (2002) demonstrated this by monitoring the extensive degradation of PABA when exposed to solar simulated light. This same study also highlighted the instability of TGA approved filters such as octyl methoxycinnamate and oxybenzone under the same exposure conditions (Figure 2.13).
A photostability study by Gonzalez et al., (2007) further highlighted the photo-instability of commercially used organic UV filters with the most prominent changes in UV protection occurring with formulations containing octyl methoxycinnamate, butyl methoxy dibenzoylmethane and oxybenzone over UVA/UVB irradiation periods of 30, 90 and 120 min. Increased understanding of the photobehaviour of organic UV filters has also led to the banning of certain combinations. For instance, octyl methoxycinnamate, avobenzone and butyl methoxy dibenzoylmethane have been shown to be incompatible with one another due to the formation of photoadducts when exposed to UV radiation. Mixtures of TiO$_2$ and ZnO with organic UV filters have also been suggested to impact the sunscreen protection efficacy after UV exposure over time. The cause for such changes in protection have been attributed to the photocatalytic activity of these inorganic UV filters, resulting in the production of ROS and oxidative damage of other sunscreens ingredients. This property is further discussed in Section 2.4.

Couteau et al., (2007) also investigated the photostability of various commonly used organic UV filters, prepared in oil/water emulsions, through the application of a spectroscopic SPF calculation. Formulations displaying significant reductions in protection,
as evidenced by a nominal decrease in SPF, included organic UV filters such as isoamyl methoxycinnamate, octyl salicylate and PEG-25 PABA. The study results also suggested that, for the irradiation time employed, octocrylene, octyl methoxy cinnamate and oxybenzone present good photostability contrary to the other studies described. This highlights the need for adopting a standardized methodology for assessing photostability, as briefly mentioned by Gonzalez et al (2007).\textsuperscript{302}

\textbf{Environmental Effects}

The increased prevalence of organic UV filters not only in personal care products, such as sunscreens, but also various textiles, plastics and paints have also increased concerns over their potential environmental impact. In particular, the contamination of aquatic environments is of major concern due to the various pathways from which these filters may enter these systems, which include through run-off from waste water treatment plants and recreational activity.\textsuperscript{306–308} \textit{In vitro} investigations of organic UV filters have shown that they may exert genotoxic effects to coral cells.\textsuperscript{309} Furthermore, it has also been shown that the occurrence and bioaccumulation of these organic filters can impact various aquatic organisms such as affecting reproduction in fish, the development of coral larvae and inducing coral bleaching.\textsuperscript{310–312} The last point in particular has led to state and national level bans of certain organic UV filtering ingredients. In 2018, Hawai‘i proposed banning the use of sunscreen products containing oxybenzone and octinoxate due to their impact on coral reefs and the frequency of sunscreen product use by tourists and locals containing these ingredients.\textsuperscript{38} Following from this in 2020, Palau became the first country to ban the use and distribution of sunscreen products containing the same organic UV filtering ingredients as those proposed by Hawai‘i due to findings of the ‘toxic sunscreen chemicals in tissues of their most famous creatures’.\textsuperscript{313} A review of both \textit{in situ} and \textit{ex situ} studies on the affects of organic UV filters on coral and reef biota was conducted by the International Coral Reef Initiative in 2018.\textsuperscript{314} It was suggested that many \textit{ex situ} studies failed to appropriately reflect realistic concentrations of the UV filters that have been found in aquatic systems. However, the levels that have been detected in studies of the Caribbean (US Virgin Islands), Mediterranean Sea (Majorca), Eastern Atlantic ocean (Gran Canaria)
and the Pacific Ocean (Hong Kong, Hawai‘i, Palau), although varying, are substantially high enough to be considered a serious environmental threat.

2.3 Health Related Issues Associated with Inorganic UV Filters

As the surface area to volume ratio of particles increases with decreasing particles size, the surface reactivity of nanoparticles increases significantly compared to their bulk forms. It is believed there is also an increase in the biological activity of these nanoparticulate materials due to their reduced size, and their use in therapeutic and cosmetic products has been a major point of discussion over the last decade. The increased production and use of nanoparticles in commercial products, not just TiO$_2$ or ZnO, is leading to a general increase in exposure to these nanomaterials, thus an understanding of the mechanisms surrounding their potential internalization and effects on the human body are essential. In some cases, sufficient evidence has been brought forward to warrant the discontinuation of nanomaterials in certain applications. For instance, the application of either TiO$_2$ or ZnO nanoparticles in spray-based sunscreen products is strictly prohibited owing to their potential internalization into the lungs and subsequent toxicological effects they may have. In this section, an overview of the biological effects TiO$_2$ and ZnO nanoparticles may have and their potential impacts on human health is given.

2.3.1 Cytotoxicity and Genotoxicity

A significant amount of effort has gone into characterising the potential toxicological effects of nanoparticulate TiO$_2$ and ZnO, particularly now with the use of such nanomaterials in commercial products becoming more common knowledge. Various in vitro and in vivo studies involving the use of mammalian cell lines and animal models have been published to investigate the effects of these nanomaterials. Furthermore, investigations surrounding the toxicological effects of these materials whilst exploiting their photocatalytic properties, termed phototoxicity, have also been performed to elucidate the potential oxidative damage induced through ROS generation.
In vitro Studies on Mammalian Cell Lines

One of the major concerns associated with both TiO$_2$ and ZnO in commercial applications is their potential oral internalization and the toxicity induced in the lungs. Anatase TiO$_2$ nanoparticles have been shown to reduce cell viability in a dose dependent manner, increase the expression of inflammatory indicators such as IL-8 and increase ROS generation in both human and murine lung cell lines.$^{47,315-317}$ Similar studies investigating the effects of rutile TiO$_2$ nanoparticles have also demonstrated similar toxicological effects, albeit, not to the same degree as anatase TiO$_2$. $^{47,318,319}$ Sayes et al., (2006) investigated the cytotoxic and inflammatory effects of anatase and rutile TiO$_2$ nanoparticles on human lung epithelial cells (A549). Cell viability assays indicating lactate dehydrogenase (LDH) release and cellular metabolic activity (MTT) were performed to assay cytotoxic effects, whilst the production of IL-8 was used as an indicator for inflammation in the cell line used. It was found that, in all assays performed, the nanoparticulate anatase TiO$_2$ resulted in greater decreases in cell metabolic activity, greater increases in LDH release and IL-8 production as compared to nanoparticulate rutile TiO$_2$ (Figure 2.14). These results suggest that the biological effects exerted by TiO$_2$ have some dependence on the phase composition of TiO$_2$, a property that has also been shown to govern its well studied photocatalytic activity and will be discussed further in Section 2.4.

![Figure 2.14:](image) (left) Mitochondrial activity, (middle) LDH release and (right) IL-8 production in A549 cells after 48 hrs exposure to anatase and rutile TiO$_2$ nanoparticles. Figure reproduced from Sayes et al.,(2006).$^{47}$

Investigations involving the use of dermal cell lines have also highlighted differences in cytotoxic and genotoxic effects between TiO$_2$ samples of varying composition. Park et al, (2011) found that TiO$_2$ exerted cytotoxic effects towards human keratinocyte (HaCaT)
It was also found that the degree of cell death induced and generation of ROS varied according to the crystal phase composition of the TiO$_2$ sample used, with mixed phases of anatase/rutile inducing greater oxidative damage and cell death as compared to the purely anatase phase. Further studies of anatase TiO$_2$ nanoparticles have shown that they may be internalized after exposure for 24 hr in in vitro grown human keratinocytes and sebocytes whilst also inhibiting cell growth, in vitro, for human fibroblasts, melanocytes, keratinocytes and sebocytes in a dose dependent manner. Murine fibroblast cells (L929) treated with anatase TiO$_2$ nanoparticles were also found to have inhibited cell proliferation as well as display evidence of organelle and membrane damage due to elevated levels of ROS as a result of TiO$_2$ internalisation. Interestingly, rutile TiO$_2$ nanoparticles have been found to have varying effects on human skin cells. The cause for these conflicting reports have been suggested to be due to differences in the physicochemical properties of the particles tested, with differences in particle size, surface area and surface chemistry being major contributing factors to the toxicity observed. Furthermore, cytotoxic effects of rutile nanoparticles may also be cell line dependent, as evidenced by differences in cell proliferation in HaCaT (human keratinocytes), A549 (human alveolar epithelial cells), U937 (human macrophage cells) and Caco-2 (human intestinal epithelial cells) cell lines and after exposure to rutile TiO$_2$ nanoparticles.

A number of in vitro studies have also demonstrated the cytotoxic and genotoxic potential of ZnO nanoparticles. The impact of ZnO on in vitro cell viability has often been attributed to the solubility of ZnO and the release of free Zn$^{2+}$, depending on the cell line in question. The presence of free Zn$^{2+}$ can disrupt the natural homoeostatic concentration of Zn in cells and lead to a loss of cell viability through oxidative stress and mitochondrial dysfunction. This dissolution mechanism for toxicity has been show to affect murine neural stem cells (C17.2), human monocyte macrophages and human alveolar adenocarcinoma cells (A549). A further study on human bronchial epithelial (BEAS-2B) cells incubated with 20 nm ZnO nanoparticles showed a concentration and time dependent dependence on cytotoxicity, with elevated levels of oxidative stress,
2.3 Health Related Issues Associated with Inorganic UV Filters

intracellular Ca\(^{2+}\) levels and membrane damage (LDH release) also being detected.\(^{331}\) This same study also highlighted that, even at sublethal concentrations, ZnO nanoparticles could modulate the expression of at least four genes involved in oxidative stress and apoptosis, thus reflecting the highly cytotoxic nature of ZnO. Size and shape dependent cytotoxicity of ZnO nanoparticles has also been demonstrated on human lymphoblastoid (WIL2-NS) cells, human neuroblastoma (SHSY5Y) cells and human alveolar adenocarcinoma (A549) cells, with the primary influence of these parameters on the specific surface area of the particles contributing strongly to Zn ion release and the toxicity displayed.\(^{324,332}\) Comparative cytotoxic studies of various metal oxides nanoparticles including TiO\(_2\), Al\(_2\)O\(_3\), SiO\(_2\) and CeO\(_2\) alongside ZnO nanoparticles have also demonstrated the highly cytotoxic nature of these ZnO nanomaterials.\(^{333–336}\)

Specific studies involving the use of sunscreen derived inorganic nanoparticles have not been thoroughly investigated, with most researchers preferring the use of other commercial TiO\(_2\) and ZnO or ‘in-house’ prepared variants. One study by Dunford et al., (1997) investigated DNA damage induced by TiO\(_2\) nanoparticles derived from commercial sunscreen products under solar-simulated light exposure.\(^{337}\) Various anatase/rutile crystal phase compositions were observed from the various sunscreen derived TiO\(_2\). Direct strand breaks in DNA derived from the plasmid pBluescript II SK\(^+\) by the sunscreen derived TiO\(_2\) nanoparticles was revealed, with greater damage observed for samples with greater anatase phase compositions. Furthermore, through the use of various free radical quenchers, it was found that the damage induced was a direct cause of OH\(^•\) presumably induced by the photoexcited TiO\(_2\). The same study also demonstrated the genotoxic effect of sunscreen derived TiO\(_2\) on human cells. Comet assays performed using a lung tissue derived fibroblasts (MRC-5) also revealed extensive oxidative damage induced by the ROS-generating ability of TiO\(_2\).

**In vivo Studies on Mammalian Models**

The main pathways of internalization as concerned with sunscreen based nanoparticles are permeation through the skin, inhalation and accidental oral ingestion. As skin permeation
of inorganic UV filtering nanoparticles is considered a major concern, it will be discussed in further detail in a Section on its own (Section 2.3.4). As such, studies primarily involving orally, inhaled or intravenously administration of TiO$_2$ and ZnO nanoparticles will be discussed here.

A significant study published in 1985 involving the administration through inhalation of TiO$_2$ fine particles (mean particle size greater than 100 nm) to rats over a 2 year period revealed the development of lung tumors.\textsuperscript{49} Although these findings were challenged by many, with some suggesting the results being due to lung overload (due excessively high dosage) as opposed to carcinogenicity by the TiO$_2$ particles, the International Agency for Research on Cancer (IARC) has since classified TiO$_2$ as a Group 2B carcinogen (materials with possible carcinogenicity towards humans).\textsuperscript{338,339} More recent studies have also now demonstrated that TiO$_2$ nanoparticles exert greater \textit{in vivo} toxic effects as compared to larger TiO$_2$ particles.\textsuperscript{340–342} Inhalation studies involving rats exposed to 0 - 50 mg m$^{-3}$ doses of TiO$_2$ nanoparticles (mean sizes of 5 nm and 25 nm, mixed anatase/rutile crystal phase) for 5 and 10 days revealed the acute induction of pulmonary inflammation.\textsuperscript{343,344} A further study by Liu \textit{et al}, (2009) demonstrated dose dependent and size dependent incidence of lesions to lung tissues of rats intra-tracheally treated with TiO$_2$ nanoparticles.\textsuperscript{345} It was found that 5 nm (anatase) TiO$_2$ nanoparticles induced more severe pulmonary toxic effects as opposed to 21 (mixed phase) or 50 nm (rutile) sized particles. It was also suggested that the smaller sized nanoparticles impaired the phagocytotic ability of alveolar macrophages at exposure doses of 50 mg kg$^{-1}$, enabling toxicological effects to occur more easily due to a decrease in efficiency of natural defensive mechanisms to dealing with foreign threats. The acute impact of particle size and, subsequently, specific surface area and crystal phase on pulmonary toxicity and alveolar macrophage activity was further demonstrated by Liu \textit{et al}, (2010).\textsuperscript{346} Interestingly, acute oral toxicity studies using rabbit and mice models suggested minimal systemic toxicity towards 25 nm, 80 nm, 129 nm and 155 nm TiO$_2$.\textsuperscript{347,348} Conversely, acute intra-peritoneal studies showed, at high doses (150 mg kg$^{-1}$), significant damage to the liver and kidneys could be induced with administration of 5 nm anatase TiO$_2$ nanoparticles, further demonstrating the size...
2.3 Health Related Issues Associated with Inorganic UV Filters

dependence of TiO\textsubscript{2} toxicity but also the dependence on the method of administration of said particles.\textsuperscript{349} This same study also reported a median lethal dose (LD\textsubscript{50}) of 150 mg kg\textsuperscript{-1} in mice used, a significantly lower dose than that reported previously by the WHO for bulk TiO\textsubscript{2} particles administered to rats (>10,000 mg kg\textsuperscript{-1}).\textsuperscript{348} Chronic exposure studies performed using inhalation and intra-gastric administration of TiO\textsubscript{2} nanoparticles and fine particles, ranging from 5 nm up to 250 nm, also suggest some moderate levels of toxicity with incidences of pulmonary lesions, spleen and lung injury, inflammation and macrophage impairment being most evident in rat, pig and mice models.\textsuperscript{49,340,350,351} Experimental studies for carcinogenicity using animal models have also demonstrated that TiO\textsubscript{2} nanoparticles may induce respiratory tract cancers and lung tumors when administered at high dosages through intra-tracheal and inhalation routes of exposure but limited carcinogenicity is observed when intra-gastric or dermal administration is used.\textsuperscript{352–356} A limited number of epidemiological studies have been carried out to assess the affect of TiO\textsubscript{2} exposure on humans and have shown that there is no significant link between exposure and risk of lung cancer.\textsuperscript{357,358} However, studies that have been performed do not specify particle size, so it is unclear as to whether chronic exposure to nanoparticulate TiO\textsubscript{2} can increase the risk of cancers and is an area that needs further investigation.

Similarly to TiO\textsubscript{2}, ZnO particles (70 nm up to 3 \(\mu\)m) have also been shown to demonstrate acute inflammatory responses and pulmonary damage to the lung tissues of rats through intra-tracheal administration and inhalation.\textsuperscript{50,359} Administration of inhaled ZnO nanoparticles (20 nm) to rats at dosage of 2.5 mg kg\textsuperscript{-1} body weight twice daily for 3 days resulted in extensive lung and liver tissue damage, further demonstrating the toxicological potential of ZnO. It has also been suggested that, due to the acidic nature of the lung lining, greater dissolution of ZnO and release of Zn\textsuperscript{2+} can result in an increase in local toxicity as a result of increased intracellular Zn\textsuperscript{2+} and metal-ion imbalances.\textsuperscript{360} Treatment of guinea pigs with 50 nm ZnO nanoparticles at the industry-standard occupational exposure dose of 5 mg m\textsuperscript{-3} through inhalation for 3 hr per day for 6 days also demonstrated the propensity for these particles to induce inflammatory response, reduced lung capacity and lung lesions.\textsuperscript{361} Furthermore, inhalation studies with human subjects re-
sulted in the expression of symptoms related to 'metal fever' due to ZnO nanoparticles at exposure levels even below 5 mg m$^{-3}$, although test group sizes were relatively small (4 and 13 participants).$^{362,363}$ Oral administration of ZnO nanoparticles to mice and rats have also highlighted the size dependent and dose dependent toxicity of ZnO in causing lung and liver tissue damage through this exposure route.$^{364–366}$ Pasupuleti et al, (2011) orally treated adult rats with 20 nm and micro-sized (greater than 100 nm) ZnO particles at doses varying from 5 up to 2000 mg kg$^{-1}$ body weight for 14 days.$^{365}$ It was found that greater incidence of lesions in the liver, pancreas, heart and stomach occurred with administration of ZnO nanoparticles at the lowest dosage level (5 mg kg$^{-1}$ body weight) and, in turn, particle number within the afflicted organs. This is particularly important in regards to the potential health effects of sunscreen based nanoparticles since, as will be further discussed later, minimal permeation of these nanoparticles occurs through the skin thus also leading to a low loading and particle number. No specific long-term carcinogenic studies on ZnO in animals could be found, whilst only a few long-term studies using Zn based chemicals or supplements are available. One epidemiological study performed on 46,974 US male volunteers supplemented with varying amounts of Zn in a 14 year-long study found that no significant correlation between Zn intake, at realistic dosages (100 mg Zn per day), and prostate cancer risk was observed after a 10-year follow up.$^{367}$ However, the lack of specific carcinogenic ZnO nanoparticle studies still warrants further investigation due to past evidence of in vitro and in vivo genotoxicity and mutagenicity.$^{368,369}$

2.3.2 Phototoxicity

Concerns over the generation of free radicals and ROS by ZnO and TiO$_2$ due to their semiconductor electronic structure has led to various biological investigations involving simultaneous exposure to these nanomaterials and UV radiation or solar-simulated sunlight exposure. A more thorough discussion of ROS generation and photocatalysis by inorganic UV filters is given in Section 2.4, whilst an overview of literature findings in regards to the phototoxic potential of ZnO and TiO$_2$ nanoparticles is given here.

The phototoxicity associated with TiO$_2$ and its crystal phases is inherently linked to its
photoactivity and physicochemical properties. Phototoxicity studies performed by Uchino et al., (2002) found a higher rate of OH• radical production through electron paramagnetic resonance (EPR) spectroscopy for anatase nanoparticles as compared to the rutile crystal phase when exposed to UVA radiation. This increased radical production also correlated to an increase in cytotoxicity towards Chinese hamster ovary (CHO) cells. Particle size and specific surface area (SSA), which are generally intimately linked, has also been shown to influence ROS generation by TiO₂ nanoparticles. Wyrwoll et al., (2008) found that the generation of OH• was highest for the smallest TiO₂ nanoparticles tested (anatase, 7-10 nm, SSA 280 m² g⁻¹), however, the overall sum of ROS generated was highest for slightly larger particles (anatase, 15-25 nm, SSA 77.6 m² g⁻¹). Furthermore, the highest degree of phototoxicity towards Daphnia magna under solar-simulated illumination was achieved with the slightly larger nanoparticles (anatase, 15-25 nm), owing to the higher concentration of ROS generated. Photocytotoxic and photogenotoxic effects of TiO₂ nanoparticles have also been demonstrated in human peripheral blood lymphocytes, human retinal pigment epithelial cells (ARPE-19), mouse lymphoma cells (L5178Y), Chinese hamster CHL/IU cells, RAW264.7 cells and HaCaT cells. It should be noted that, in all these studies mentioned, the TiO₂ nanoparticles tested were uncoated, whilst sunscreen based TiO₂ nanoparticles are nearly always coated with some form of inert material that aims to inhibit ROS generation. As such, specific phototoxic studies investigating the effects of coated TiO₂ nanoparticles are of more significant relevance here. Horie et al., (2010) evaluated the impact of cosmetic grade rutile TiO₂ nanoparticles (5-15 nm × 20-90 nm) coated with Al(OH)₃ on the HaCaT and A549 cell lines, showing minimal influence in toxicity towards the cells in absence of UV light. A further study by Al-Abed et al., (2016) investigated the phototoxic potential of Al(OH)₃ coated rutile TiO₂ (mean size 60 nm) but with the additional step of artificial ageing under highly chlorinated conditions to simulate the conditions of sunscreen users in swimming pool water. It was found that, whilst there was no significant phototoxicity towards human retinal pigment epithelial cells (ARPE-19) for un-aged samples, a significant difference between UVA irradiated and non-irradiated aged samples did occur. This was presum-
ably due to degradation of the Al(OH)$_3$ coating and exposure of the core TiO$_2$. Tang et al., (2018) conducted a study on the photocatalytic production of hydroxyl radicals for a range of commercial TiO$_2$ nanoparticles and their potential phototoxicity.$^{378}$ The samples were obtained from a variety of cosmetic manufacturers and include TiO$_2$ nanoparticles of varying crystal phase composition, particle size and coating types ranging from alumina, silica, silane and combinations of these. *In vitro* phototoxicity (5 J cm$^{-2}$ UVA dose) assays included the neutral red uptake (NTU) assay on murine embyronic fibroblasts (BALB/c 3T3) for cell proliferation effects and malondialdehyde (MDA) supplemented erythrocytes from rabbits for membrane integrity assessment. The OH• radical rate generation of the tested samples were also assessed using EPR and the DMPO spin-adduct. It was found that samples consisting of anatase and anatase/rutile phase compositions exhibited the highest levels of phototoxicity, causing extensive cell membrane damage through photo-induced lipidperoxidation (Figure 2.15). The most destructive sample tested consisted of a mixed anatase/rutile phase and silica/alumina surface coating. Furthermore, the phototoxicity it displayed did not correlate with increased levels of OH• generation, as compared to the other samples, implying that the phototoxicity mechanism for the core TiO$_2$ may be modified as a result of the coating material.

![Figure 2.15: MDA levels, indicated of cell membrane damage, measured in the supernatants of erythrocytes treated with phosphate-buffered saline (NC) or with TiO$_2$ nanoparticles (100 µg mL$^{-1}$)(Physical parameters listed in Table B.3).● Significant difference from the control (NC) without UV exposure ($p<0.05$). †† Significant difference from control with UV exposure ($p<0.01$). Figure reproduced from Tang et al., 2018.]

Phototoxicity exerted by ZnO nanoparticles has also been demonstrated under UV irradia-
tion towards HaCaT, BALB/c 3T3a, A549 cells and human foreskin fibroblasts (AG01518).\textsuperscript{52,379–381}

The phototoxic properties of ZnO have also been shown to be particle size dependent, dose dependent and time dependent with decreasing particle size and increasing dose/time leading to greater generation of ROS, release of free Zn\textsuperscript{2+} and, subsequently, phototoxicity.\textsuperscript{380,382,383} Phototoxicity studies of ZnO nanoparticles have also been performed in unison with TiO\textsubscript{2} nanoparticles. Pre-UV irradiated suspensions of ZnO nanoparticles (<50 nm) and TiO\textsubscript{2} nanoparticles (anatase, <25 nm) were shown to induce significant phototoxic effects towards Artemia salina shrimp cysts.\textsuperscript{384} Greater toxicity was observed in the case of the ZnO nanoparticles and was attributed to the two-fold action of both ROS generation and ZnO dissolution. Gopalan \textit{et al}, (2009) also investigated specific photogenotoxicity of ZnO and TiO\textsubscript{2} nanoparticles (both within the 40-70 nm size range) towards human sperm and lymphocytes using the Comet assay.\textsuperscript{385} The ZnO nanoparticles were found to induce greater toxicity in both cell types in the way of DNA damage, both with and without UV irradiation, as compared to the TiO\textsubscript{2} nanoparticles, which only showed significant phototoxicity towards the tested lymphocytes.

Although it is highly recommended by the TGA that all current and new organic and inorganic based UV filters developed by manufacturers have the appropriate cytotoxic/genotoxic and phototoxic properties of their material evaluated prior to submission, certain information may be omitted or excepted if it can be shown said material does not permeate through the skin and reach viable cells. This has caused a lot of distress in consumers surrounding the use of nanoparticles in cosmetic and therapeutic products and much effort has gone into assessing the permeation potential of these particles. The findings of such studies will be discussed further in Section 2.3.4.

2.3.3 Environmental Effects

Global production of nano-TiO\textsubscript{2} and nano-ZnO has been estimated to be between 550 - 5500 t and 55 - 550 t per year, respectively, with 60\% of TiO\textsubscript{2} and 80\% of ZnO thought to be used in cosmetic products.\textsuperscript{386} The primary ecosystems most prone to nanoparticulate ZnO and TiO\textsubscript{2} exposure are aquatic systems due to their use in various therapeutic
and cosmetic sunscreens. An estimation of the release of these nanoparticles into aquatic systems was suggested by Wong et al., (2010) to be as high as 250 t per year which, considering the chemical inertness of TiO$_2$ in particular, may pose a significant cause of concern to aquatic biota.\textsuperscript{387} Exposure of various aquatic organisms to TiO$_2$ and ZnO nanoparticles has been demonstrated to have negative effects on the health of such organisms.\textsuperscript{387–389} In particular, coral reefs exposed to uncoated TiO$_2$ and ZnO nanoparticles have been shown to undergo coral bleaching processes.\textsuperscript{390} Notably, ZnO nanoparticles were shown to induce irreversible expulsion of Acropora coral microbiota, which are necessary for providing the coral with much of its energy input. Other studies on the Montastraea faveolata stony coral exposed to TiO$_2$ nanoparticles, whilst still displaying microbiota expulsion, show some levels of acclimation and recovery.\textsuperscript{391} Regardless, the large input of either inorganic UV filter into aquatic systems needs to be constantly monitored or require modifications to minimize their impact on marine organisms.

### 2.3.4 Dermal Permeation of Inorganic UV Filters

The most pressing concern associated with the use of nanoparticles in sunscreen products is their potential permeation through the skin and subsequent oxidative damage they may induce to viable cells through photocatalyzed ROS generation. The weight of evidence presented by various ex vivo permeation studies through animal and human skin models have suggested sunscreen based nanoparticles remain on the surface of the skin and are unable to penetrate deep enough to reach viable cells.\textsuperscript{54,392,393} However, it is also important to mention that conflicting reports highlight that various factors related both to the skin model and the nanoparticle characteristics can impact the depth of penetration. Physicochemical properties of the nanoparticles, such as their size, shape and surface properties, can influence their permeation. In addition, the condition of the skin model, site of application, origin of the skin and method of analysis can also influence the results obtained from such studies.

Human skin operates as the ‘first line of defence’ against environmental factors, both chemical and physical, and constitutes for approximately 16\% of a human's total body
weight. The layers of most concern associated with phototoxicity and nanotoxicity are those making up the epidermis. The epidermis is the outermost layer of the skin, lying atop the dermis, and can be further sectioned into a number of sub-layers (Figure 2.16). These include the stratum corneum, stratum lucidum, stratum granulosum, stratum spinosum and stratum basale. As mentioned previously, the major cell type in the epidermis are the keratinocytes which, starting from the stratum basale, differentiate into more specialized versions through each subsequent layer. The stratum basale differs from the other layers of the epidermis as it consists primarily of a single layer of cells, called basal cells, and is intimately linked to the dermis through connective collagen fibers. These basal cells are precursor stem cells for the keratinocytes of the epidermis and allow the continual replenishment and shedding of dead skin cells. Also present in the stratum basale are Merkel cells and melanocytes which are responsible for sensory stimulation and the production of the melanin pigment, respectively. The transition from living to non-living cells generally occurs in the stratum granulosum. Keratinocytes in these layers that have been pushed up from the stratum spinosum undergo morphological changes, becoming flatter and developing thicker cell membranes. They also produce a large amount of keratin, the primary protein component of hair. In addition, the nuclei and organelles of these cells disintegrate. This signifies the end of the keratinocyte differentiation pathway and the transition to non-viable cells known as corneocytes. These corneocytes are the major cell population of the stratum corneum. Corneocytes densely packed in the stratum corneum are enveloped in a cross-linked protein shell, chemically bound by a lipid monolayer that enables interlocking between different corneocytes in the lipophilic lipid matrix. These cornified envelopes are important in the functionality of the skin barrier as they inhibit the partitioning of foreign agents through the skin and are in most intimate contact with inorganic UV filter nanoparticles when sunscreen are topically applied.
Figure 2.16: (left) Diagram and (right) microscope image of human skin, likely from the palms of the hands or soles of the feet, detailing the layered structure of the epidermis and dermis. Figures reproduced from (left) Wickett et al., (2006) and (right) Wbemsmith, (2007).

Figure 2.17 highlights the potential cutaneous penetrative pathways through the stratum corneum including the transcellular/paracellular routes and transappendagael routes through sweat pores, hair follicles and sebaceous glands. The degree to which foreign substances permeate through the skin may be enhanced due to the exposure of skin to certain environmental or pathological conditions. A disruption in the skin barrier through physical damage or disease can lead to an alteration in the rigidity of tight junctions or lipid composition and organization, facilitating the passage of previously non-penetrative substances. Exposure to UV radiation can also disrupt the skin barrier. Jiang et al., (2007) studied the effect of UVB radiation on adult hairless mice. The mice were exposed to a single dose of UVB radiation equivalent to seven and half times the MED for humans. Transepidermal water loss (TEWL) was found to significantly increase after UVB exposure, correlating with an observed increase in intercellular permeability towards a water-soluble lanthanum tracer, thus highlighting perturbation of the epidermal barrier. Barrier disruption due to UV exposure has also been studied in human populations. Liu et al., (2010) also reported dose dependent and gender dependent changes in TEWL for a group of Chinese males and females after daily sun exposure over a period of three months. These environmental and pathological conditions could thus impact the
permeation of sunscreen based nanoparticles, providing alternate routes of penetration to the viable dermis. This was demonstrated by Mortensen et al., (2008) who investigated the *in vivo* penetration of carboxylated-quantum dots (20-33 nm) applied in formulation to mice dosed with sufficient UVA/UVB radiation to induce erythema.\(^{403}\) It was found that 24 hr after application, the penetration levels of the quantum dots were, qualitatively, higher than those applied to non-irradiated mice, thus demonstrating the impact of UV radiation of barrier function and the potential for nanoparticle penetration enhancement.

![Diagram of the epidermis and cutaneous penetration pathways](image)

**Figure 2.17:** Layered structure of the epidermis and the potential pathways for cutaneous penetration including the a) paracellular, b) transcellular and transappendagael routes. The transappendagael routes include c1) hair follicles, c2) sweat pores and c3) sebaceous glands. Figure reproduced from Smijs et al., (2011).\(^{7}\)

The Australian TGA have conducted a safety review of TiO\(_2\) and ZnO nanoparticles in sunscreen products, assessing various physicochemical properties and interactions with biological systems, including skin permeation.\(^{54}\) It is in their opinion that the weight of literature evidence suggests these sunscreen based nanoparticles do not penetrate the skin sufficiently to reach viable cells, thus pose no significant threat. Similarly, the EU SCCS also takes a similar stance on the safety of these two materials and have allowed the continued use of these nanoparticulate materials. Despite these opinions, their is still some uncertainty in the scientific community surrounding the safety of these inorganic nanoparticles due to conflicting experimental evidence and lack of mechanistic explanations for the behaviour of these nanoparticles on the skin. In their report, the TGA also drew attention to the fact that different test methodologies can lead to differences in permeation potential for the same material. For instance, studies using isolated human or animal epi-
2.3 Health Related Issues Associated with Inorganic UV Filters

dermis/stratum corneum films could yield higher rates of absorption as compared to full thickness skin.\textsuperscript{404,405} Furthermore, immersion studies of skin substrates can result in significant swelling, thus enabling easier penetration of foreign substances between swollen corneocytes.\textsuperscript{406} Tape stripping methods for the detection of contaminants through skin layers can also introduce artefacts due to the presence of hair follicles which have been show to be sites of nano- and micro-particle accumulation.\textsuperscript{407} The choice of skin model may also impact the permeation results. Various \textit{in vivo} penetration studies have been carried out on a variety of human, murine and farm animal subjects and the general degree of skin permeation of nanoparticles varies from animal to animal as follows: rabbit skin>pig skin>monkey skin>human skin.\textsuperscript{408} Therefore, extrapolation of skin penetration through animal models other than humans should be approached with caution.

A number of \textit{in vitro} skin permeation studies using healthy, undamaged human skin substrates have shown that TiO\textsubscript{2} and ZnO nanoparticles, of varying size and composition, primarily localize in the stratum corneum and/or hair follicles.\textsuperscript{409–413} Similarly, \textit{in vivo} studies on human volunteers, evaluating the permeation and penetration of TiO\textsubscript{2} and ZnO nanoparticles of varying size and composition, have also been conducted.\textsuperscript{407,414–417} Using a variety of retrieval and detection methodologies, the majority of studies viewed have suggested minimal penetration into the stratum corneum occurs for both metal oxides. Studies aiming to better replicate real-life conditions, including modelling skin flexion and using pre-damaged (UV exposed or abraded) have also produced results indicating minimal penetration into the stratum corneum, although studies specifically using human subjects are lacking.\textsuperscript{418–420} There are of course studies that suggest otherwise. One \textit{in vivo} study suggested that Zn\textsuperscript{2+} levels detected in blood and urine samples were elevated in human volunteers applying ZnO based sunscreens over a period of 5 days, however, whether the Zn\textsuperscript{2+} originated from the ZnO nanoparticles could not be elucidated.\textsuperscript{421} Another study by Zhang \textit{et al} (2018) showed that dermal exposure of mice to TiO\textsubscript{2} nanoparticles (15-40 nm) at loadings between 20-500 mg kg\textsuperscript{-1} per day for 42 days led to the expression of inflammatory markers, including IL-8, and increased levels of ROS and 8-hydroxy-2’-deoxyguanosine (one of the major products of DNA oxidation) in the mice
blood serum.\textsuperscript{422} This would imply some level of skin penetration by the TiO$_2$ nanoparticles used however, as mentioned previously, the susceptibility of skin models such as mice towards metal oxide nanoparticles is much higher than that of humans, so the results produced may not necessarily translate in human subjects.

\textbf{Figure 2.18:} Multiple-photon second harmonic generation (MP-SHG) and fluorescence lifetime imaging (MP-FLIM) images of cryosectioned human skin after 48 hrs of applying ZnO nanoparticles in sunscreen formulation. (\textit{top-left}) MP-SHG signal of ZnO nanoparticles. (\textit{top-right}) Transmission image of skin labelling stratum corneum (SC) and the viable epidermis (VE). (\textit{bottom-left}) MP-FLIM signal from ZinPyr-1 (ZP1) fluorescent dye for detecting labile Zn. (bottom-right) Overlay of images. Figure reproduced from Mohammed \textit{et al}, (2020).\textsuperscript{423}

Although these conflicting reports do exist, the majority consensus is that there is minimal penetration into the epidermis and that the health benefits afforded by having these nanoparticles in sunscreen products still outweigh their potential risks.\textsuperscript{424} In spite of these opinions, it is abundantly clear that a standardized methodology for assessing skin penetration by these nanoparticles is needed. In particular, \textit{in vitro} methods employing Franz-type diffusion cells and excised skin are the furthest removed from real-world conditions, thus the validity of results obtained from such methods is highly questionable. Ideally, \textit{in vivo} testing on human subjects offers the most representative model for real-world application however the ethical issues surrounding such studies may hamper such progress particularly in investigating the effects of pre-damaged skin on inorganic UV filter penetration, which is currently severely lacking in data. Although their use in sun-
screen products has continued despite ambiguity surrounding their safety, both TiO₂ and ZnO are photocatalyst materials and their propensity to produce ROS when exposed to UV radiation should still be addressed to further minimize their potentially detrimental health effects.

### 2.4 Photocatalysis by Inorganic UV Filtering TiO₂ Nanoparticles

As mentioned in the prior section, modern formulations will often contain inorganic UV filters in the form of nanoparticles. The use of these materials in this size range modifies their interaction with light by increasing their transparency in the visible light region and improving their absorption across the UVA and UVB wavelength regions. However, an additional side effect of this size reduction is their increased propensity to produce free radical species. The reason for this is due to the photocatalytic nature of TiO₂ and ZnO.

Photocatalysts may be defined as stable semiconductors capable of initiating surface based chemical reactions due to the production of photoexcited charge carriers. Both TiO₂ and ZnO are photocatalysts that have long been researched in applications that manipulate this property. In particular, TiO₂ nanoparticles have been used as a reference photocatalyst materials for many research fields due to its apparently high photocatalytic activity relative to other semiconductor materials. The use of TiO₂ in nanoparticulate form for catalysis dates back to the development of the first dye-sensitized solar cells by Gratzel et al., (1991) owing to the high surface area at this size range and the high efficiency of photon to current conversion of TiO₂. In this section, an overview of the photocatalytic mechanism of TiO₂ nanoparticles and methodology for modifying this property is given. Only TiO₂ as an inorganic UV filter is considered from here due to it forming the basis of the thesis work.
2.4 Photocatalysis by Inorganic UV Filtering TiO$_2$ Nanoparticles

2.4.1 General Photocatalysis Mechanism

A number of steps are involved in the process of photochemically degrading adsorbed molecules by photocatalysis (Figure 2.19). For excitation to occur, photons of sufficient energy are needed to excite an electron from a semiconductor’s valence band to its conduction band. The separation between these two bands is termed the band gap and can vary depending on the semiconductor composition, particle size, crystallinity and defect structure. The result of this excitation process is a negatively charged electron (e$^-$) elevated to a higher energy state in the conduction band and a positively charged hole (h$^+$) in a lower energy state of the valence band.

Migration of these photoexcited charge carriers to the surface of the catalyst enables interaction with chemically adsorbed molecules to occur. The major limiting factor for this interaction however is the effect of recombination and charge trapping at defect sites within the bulk of the semiconductor structure. Depending on the band gap energy and band positions of the semiconductor material, interaction of these photoexcited charge carriers with adsorbed molecules can result in their degradation through oxidative/reductive processes. For oxidation to occur, the conduction band minimum (relative to vacuum) for
the semiconductor must be higher than the reduction potential of the adsorbed molecule. Conversely, for reduction to occur, the valence band maximum must sit at an energy lower than that of the oxidation potential. Figure 2.20 highlights the relative band gaps and band positions of various semiconductor materials and the redox potentials for water. The overall efficiency of the photocatalytic process for a given semiconductor photocatalyst is termed the photonic efficiency $\zeta$. $\zeta$ is given as the rate of degradation product formation divided by the incident photon flux and has been found to be relatively small for most semiconductor materials ($< 10\%$) due to the rapid recombination of $e^-/h^+$ pairs.

Figure 2.20: Band gaps and band edge positions for different semiconductor materials relative to the vacuum level. The red dashed area indicates the redox potentials for water photolysis. Figure reproduced from Batzill et al, (2011).

The formation of the OH$^*$ ROS is the major cause of photo-induced degradation observed in photocatalytic degradation experiments. Upon photoexcitation, OH$^*$ may be formed at the surface the excited catalyst or free in solution. The reactivity of these two species also differs as a result of the difference in their spatial environments. Typically, OH$^*$ produced at the surface of the catalyst is limited to interacting with surface-bound molecules whilst those free in solution can interact with molecules present in the bulk. This in turn has an effect on the rate of decomposition of a particular substance and the photocatalytic activity of the semiconductor material.

One of the main reasons for the application and study of TiO$_2$ nanoparticles, and nanomaterials in general, is due to its relatively high $\zeta$ compared to other semiconductor nanomaterials ($10\%$). Combined with its relatively low cost of preparation, chemical inertness, photostability and UV absorptive properties, the commercial opportunities for
2.4 Photocatalysis by Inorganic UV Filtering TiO$_2$ Nanoparticles

TiO$_2$ nanomaterials in various applications, such as in sunscreens, is apparent.

2.4.2 Photocatalysis by TiO$_2$ Nanoparticles

TiO$_2$ Surface Adsorption

An important consideration for photocatalysis in TiO$_2$, as well as all other semiconductor materials, is the surface adsorption of chemical species. TiO$_2$ surfaces are often composed of defect sites known as oxygen vacancies, which are formed by the transfer of unpaired electrons in O 2$p$ orbitals to vacant Ti 3$d$ orbitals, accompanied by the removal of an oxygen atom.$^{430,431}$ The result of these oxygen vacancies leads to an accumulation of charge near the surface of the catalyst which is thought to have an impact of the adsorption behaviour of molecules. The adsorption of H$_2$O at the surface of TiO$_2$ is of most immediate relevance owing to its abundance in cellular environments and use in sunscreen products. Experimental evidence and theoretical calculations have shown that H$_2$O adsorbs through a dissociate process at oxygen vacancy sites on the surface of TiO$_2$.$^{432–434}$ In this process, oxygen vacancy sites are paired with the hydroxyl groups of water molecules and the excess charge present in Ti 3$d$ orbitals due to these oxygen vacancies is transferred to the $\pi$ molecular orbitals of OH.$^{435}$

Charge Carrier Generation and Recombination in TiO$_2$

Excitation of TiO$_2$ with photons of sufficient energy to generate photoexcited charge carriers is dependent on a few factors, including the crystal phase composition of the material. The two main phases of TiO$_2$, rutile and anatase, have bulk band gap energies of 3.0 and 3.2 eV, respectively.$^{53,436}$ The positioning of these band gap energies at the boundary of UV and visible light radiation also contributes to their use in sunscreen products. Another factor that may affect this band gap energy is the size of the semiconductor particle. Modification of a materials dimensions to the nanoscale can induce a phenomenon known as quantum confinement. As the size of the semiconductor particle is reduced, spatial confinement of charge carriers ($e^-/h^+$) becomes more prominent. As a result, the energy states comprising the valence and conduction bands become discrete as opposed
to the continuous band structure of the corresponding bulk material. This also leads to an increase in band gap energy with decreasing particle size and is a property that can be exploited to tailor the band gap properties of various semiconducting materials. However, variance of the TiO$_2$ band gap, particularly for the anatase crystal phase, has been shown to be relatively minimal even down particle diameters of 1.5 nm.$^{437}$ In certain experimental cases, a reduction in particle size below 100 nm first resulted in a red-shift in band gap energy before widening again below 29 nm.$^{438}$ The explanation given for the apparent red-shift was attributed to bulk defects in the material which allowed delocalization of the LUMO and creation of shallow trap sites within the band gap whilst the blue-shift that occurred below a certain threshold particle size was due to shifting of these trap sites to higher energies (size quantization effect). What can be drawn from literature however is that the main modification of the TiO$_2$ band gap occurs through modification of the crystal phase, as mentioned previously, or through the introduction of foreign elements in a process known as doping (Section 2.5).

Generation of $e^-/h^+$ pairs in TiO$_2$ nanoparticles generally occurs near the surface of the particle, owing to the small penetration depth of the UV radiation needed to excite the material. This could be a contributing factor in the prominent photocatalytic properties the material displays. However, recombination of these photoexcited charge carriers is a limitation that affects not only TiO$_2$ but all photocatalyst semiconductor materials. Photoluminescence and time-resolved absorption spectroscopy methods have been employed and demonstrated the lifetime of such charge carriers in TiO$_2$, showing longer lifetimes for photoexcited electrons in the anatase crystal phase as compared to the rutile phase.$^{439}$ Dozzi et al., (2013) showed evidence of the impact of this difference in lifetimes for photoexcited electrons through the enhanced photocatalytic degradation rate of formic acid by fluorine-doped TiO$_2$ and analysis of the photoluminescent signal generated by trapped electrons.$^{440}$ Little difference was observed between lifetimes for photoexcited holes across the two different crystal phases.
2.4 Photocatalysis by Inorganic UV Filtering TiO$_2$ Nanoparticles

ROS Generation by Photoexcited TiO$_2$

Following excitation and the generation of photoexcited charge carriers in TiO$_2$, redox reactions with surface bound molecules may occur. In the context of an aqueous medium, the relevant ROS generating reactions that may occur at the surface of a TiO$_2$ particle are as follows:

\[
\text{TiO}_2 + hv \rightarrow \text{TiO}_2(e^-_{CB} + h^+_V) \quad (2.6)
\]

\[
\text{TiO}_2(h^+_V) + \text{H}_2\text{O} \rightarrow \text{TiO}_2 + \text{H}^+ + \text{OH}^* \quad (2.7)
\]

\[
\text{TiO}_2(e^-_{CB}) + \text{O}_2 \rightarrow \text{TiO}_2 + \text{O}_2^{*-} \quad (2.8)
\]

Subsequent dissociation of these free radical species from the surface of the TiO$_2$ particle gives these species free reign to react with other molecules present within the same chemical environment as the photocatalyst. Along with direct charge transfer reactions that may occur with chemically adsorbed molecules, this photocatalytic behaviour of TiO$_2$ and its ability to mineralize chemical compounds through surface mediated redox reactions, particularly organic compounds, is the reason behind its application in various photocatalysis application ranging from H$_2$ production, waste water purification and dye sensitized solar cells. It is also this property, along with the reduction in particle size to below 100 nm, that has raised concerns over its use in sunscreen products and the oxidative damage it may cause to other ingredients in the formulation and to the consumers using such products.

2.4.3 Consequences of a Photocatalyst in Sunscreen Products

Photostability of sunscreen formulations and active ingredients in such products is of major importance in regards to their ability to provide UV protection over the full duration expected. Many organic UV filters that were once incorporated in sunscreens have since been removed from commercial use due to photostability issues such as with PABA derived UV filters and those outlined earlier in Sections 2.2.4 and 2.2.5. Furthermore,
certain organic filters such as octyl methoxycinnamate and octocrylene have also been found to be responsible for the oxidative damage of other formulation ingredients through the generation of singlet oxygen (\(1^O_2\)). Adding to these concerns are the photoreactivity of both inorganic UV filters, ZnO and the highly photoactive TiO₂.

Both inorganic UV filters, as previously discussed, are photocatalytic by nature and can have a catastrophic impact on the efficacy of sunscreen formulations, particularly if left uncoated or in formulations lacking additional antioxidant ingredients. This issue is further propagated with the use of these compounds as nanoparticles, resulting in increased surface reactivity and photocatalytic activity. The interaction of organic UV filters with TiO₂ has been extensively studied. A study performed by Ricci et al., (2003) investigated the mineralization behaviour of organic UV filters in the presence of anatase TiO₂ nanoparticles (mean size 32 nm) and irradiated with UVA radiation (\(\lambda = 366 \text{ nm}\)). It was found that direct mineralization of the tested UV filters, which include octocrylene, oxybenzone, octyl salicylate and E-methoxycinnamic acid, occurred due to the presence of photoexcited TiO₂. In addition, photodegradation experiments in which the TiO₂ photocatalyst particles were separated from the organic UV filters by micelle encapsulation using sodium dodecyl sulfate (SDS) yielded enhanced degradation rates compared to those experiments performed in absence of SDS (Figure 2.21). The reason suggested was due to the production of ROS species initially by TiO₂ and H₂O and then the generation of highly reactive carbon-centred radicals by the subsequent ROS. Following on from this work, additional investigations on the photodegradation of organic UV filters and other sunscreen formulation ingredients by TiO₂ materials were performed, emphasizing the potential dangers of reduced sun protection when using TiO₂ and TiO₂ nanoparticles in sunscreen formulations. There was also an increased drive in developing methods for manipulating the photocatalytic behaviour of TiO₂, leading to the development of the modern sunscreen based TiO₂ nanomaterials that are used in formulations today.
2.5 Routes for Inhibiting Photocatalysis in TiO$_2$

Many different commercial varieties of TiO$_2$ and ZnO nanoparticles exist, some of which are used in sunscreen formulations. More often than not, these materials are coated with inert materials such as those mentioned previously without greatly impacting the UV attenuative properties of the core material. Furthermore, the crystal phase composition is carefully manipulated to suit the application needs. Table 2.3 lists examples of some manufactured TiO$_2$ and ZnO nanoparticles used in commercial products, including cosmetics, along with some of their physical properties. In this Section, an overview of different methods employed in manipulating and reducing the photocatalytic behaviour of TiO$_2$ nanomaterials is given, highlighting their impact on UV protection and their application in research and commercial products, including sunscreens. Due to the interlinking nature of certain materials properties, such particle size and surface area, the methods that shall be described and reviewed here will focus on those used specifically in photocatalysis research and commercial application of TiO$_2$. 

**Figure 2.21:** Generation of carbon-centred radicals through the photoexcitation of TiO$_2$ in the presence of SDS using fluorescence spectroscopy and 4-(3-hydroxy-2-methyl-4-quinolineoxy)-2,2,6,6-tetramethylpiperidine-1-oxyl as the free radical probe. The curves shown are from degradation experiments performed, from top to bottom, with: [SDS]=6.5x10$^{-4}$ M/[TiO$_2$]=0.0 mg mL$^{-1}$, [SDS]=6.5x10$^{-4}$ M/[TiO$_2$]=0.5 mg mL$^{-1}$, [SDS]=6.5x10$^{-4}$ M/[TiO$_2$]=1.0 mg mL$^{-1}$ and [SDS]=1.9x10$^{-4}$ M/[TiO$_2$]=1.0 mg mL$^{-1}$. Figure reproduced from Ricci et al, (2003).
### Table 2.3: List of commercial TiO$_2$ and ZnO nanoparticles.$^{59,447–449}$ TMCS and PMMA refer to trimethoxycaprylylsilane and polymethyl methacrylate.

<table>
<thead>
<tr>
<th>Commercial Name</th>
<th>Composition (%)</th>
<th>Crystal Phases (%)</th>
<th>Specific Surface Area</th>
<th>Mean Particle Size (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maxlight F-TS20</td>
<td>TiO$_2$ (75), SiO$_2$ (25)</td>
<td>Rutile (100)</td>
<td>55</td>
<td>100</td>
</tr>
<tr>
<td>MT-100Z</td>
<td>TiO$_2$ (75), Al$_2$O$_3$/Stearic Acid (25)</td>
<td>Rutile (100)</td>
<td>40-60</td>
<td>15</td>
</tr>
<tr>
<td>T-Lite SF</td>
<td>TiO$_2$ (84), Al(OH)$_3$ (7), dimethicone (4.5)</td>
<td>Rutile (100)</td>
<td>76</td>
<td>30-60 x 10</td>
</tr>
<tr>
<td>T-Lite SF-S</td>
<td>TiO$_2$ (78), Al(OH)$_3$ (3.5), SiO$_2$ (7.5), dimethicone (5.5)</td>
<td>Rutile (100)</td>
<td>71</td>
<td>30-60 x 10</td>
</tr>
<tr>
<td>Eusolex T-AVO</td>
<td>TiO$_2$, SiO$_2$</td>
<td>Rutile (100)</td>
<td>40-90</td>
<td>&lt;200</td>
</tr>
<tr>
<td>Dupont R-900</td>
<td>TiO$_2$</td>
<td>Rutile (100)</td>
<td>43</td>
<td></td>
</tr>
<tr>
<td>PW Covasil S-1</td>
<td>TiO$_2$ (&gt;90), TMCS (&lt;5), PMMA (5)</td>
<td>Anatase (80), Rutile (20)</td>
<td>40</td>
<td>28-32</td>
</tr>
<tr>
<td>Tego Sun TS Plus</td>
<td>TiO$_2$ (&gt;50), SiO$_2$ (10-25), TMCS (4.5)</td>
<td>Anatase (80), Rutile (20)</td>
<td>60</td>
<td>28-32</td>
</tr>
</tbody>
</table>
### 2.5 Routes for Inhibiting Photocatalysis in TiO$_2$

<table>
<thead>
<tr>
<th>Product</th>
<th>TiO$_2$ Phase</th>
<th>Anatase (%), Rutile (%)</th>
<th>Anatase (100%)</th>
<th>Rutile (100%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aerioxide P25</td>
<td>TiO$_2$ (100)</td>
<td>Anatase (80), Rutile (20)</td>
<td>53</td>
<td>25</td>
</tr>
<tr>
<td>Millenium PC 500</td>
<td>TiO$_2$ (100)</td>
<td>Anatase (100)</td>
<td>287</td>
<td>5-10</td>
</tr>
<tr>
<td>Millenium PC 50</td>
<td>TiO$_2$ (100)</td>
<td>Anatase (100)</td>
<td>45</td>
<td></td>
</tr>
<tr>
<td>Kerr-McGee</td>
<td>TiO$_2$ (100)</td>
<td>Anatase (100)</td>
<td>90</td>
<td>20</td>
</tr>
<tr>
<td>Hombikat UV 100</td>
<td>TiO$_2$ (100)</td>
<td>Anatase (100)</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Tranox A-K-1</td>
<td>TiO$_2$</td>
<td>Anatase (100)</td>
<td>90</td>
<td>20</td>
</tr>
<tr>
<td>Zinc Oxide NEUTRAL</td>
<td>ZnO ($\geq$ 95)</td>
<td></td>
<td>30-70</td>
<td>41</td>
</tr>
<tr>
<td>Tego Sun Z500</td>
<td>ZnO (&gt;99.5)</td>
<td></td>
<td></td>
<td>10-60</td>
</tr>
<tr>
<td>NANOX 200</td>
<td>ZnO (99)</td>
<td></td>
<td>17</td>
<td>&lt;200</td>
</tr>
<tr>
<td>Z COTE MAX</td>
<td>ZnO, dimethoxy-diphenylsilane, triethoxy-caprylylsilane</td>
<td>Wurtzite</td>
<td>12-24</td>
<td></td>
</tr>
<tr>
<td>Zinc Oxide NDM</td>
<td>ZnO (&gt;90), dimethicone (&lt;10)</td>
<td></td>
<td>10-70</td>
<td>&lt;50</td>
</tr>
<tr>
<td>Z-Ald</td>
<td>ZnO (100)</td>
<td>Wurtzite</td>
<td>12</td>
<td>42-79</td>
</tr>
</tbody>
</table>

#### 2.5.1 Crystal Phase Composition

The identify of the crystal phase of TiO$_2$ and its composition can have an impact on its photocatalytic properties. It is often suggested that the anatase crystal phase predominantly leads to greater photocatalytic activity, particularly towards the degradation of...
2.5 Routes for Inhibiting Photocatalysis in TiO$_2$

organic chemicals and molecules. Much debate and research has been conducted to investigate the cause for this phenomenon without a decisive explanation. However, studies have brought to light some possible explanations such as the larger band gap of anatase, the longer life-time of photoexcited charge carriers and differences in their mobility between phases as well as differences in surface properties.$^{425,450,451}$ Jiang et al, (2008) investigated the affect of particle size and crystal phase composition of TiO$_2$ on their ROS generating capacity.$^{452}$ A series of TiO$_2$ nanoparticle samples were prepared through a number of gas phase synthesis methods to produce powders of varying size and crystal phase composition. ROS generation was determined using a fluorescent dye which, upon oxidative modification produces a fluorescent derivative that may be differentiated through spectrofluorometry. It was found that amorphous and anatase particles, greater than 30 nm in size, produced the highest rates of ROS production (Figure 2.22). At the opposite end of ROS production was the rutile phase. The cause of these variances observed in this instance was suggested to be due to differences in the number of surface defect sites per unit area between the amorphous, anatase and rutile samples, with the general observation being that samples with larger particle sizes (and thus lower surface areas), displayed lower rates of ROS generation due to a reduction in these defect sites.

![Figure 2.22: ROS generated by TiO$_2$ nanoparticles of varying particle size and phase composition (left) before and (right) after surface area normalization. Figure reproduced from Jian et al, (2008).$^{452}$](image)

There is also substantial evidence to suggest certain compositions of anatase-rutile mixtures can outperform either single phase.$^{453,454}$ AEROXIDE® TiO$_2$ P25 (also known as Degussa P25), a popular reference material used in photocatalysis studies, is a TiO$_2$
nanopowder consisting of an anatase-rutile ratio of 4:1. This same ratio of anatase-rutile has also been found in certain sunscreen products containing TiO$_2$ which were shown to cause accelerated damage to the surface coatings of UV-resistant fluoropolymer-coated steel panels used in outdoor roofing applications.$^{53}$ A long-term study investigating the weathering affects of various commercial sunscreen formulations was performed on these coated steel panels by exposing the panels to outdoor conditions over a period of 6 or 12 weeks. It was found that a decrease in gloss, corresponding to a degradation in the surface coating, occurred for formulations containing TiO$_2$. Separation of these particles from the formulation and analysis using x-ray diffraction revealed a crystal phase composition similar to that of P25, the commercial photocatalyst mentioned previously. Subsequent electron paramagnetic resonance (EPR) spectroscopy was performed and revealed that a similar ROS generation rate for commercially used TiO$_2$ particles existed compared to that of P25. Such findings have eventuated in modification of the materials properties criteria in regards to the photocatalytic activity of inorganic UV filters by governing cosmetic and health regulating institutions, such as the SCCS (Section 2.2.4).

![Figure 2.23: Bar graph representation of EPR spectrum intensities highlighting the generation of the DMPO-spin adduct (spin trap for the OH• radical). Samples F and G refer to inorganic TiO$_2$ UV filters found in commercial sunscreens where F is purely rutile whilst G is an anatase/rutile mixture. Figure reproduced from Barker et al., (2008).$^{53}$](image)

### 2.5.2 Surface Passivation by Inert Coating

Surface passivation and manipulation of the surface chemistry of TiO$_2$ nanoparticles is an important step in developing photoinactive nanoparticles that can be considered 'safe' for use in commercial products. In regards to sunscreen based TiO$_2$, approved coating materials and compositions must be applied that demonstrate an ability to reduce the photocat-
alytic activity of the core material without compromising the effectiveness of the product or impart further toxicological effects. Although the use of specifically listed coating materials is recommended for use when developing commercial TiO$_2$ based materials, the SCCS’s stance on new and alternative coatings is as follows:

"Other cosmetic ingredients applied as stable coatings on TiO$_2$ nanomaterials can also be used, provided they can be demonstrated to the SCCS to be safe and the coatings do not affect the particle properties".455

The Australian TGA has a similar stance on coating materials, declaring that active ingredients for sunscreen products with new coating variants require adequate safety data and characterisation to assess suitability for use in sunscreen products.

Mentioned in an earlier section (Section 2.2.4), the types of coating materials applied include organic coatings based on alkoxytitanates, polysiloxanes, silanes and inorganic coatings based upon alumina, silica or zirconia (Table 2.3). Specialized coating materials using various polymers have also been investigated with the aim of minimising or removing the propensity of TiO$_2$ to generate ROS and prevent their interaction with other formulation ingredients. Often, coatings are also applied for reducing particle agglomeration and thus improving suspension stability and the shelf-life of commercial emulsions.

The photocatalytic activity of dimethicone and silica coated variants of TiO$_2$ towards isopropanol oxidation was investigated by Mitchnick et al, (1999).456 It was found that the highest oxidation rate, and thus highest photocatalytic activity, was achieved for uncoated TiO$_2$, whilst the dimethicone and silica coated TiO$_2$ showed reductions in oxidation rates of 45% and 57%, respectively. However, investigations by Rampaul et al, (2007) found that dimethicone coated TiO$_2$ rapidly degraded methylene blue dye under UVA illumination at a similar rate to that of the known photocatalyst Degussa P25 and induced cell death in human skin cells.457 A possible reason for the difference in photocatalytic performance here as compared to Mitchnick, (1999) could be due to the crystal phase composition of the TiO$_2$ used, with Rampaul, (2007) using TiO$_2$ with 80-90% anatase phase whilst the crystal phase composition in the Mitchnick study is not mentioned. Regardless, the re-
2.5 Routes for Inhibiting Photocatalysis in TiO$_2$

Results suggest that certain coating materials are insufficient in reducing the photocatalytic performance of certain TiO$_2$ crystal phase compositions. Further evidence of this was shown by Carlotti et al., (2009).$^{59}$ In this work, the linoleic acid peroxidation by various commercial TiO$_2$ nanoparticle products when exposed to a UVB lamp was investigated. Ranked in order of activity, the commercial products that displayed the most substantial linoleic acid peroxidation activity included PW Covasil S-1, Aeroxide P25 and T-Lite SF (Figure 2.24).

![Figure 2.24](image)

Figure 2.24: Malondialdehyde production as a result of linoleic acid peroxidation after 2 hrs of UVB irradiation in absence and in the presence of 0.05% w/w (white bars) or 1.0% (grey bars) TiO$_2$ based sample. Figure reproduced from Carlotti et al., (2009)$^{59}$

Of these substantially active materials, PW Covasil S-1 consisted of a crystal phase composition similar to that of the known photocatalyst Aeroxide P25 (approximately 80% anatase, 20% rutile). The coating composition for this sample consisted of a combination of PMMA and TMCS (Table 2.3). The crystal phase composition of T-Lite SF on the other hand is purely rutile, however, the coatings employed were low loadings of Al(OH)$_3$ and dimethicone. The remaining commercial samples tested consisted of samples with TiO$_2$ phases primarily of the rutile phase except for Tego Sun TS Plus which, again, had a similar crystal composition to that of Aeroxide P25. Despite this problematic phase composition, the activity of the sample was greatly reduced compared to its uncoated variant, which could be attributed to the SiO$_2$ coating used. This could also explain why the commercial product T-Lite SF-S was amongst the samples with reportedly low photocatalytic activity. Both T-Lite SF and T-Lite SF-S consist purely of the rutile crystal phase and have similar particle morphologies and sizes, yet the activity determined was vastly different. The major difference between the two is in the coating composition employed, with T-
2.5 Routes for Inhibiting Photocatalysis in TiO$_2$

Lite SF-S containing an additional coating component of SiO$_2$ as compared to the T-Lite SF coating composition of Al(OH)$_3$ and dimethicone. It is therefore evident that both the type of coating and the crystal phase composition work hand in hand in reducing the photocatalytic activity of the core material and need to be carefully considered depending on the application in which they are employed.

For sunscreen based products, ideally the coating material employed should display inhibitory effects for all photocatalytically active TiO$_2$, regardless of the crystal phase, yet it has been shown that this is not the case. It is also important that the UV filtering capabilities of the core TiO$_2$ are not impacted or diminished and in fact are, ideally, improved. A more specialized coating material that has been investigated but not yet commercialized is lignin. Lignin is a biopolymer naturally produced in plants and as a by-product in the production of paper. Studies have also suggested lignin can act as a free radical scavenger, making it an ideal ingredient for cosmetic formulations and other topical products such as sunscreens.$^{458,459}$ Investigations of lignin/TiO$_2$ based nanocomposites have yielded materials displaying substantially reduced photocatalytic activity, towards both anatase and rutile crystal phases, whilst also serving to maintain and improve the UV attenuative properties of the core material.$^{460,461}$ Direct grafting of antioxidant compounds to the surface of TiO$_2$ nanoparticles have also been investigated as a means of inhibiting free-radical production through novel methodologies.$^{462,463}$ Yet the use of organic based antioxidants presents in itself compatibility issues with other formulation ingredients in a similar manner to those for organic UV filters outlined in Section 2.2.5. Although manufacturers of cosmetic and therapeutic UV filters prepare these materials to address issues surrounding the use of nanoparticulate TiO$_2$, certain drawbacks have been highlighted with the current coating materials used, as outlined above.

2.5.3 Elemental Doping

Doping of TiO$_2$ has been extensively investigated, primarily with the view of improving the visible light responsiveness of the material and the aim of driving future application into solar cells and visible light driven catalysis. Few research works specifically study
the effect of doping into TiO$_2$ with the purpose of minimizing photocatalytic activity and fewer still report such findings. Implantation of metal or non-metal ions into interstitial sites or as substitutional dopants into the TiO$_2$ crystal lattice has been shown to modify the electronic properties of the core material.\[^{464-467}\] These modifications can have ramifications on the photocatalytic and light absorptive properties of TiO$_2$ and can influence particle growth, crystal phase expression and crystallinity.\[^{468-470}\]

Various metal dopants have been investigated for improving the photocatalytic activity of TiO$_2$ nanoparticles including transition metals such as cobalt (Co$^{2+}$), barium (Ba$^{2+}$), nickel (Ni$^{2+}$), copper (Cu$^{2+}$), zinc (Zn$^{2+}$) and iron (Fe$^{3+}$) as well as various rare-earth metals such as lanthanum (La$^{3+}$), cerium (Ce$^{4+}$), samarium (Sm$^{3+}$), europium (Eu$^{3+}$) and ytterbium (Yb$^{2+}$).\[^{471-481}\] Such dopants have been shown to improve the photocatalytic activity of TiO$_2$ whilst under visible light illumination by shifting the absorption characteristics of the material to the lower energy visible light region. The cause for this shift in the absorption band of metal doped TiO$_2$ has been ascribed to be due to a shrinking of the band gap as a result of the introduction of mid-band gap impurity states that can act as trap sites for photoexcited species. This trapping behaviour in turn can reduce recombination rates of $e^-/h^+$ pairs and improve the photocatalysis efficiency. Yan et al, (2012) found that doping TiO$_2$ with cerium (Ce$^{4+}$) at a Ce:Ti ratio of 0.33% resulted in an improvement in visible light catalysis and red-shift in absorption properties.\[^{480}\] The reason suggested for these results was attributed to the presence of additional electronic states just above the valence band of TiO$_2$, which aided in capturing photoexcited h$^+$ and decreasing recombination. It’s important to note however that the affect on photocatalytic activity is also dependent on the dopant loading concentration and the synthesis method employed, which can affect the type of doping that occurs. Although some reports have shown Fe$^{3+}$ can improve the photocatalytic activity of TiO$_2$, others have shown that excessive doping can impact the particle growth, specific surface area and, subsequently, the photoactivity.\[^{482}\] Li et al, (2008) also contributed to the idea of needing to optimize the dopant concentration for improving photocatalytic performance with their work on Fe-doped TiO$_2$ prepared through a hydrothermal method.\[^{483}\] They suggested that, al-
though doping led to a narrowing of the band gap, even with increasing dopant load, the same increase in photocatalytic performance wasn’t achieved due to the location of the $\text{Fe}^{3+}$ dopant deeper within the bulk of the TiO$_2$ particles as opposed to the surface. What this means is that, although trapping of photoexcited charge carriers could occur at these sites, migration to the surface could not be achieved efficiently, thus could not contribute effectively to the photocatalysis.

One particularly interesting transition metal dopant for TiO$_2$ in sunscreen products is manganese. Wakefield et al., (2004) synthesized 1% w/w Mn-doped TiO$_2$ through a sol-gel method and found that the doped materials displayed enhanced UVA protection and provided broad UV protection relative to undoped TiO$_2$. Additionally, the free radical generation rate of the doped material was found to be reduced, in turn, leading to a reduction in photocatalytic activity. The reason for this decrease was suggested to be due to a form of free radical scavenging effect instilled by the presence of surface $\text{Mn}^{3+}/\text{Mn}^{4+}$ species. The significance of this finding ultimately culminated in the commercialization of this doped material under the trade name Optisol$^{\text{TM}}$. Non-sunscreen specific cosmetic products and sunscreens in the EU may be found containing this UV filter.

Non-metal doping of TiO$_2$ nanoparticles has also been extensively studied particularly with nitrogen, sulfur, phosphorus and carbon. In all cases however, the primary purpose of the non-metal doping process is for improvement in photocatalysis, particu-
larly driven by visible light excitation. N doping into TiO$_2$ was one of the first non-metal dopants employed for increasing the TiO$_2$ photocatalysis under visible light illumination.$^{489}$ It is also regarded as one of the most efficient dopants however the exact mechanism for the improvements in photocatalytic performance is uncertain.$^{467,489,490}$ It has been suggested that N doping can shrink the band gap of TiO$_2$ due to hybridisation of lower energy N 2$p$ states and higher energy O 2$p$ states, thus allowing visible light absorption.$^{489}$ Another approach is that N doping introduces an impurity energy level just above the valence bond from which electrons may be excited from to the conduction band by visible light illumination. Zhao et al (2007) also made the suggestion that the dopant position influences the modification mechanism, as with metal dopants previously discussed.$^{491}$ It was suggested that substitutionally doped N introduced shallow acceptor states above the valence band whilst interstitially doped N created isolated impurity states from which electrons could be excited from.

Again, as with metal doping, a saturation point is reached with non-metal doping whereby the introduction of additional dopants leads to a gradual decrease in photocatalytic performance. Unfortunately, this also tends to coincide with a decrease in the absorptive performance of the core TiO$_2$ thus is not a particularly viable approach in mitigating the photocatalytic potential of sunscreen based TiO$_2$, without some compromise.

### 2.6 Emerging Nanomaterials as Possible UV Filters

Maybe in part due to the limited number of different inorganic UV filters currently approved by global regulatory bodies or due to the shortcomings of either ZnO or TiO$_2$, significant effort has gone into investigating and developing alternative inorganic based UV filtering nanomaterials. The key characteristics needed to be displayed by any potential UV filtering ingredient include high UV attenuation (UVA, UVB or both), photostability, low photocatalytic activity, low persistence in skin and low toxicity, although adherence to commercial requirements must also be considered such as the ease of synthesis scalability.
2.6 Emerging Nanomaterials as Possible UV Filters

**Cerium Oxides**

A promising metal oxide material that has garnered significant attention is cerium oxide (CeO\(_2\)), most notably in the form of nanoparticles. As with TiO\(_2\) and ZnO, CeO\(_2\) is a semiconductor material with a relatively wide band gap (3.19 eV for bulk crystals) and has been shown to display UV absorptive properties.\(^{492}\) In addition, manipulation of the size of CeO\(_2\) has been shown to enable tailoring of these optical properties, with decreasing particle size leading to an increase in transparency across visible light wavelengths and a widening of the band gap.\(^{493,494}\) Doping has also proven to be an effective means of modifying the UV absorptive properties of CeO\(_2\), enabling a shift in the absorbance band to more biologically relevant wavelengths in the UVB and UVA wavelength regions. Yabe *et al.*, (2001) investigated the UV-shielding properties of CeO\(_2\) nanoparticles doped with various metal ions including Mg\(^{2+}\), Ca\(^{2+}\), Sr\(^{2+}\), Ba\(^{2+}\) and Zn\(^{2+}\).\(^{495}\) It was found that doping with 20 mol\% of either Ca\(^{2+}\) or Zn\(^{2+}\) resulted in a reduction in photocatalytic activity and increase in visible light transparency. A further study by Truffault *et al.*, (2010) on co-precipitated Ca-doped CeO\(_2\) nanoparticles also demonstrated modification of the optical properties of CeO\(_2\) with a blue-shift and increase in UVB absorbance being absorbed for 10 mol\% Ca-doped CeO\(_2\), as compared to pure CeO\(_2\).\(^{61}\) Incorporation of these 10 mol\% Ca-doped CeO\(_2\) nanoparticles into sunscreen emulsion was also performed by this same group and a comparative study to TiO\(_2\)/ZnO nanoparticle emulsions on the SPF and UVAPF was conducted.\(^{496}\) Mixed formulations consisting of the doped CeO\(_2\) and TiO\(_2\) were prepared and compared to an emulsion consisting of TiO\(_2\) and ZnO. Although no significant difference in UVAPF was determined, it was found that the Ca-doped CeO\(_2\)/TiO\(_2\) emulsion displayed a higher SPF rating as compared to the TiO\(_2\)/ZnO emulsion. In addition, the critical wavelength of the emulsion containing Ca-doped CeO\(_2\) was determined to be 373 nm, implying broad spectrum protection.
CeO$_2$ nanoparticles have also garnered recent interest due to their potential to behave as antioxidants in biological systems. CeO$_2$ nanoparticles in the size range of 6 and 12 nm were shown to have a little toxicological effect on mouse nerve (HT22) and macrophage (RAW164) cell lines when incubated together for 24 hr up to concentrations of 100 $\mu$g/mL.\(^{497}\) The effect of CeO$_2$ nanoparticles (4-5 nm) and TiO$_2$ nanoparticles (7-10 nm) on the modulation of immune response by human dendritic cells was performed by Schanen et al (2013).\(^{498}\) In comparison to the TiO$_2$ nanoparticles tested, which were found to induce a pro-inflammatory response through Th1 activation, the CeO$_2$ nanoparticles were demonstrated to have the opposite effect by inducing the production of IL-10, indicative of Th2 activation. The disparity in activation pathways between the two metal oxides was suggested to be due to differences in surface reactivity and ROS generation capacity. Subsequent examination of the intracellular oxidative stress levels of the tested human dendritic cells when exposed to these nanoparticles revealed that the CeO$_2$ nanoparticles triggered little to no ROS generation and were capable of inhibiting ROS production in cells exposed to H$_2$O$_2$. In contrast, the TiO$_2$ nanoparticles were shown to generate ROS in a dose-dependent manner. Thus, it was demonstrated that CeO$_2$ nanoparticles may display potent antioxidant properties and catalase-like mimetic activity. CeO$_2$ nanoparticles often have a non-stoichiometric surface consisting of Ce atoms in a combination of the 3+ and 4+ oxidation states.\(^{497}\) Evidence from X-ray photoelectron spectroscopy (XPS)
studies have shown that the ratio of these two oxidation states can be tuned with particle size, whereby, decreasing particle size leads to an increase in Ce\(^{3+}\) relative to Ce\(^{4+}\).\(^{499}\) The mixed valent state of this compound leads to the presence of oxygen vacancies, so as to maintain charge neutrality, and has been suggested to be responsible for the unique redox properties displayed by the compound.\(^{500}\) The combination of both these antioxidant and UV absorptive properties highlight the potential of CeO\(_2\) nanoparticles as an inorganic UV filter in sunscreen products, however, further toxicological characterisation of these metal oxide nanoparticles is needed. In particular, evaluation of the dermal permeation of these particles is essential and currently lacking. An in vitro Franz-diffusion cell study using damaged and intact human skin exposed to CeO\(_2\) nanoparticles (17±5 nm) has shown that little dermal penetration occurs, with the little amount of Ce detected through energy dispersive spectroscopy more likely due to ionized CeO\(_2\) and not CeO\(_2\) nanoparticles.\(^{501}\)

**Iron Oxides**

The use of natural minerals containing iron oxides as a skin protecting agents is no novel idea (Section 2.2.1). However, the application of iron oxide nanoparticles, in particular those with the hematite (\(\alpha\)-Fe\(_2\)O\(_3\)) crystal phase, in cosmetic products is a modern development. Specific application of \(\alpha\)-Fe\(_2\)O\(_3\) in sunscreen products is limited due to the intense colouring of formulation containing these pigments (band gap values around 2.2 eV), however, this has not dampened efforts into investigating and characterising \(\alpha\)-Fe\(_2\)O\(_3\) and modified \(\alpha\)-Fe\(_2\)O\(_3\) nanoparticles as a potential inorganic UV filter.\(^{502}\) Truffault et al, (2011) prepared chemically precipitated \(\alpha\)-Fe\(_2\)O\(_3\) nanoparticles through post-synthesis calcination.\(^{503}\) Measurement of the in vitro UVAPF for emulsions containing these nanoparticles yielded higher UVA protection as compared to emulsions containing either TiO\(_2\) or ZnO at the same mass loadings. Modification of \(\alpha\)-Fe\(_2\)O\(_3\) through doping with Ce\(^{3+}\) has also been shown to aid in improving UVA and UVB attenuation and shrinking the optical band gap.\(^{502}\) Nanocomposite materials consisting of varying loadings of CeO\(_2\) with \(\alpha\)-Fe\(_2\)O\(_3\) can also modify the absorption profile of the core iron oxide material, leading to an increase in UV attenuation.\(^{62}\) Simultaneously, a reduction in
the photocatalyzed degradation of crystal violet dye, owing to the presence of the CeO$_2$ nanoparticles, was also observed.

Another nanocomposite consisting of $\alpha$-Fe$_2$O$_3$ with hydroxyapatite (Ca$_{10}$(PO$_4$)$_6$(OH)$_2$) has also been developed from waste cod fish bones and investigated for its UV protective potential.$^{504}$ Sunscreen formulations incorporating the nanocomposite materials were shown to display broad spectrum protection across both the UVA and UVB wavelength regions whilst also displaying a high level of photostability and low skin irritability potential. Skin permeation studies of $\alpha$-Fe$_2$O$_3$, as with CeO$_2$ nanoparticles, are limited, with most investigations focussed on superparamagnetic iron oxide nanomaterials (those with either the maghemite ($\gamma$-Fe$_2$O$_3$) or magnetite (Fe$_3$O$_4$) crystal phases). Studies involving these magnetic iron oxide nanomaterials present conflicting results in regards to their ability to permeate skin, with some suggesting extensive translocation to the viable epidermis and dermis whilst others showing minimal permeation through the stratum corneum.$^{505-507}$ However, as suggested by one author, the differences in the results presented in these studies could be contributed to the differences in the surface modifications of the particles used, the skin model employed and application conditions. Concerns with the cytotoxic and genotoxic potential of iron based nanomaterials have arisen due to their potential to generate ROS through the Fenton reaction (Equation 2.5). However, not all
iron-based materials are toxic or generate ROS as it has been shown that these properties are strongly dependent on the coordination of surface bound iron ions which can be manipulated through the synthesis method employed to produce the material.\textsuperscript{508–510} 

\textit{In vitro} investigations into the cytotoxicity of iron oxides also yield a range of results. In one study, $\alpha$-Fe$_2$O$_3$ nanoparticles were shown to have size-dependent and synthesis-dependent cytotoxic and ROS-generative activity towards cultured epithelial canine kidney cells (MDCK).\textsuperscript{511} It was suggested in this work that localized defect states (termed T-defects), introduced by specific synthesis conditions and particle size parameters, impart certain free-radical scavenging properties similar to that of CeO$_2$, thus resulting in improved biocompatibility towards MDCK cells. Another work by Freyria \textit{et al}, (2012) also investigated structural defects in hematite particles, ranging from nanometric (90 nm) to micrometric (2 \(\mu\)m), and any potential correlation in toxicity towards murine alveolar macrophages (MH-S) and human lung epithelial cells (A549). Minimal change in surface defect states corresponding to the presence of surface bound Fe$^{2+}$ between the particle samples tested also correlated with minimal changes in cytotoxic and genotoxic potential, as measured through \textit{in vitro} LDH release and Comet assays, and overall low toxicity. However, it was stipulated that further decreases in particle size may lead to an increase in surface Fe$^{2+}$ defect states, which may promote ROS generation through the Fentation reaction (Equation 2.5), thus resulting in greater toxicity through oxidative cellular damage.

\textbf{Other Potential Organic/Inorganic and Hybrid UV Filters}

Tin oxide (SnO$_2$) nanoparticles have also been demonstrated to display optical properties suitable for UV filtering. With a wide band gap of 3.60 eV for bulk SnO$_2$, nanoparticulate SnO$_2$ has great transparency in the visible light range and generally appears white or pale yellow in powder form, thus appeasing cosmetic aesthetic requirements similar to that of TiO$_2$ and ZnO. Doping of SnO$_2$ with Ti$^{4+}$ has also been shown to manipulate the band gap properties of the material, with increasing Ti content resulting in further widening of the SnO$_2$ band gap.\textsuperscript{512} Although the photocatalytic degradation of methylene blue dye of the doped material was observed to increase under UV irradiation, the photocatalytic
activity on the whole for both doped and undoped SnO$_2$ was significantly less than that of commercial ZnO and TiO$_2$ nanoparticles.

To address consumer concerns over the skin penetration of sunscreen active ingredients, methods for encapsulating UV filters have been developed. Deng et al, (2015) developed so-called 'bioadhesive' polylactic acid (PLA) nanoparticles able to encapsulate the organic UV filter, Padimate O. They demonstrated that these PLA nanoparticles remained adhered to the outer layers of the stratum corneum and prevented epidermal or follicular penetration of the encapsulated organic UV filter. Furthermore, the UV protective properties of the PLA-encapsulated Padimate O were shown to provide greater protection against double-stranded DNA breaks in vivo on murine models as compared to a commercial sunscreen formulation. Hybrid organic/inorganic UV filter materials have also been investigated for encapsulation of UV filter ingredients, such as with the encapsulation of octyl salicylate by an organic/inorganic polysilisesquioxane/silica shell. Although leaching and photodegradation of the organic UV filter was prevented such complexities in the synthesis method would like hamper any commercial viability.
Chapter 3

Experimental Methods

3.1 Synthesis of Nanomaterials

3.1.1 Synthesis of Spray-Dried Chitosan and Chitosan/TiO$_2$ Nanocomposite Particles

For the preparation of the chitosan and chitosan/TiO$_2$ nanocomposite materials, desired quantities of chitosan powder (from Shrimp shells, $\geq$75% deacetylated, Sigma Aldrich) and commercial photocatalyst TiO$_2$ powder (P25, Degussa Evonik) were weighed and transferred to a beaker containing a solution of 3% v/v aqueous acetic acid (CH$_3$COOH, Sigma Aldrich) in deionized (DI) water such that the theoretical weight ratios of chitosan to TiO$_2$ were 2:1, 1:1 and 1:0 (in the case of the purely chitosan sample). The solution was left to stir overnight so as to ensure homogeneity before being spray-dried. As seen in Figure 3.1, the suspension is fed through a 0.7 mm spray drying nozzle with the aid of a peristaltic pump at a flow rate of 100 mL hr$^{-1}$. The nozzle is connected to an air pump system that atomizes the solution, whilst a hot air stream (inlet temperature of 120°C and outlet temperature of 40°C) is applied in co-current flow, leading to the drying of the polymer nanocomposite droplets, and subsequently to solid particle formation. The resultant chitosan and chitosan/TiO$_2$ nanocomposite particles were cross-linked via a vapour phase process using a heated vacuum desiccator system (JP Selecta S.A.) set at 25°C and in the presence of glutaraldehyde (OHC(CH$_2$)$_3$CHO, 50% in H$_2$O, Sigma Aldrich) for 48 hr.
3.1 Synthesis of Nanomaterials

Figure 3.1: Schematic representation of the spray drying process used to produce the chitosan and chitosan/TiO₂ nanocomposite particles.

Table 3.1: Sample details and coding used for the samples prepared and described in Sections 3.1.1 and Chapter 4.

<table>
<thead>
<tr>
<th>Sample Details</th>
<th>Sample Code</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chitosan</td>
<td>CHI</td>
</tr>
<tr>
<td>50 wt% chitosan, 50 wt% TiO₂</td>
<td>1:1 CHI/TiO₂</td>
</tr>
<tr>
<td>67 wt% chitosan, 33 wt% TiO₂</td>
<td>2:1 CHI/TiO₂</td>
</tr>
</tbody>
</table>

3.1.2 Synthesis of CeO₂ Decorated Commercial TiO₂ Nanoparticles

The synthesis of the CeO₂ decorated TiO₂ nanoparticles follows a similar process previously outlined by Cardillo et al.² In summation, a suspension of the core TiO₂ nanoparticles (0.5 g of P25) was prepared in 50 mL of DI water. Relative amounts of cerium (III) nitrate hexahydrate (Ce(NO₃)₃·6H₂O, 99%, Sigma Aldrich) were added so as to yield relative ratios of the number of Ce atoms to the number of Ti atoms (atomic concentration; at%) of 2.5, 5 and 10 at%. The suspension was heated to 60°C before 1 mL of concentrated ammonium hydroxide (NH₄OH, 28 - 30% NH₃ basis, Sigma Aldrich) was
3.1 Synthesis of Nanomaterials

added drop wise, followed by the addition of 1 mL of hydrogen peroxide (H\textsubscript{2}O\textsubscript{2}, 30 wt% in H\textsubscript{2}O, Sigma Aldrich). The precipitants were collected via centrifugation (12,840 × g for 10 min) and washed several times with DI water and ethanol (EtOH, absolute, Chem-Supply) before being dried at 100°C overnight and ground into a fine powder. A sample of purely CeO\textsubscript{2} was prepared in the same manner as described but in absence of the core TiO\textsubscript{2} nanoparticles.

Table 3.2: Sample details and coding used for the samples prepared and described in Sections 3.1.2 and Chapter 5.

<table>
<thead>
<tr>
<th>Sample Details</th>
<th>Sample Coding</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pristine CeO\textsubscript{2}</td>
<td>CeO\textsubscript{2}</td>
</tr>
<tr>
<td>Degussa Evonik TiO\textsubscript{2} nanoparticles</td>
<td>TiO\textsubscript{2} (P25)</td>
</tr>
<tr>
<td>2.5 at% CeO\textsubscript{2} decorated TiO\textsubscript{2} (P25)</td>
<td>2.5% CeO\textsubscript{2}/TiO\textsubscript{2}</td>
</tr>
<tr>
<td>5 at% CeO\textsubscript{2} decorated TiO\textsubscript{2} (P25)</td>
<td>5% CeO\textsubscript{2}/TiO\textsubscript{2}</td>
</tr>
<tr>
<td>10 at% CeO\textsubscript{2} decorated TiO\textsubscript{2} (P25)</td>
<td>10% CeO\textsubscript{2}/TiO\textsubscript{2}</td>
</tr>
</tbody>
</table>

3.1.3 Synthesis of Rutile TiO\textsubscript{2} Nanorods and CeO\textsubscript{2}/Rutile TiO\textsubscript{2} Nanocomposite Particles

Preparation of the CeO\textsubscript{2}/rutile TiO\textsubscript{2} nanocomposite involved a multi-step method involving precipitation and hydrothermal reaction methods. A schematic representation of the reactions involved is highlighted in Figure 3.2.

Hydrothermal Synthesis of Rutile TiO\textsubscript{2}

The rutile TiO\textsubscript{2} nanorods were synthesized through a two-step process based upon a similar procedure previously outlined by Bu et al.\textsuperscript{513} The first step involved the generation of amorphous TiO\textsubscript{2} from the precursor source, titanium butoxide (TBT, 97%, Sigma Aldrich). Typically, 10 mL of TBT was dissolved in 40 mL of warmed EtOH. Separately, a solution of 0.5 M NH\textsubscript{4}OH was prepared. To the dissolved TBT, 75 mL of the 0.5 M NH\textsubscript{4}OH was added drop-wise under vigorous stirring. The resultant suspension was stirred a further 30 min before being collected via centrifugation (12,840 × g for 10
3.1 Synthesis of Nanomaterials

min) and washed multiple times with DI water and EtOH. The precipitant obtained was then dried at 90°C for 12 hr then ground into a powder with a mortar and pestle. The second step of the synthesis involved the hydrothermal synthesis of the rutile TiO\textsubscript{2} nanorods from acidic media. A suspension of the amorphous TiO\textsubscript{2} was prepared in 10 mL of nitric acid (HNO\textsubscript{3}, 70%, Sigma Aldrich) at various concentrations and sonicated for an hour (Branson 3800, Ultrasonics Corp.). The concentration of acid used was adjusted through 3 – 16 M by diluting in DI water. After sonication, the suspension was transferred to a 45 mL Teflon cup and sealed in an acid digestion vessel (Parr Instruments). The vessel was then transferred to an oven and heated for 24 hr at either 150°C or 180°C so as to assess the temperature effects on the resultant material. After cooling back to room temperature, a white precipitate was obtained. The suspended precipitate was carefully diluted in DI water to reduce the acid concentration before being separated via centrifugation (12,840 × g for 10 min). The separated solid was further diluted with DI water and EtOH before being dried in air at 100°C for 12 hr. A fine powder was obtained after crushing the dried product with a mortar and pestle.

Figure 3.2: Schematic representation of the HTIO\textsubscript{2} and CTIO\textsubscript{2} synthesis methods.
3.2 Materials Characterisation

Precipitation of the CeO₂/Rutile TiO₂ Nanocomposite

As in Section 3.1.2, a suspension of the rutile TiO₂ nanorods (HTIO2, Table 3.3, 0.5 g) was prepared in 50 mL of DI water. To the suspension, an amount of Ce(NO₃)₃·6H₂O was added so as to give a relative weight percentage of Ce/Ti of 7.5 wt%. The suspension was then heated to 60°C before the addition of, firstly, 1 mL of concentrated NH₄OH and 1 mL of H₂O₂ with rapid stirring. The resulting precipitate was collected via centrifugation (12,840 × g for 10 min) and washed several times with DI water and EtOH before being dried at 100°C overnight. The final nanocomposite, henceforth denoted CTIO2, was obtained by grinding the dried product into a fine powder.

Table 3.3: Sample details and coding used for the samples prepared and described in Sections 3.1.3, 3.1.3 and Chapter 6.

<table>
<thead>
<tr>
<th>Sample Details</th>
<th>Sample Coding</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rutile TiO₂ nanoparticles</td>
<td></td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>HNO₃ concentration (M)</td>
</tr>
<tr>
<td>180</td>
<td>3</td>
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<tr>
<td>180</td>
<td>6</td>
</tr>
<tr>
<td>180</td>
<td>16</td>
</tr>
<tr>
<td>150</td>
<td>16</td>
</tr>
<tr>
<td>CeO₂/TiO₂ nanocomposite</td>
<td>CTIO2</td>
</tr>
</tbody>
</table>

3.2 Materials Characterisation

A number of techniques were employed to characterise and assess the morphological, optical, thermal, photocatalytic and toxicological properties of the nanomaterials synthesized. This section provides a description of the experimental protocol, parameters and conditions employed using these instrumental and experimental techniques.
3.2 Materials Characterisation

3.2.1 X-Ray Diffraction

X-ray radiation is a subset of electromagnetic radiation spanning the wavelength range of 0.01 to 10 nm. This type of radiation is used in X-ray crystallography as an analytical means of differentiating between different crystal structures due to having wavelengths close in length to the separation between different crystal planes making up the crystal structure.\(^\text{514}\)

Long range periodicity in crystals are described by translational vectors and is governed by one of seven symmetry systems. Local variation in crystal structure about particular atoms in the crystal structure can also lead to localized/point symmetries. The combination of both translational and point symmetry elements leads to further new spatial symmetry elements and differences from one crystalline compound to another. Generation of a diffraction pattern by x-ray radiation passing through a crystalline solid can be used to differentiate between different crystal structures or crystal phases and can be semi-qualitatively used to determine the presence of particular elements/compounds in a given sample. The angular positions at which these diffraction events occur is defined by the Bragg law:

\[
2d \sin(\theta) = n\lambda
\]

(3.1)

where \(d\) is the interplanar spacing between diffracting planes (nm) with Miller indices \((hkl)\), \(\theta\) the angle of the incident x-ray beam (°), \(n\) a integer value defining the diffraction order and \(\lambda\) the wavelength of the incident x-ray beam. Determining the angular position of diffraction allows the calculation of the lattice parameters (translational vector lengths, \(a, b, c\) and the vector angles \(\alpha, \beta, \gamma\)) that define the basic repeating unit of the crystal structure being examined, termed the unit cell. In this manner, the diffraction pattern obtained for a particular crystal structure can be used as a fingerprint for crystal phase identification, with reference to standard Joint Committee for Powder Diffraction Standards (JCPDS) files for that crystal phase in question.

X-ray diffraction (XRD) was performed on the powdered forms of the nanomaterials synthesized in order to characterise the crystal phase composition and crystallinity. The XRD patterns for the as-prepared samples were obtained using a GBC Mini-Materials Analyser.
3.2 Materials Characterisation

(MMA) X-ray Diffractometer (XRD) (GBC Scientific Equipment) coupled with a Cu Kα x-ray source. Samples for analysis were prepared by mixing a small portion of powder sample with ethanol in a agate mortar and pestle to make a slurry. The slurry was then drop cast onto a quartz glass slide to form a thin film and allowed to dry before analysis. Diffraction scans were obtained between 2θ = 20 - 90° at a scan rate of 1.5° min⁻¹ and step size of 0.020. The mean crystallite sizes were also approximated using the Scherrer equation:

\[ \tau = \frac{\kappa \lambda}{\beta \cos \theta} \]  

(3.2)

Where \( \tau \) is the mean crystallite size in the direction normal to the diffraction plane \( h k l \) (nm), \( \kappa \) a constant shape factor (0.9 used for unknown particle sizes), \( \lambda \) the wavelength of incident X-ray radiation (nm), \( \theta \) the angle of diffraction (radians) and \( \beta \) the full width half maximum or line broadening of the selected peak, taking into account the observed broadening of the sample and the broadening due to the instrumental arrangement (radians). The diffraction patterns obtained were examined and fitted using the Match! software package.

Figure 3.3: GBC Mini-Materials Analyser X-ray Diffractometer (interior) and sample holder.
3.2 Materials Characterisation

3.2.2 Electron Microscopy

Scanning Electron Microscopy

Scanning electron microscopy (SEM) is a powerful tool for viewing topographical and morphological features of a sample at extremely high magnification (ranging from $20 \times$ to $>500,000 \times$) with nanoscale resolution (1 nm imaging). The use an electron beam probe scanning across the sample enables the indirect viewing of the sample surface and its morphological/topographical features. Interaction of the incident electron beam with a sample can result in the production of a variety of signals depending on the interaction volume. This interaction volume is dependent on a few factors including:

- The atomic number of the element(s) present in the sample: higher atomic number elements absorb or impede more electrons, reducing the interaction volume.
- Acceleration voltage used: a higher energy will result in greater interaction volume.
- Angle of the incident electron beam: a greater angle from normal results in a smaller interaction volume.

SEM images are produced due to secondary electron emission from the sample. Secondary electrons (SE) originate from or close to the sample surface and are due to inelastic collisions between the primary electron beam (and also some backscattered electrons) and electrons orbiting the specimen atoms. These orbiting electrons are sufficiently excited to overcome the work-function for that atom and are ejected with low kinetic energies (2-5 eV). Because such a small amount of energy is lost from the initial electron beam (usually accelerated at energies between 5-50 keV), multiple SE’s can be produced by a single incident electron. As a result of the low energy of SE’s, their mean free path is quite small as they themselves are quite easily scattered and so, as mentioned earlier, only those ejected near the surface of the sample can be collected and analysed. Changes in topography of the sample will result in a change in the number of SE’s produced. In this manner, as the incident electron beam is rastered across the specimen surface, a contrast image can be produced based upon the intensity/count of ejected secondary electrons.
3.2 Materials Characterisation

**Figure 3.4:** (left) Interaction volume generated by incident electron beam and generation of secondary electrons (SE). (right) JEOL JSM-7500FA field emission electron microscope. Figure (right) reproduced from JEOL.515

SEM images were obtained using a JSM-7500FA field emission electron microscope (JEOL). Samples for imaging were prepared by spreading a small quantity of powdered sample onto a small section of double-sided sticky carbon tape attached to an aluminium stub. To improve image quality and reduce charging effects, the samples were coated with a thin layer of platinum (Pt) using a Sputter Coater (Dynavac). The conditions for imaging generally consisted of using an accelerating voltage of 5 kV, emission current of 10 μA and spot size of 8.

**Transmission Electron Microscopy**

As the name suggests, transmission electron microscopy (TEM) operates on the basis of the detection of transmitted electrons through the sample, much like a light microscope that uses visible light. However, TEM microscopes are capable of producing images with much higher resolution and magnification. The reason for this, as with SEM, is due to the use of very high energy electrons with very small wavelengths as governed by the de Broglie equation:

\[ \lambda = \frac{hc}{E} \]  

(3.3)

Where \( \lambda \) is the electron wavelength (nm), \( h \) the Planck constant \( (6.626 \times 10^{-34} \text{ Js}) \) and \( E \) the electron energy \( (\text{Js nm}^{-1}) \). Thus, at high electron voltages, atomic resolution may be achieved, provided the atomic column being viewed is some low-indexed projection with the atoms sitting atop each other. Because the technique is reliant on transmittance,
samples for analysis must be sufficiently thin ($\leq 100$ nm) to allow an adequate number of electrons to be transmitted and thus generate an image. As the sample thickness increases, a greater degree of electron energy is lost since they are susceptible to scattering by matter. This can cause different wavelength electrons to reach the detector at the same time over a single spot resulting in an effect called chromatic aberration, which causes the image of the sample to appear blurred and unfocused.

Since the materials being dealt with in this thesis are nanometric in their dimensions, no special preparation methods are required such as electropolishing, ion milling or mounting in specific resins. Samples for imaging were prepared by first dispersing sample powder in EtOH and soninating for 1 hr. Two drops of the sample suspensions were then drop cast onto holey carbon-coated 200 mesh copper TEM grids using a disposable Pasteur pipette. The grids were allowed to dry overnight before being used for imaging. TEM images were obtained using a JEM-ARM200F scanning transmission electron microscope (JEOL), fitted with an Orius CCD camera (Gatan) and operating at 200 kV. The images obtained were processed and analysed using the Gatan Digital Micrograph software package.

![JEOL JEM-ARM200F scanning transmission electron microscope. Figure reproduced from JEOL, 2019.](image)

### 3.2.3 Energy Dispersive X-Ray Spectroscopy (EDS)

EDS involves the generation of characteristic x-rays by elements present in the sample when excited by the incident electron beam. Electrons that are ejected from the shell(s) of
an atom by the incident electron beam leave behind an electron hole. Electrons in higher energy states may drop-down in energy to occupy this vacancy (and to reduce the overall energy of the ionized atom). In the process of doing so, this higher energy state electron needs to lose some energy to be able to occupy a lower energy state vacancy. As a result, an x-ray is usually emitted. Because the separation between atomic shells/subshells is variable and unique to different elements, the x-rays produced are also characteristic of that element. In this manner, it is possible to collect these characteristic x-rays produced and assign the element of origin based upon the energy of the detected x-ray. The intensity of characteristic x-rays is also indicative of the quantity of that particular element, and so, EDS can be used both qualitatively and quantitatively. Much higher incident electron beam energies are usually used when performing EDS as compared to SEM imaging so as to ensure the elements of interest are excited.

![Diagram of electron energy levels and characteristic x-ray generation](image)

**Figure 3.6:** (left) Characteristic x-ray generation and (right) EDS mapping of a chitosan/TiO\textsubscript{2} nanocomposite material.

To complement the obtained XRD data, EDS analysis was performed in conjunction with SEM/TEM analysis so as to assess the atomic composition of the prepared nanoparticles and to highlight any sources of contamination that may be present. EDS analysis was performed using one of two electron microscopes. A JSM-7500FA field electron microscope coupled with an X-Flash 4010 10 mm\textsuperscript{2}, 127 eV SDD energy dispersive X-ray detector (Bruker) was used for routine EDS measurements. These measurements were performed with an acceleration voltage of 15 kV, probe current of 20 µA and spot size of 14 so to achieve an adequate signal intensity between 2000 - 3000 counts s\textsuperscript{-1}. A JEM-ARM200F
scanning transmission electron microscope fitted with a Centurio SDD detector (JEOL) with a 100 mm$^2$ detection area was used to perform high-resolution EDS mapping. Post data acquisition analysis was performed using NSS 3 X-ray microanalysis software (ThermoFischer Scientific).

### 3.2.4 Electron Energy Loss Spectroscopy

As has been mentioned in previous sections, various interactions of the electron beam in electron microscopy techniques can lead to a variety of signals brought about by various interactions with sample atoms. One such interaction is the inelastic scattering of electrons from the incident electron beam by sample atoms. Electron energy loss spectroscopy (EELS) utilizes this interaction to provide details about the local environment of atomic electrons which, in turn, provides information about the physical and chemical properties of the sample being examined.$^{517}$ The low energy-loss region (>50 eV) can be used to provide information about the electronic band structure properties of the material being examined, such as the band gap and surface plasmons. Peaks within the higher energy loss region (>50 eV) are usually assigned to ionization edges, whereby, core shell electrons in the sample are excited above the work function due to the high energy incident electron beam. Using these characteristic ionization edges and having adequate energy-filters in place, compositional information may obtained from the sample, enabling a means of quantifying and mapping the distribution of elements present in the sample. In addition to this compositional information, EELS can also be used to differentiate between different crystal structures of the same compositional compound due to slight variations in the local chemical environmental. These variations can be exploited and used in combination with compositional mapping to identify changes in crystal structure across a sample due to variations in the so-called ‘fine’ structure of element specific electron energy loss regions.
3.2 Materials Characterisation

![Figure 3.7](left) EELS spectra example highlighting the low loss (top) and core loss (bottom) regions. (right) Experimental EELS Ti L\(_{2,3}\) main edges for different titania crystal phases. Figures reproduced from Gloter *et al.*, 2009\(^{518}\) and Egerton *et al.*, 2005.\(^{519}\)

EELS spectra and mapped images were collected using a JEM-ARM200F scanning transmission electron microscope fitted with a Quantum 963 SE image filter and UltraScan 1000XP charge-coupled-device (CCD) camera (Gatan). Post-imaging analysis was performed using the Gatan Digital Micrograph software package.

### 3.2.5 X-ray Photoelectron Spectroscopy

X-ray photoelectron spectroscopy (XPS) is a surface analysis technique used to investigate the surface composition of a sample at a depth of 1-10 nm. As with EDS, the elemental composition of a particular sample can be investigated however, unlike EDS which typically has a depth profile of approximately 1 \(\mu m\), XPS can be used to probe surfaces from only a few atomic layers (1 nm or less) to hundreds of atomic layers (100 nm) thick. The underlying principle of XPS is the photoelectron effect, whereby, an electron bound to an atom or ion (usually at the core level) is ejected by an incident photon of sufficient energy. The energy of the ejected electron is then measured as it provides information pertaining to the type of atom or ion the electron was emitted from and, to some extent, the nature of the bonding.
XPS spectra were collected using a SPECS PHOIBOS 100 Analyzer under high vacuum and base pressure below $10^{-8}$ mbar. An Al Kα radiation source, operated at 12 kV and 120 W, was used to supplement photons with an energy of 1486.6 eV. The XPS binding energy spectra for selected samples were recorded with a pass energy of 20 eV in a fixed analyzer transmission mode. Subsequent analysis of the XPS data obtained was performed using the CasaXPS software package.

### 3.2.6 Fourier Transform Infrared Spectroscopy

Fourier transform infrared (FTIR) spectroscopy investigates the vibration and rotational behaviour of molecules when exposed to infrared radiation. Bonds and angles between atoms are non-rigid and are susceptible to external forces that can cause stretching and twisting without breaking of such chemical bonds. Certain frequencies of light can cause susceptible molecules to oscillate in a particular manner depending on the bonding structure of the molecule. A number of different frequencies can induce such movement and are termed normal modes of vibration. Generally, frequencies of light with the same frequency as the normal modes of vibration occur within the infrared region of the electromagnetic spectrum. This forms the basis of FTIR spectroscopy, whereby the transmittance of infrared radiation across various wavelengths through a sample is determined. For an absorption event to occur, and thus be 'IR-active', an electric dipole moment must
be induced in the molecule upon excitation. Thus different groups of atoms, or functional
groups in organic compounds, often absorb across different regions in the IR wavelength
range, thus enabling identification of certain groups in such organic compounds. Al-
though the wavelengths at which absorption occurs are specific for specific normal modes
of vibration, IR spectra often display broad shaped peaks, in addition to sharp transitions.
The reason for this is due to overtones and combinations of fundamental normal modes
in complex compounds, such as polymers. Polymers would be expected to have tens of
thousands of normal modes due to being composed of tens of thousands of atoms, how-
ever, their spectra are not as complex as would be expected and often display some broad
infrared absorption peaks. The reason for this is due to a phenomena known as ’group vi-
brations’, which is brought about by the presence of similar groups of atoms in repeating
polymer units but in slightly different chemical environments, leading to slight shifts in
the absorption wavelength and broadening of the overall peak.

FTIR spectra were obtained using a IRAffinity-1S FTIR spectrophotometer coupled with
a MIRacle-10 Single Reflection Horizontal Attenuated Total Reflectance (ATR) accessory
(Shimdazu). Dried sample powders were used for analysis and loaded onto the crystal
plate of the MIRacle-10. The sample was clamped under pressure to ensure good contact
with the plate and incident IR beam. Spectra were collected between 400 - 4000 cm$^{-1}$ at
a resolution of 2 cm$^{-1}$, averaged across 64 scans.

3.2.7 Raman Spectroscopy

As with FTIR, Raman spectroscopy involves the interaction of infrared radiation with the
chemical structure of a compound. In this instance however, the interaction of interest is
the scattering of the incident infrared radiation. Scattering of the incident light can result
in a change in the frequency of the light or no change at all. In absence of a frequency
change, the scattering is termed 'Rayleigh' scattering, whilst changes in light frequency
is termed 'Raman' scattering. The change in frequency observed typically corresponds to
the frequency of one of the vibration modes of the compound.$^{52}$ Furthermore, scattering
of the incident infrared light can result excitation and relaxation to different ground state
vibration states (Figure 3.9). Raman spectra were collected using a LabRAM HR Evolution Raman spectrophotometer (Horiba). Spectra were collected between 100 - 800 cm\(^{-1}\) using a 532 nm laser.

![Figure 3.9: Energy diagram detailing Rayleigh and Raman scattering events and the electronic transitions that occur.](image)

### 3.2.8 Nitrogen Adsorption/Desorption Analysis

Gas adsorption is a technique used to characterise the physical surface properties and textures of porous solids and fine powders.\(^{522}\) Measurement of the physical adsorption and subsequent desorption of an inert gas at various relative pressures and constant temperature produces an isotherm which can provide details in regards to a sample’s adsorption capacity, surface area and porosity. Differences in sample morphology, porosity, size and chemistry can lead to differences in the isotherm curve obtained, as shown in Figure 3.10. From the isotherm obtained, a measure of the specific surface area for the sample may be obtained with the application of the Bruneaur-Emmett-Teller (BET) equation:

\[
\frac{p/p^o}{n(1 - p/p^o)} = \frac{1}{n_mC} + \frac{C - 1}{n_mC}(p/p^o)
\]  

(3.4)

where \(n\) is the specific amount adsorbed at a relative pressure of \(p/p^o\), \(n_m\) the specific monolayer capacity and \(C\) a parameter exponentially related to the energy of the monolayer formation. Calculating for \(n_m\) using Equation 3.4, the specific surface area (SSA)
3.2 Materials Characterisation

can then be determined using:

\[
SSA = n_m N \sigma_m / m
\]  \hspace{1cm} (3.5)

where \( m \) is the mass of the sample, \( N \) is Avogadro’s number and \( \sigma_m \) the molecular cross-sectional area of the adsorbate gas used. Calculation of the specific surface area of the prepared nanoparticles was assessed through nitrogen adsorption/desorption methods using a Tristar II 3020 Gas Sorption system (Micromeritics). Initially, clean sample tubes were prepared by washing with detergent, hot water and EtOH before being dried under vacuum at 150°C for 4 hr. Samples were loaded into the cell such that the cell bulb was at least half-filled or with a mass such that the expected specific surface area of the sample was enough to provide a total surface area in the cell between 10 - 100 m². The samples were degassed at 120°C overnight prior to analysis using a Vacuum Degassing Station (Micromeritics). After degassing, the cells with sample were weighed to determine the dried sample mass and installed into the analysis station (Figure 3.4). The adsorbate gas used was nitrogen (N₂), with an assumed \( \sigma_m \) of 0.162 nm², and the measurements performed at constant liquid nitrogen temperature (77 K). Specific surface area values were calculated using the isotherm data points between \( p/p^o \) 0.05 - 0.3, standard for isotherm Types II and IV(a).
3.2 Materials Characterisation

Figure 3.10: Micromeritics Vacuum Degassing Station and Tristar II 3020 Gas Sorption systems. Classification of physisorption isotherms. Graphical figure reproduced from Thommes, 2015.522

3.2.9 Thermal Analysis

The thermal properties of the materials prepared in this work were investigated using a TGA/DSC 1 thermal analysis system (Mettler Toledo)(Figure 3.11). Samples for analysis were weighed into 600 µL alumina crucibles such that the mass of the samples were between 15 - 50 mg. Samples were loaded onto the TGA/DSC 1 system’s autosampler and the heating program desired loaded onto the Mettler Toledo thermal analysis software program. Typically, samples were treated between 40 - 800°C at a rate of 20°C min⁻¹ and under normal air atmosphere. For certain materials, the heating rates were cycled between 10 - 25°C min⁻¹ for further analysis. Prior to any sample measurements under a particular heating regime, a ‘blank’ measurement was performed using an empty alumina crucible. A small contribution in weight change (and heat flow) is observed for the ‘blank’ and must be subtracted from any sample measurements performed under the same heating regime. A brief outline of the different analysis techniques is given in the following sections.
Thermogravimetric Analysis

Thermogravimetric analysis (TGA) systems are used to measure changes in the mass of a sample over a range of temperatures, whether it be heating, cooling or at a static temperature, over time. The primary use of this technique is to assist in determining the composition of a material/compound. The usual causes for loss of mass in a sample during heating are due to processes such as decomposition, reduction, pyrolysis or evaporation, however, a sample may also gain mass as a result of oxidative or absorptive processes.

In this work, TGA curves were used to assess the mass loss observed for samples, as a function of temperature, and to give an indication of sample purity and thermal stability. For certain samples, TGA curves were converted to differential thermogravimetric (DTG) curves for further subsequent analysis. DTG curves allow for the accurate determination of temperatures from which the greatest change in mass occurs by taking the derivative of the TGA curve. In this manner, the peak decomposition rate may be determined. Conversion of TGA curves to DTG curves was performed using the Mettler Toledo software.
Differential Scanning Calorimetry

Differential scanning calorimetry (DSC) measures differences in the heat transmittance required to increase the temperature of a sample relative to a reference such that both sample and reference are at the same temperature. When the sample undergoes a phase transition (or other thermal process) more or less heat will flow into the sample compared to the reference to ensure the temperatures of the two are equivalent. For example, when a solid melts to form a liquid, the process is endothermic meaning heat is absorbed by the sample. This thus requires an additional amount of heat flow to the sample to increase its temperature to match that of the reference which should be increasing linearly. Crystallization is another thermal process that can be observed through DSC. Crystallization is an exothermic process and thus requires less heat to raise the sample temperature relative to the reference. Collection of DSC data occurs concurrently with the collection of the TGA curves and was obtained for all tested samples.

3.2.10 Ultraviolet-Visible Absorption Spectroscopy

Ultraviolet-visible (UV-Vis) absorption spectroscopy was employed to assess the absorptive properties of the prepared materials.

Samples for analysis were prepared by first weighing 2.5 mg of dry powdered sample into a glass sample tube. To the weighed powders, 10 mL of EtOH was added to yield a sample concentration of 250 mg L$^{-1}$. The samples were then suspended using an ultrasonication bath (Branson) for 1 hr. A series of dilutions were prepared in EtOH but transferring aliquots of the 250 mg L$^{-1}$ to separate volumes of EtOH in 15 mL Falcon centrifuge tubes. In this manner, concentrations of 10, 20, 30, 40 and 50 mg L$^{-1}$ were prepared. Prior to analysis, these diluted samples were sonicated a further 30 min. Absorbance measurements were performed using a UV-1800 spectrophotometer (Shimadzu). Data was collected between 200 - 800 nm at a step size of 1 nm. Baselining was performed in absence of any sample or cuvette and background measurements obtained for cuvette and
3.2 Materials Characterisation

solvent contributions to the absorption spectra. Calculation of the extinction coefficient for each of the prepared nanoparticle samples was determined from the relation between the absorbance measured and concentration, in accordance with the Beer-Lambert law:

\[ A = \varepsilon cl \]  \hspace{1cm} (3.6)

where \( A \) is the absorbance (a.u.), \( \varepsilon \) the extinction coefficient (L mg\(^{-1}\) cm\(^{-1}\)), \( c \) the concentration (mg L\(^{-1}\)) and \( l \) the path length (cm). By plotting the absorbance against the concentration of the sample tested, the extinction coefficient may be obtained from the resulting slope. In addition to the extinction coefficient, for semiconducting materials, the band gap may also be determined. Due to the quantized nature of energy levels in semiconducting nanomaterials, there exists an absorption edge from which incident photons of sufficient energy can excite electrons from the valence band of the semiconductor to its conduction band. The energy at which this transition may occur can be estimated using the Tauc equation:\textsuperscript{524}

\[ (\alpha hv)^{1/n} = B(hv + E_g) \]  \hspace{1cm} (3.7)

where \( hv \) refers to the photon energy, calculated from the incident photon wavelength (\( \lambda \)), \( B \) is a constant, \( E_g \) the band gap (eV), \( n \) a value related to the nature of the band gap transition and \( \alpha \) the absorption coefficient (or attenuation). The value of \( n \) can take on values between 0 - 3 corresponding to different types such as direct, indirect, allowed, forbidden or combinations of each. The absorption coefficient, as a function of wavelength (\( \alpha(\lambda) \)) can be calculated from absorption spectra data through the following equation:

\[ \alpha(\lambda) = \frac{(2.303 \times 10^3)A(\lambda)\rho}{cl} \]  \hspace{1cm} (3.8)

where \( A(\lambda) \) (a.u.) is the absorption of the sample as a function of the wavelength, \( \rho \) the density of the sample (mg cm\(^{-3}\)), \( c \) the concentration (mg cm\(^{-3}\)) and \( l \) the pathlength (cm). By plotting \( (\alpha hv)^{1/n} \) against \( hv \) and extrapolating the linear portion of the curve obtained to the x-axis, an estimation of the optical band gap may be obtained.
3.2 Materials Characterisation

Figure 3.12: (left) Absorption plots for a commercial TiO$_2$ powder (P25) at varying concentrations. (right) Relationship between the peak absorbance and concentration, validating the Beer-Lambert law.

3.2.11 Ultraviolet-Visible (UV-Vis) Diffuse Reflectance Spectroscopy

For certain materials, the impact of scattering caused by agglomerated particles in suspension can outweigh the absorbance, leading to an obscuring of the absorption edge and optical band gap. In the case of nanoparticles, this scattering is governed by the Rayleigh scattering equation:

$$I = I_o \frac{1 + \cos^2 \theta}{2R^2} \left( \frac{2\pi}{\lambda} \right)^4 \left( \frac{n^2 - 1}{n^2 + 2} \right)^2 \left( \frac{d}{2} \right)^6$$  \hspace{1cm} (3.9)

where $I$ and $I_o$ are the intensity and initial intensity of the scattered light, $\theta$ the scattering angle of the scattered light, $R$ the distance from the observer and scattering particle, $\lambda$ the wavelength of incident light, $n$ the refractive index and $d$ the diameter of the scattering particle. Simplification of this equation leads to the following expression:

$$I \propto \frac{d^6}{\lambda^4} I_o$$  \hspace{1cm} (3.10)

which suggests that as the particle or agglomerate size increases as does the degree of scattering. This effect can be observed in Figure 3.12 for TiO$_2$ nanoparticles, whereby, there is an observable amount of 'absorbance' measured within the visible light region, contradicting the supposed band gap values of 3.02 and 3.20 eV for the anatase and rutile crystal phases of the material. The reason for this continual absorbance in this region is
3.2 Materials Characterisation

due to the agglomeration of the nanoparticles when in solution to form larger structures that scatter longer wavelengths of light as compared to when in their individual nanoparticulate form. Thus diffuse reflectance measurements were obtained to better assess the optical band gap for highly light scattering samples.

Samples for analysis were prepared by applying a small quantity of powdered sample to a transparent quartz microscope slide. The powder was evenly spread across the quartz and a second quartz slide applied and taped down to keep the powder spread uniformly and with no cracks. Reflectance spectra were collected using a UV-3600 spectrophotometer (Shimadzu) coupled with an integrating sphere within the 200 - 800 nm range and step size of 1 nm. Because the samples appear opaque when prepared in the quartz slides, the samples are positioned in the back of the integrating sphere. The photons of light collected in this configuration are those that are reflected and scattered back into the integrating sphere. These photons continue to reflect off the surfaces of the sphere until they exit through the detector port. In this manner, scattered transmitted light is unused and the transformed absorbance data is free of scattering effects around the band edge positions for semiconducting materials.

![Figure 3.13: (left) Diffuse reflectance plot for a commercial TiO$_2$ powder (P25). (right) Calculated band gap using the Kulbelka Monk and Tauc relationships.](image)

The process of relating the observed reflectance of the measured sample to the absorption
requires intermediate transformation using the Kubelka-Munk function:

\[ F(R) = \frac{(1 - r)^2}{2r} \approx \frac{\alpha}{s} \]  

(3.11)

Where \( \alpha \) is the absorption coefficient, \( s \) the scattering coefficient, \( r \) the measured reflectance at a particular wavelength and \( F(R) \) the Kubelka-Monk function. \( F(R) \) is then used in place of the absorption coefficient, \( \alpha \), in the Tauc equation (Equation 3.8), thus enabling calculation of the optical band gap through a similar process as that detailed in Section 3.2.10.

### 3.3 Assessment of Photocatalytic Activity

Evaluation of the photocatalytic potential of the prepared nanoparticles was performed via the photo-induced degradation of the aqueous triarylmethane dye, crystal violet (CV) \((C_{25}H_{30}ClN_{3} \geq 90\% \text{SigmaAldrich})\). Such an experimental approach towards the approximation of the photocatalytic activity of inorganic nanoparticles, including TiO\(_2\) and ZnO, is often reported in literature, and so, is a suitable technique to employ.

#### 3.3.1 Experimental Procedure

For each degradation experiment, a new 1000 mg L\(^{-1}\) suspension of the tested sample was prepared by weighing 3 mg of dried sample into a glass sample tube, diluting in DI water and sonicating for 1 hr. A stock solution of CV at a concentration of 500 mg L\(^{-1}\) and the suspended particles were used to prepare the final reaction mixture. To a 100 mL volumetric flask, 1 mL of the CV stock and 0.5 mL of the suspension were added and the flask filled to the mark. The final concentrations of both the CV dye and the tested sample were 5 mg L\(^{-1}\).

Two different light sources and, subsequently, experimental set-ups were used to induce excitation in the nanoparticles being examined and to induce photo-oxidative damage to the target dye, as in accordance with the scheme shown in Figure 3.14. In the first instance, a Rayonet photocatalytic reactor, fitted with 350 nm (8x, 24W) and 300 nm (8x,
21W) phosphor-coated lamps was used as the UV radiation source. A quartz beaker (100 mL) was used to contain the reaction mixture and to enable transmittance of the incident UV radiation. A magnetic stir bar and inbuilt stirring system in the reactor enabled continual stirring of the tested sample suspension so as to inhibit sedimentation. Furthermore, the experiments were conducted within a fumehood so as to minimize exposure to any photo-generated ozone (O$_3$) that may be produced by the high energy UV light sources and oxygen present in the atmosphere. The purpose of using purely UV sources with intensities substantially higher than that reflected in ambient UV measurements is to better reflect acute photocatalytic effects of the tested nanoparticles.

The second dye degradation based set-up involved the use of a simulated solar radiation emitting source (filtered 1000 W xenon lamp), calibrated using a silicon photovoltaic cell, to reflect natural sunlight at an intensity of 1 sun as according to the ASTM E 892 standard outlined by the American Society for Testing and Materials (ASTM). In this case, the dye/nanoparticle suspension was prepared and transferred to a transparent PMMA glass reactor vessel and stirred with a magnetic stir bar and stir plate. By irradiating the target suspension with simulated solar light, a better reflection of the expected ambient conditions for a consumer using a sunscreen product outdoors may be achieved and thus, a closer approximation of the solar photocatalytic activity of these UV filtering materials can be obtained.

For both methods, the photo-induced oxidative degradation of the dyes used is fit to the Langmuir-Hinshelwood model:

$$ r = \frac{dC}{dt} = \frac{kKC}{1 + KC} $$  \hspace{1cm} (3.12)

where $r$ is the oxidation rate (mg L$^{-1}$min$^{-1}$), $C$ the concentration of the dye (mg L$^{-1}$), $t$ the irradiation time (min), $k$ the rate constant (mg L$^{-1}$min$^{-1}$) and $K$ the adsorption coefficient (L mg$^{-1}$). When the initial concentration of the dye ($C_o$) is substantially small (in the order of mM), the above expression can be simplified to follow a pseudo first-order
rate equation:

$$\ln\left(\frac{C_0}{C}\right) = kKt = k_{app}t$$  \hspace{1cm} (3.13)

In this manner, a plot of $\ln(C_0/C)$ against $t$ yields a plot where the gradient corresponds to the apparent rate constant, $k_{app}$, for the photo-mineralization of the dye.

**Figure 3.14**: Assessment of photocatalytic activity scheme using crystal violet as the degradation target.

### 3.3.2 Data Representation and Statistical Analysis

Each nanoparticle and nanocomposite sample was tested in three separate experiments, either for UV or solar-simulated light exposure or both, and the mean degradation at each
time interval taken. Rate constants are presented as the mean ± standard error of mean (SeM).

## 3.4 In Vitro Cytotoxicity towards Human Keratinocytes (HaCaT)

Because we are concerned with the effects of inorganic UV filtering nanoparticles on human health when applied to skin in a sunscreen formulation, it would be appropriate to therefore use a cell line that reflects cellular structures that said particles may interact with. As such, the cell line chosen for these assays was the HaCaT cell line, a spontaneously transformed human epithelial cell line originating from human adult skin. This immortalized cell line is a useful representation of the human keratinocyte cell type, which is the predominant cell type found in the epidermis, the outermost layers of skin. Therefore, its use as a means of modelling the possible toxicological effects of inorganic nanoparticles applied to the skin is obvious.

### 3.4.1 Cell Culture

The HaCaT cell line was used for all culture experiments and were originally provided by Dr. J. Guy Lyons (University of Sydney). Short Tandem Repeat Profiling (Garvan Institute of Medical Research) verified the identity of the cells. The cells were maintained in phenol red Dulbecco’s Modified Eagle Medium/Nutrient Mixture F-12 (DMEM/F12, Thermo Fisher Scientific) supplemented with 10% (v/v) heat inactivated fetal bovine serum (FBS, Bovogen Biologicals), 100 U mL$^{-1}$ penicillin/100 µg m$^{-1}$ streptomycin (Thermo Fisher Scientific) and 2 mM GlutaMAX$^{TM}$ (Thermo Fisher Scientific) and incubated at 37°C with 5% (v/v) CO$_2$ (Hercell 150i cell culture incubator, Thermo Fisher Scientific) in 75 cm$^2$ tissue culture flasks (Greiner Bio-One). Cells were passaged twice weekly when the confluency of cells had reached $\geq$90%. Cells were routinely negative for mycoplasma (MycoAlert Mycoplasma Detection Kit, Lonza).

Prior to subculturing, the cells were examined using a light microscope to assess con-
fluency and if any contamination may be present. After which, the confluent cells were transferred to a Bio-safety cabinet (BSC) with aseptic measures taken. The old cell culture medium was decanted from the flask before rinsing the cells with three 3 mL washes of Dulbecco’s phosphate buffered saline (DPBS, no \( \text{Ca}^{2+} \) or \( \text{Mg}^{2+} \), Thermo Fisher Scientific). Following this, 3 mL of 0.25% trypsin-ethylenediaminetetraacetic acid (EDTA) (Thermo Fisher Scientific) was added to the adherent cells and the flask placed in the incubator for 8 min so as to enzymatically detach the cells from the flask surface. After detachment, an additional 7 mL of fresh complete medium was added to the flask before transferring the contents to a 50 mL Falcon centrifuge tube. The cell suspension was centrifuged using a Heraeus Multiufge X3 centrifuge (Thermo Fisher Scientific) at 300 \( \times \) g for 5 min. The media/trypsin-EDTA mixture was decanted and the resulting pellet resuspended in 10 mL of complete medium.

Cell counts were performed during each passage so as to determine the seeding number needed for future passages and for determining the cell concentration for cytotoxic testing. 50 \( \mu \text{L} \) of the resuspended cells were transferred to a 1.5 mL Falcon tube and mixed with 50 \( \mu \text{L} \) of trypan blue dye (0.4%, Sigma Aldrich). From this, 10 \( \mu \text{L} \) was transferred to either side of a haemocytometer (Neubauer), cleaned and sterilized with 70% (v/v) EtOH. The haemocytometer consisted of two gridded counting chambers from which a total of eight \( 1 \text{ mm}^2 \) square grids were used for cell counting. Cells that appeared blue in colour were not included in the count as the colouration indicates permeation of the trypan blue dye into the cell and non-viability. The cell concentration and cell number for the passage are given by the following equations:

\[
[\text{Cells}] = \bar{x}_{\text{count}} \times 2 \times 10^4
\]  

(3.14)

\[
N_{\text{Cells}} = [\text{Cells}] \times V_{\text{resus}}.
\]  

(3.15)

where \([\text{Cells}]\) corresponds to the concentration of cells (cells \( \text{mL}^{-1} \)), \(N_{\text{Cells}}\) the cell number (cells), \(\bar{x}_{\text{count}}\) the average cell count determined using the haemocytometer and \(V_{\text{resus}}\).
the volume the cells were initially suspended in (usually 10 mL). The seeding volume needed for obtaining cells at a confluency of approximately 90% after a particular number of days was determined by first calculating the seeding number:

\[
S_N = \frac{N_{\text{Cells}}}{2^{(24 \times N_{\text{Days}})/DT}}
\]  

(3.16)

where \(S_N\) is the seeding number (cells), \(N_{\text{Cells}}\) the number of cells at 90% confluency (approximately \(15 \times 10^6\) cells \(\text{mL}^{-1}\) based on previous cell counts), \(N_{\text{Days}}\) the number of days between passages and \(DT\) the doubling time of the cells. For the HaCaT cell line, the doubling time was varied between 22-24 hr based observations and cell counts. The seeding volume needed could then be calculated based on the seeding number needed and the concentration of cells determined for a particular passage day. Seeding numbers calculated are shown in Table C.1.

### 3.4.2 Cell Number Optimization

The *in vitro* toxicity of the prepared samples was assessed with the cell proliferation MTS assay using the CellTiter 96®AQueous One Solution Cell Proliferation Assay kit from Promega. The MTS assay makes use of the tetrazolium compound [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt] (MTS) and electron coupling reagent, phenazine ethosulfate (PES) to assess the number of viable cells in proliferation based upon mitochondrial functionality. As highlighted in Figure 3.15, metabolically active cells produce reduced and/or phosphonated forms of nicotinamide adenine dinucleotide (NAD) coenzymes as NADH/NADPH. These electron rich donors coordinate with the electron coupling reagent PES (which is mixed in with the MTS reagent) to reduce the MTS salt into a formazan product. This formazan product is a coloured compound with an absorption maximum occurring at \(\lambda = 490\) nm, with the intensity of the absorbance correlating to the cell viability (percentage of living cells).
Before performing the assays, the cell concentration needed to produce adequate absorbance (close to 1 a.u.) in absence of cytotoxic effects was determined. For these optimization experiments, confluent cells (≥90%) were treated in accordance with standard subculturing protocol as previously outlined. After determining the cell concentration, 100 µL aliquots of the cells were transferred to all wells of the 2nd column of a 96-well flat bottom plate. An equal volume of complete medium was added to all the wells of the 1st column of the same plate. These two columns were used as positive and negative controls for the experiment. From columns 3 - 12 and rows A - D, a serial dilution of the cells, with known concentration, was performed. This was performed by first adding 100 µL of complete medium to wells in rows A - D and in columns 3 - 11. A 100 µL of cells was added to column 12, rows A - D. For the serial dilution, 100 µL of cells was added to column 11, rows A - D, and mixed with the multichannel pipette by drawing and expelling three times. 100 µL was then drawn from these wells and transferred to the next column and the process repeated. In this manner, each consecutive column for rows A - D had a cell seeding half that of its preceding wells. For rows E - H, columns 3 - 12, 100 µL of media were added for determination of background effects from the media. Figure 3.16 highlights the plate design and visual representation of the cell optimization experiments. The plated cells were incubated for 48 hr total, 24 hr to allow cell adherence.
and an additional 24 hr to take into account incubation with the prepared samples being tested. Four hours prior to the end of this incubation period, 20 µL of MTS reagent was added to each well used and incubated to the end of the 48 hr period at 37°C. During this time, the MTS salt is converted to a coloured formazan product, with the intensity of the colouration dependent on the number of viable cells with functioning metabolic activity. After incubation, the plate was centrifuged at 750 \times g for 10 min. Then, 80 µL of supernatant was transferred to a new 96-well plate and the absorbance at \( \lambda = 490 \) nm read using a SpectraMax 384 Plus microplate reader (Molecular Devices).

**Figure 3.16:** Experimental plate design for the cell optimization experiments.
3.4 Cytotoxicity in Absence of UV Light

The HaCaT cells were seeded at an optimal concentration, based upon the cell optimization experiments performed in the previous section \((10 \times 10^3 \text{ cells well}^{-1})\), in 96-well plates and incubated at 37°C and 5\% (v/v) CO\(_2\) for 24 hr. Prior to preparing the sample suspensions for the cytotoxicity assays, the samples were decontaminated under UVC radiation using the inbuilt UV function of a BSC for 20 min. After decontamination, 5 mL of complete medium was added to 2.5 mg of sample powder, so as to yield a suspension concentration of 500 mg L\(^{-1}\). The samples being tested were then sonicated for 1 hr in a sonication bath (Branson 3800, Ultrasonics Corp.). Once sufficiently sonicated, aliquots of media from the 96-well plates containing the seeded HaCaT cells were removed and replaced with aliquots of the sample suspension such as to yield nanoparticle concentrations of 1, 3, 10, 30, 100 and 300 mg L\(^{-1}\). After incubating the cells with the test samples for 24 hr, the 96-well plate was centrifuged at 750 \(\times\) g for 10 min and 80 \(\mu\)L of supernatant from each well used transferred to a new 96-well plate. The absorbance was read at 490 nm using a plate reader, as before for the cell optimization experiments. The cell viability (as a percentage) was determined as the ratio of the net absorbance for treated cells at a particular sample concentration to the net absorbance of the control (no sample present). Figure 3.17 details the plate design employed for these experiments. Each assay was performed in triplicate for each tested nanoparticle or nanocomposite sample and repeated in three separate experiments.
3.4 In Vitro Cytotoxicity towards Human Keratinocytes (HaCaT)

3.4.4 Cytotoxicity in the Presence of UV Light

The MTS cell proliferation assays were also performed in the presence of solar simulated light. Because the light source also emits UV radiation, the photocatalytic effects of the tested samples on the treated HaCaT cells could be examined. Figure 3.18 details the experimental set up and plate design employed for these experiments.

The light source used in these experiments was a 300 W Ultra-Vitalux sunlamp (OS-RAM). The emission profile is detailed in Figure C.1. A UVA/B meter (Sper Scientific) was used to measure the intensity output of the lamp. Due to the radial nature of the light source, the emission intensity of the lamp varied greatly across the 96-well plate. As such, prior to testing of samples and their effects on HaCaT cells, a degradation experiment using similar components to those outlined in Section 3.3 was used to assess the intensity distribution of the lamp. For these experiments, CV dye was used as the degradation target and P25 (TiO$_2$) as the photocatalyst. CV dye was dissolved in DPBS at a concentration of 5 mg L$^{-1}$ and added to all wells (100 µL) across a 96-well plate.
suspension of P25 in DPBS was prepared and sonicated for 1 hr and added to each well so as to yield a concentration of 500 mg L\(^{-1}\). A high concentration of photocatalyst was chosen so as to ensure adequate degradation within the time-frame of exposure chosen. The lamp was allowed to pre-heat for 2 hr to allow stabilization of the light emission. The 96-well plate containing CV and P25 were placed atop an iceblock, to minimise heating effects, and centred beneath the light source. The plate was exposed for 15 min at a chosen intensity of 6 mW cm\(^{-2}\). The absorbance of the CV dye was measured at 590 nm using a plate reader and mapped distribution of the degradation variance across the plate assessed.

**Figure 3.18:** Solar simulated light exposure set up (*top*) and experimental plate design (*bottom*) for the *in vitro* MTS cell proliferation assays under UV exposure.

Based upon the results obtained from the intensity distribution experiments, nanoparticle samples were tested using a reduced number of columns as shown in Figure 3.18. An initial cell optimization experiment was performed in a similar manner to those for the cell proliferation assays in absence of UV. For these experiments, HaCaT cells were seeded at varying concentrations in complete medium and allowed to grow/adhere to the bottom of
3.4 In Vitro Cytotoxicity towards Human Keratinocytes (HaCaT)

the wells of a 96-well plate for 24 hr in an incubator (37°C and 5% (v/v)). After incubation and prior to light exposure, the initial media used was removed and replaced with 100 µL of DPBS. The reason for this is due to the absorptive properties of phenol red, which can reduce the expected light output reaching the cells. In addition, phenol red free media similarly could not be used due to its absorbance across the UV region, thus DPBS was chosen due to its lack of absorbance (Figure C.2). Once all media containing wells had been replaced with DPBS and allowed to incubate for 1 hr, the plate was exposed to the simulated solar light lamp at a UVA/UVB intensity of 6 mW cm\(^{-2}\) for 5 or 15 min. After the exposure period, the 100 µL of DPBS in each well was replaced with fresh phenol red media and returned to the incubator for 24 hr. MTS reagent was again added 4 hr prior to the conclusion of this incubation period and the absorbance read at 490 nm using a plate reader, after centrifugation and aliquoting of 80 µL to a new plate.

For the nanoparticle treated experiments, three plates were used concurrently. A control plate containing a column each of cells only and complete medium was prepared and treated in the same manner as the test plates except for light exposure. The two other plates consisted of the same number of wells and columns used for the initial cell optimization as shown in Figure 3.18 and were treated in the same manner except for the time of exposure (the difference being 5 and 15 min exposure periods). Cells for each plate were seeded at a concentration based upon the cell optimization results (30\(\times\)10\(^3\) cells well\(^{-1}\)). The cells were incubated for 24 hr (at 37°C, 5% (v/v) CO\(_2\)) to allow adherence to the bottom of the wells. Sample nanoparticle suspensions were prepared in DPBS and sonicated for 1 hr. Decontamination procedures for the samples were the same as those used for cell proliferation assays in absence of solar simulated light. Aliquots were removed from the test plates and replaced with volumes of the sample suspensions so as to yield concentrations either 25, 50 or 100 mg L\(^{-1}\) and a total volume of 100 µL well\(^{-1}\). After the addition of the nanoparticles, the plates were returned to the incubator for 1 hr so as to allow the nanoparticles to settle and increase their interaction with the cell layer at the bottom of each test well. Plates were then exposed to the simulated light lamp for the time periods mentioned previously. After the exposure period, the DPBS was
removed and 100 µL of fresh complete medium was added to each test well. All three plates (control and the two exposure plates) were incubated for a further 24 hr (37°C, 5% (v/v) CeO₂). After the incubation period, each plate was centrifuged at 750 × g for 10 min and 80 µL of each well used transferred to new 96-well plates. The absorbance at 490 nm was read for each plate and the cell viability (%) calculated in similar manner to that for the non-irradiated cell proliferation experiments. In this instance however, the control plate not exposed to the simulated light was used as the control for the calculation. Each nanoparticle and nanocomposite tested were tested in triplicate per experiments and three experiments performed for each concentration tested.

### 3.4.5 Data Representation and Statistical Analysis

Data is presented as mean ± SeM. One-way ANOVA and Tukey post-hoc statistical analysis was performed to assess statistical differences between the nanoparticle and nanocomposites samples tested using OriginPro. Statistical significance was determined at the 95% and 99% confidence levels ($p < 0.05$ and $p < 0.01$, respectively).
Chapter 4

Suppression of the Photocatalytic Activity of TiO$_2$ Nanoparticles Encapsulated by Chitosan through a Spray-Drying Method with Potential for use in Sunblocking Applications

The following chapter describes and discusses the research reported in an article published in the journal *Powder Technology*. Abbreviations used throughout this chapter have been previously outlined in Section 3.1.1.

4.1 Introduction

Solar UV radiation exposure, particularly to wavelengths in the UVA (320 - 400 nm) and UVB (290 - 320 nm) regions, is a known cause of skin cancers and has been proven to cause DNA damage both directly and indirectly through the production of ROS and induction of oxidative stress. The use of UV filtering products such as sunscreens is the primary means of protection employed. These products contain organic and inorganic compounds, which can protect the skin against UV radiation through modes of absorption, scattering or reflection. The two mineral compounds TiO$_2$ and ZnO are extensively used in sunscreen products as inorganic UV filters due to their broadband protection across the
4.1 Introduction

UVA and UVB regions, as well as their ability to produce high SPF products. Additionally, modern sunscreen products may now contain these materials as nanoparticles due to the increased absorbance of UV radiation they display comparatively to larger particles as a result of size quantization.\(^43\) Both TiO\(_2\) and ZnO are semiconductor materials which, when illuminated by electromagnetic radiation of energy equal to or greater than their Eg, can result in the production of photoexcited electron (e\(^-\))/ hole (h\(^+\)) pairs. In the context of a biological system, these photoexcited species can interact with molecules adsorbed to the surface of these particles such as H\(_2\)O, a major constituent of human cells, producing ROS, which can go on to cause cellular and potentially mutagenic damage. Some of these ROS include OH\(^\bullet\) and O\(_2\)\(^{\bullet-}\) radicals and are due to interfacial redox reactions between the e\(^-\)/h\(^+\) pairs and adsorbed H\(_2\)O molecules. One study on the photooxidative ability of these photocatalysts involved the investigation of various sunscreen products containing TiO\(_2\) or ZnO and their effect when applied to steel sheets pre-painted with highly durable coatings such as fluoropolymer coating types.\(^{53}\) After performing a series of “accelerated weathering” experiments, it was found that formulations containing these inorganic components resulted in severe degradation of the panels in terms of gloss and surface roughness. In addition, it was found through X-ray diffraction that, for a particular cream, the active UV filtering TiO\(_2\) ingredient shared a similar mixed anatase/rutile crystal structure to that of the known commercial photocatalyst TiO\(_2\) powder, P25. P25 has been extensively studied for use in applications such as dye-sensitized solar cells, self-cleaning glass and water purification owing to its photocatalytic nature and ability to generate free-radicals.\(^{55,56,532}\) As such, despite the inherent benefits of nanoparticles in sunscreen products, there has been concern as to the potential of these materials to penetrate past the skin and to induce oxidative stress due to their known photocatalytic activity. In a review on the safety of TiO\(_2\) and ZnO nanoparticles in sunscreens, it was concluded that the weight of evidence suggests that these nanoparticles remain on the surface of the skin and the outer layer of the stratum corneum, where they can only interact with non-viable cells, however there is conclusive in vitro evidence that, whilst in the presence of UV radiation, these materials bring about the production of ROS, which can potentially lead to the
4.1 Introduction

damaging of cells. In addition, studies have shown ZnO to display cytotoxicity to cells even in the absence of UV radiation through ROS generation. As such, there has been an emphasis on developing and investigating alternative materials for potential use as UV filtering additives in sunscreen products. Some potential candidates include CeO₂, Fe₂O₃ and SnO₂. Developing methods for reducing the production of ROS and thus reducing the photocatalytic activity of TiO₂ and ZnO is an additional approach being explored and include methods of doping with foreign elements and coating/encapsulating with ceramic or polymeric materials. Wakefield et al synthesized manganese (Mn) doped TiO₂ nanoparticles through a sol gel method with increased UVA attenuation. Additionally, the free radical production was observed to be inhibited and was attributed to a free radical scavenging effect. Commonly used coating materials include wide Eg metal oxides, such as SiO₂ and Al₂O₃ however, conflicting reports have shown that such composites could in fact enhance the photoactivity, thus alternative materials such as polymers have also been investigated. One promising coating/encapsulating material is the natural polymer chitosan. Chitosan is a non-toxic, biocompatible and biodegradable polysaccharide that has gained interest for use in biomedical applications such as drug delivery, artificial skin and wound dressing. Studies involving chitosan as a coating material have also been reported and have yielded promising results in the context of UV filtration. For example, an investigation into the photocatalytic activity of chitosan/ZnO composite nanoparticles synthesized through ionotropic gelation had been investigated and reported to exhibit a quenching effect on the free radical production of ZnO highlighting its potential suitability for use as a UV filtering additive in cosmetic products, such as sunscreens. Work on the development of chitosan/TiO₂ composites has also been reported but such findings generally involve chitosan as a form of scaffolding for the TiO₂ particles for use in applications such as tissue engineering and ultrafiltration. One reported wet chemical approach resulted in the development of TiO₂ coated chitosan particles with enhanced photocatalytic activity, relative to bare TiO₂, for use in antimicrobial and photocatalytic applications, with the lack of photocatalytic inhibition being due to the significant presence of surface TiO₂ particles. In the context of safe UV
4.2 Results and Discussion

4.2.1 SEM/TEM Microanalysis of Particle Size and Morphology

SEM/TEM micrographs of the chitosan/TiO$_2$ composites were obtained so as to ascertain the morphological profile of the spray dried particles and to assess the loading effects on the particle sizes obtained and the effectiveness of the encapsulation process. As evidenced from SEM (Figure 4.1) and TEM (Figure 4.2), the TiO$_2$ loading amount has an impact on the particle morphology and particle sizes of the spray-dried composite particles. In absence of the TiO$_2$ nanoparticles, the CHI particles formed are spherical and symmetric in shape but relatively inhomogeneous in size. With the incorporation of the TiO$_2$ nanoparticles, it is evident there is an increase in the size of the composite particles formed and, whilst still primarily spherical, the surfaces of the particles appear deformed and rough due to the presence of TiO$_2$ decorating the outer layer of the polymer shell. This surface roughness is much more evident in the case of the 1:1 CHI/TiO$_2$ sample due to the higher ceramic particle loading, relative to the 2:1 CHI/TiO$_2$ sample.
4.2 Results and Discussion

Figure 4.1: SEM images and EDS maps of the spray dried CHI (top), 2:1 CHI/TiO$_2$ (middle) and 1:1 CHI/TiO$_2$ (bottom) nanocomposite particles. The EDS maps shown are for the elements Ti (red) and oxygen (green). The scale bar shown in the SEM images (left) corresponds to 1 µm.

In addition to the change in particle morphology it can be seen through TEM (Figure 4.2) of the 1:1 CHI/TiO$_2$ sample regions in which the ceramic nanofiller decorates the external layer of the polymer matrix that perhaps suggests an optimal loading amount exists between the 1:1 and 2:1 CHI/TiO$_2$ samples. The particle diameters were measured from the SEM images obtained and the mean values listed in Table 4.1. As mentioned previously, the mean particle sizes increase from the CHI sample ($\bar{x} = 1.40 \pm 0.4 \mu m$) to the 2:1 CHI/TiO$_2$ sample ($\bar{x} = 2.08 \pm 0.3 \mu m$) and then finally the 1:1 CHI/TiO$_2$ sample ($\bar{x} = 2.52 \pm 0.3 \mu m$), in accordance with the TiO$_2$ loading. Further characterization of the positioning of the encapsulated TiO$_2$ nanoparticles was performed using an EDS mapping technique. Figure 4.1 displays the mapping images obtained, highlighting the distribution of titanium (Ti) throughout the spray-dried chitosan and nanocomposite particles. For the purely chitosan sample (Figure 4.1 (top)), the mapping of Ti resulted in a random
distribution, indicating no localized concentration of Ti atoms in the CHI particles and is attributed to general background noise. For the composite samples (Figure 4.1 (middle-bottom)), it is evident that the distribution of Ti atoms are concentrated and localized within the particles positioned in the foreground and background of the corresponding grey-scale images, implying that the spray-drying technique was a successful approach, to an extent, in encapsulating and concentrating the core TiO$_2$ nanoparticles.

Figure 4.2: TEM micrographs obtained for the (top-left) CHI, (top-right) 2:1 CHI/TiO$_2$, (bottom-left) 1:1 CHI/TiO$_2$ and (bottom-right) pristine commercial TiO$_2$ nanoparticles.

Figure 4.3 highlights the XRD patterns obtained for the pristine TiO$_2$ nanoparticles, chitosan microparticles and the nanocomposite particles. The chitosan microparticles exhibit a broad diffraction peak around $2\theta^o$, corresponding to the chitosan crystalline structure-II.$^{544,545}$ Moreover, the diffraction pattern of the pristine TiO$_2$ nanoparticles suggests a mixture of the anatase and rutile crystal phases of TiO$_2$, with the major peaks for each phase appearing at $2\theta = 25^o$ and $27^o$, as expected for commercial P25.$^{546}$ For the nanocomposite microparticles, no clear changes in the diffraction patterns was noticed when compared to the pristine raw materials (ceramic nanopowder and chitosan), suggesting that the chitosan encapsulation or the processing method has little to no effect on the crystal phase of the incorporated TiO$_2$ nanoparticles.
4.2 Results and Discussion

Figure 4.3: XRD patterns for the raw chitosan starting material, pristine TiO$_2$ nanoparticles and nanocomposite powders prepared.

4.2.2 Chemical and Thermal Analysis

Figure 4.4 displays the FTIR spectra obtained for the spray-dried chitosan and nanocomposite particles, as well as the pristine TiO$_2$ nanoparticles. In the case of the chitosan containing materials, characteristic peaks may be observed including absorption bands between 3305-3280 cm$^{-1}$, 2888-2875 cm$^{-1}$, 1558-1550 cm$^{-1}$, 1421-1410 cm$^{-1}$ and 1065-1050 cm$^{-1}$ corresponding to -OH, -C-H, -NH, -CH, and C-O vibrational modes.$^{545,546}$ In addition to these characteristic peaks, an absorption band can also be observed in all chitosan containing samples in the range of 1652-1645 cm$^{-1}$ which is associated with the amide II carbonyl stretch of the chitosan precursor structure, chitin (Figure D.1),$^{547,548}$ and is to be expected considering the starting raw chitosan material only consisted of a deacetylation degree of $\geq$75%.$^{549,550}$ The presence of the TiO$_2$ in the composite materials is also further supported due to the occurrence of strong Ti-O stretch bands (627-610 cm$^{-1}$) in both the 2:1 and 1:1 composite samples, coinciding with the same band in the pristine TiO$_2$ spectrum and the results obtained through SEM and EDS (Figure 4.1).
4.2 Results and Discussion

Figure 4.4: FTIR spectra for the pristine TiO$_2$ (P25) nanoparticles as well as the spray-dried CHI, 1:1 CHI/TiO$_2$ and 2:1 CHI/TiO$_2$ particles.

Figure 4.5 (top-left) highlights the TGA curves obtained for the chitosan and composite samples heated at a rate of 20°C min$^{-1}$. In the case of the CHI sample, three main weight loss steps can be observed. The first occurs between 40°C - 110°C, corresponding to a weight loss of 5.5% and is attributed to the loss of adsorbed water, due to the hydrophilic nature of chitosan. The second step occurs between 220°C - 350°C, from which a further loss of 40.5% is observed. This weight loss is often attributed to the random splitting of the chitosan polysaccharide structure during decomposition and the removal of degradation by-products such as acetic, butyric and low mass fatty acids.$^{547,548}$ The final stage, occurring between 350°C - 750°C, arises from the presence of residual cross-linked chitosan chains and is connected with the remaining sample weight loss (45.6%), leaving a residual mass of 8.4%.$^{551}$ The onset of degradation ($T_{\text{onset}}$) for the 2:1 (228°C) and 1:1 (236°C) CHI/TiO$_2$ samples occurs earlier than that of the CHI sample (269°C) suggesting incorporation of the inorganic TiO$_2$ nanoparticles leads to a decrease in thermal stability, contrary to previously reported findings, but can be attributed to the thermal conductivity of the ceramic TiO$_2$ nanoparticles, resulting in an enhancement in the rate of heating of the polymeric components of the nanocomposite particles.$^{552}$ As with the CHI sample, the second degradation stage, corresponding to the decomposition of cross-linked chitosan
chains, also appears in the nanocomposite samples. Additionally, the decomposition of
the chitosan component of the nanocomposite samples appears to end at a lower temper-
ature (585°C) than that of the purely chitosan sample (725°C), further highlighting the
reduced thermal stability of the nanocomposite materials. The activation energy (\(E_a\)) for
the onset of decomposition for the spray-dried chitosan and nanocomposite samples were
calculated using the Kissinger mathematical method:

\[
\ln\left(\frac{\beta}{T_p^2}\right) = \frac{\ln(AE_a)}{R} + \ln[n(1 - \alpha_p)^{1-n}] - \frac{E_a}{RT_p}
\]  

(4.1)

where \(A\) is the pre-exponential factor (min\(^{-1}\)), \(R\) the ideal gas constant (8.31 J mol\(^{-1}\)K\(^{-1}\)), \(\beta\) the heating rate and \(\alpha_p\) and \(T_p\) the degree of conversion and temperature at
the maximum weight loss.\(^{553}\) From the plot of \(\ln(\beta/T_p^2)\) against \(1/T_p\), at heating rates
between 10°C min\(^{-1}\) and 25°C min\(^{-1}\), the \(E_a\) can be calculated from the slope of the line
produced (Figure 4.5 (bottom-left)). The values obtained for the spray-dried chitosan and
composite samples are listed in Table 4.1 and correlate with the initial onset of degradation
for the spray-dried samples, in that, the CHI sample displays the highest degree of thermal
stability (\(E_a = 183 \text{ kJ mol}^{-1}\)) followed by the 1:1 (\(E_a = 119 \text{ kJ mol}^{-1}\)) and the 2:1 (\(E_a
= 95 \text{ kJ mol}^{-1}\)) CHI/TiO\(_2\) samples. The loading ratios for the composite particles were
also estimated from the 20°C min\(^{-1}\) TGA curves obtained by subtracting the residual
mass percentage of the purely chitosan sample from those of the composite samples.
In this way, the percentage of TiO\(_2\) in the composite samples were determined to be
32% (2:1 CHI/TiO\(_2\)) and 47% (1:1 CHI/TiO\(_2\)), which agree well with the desired loading
amounts.
4.2 Results and Discussion

Figure 4.5: (top-left) TGA curves for the spray-dried samples and corresponding (top-left) derivative curves obtained at a heating rate of 20°C min$^{-1}$. (bottom-left) Kissinger plots and (bottom-right) influence of TiO$_2$ (P25) loading on the activation energy ($E_a$) for the spray-dried materials.

Table 4.1: Experimental results obtained from the SEM/TEM and thermal analysis for the spray-dried particles and commercial TiO$_2$ (P25) nanoparticles. The SEM particle size data represents mean ± standard deviation (SD) (count = 100).

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mean Particle Size (SEM) ($\mu$m)</th>
<th>$T_{onset}$ ($^\circ$C)</th>
<th>$E_a$ (kJ mol$^{-1}$)</th>
<th>Residual Mass (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHI</td>
<td>1.4±0.4</td>
<td>279</td>
<td>183</td>
<td>9</td>
</tr>
<tr>
<td>2:1 CHI/TiO$_2$</td>
<td>2.1±0.3</td>
<td>245</td>
<td>95</td>
<td>41</td>
</tr>
<tr>
<td>1:1 CHI/TiO$_2$</td>
<td>2.5±0.3</td>
<td>241</td>
<td>119</td>
<td>55</td>
</tr>
<tr>
<td>TiO$_2$ (P25)</td>
<td>40±20 (nm)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
4.2 Results and Discussion

4.2.3 Optical Absorbance and Photocatalytic Activity

Diffuse reflectance spectra were obtained so as to ascertain the effect of the chitosan on the optical properties of the encapsulated TiO$_2$ nanoparticles. Figure 4.6 (left) highlights the absorption spectra obtained for the nanocomposite particles as well as the purely chitosan particles and pristine TiO$_2$ nanoparticles. In the case of the TiO$_2$ nanoparticles, the absorption edge for the material begins at 405 nm and peaks at 310 nm, corresponding to the UVB region, as has been previously reported.$^{554}$ For the CHI sample, the primary absorption band is observed in the UV region and peaks at 305 nm, however, steady absorption is observed across the visible light region, with smaller absorption peaks seen at 445 nm, 525 nm and 665 nm. The absorption features seen at 305 nm, 445 nm and 525 nm could be attributed to electronic transitions occurring from $\sigma \rightarrow \sigma^*$ and $\pi \rightarrow \pi^*$ molecular orbitals owing to the mixture of $sp^3$ and $sp^2$ hybridized bonds present as a result of the less than 100% deacetylation degree of the chitosan.$^{555}$ Transitions occurring from non-bonding ($n$) orbitals may also arise due to the presence of atoms such as oxygen and nitrogen in the chitosan structure that have lone pairs of electrons capable of undergoing such transitions, and could explain the appearance of the absorption peak at 665 nm as being a $n \rightarrow \pi^*$ transition.$^{556,557}$ In the case of the nanocomposite materials, we can see that the UV absorption edges appear red-shifted compared to the pristine TiO$_2$ nanoparticles, with broad absorption bands peaking between 320-325 nm, within the UVA region. In addition to the shift into the UVA region, translation of pure chitosan visible light absorption features can also be observed, with the features being more prominent in the case of the 2:1 CHI/TiO$_2$ sample due to the higher concentration of chitosan present. Despite the non-white appearance of the composite powders, the pale yellow/brown appearance brought about by the chitosan absorption features could still be quite appealing in cosmetic cream formulations due to the closer appearance to skin tones.
4.2 Results and Discussion

Figure 4.6: (left) Absorption plots for the spray-dried and commercial samples obtained through diffuse-reflectance spectroscopy. (right) Relative decrease in absorbance of crystal violet dye as a function of UV irradiation time in the presence of the spray-dried and commercial samples.

The photocatalytic activity of the spray-dried chitosan, nanocomposite particles and the pristine TiO$_2$ nanoparticles, were evaluated by measuring the degradation of CV under UV irradiation over a period of 2 hr. Figure 4.6 (right) and Table 4.2 highlight the photodegradation efficiencies and rate constants for the degradation of the CV dye after UV irradiance in the presence of the as-prepared materials. It is clear that the incorporation of the chitosan layer in the nanocomposite particles significantly impacts the degradation efficiency of the TiO$_2$ nanoparticles. It can be seen that the degradation efficiency decreases in accordance with the content of chitosan, whereby, the pristine TiO$_2$ nanoparticles display the highest degradation efficiency (97.2±0.03%) followed by the 1:1 (69±6%), 2:1 (50±10%) CHI/TiO$_2$ and CHI (18±3%) samples (Table 4.2). A possible reason for the substantial decrease in photocatalytic activity of the composite materials could be associated with the inhibition of free-radical production due to the external layer of chitosan polymer. It has been previously reported that the application of an inert coating layer to photocatalytic metal oxide particles can act as a means of blocking the migration of photogenerated charge carriers to the surface of the excited particle, thus preventing interfacial charge transfer reactions from occurring. Another factor affecting the reduced degradation rates for the composite materials could also be the agglomeration of the encapsulated TiO$_2$ particles, thus reducing the total surface area available for chemi-
4.3 Conclusion

Chitosan and chitosan/TiO$_2$ nanocomposite particles were successfully produced through the use of a spray-drying technique and evaluated for the possible application of chitosan as a coating agent for inorganic TiO$_2$ nanoparticles in UV filtering applications. The morphology and mean particle sizes of the synthesized materials were characterized through the use of SEM and TEM micrographs and showed that an increase in TiO$_2$ loading yields an expansion in mean particle size as well as presence of surface TiO$_2$ particles when the

<table>
<thead>
<tr>
<th>Sample</th>
<th>Dye degradation (%)</th>
<th>Rate constant $k_{app}$ ($\times 10^{-3}$)(min$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHI</td>
<td>18±3</td>
<td>1.6±0.2</td>
</tr>
<tr>
<td>2:1 CHI/TiO$_2$</td>
<td>50±10</td>
<td>5.3±0.3</td>
</tr>
<tr>
<td>1:1 CHI/TiO$_2$</td>
<td>69±6</td>
<td>9.8±0.7</td>
</tr>
<tr>
<td>TiO$_2$ (P25)</td>
<td>97.2±0.03*</td>
<td>54±2*</td>
</tr>
</tbody>
</table>

Table 4.2: Photocatalytic degradation efficiencies and rate constants for the spray-dried particles and commercial TiO$_2$ (P25) nanoparticles. The errors shown are taken as the SeM between three separate experiments. *These values were calculated based on the data obtained up until 60 min of UV exposure.

Chitosan and chitosan/TiO$_2$ nanocomposite particles were successfully produced through the use of a spray-drying technique and evaluated for the possible application of chitosan as a coating agent for inorganic TiO$_2$ nanoparticles in UV filtering applications. The morphology and mean particle sizes of the synthesized materials were characterized through the use of SEM and TEM micrographs and showed that an increase in TiO$_2$ loading yields an expansion in mean particle size as well as presence of surface TiO$_2$ particles when the
4.3 Conclusion

Loading exceeds the capacitive amount for the spray-dried chitosan particles. The thermal properties of the chitosan and composite samples were analysed using TGA/DTA methods and showed that the thermal stability of the composites was decreased relative to that of the purely chitosan sample, whilst FTIR analysis displayed absorption peaks corresponding to characteristic chitosan and TiO$_2$ vibrational modes in the case of the composite particles. Diffuse reflectance spectra for the synthesized materials and pristine TiO$_2$ nanoparticles were obtained and showed that the primary UV absorbance band in the composite samples was slightly red-shifted into the UVA region whilst also displaying additional, smaller, visible light region absorption peaks as a result of the chitosan coating leading to a pale-yellow tone for the composite powders. The photocatalytic activity of the spray-dried materials were evaluated and the activity of the composite chitosan/TiO$_2$ particles was found to be significantly reduced in comparison to that of the unbound TiO$_2$ nanoparticles, highlighting the potential for this chitosan coating process for use in the industrial manufacturing of inorganic TiO$_2$ containing sunscreen products.
Chapter 5

Development of CeO$_2$ Nanodot Encrusted TiO$_2$ Nanoparticles with Reduced Photocatalytic Activity and Increased Biocompatibility towards the Human Keratinocyte Cell Line

The following chapter describes and discusses the research reported in an article published in the *Journal of Materials Chemistry B*. 562 Abbreviations used throughout this chapter have been previously outlined in Section 3.1.2.

5.1 Introduction

The detrimental effects of extensive solar ultraviolet (UV) exposure have long been known and include erythema (sunburn), pre-mature skin aging and skin cancer. 304, 563, 564 To counteract such adverse effects, the application of sunscreen products containing active UV filtering ingredients is a common means of protection. Such products may contain a combination of inorganic and organic compounds that provide protection through processes of absorption, scattering and reflection of incident UV radiation. 243 Of the inorganic compounds, the mineral compounds of TiO$_2$ and ZnO are most regularly used. Initially incorporated into formulations as pigmented grade particles, recent developments
5.1 Introduction

in technology has led to the increased use of nanoparticle materials in the nanoscale size range of 20-50 nm. This in turn has provided sunscreen products with the ability to provide enhanced UV protection, as well as increased cosmetic acceptability of such products by offering transparency in the visible light region. Despite concerns over the potential penetrative ability of these nanoparticles, various dermal penetration studies have concluded that these particles, when in the region of 20-50 nm in size, do not penetrate past the stratum corneum nor reach viable skin cells. There is, however, conclusive in vitro evidence that shows these materials, as nanoparticles, can impart cytotoxic and genotoxic effects on human cell lines, particularly when exposed to UV radiation. When excited by UV radiation these materials may instigate the production of free radical species, such as ROS, through the generation of $e^-/h^+$ pairs. Particularly in the case of TiO$_2$, a well-known and thoroughly used photocatalyst in applications such as dye-sensitized solar cells and water splitting, such photocatalytic ability can severely impact the photoprotective ability and length of protection provided by sunscreen products due to potential photodegradation of other organic UV filtering ingredients. The production of ROS species can also induce states of oxidative stress in cells if internalized, leading to potential mutagenic effects and premature cell death. To counteract these issues, sunscreen manufacturers may incorporate antioxidant compounds or apply inert coatings to the inorganic UV filtering nanoparticles as a means of scavenging and/or minimizing any free radicals produced and potential interactions with other UV filtering ingredients. The issues with these strategies, however, are that the antioxidant compounds used are typically organic, which could increase the probability for an allergic reaction to occur when applied to sensitive skin, whilst coating of TiO$_2$ with materials such as SiO$_2$ and Al$_2$O$_3$ does not necessarily enhance the efficacy of the overall formulation. For instance, various studies have investigated the benefit of applying a silicon-based coating to the surface of photoactive TiO$_2$ nanoparticles, with the subsequent photocatalytic activity appearing to be reduced. Despite this reduction, excessive coating can lead to a decrease in the UV absorptive ability of the core TiO$_2$ particles, thus being detrimental to the overall effectiveness of its use in sunscreen products. Because of the above, there
is still critical need to develop methods or materials that suppress or completely mitigate the photocatalytic ability of these photoactive nanoparticles whilst also simultaneously maintaining or improving the UV attenuation and photostability of the subsequent sunscreen formulation, ideally through some form of free radical scavenging process. Minimisation or removal of the cytotoxicity and phototoxic potential of these sunscreen-based materials is also an essential component of increasing consumer safety. A promising candidate material that could act as both part coating and antioxidant are CeO\(_2\) nanoparticles. CeO\(_2\) nanoparticles have been investigated previously specifically for potential use as a UV filter in sunscreen products in part due to its UV absorbing ability, as a result of its wide band gap (3.19 eV). It has also been shown to display free-radical scavenging properties owing to its potential to cycle between the Ce\(^{3+}/Ce^{4+}\) oxidation states through redox mediated processes. In vitro studies involving human cell lines have also shown that CeO\(_2\) imparts relatively low cytotoxic responses and minimal intracellular ROS production, further evidencing its potential in biological oriented applications. It has also been shown through biological studies to act as a photo-protectant, specifically against UVA. Composites of CeO\(_2\) with TiO\(_2\) have been previously investigated, primarily for use in applications such as visible-light driven photocatalyst and typically involve the formation of core-shell or doped structures. However, there are limited reports of this composite material for targeted use in UV filtering applications. One reported study though incorporates CeO\(_2\) as a partial coating for Fe\(_2\)O\(_3\) nanoparticles which yielded composite materials displaying improved UV absorbance selectivity and reduced photocatalytic activity through free-radical scavenging. In this manner, the current Chapter presents a material based upon TiO\(_2\) nanoparticles encrusted with CeO\(_2\) nanodots for the purpose of minimizing free-radical production of the core TiO\(_2\) nanoparticles upon UV radiation exposure whilst also maintaining UV attenuating efficiency and reducing any potential cytotoxic and phototoxic effects on the HaCaT human keratinocyte cell line.
5.2 Results and Discussion

5.2.1 Materials Characterisation

Figure 5.1 highlights the XRD patterns obtained for the composite and pristine CeO\(_2\)/TiO\(_2\) samples prepared. For the pristine TiO\(_2\), the diffraction pattern obtained corresponds to a mixed phase of anatase (PDF card 03-065-5714) and rutile (PDF card 03-065-1119) crystal forms, as has been previously reported for Degussa P25 TiO\(_2\).\(^{63}\) The peak broadening observed for the anatase and rutile reflections in each of the TiO\(_2\) containing samples is also indicative of the nanocrystalline nature of the core material, as evidenced by the mean crystal size of 27±3 nm, as calculated from the Scherrer equation (Equation 3.2) and the full-width half maximum (FWHM) of the anatase (101) reflection. As for the pristine CeO\(_2\) sample, the pattern obtained was identified as the cubic (fluorite) (PDF card 01-089-8436) crystal phase, with broad diffraction peaks similarly due to the nanocrystalline nature of the particles produced (4.8±0.9 nm).\(^{589}\) In the case of the nanocomposite samples, there is little variation between the patterns obtained, particularly in the case of the 2.5 at% and 5 at% samples, and no evidence of a secondary phase corresponding to CeO\(_2\) is evident. However, for the 10 at% composite sample, a shoulder appears off the (110) rutile reflection at approximately 2\(\theta\) = 28°, corresponding to the (111) CeO\(_2\) crystal plane, likely a result of the increased CeO\(_2\) loading.
5.2 Results and Discussion

**Figure 5.1:** XRD patterns for the as-prepared composites as well as for pristine TiO$_2$ (P25) and CeO$_2$. Peaks indexed for the TiO$_2$ and CeO$_2$ samples according to the following PDF cards: Anatase (03-065-5714), Rutile (03-065-1119), CeO$_2$ (01-089-8436).

Surface composition analysis performed with high resolution XPS further reveals the presence of Ce in the composite samples. Figure 5.2 highlights the Ti 2p and Ce 3d spin-orbit splitting regions for each of the nanocomposite and pristine CeO$_2$/TiO$_2$ samples. Peak deconvolution of the Ti 2p region for pristine TiO$_2$ (Figure 5.2 (top-left)) reveals the presence of a doublet pair corresponding to the 2p$_{1/2}$ and 2p$_{3/2}$ degenerate electron spin states of the Ti$^{4+}$ ion. In addition, the energy separation ($\Delta E = 5.91$ eV) between the two peak positions for the 2p$_{1/2}$ (463.9 eV) and 2p$_{3/2}$ (457.9 eV) peaks agree well with those previously reported for P25. No peaks due to splitting of Ce 3d orbitals was observed for the pristine TiO$_2$, as expected. In the case of the pristine CeO$_2$ sample (Figure 5.2 (bottom-right)), a six peak splitting pattern was observed. Peak deconvolution yielded three pairs of d orbital emission doublets with spin states of $j = 3/2$ or $5/2$ attributed to tetravalent Ce (Ce$^{4+}$) along with two doublet pairs attributed to trivalent Ce (Ce$^{3+}$). The presence of these mixed oxidation states in nanoparticulate CeO$_2$ has been previously reported, although no evidence of Ce$_2$O$_3$ is observed through XRD (Figure 5.1). This could be due to a combination of the small crystal size of the material, leading to significant peak broadening, and a low amount of Ce$_2$O$_3$ relative to CeO$_2$, resulting
in a masking of the Ce\textsubscript{2}O\textsubscript{3} contributions to the XRD pattern\textsuperscript{593,594}. As is expected, no evidence of Ti is observed in the pristine CeO\textsubscript{2} sample.

Figure 5.2: Narrow XPS spectra and fitted peaks of the Ti 2\textit{p} (left) and Ce 3\textit{d} (right) regions for the (top) pristine TiO\textsubscript{2} and (bottom) pristine CeO\textsubscript{2}. Each spectra includes lines for the raw data, fitted peaks and envelope for each peak fit (excluding spectra where no peaks were observed).

XPS spectra of the nanocomposite samples highlight peaks from both the Ti 2\textit{p} and Ce 3\textit{d} orbitals of TiO\textsubscript{2} and CeO\textsubscript{2} (Figure 5.3). In addition, the intensity of both the Ti 2\textit{p} and Ce 3\textit{d} peaks vary according to the loading of CeO\textsubscript{2}. As the loading of CeO\textsubscript{2} increases, the intensity of the Ce 3\textit{d} peaks increases whilst, conversely, the Ti 2\textit{p} peak intensities decrease accordingly. Furthermore, it is evident in the 10 at\% CeO\textsubscript{2}/TiO\textsubscript{2} XPS spectra that Ce is present as a mixture of the 3+/4+ oxidation state. Although deconvolution yielding peaks due to Ce\textsuperscript{3+} spin states become less clear in the 2.5 at\% and 5 at\% nanocomposite samples, considering that the synthesis method employed for preparing the composites relative to the pristine CeO\textsubscript{2} is the same, it can be inferred that the Ce present in these
samples also exists as some ratio of the 3+/4+ oxidation states.

![Figure 5.3](image)

**Figure 5.3:** Narrow XPS spectra and fitted peaks of the Ti 2p (left) and Ce 3d (right) regions for the (top) 2.5%, (middle) 5% and (bottom) 10% CeO2/TiO2 composites. Each spectra includes lines for the raw data, fitted peaks and envelope for each peak fit (excluding spectra where no peaks were observed).

Table 5.1 and Figures 5.4 and 5.5 list and highlight the results obtained from the EDS/TEM analysis. The Ce atomic concentrations for the composite samples were determined to be 1.4±0.3, 4±1 and 11±5 at% for the 2.5, 5 and 10 at% composite samples, which are in
reasonable agreement with the desired concentrations. In addition to the Ce content, the mapped images also provide an indication of the quality of the coating process.

![Figure 5.4: TEM micrographs and EDS mapped images of the 10% composite CeO$_2$/TiO$_2$ sample where (left) corresponds to the base dark field image, (middle) the Ti content and (right) the Ce content.](image)

For each sample, it is evident from the images obtained (Figure 5.5) that the CeO$_2$ nanoparticles tended to precipitate as clusters as opposed to a layered coating atop the core TiO$_2$ particle surface. This was expected, particularly for the 2.5 and 5 at% CeO$_2$/TiO$_2$ samples, due to low CeO$_2$ loading applied. However, this was also observed for the higher concentration 10 at% CeO$_2$/TiO$_2$ sample where this clustering or agglomeration was most prominent, suggesting even at 10 at%, higher concentrations of CeO$_2$ would be needed for full coverage. The addition of some form of surfactant or binding agent to the surface of the core TiO$_2$ nanoparticles prior to precipitation may have aided in reducing agglomeration of the CeO$_2$ nanoparticles and in obtaining a more uniform coating. However, complete coverage of the core TiO$_2$ nanoparticles could also affect the performance of the nanocomposite materials in terms of UV attenuation. Reducing the surface area of exposed TiO$_2$ in the composite samples would likely result in reduced UV absorption performance, particularly in the important UVB (290 – 320 nm) and UVA (320 – 400 nm) wavelength bands associated with sun damaging effects.
Table 5.1: Band gaps \( (E_g) \), extinction coefficients \( (\varepsilon) \) and Ce loading for the as-prepared samples. \( \varepsilon \) values correspond to extinction coefficients calculated at the wavelengths of maximum absorption for each sample at a concentration of 30 mg L\(^{-1}\). The errors shown are the standard deviation between triplicate measurements.

<table>
<thead>
<tr>
<th>Sample</th>
<th>( E_g ) (eV)</th>
<th>( \varepsilon ) (L mg(^{-1}) cm(^{-1}))</th>
<th>Ce/Ti (at%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TiO(_2) (P25)</td>
<td>3.30±0.02</td>
<td>53.8±2.0</td>
<td>-</td>
</tr>
<tr>
<td>2.5% CeO(_2)/TiO(_2)</td>
<td>3.23±0.01</td>
<td>11.7±0.6</td>
<td>1.4±0.3</td>
</tr>
<tr>
<td>5% CeO(_2)/TiO(_2)</td>
<td>3.26±0.02</td>
<td>1.3±0.2</td>
<td>4±1</td>
</tr>
<tr>
<td>10% CeO(_2)/TiO(_2)</td>
<td>3.21±0.01</td>
<td>0.9±0.1</td>
<td>11±5</td>
</tr>
<tr>
<td>CeO(_2)</td>
<td>3.28±0.01</td>
<td>0.5±0.1</td>
<td>-</td>
</tr>
</tbody>
</table>

Figure 5.5: Bright field (left) and corresponding dark field (right) images of the (top) 2.5 at\%, (middle) 5 at\% and (bottom) 10 at\% CeO\(_2\)/TiO\(_2\) composite samples.
5.2 Results and Discussion

The mean size of the CeO$_2$ nanodots formed on the surface of the core TiO$_2$ nanoparticles in the 10 at% CeO$_2$/TiO$_2$ composite sample were measured and averaged. The mean size calculated for this sample corresponded to 4.6±0.8 nm (Figure 5.6). This value also corroborates with the mean crystallite size of 4.8±0.5 nm, calculated from the pristine CeO$_2$ XRD pattern using the Scherrer equation. It has been reported that the size of CeO$_2$ nanoparticles is important in terms of its redox activity and contributes to the coexistence of the 3+/4+ oxidation states of Ce and the presence of Ce$^{3+}$ surface sites and oxygen vacancies.$^{500}$ This is thought to bring about the prominent antioxidant properties of these nanoparticles and their ability to scavenge ROS.$^{595}$ It has been predominantly found that, as the size of the CeO$_2$ nanoparticle decreases, an increase in the antioxidant activity is observed.$^{62,500,596}$ In the case of the 2.5 at% and 5 at% CeO$_2$/TiO$_2$ samples the particles of CeO$_2$ present to be smaller than those found in the 10 at% sample, suggesting sizes below the approximately the mean of approximately 5 nm. This could lead to a further increase in the presence of surface Ce$^{3+}$ sites that contribute to the ROS scavenging ability of theses materials at these loading concentrations.
5.2 Results and Discussion

Figure 5.6: HRTEM images of the 10 at% CeO$_2$/TiO$_2$ nanocomposite sample obtained in (top-left) dark field and (top-right) bright field imaging modes. (bottom) Particle size distribution of the CeO$_2$ nanoparticles present on the surface of TiO$_2$ nanoparticles in the 10 at% composite sample.

5.2.2 Optical Properties and Photocatalytic Performance

Dilute UV-Vis absorption spectroscopy was performed so as to ascertain the effects of ceria loading on the optical absorption properties of the nanocomposite materials. Figure 5.7 (left) highlights the absorption spectra obtained for the composite materials, as well as the pristine CeO$_2$ and TiO$_2$ nanoparticles for 30 mg L$^{-1}$ suspensions prepared in ethanol. Values for $\varepsilon$ were calculated from calibration curves (Figures 5.7 (right) and E.2) for each sample and were determined to be 44±1, 21.9±0.7, 28.6±0.6, 20.0±0.4 and 10.9±0.3 $\times 10^{-3}$ L mg$^{-1}$cm$^{-1}$ for TiO$_2$ (P25), the 2.5, 5 and 10 at% CeO$_2$/TiO$_2$ nanocomposites and CeO$_2$, respectively. For the pristine CeO$_2$ nanoparticles, the absorbance and extinction coefficient ($\varepsilon = 10.9\pm0.3 \times 10^{-3}$ L mg$^{-1}$cm$^{-1}$) relative to the other samples is considerably lower than that of the other nanoparticle and nanocomposite samples is considerably lower, with the major absorbance peaking at the higher energy end of the
UVB region (305 nm). For each of the TiO$_2$ containing samples tested, the primary absorption band was observed within the UVB region, with the major peak absorption spanning between 290–320 nm, although substantial absorbance is also observed within the UVA region, accounting for its commercial use in commercial UV filtering products. The lower absorbance and extinction values for the nanocomposite samples, as compared to the pristine TiO$_2$ (P25) nanoparticles would indicate minimal synergistic effect from the CeO$_2$ coupling concerning these optical properties. Furthermore, as mentioned previously, the lower optical performance for the nanocomposite samples could be attributed to the reduction in TiO$_2$ surface area exposed to the incident light source, thus lower absorption contributed by the TiO$_2$. Despite displaying lower absorbance than the pristine TiO$_2$ (P25), the nanocomposite samples still display substantial UV absorption, highlighting their promise as UV protection agents. They also display a higher degree of transparency in the visible light region (400 – 700 nm) compared to the pristine TiO$_2$ (P25), making them more cosmetically advantageous for use in sunscreens formulations. Notably, the extinction coefficient increased between 2.5–5 at% CeO$_2$ loading but decreased between the 5 and 10 at% samples. The increase and decrease suggests that some optimal CeO$_2$ loading amount aids in improving the UV attenuation of the core material, as evidenced by the improvement between the 2.5 at% and 5% samples in absorbance across the UV region. However, further loading of CeO$_2$ in the 10 at% samples increases the surface coverage of the core TiO$_2$ nanoparticles. As such, any synergistic effects imparted by the CeO$_2$/TiO$_2$ coupling towards UV attenuation is being mitigated by the reduction in available TiO$_2$ surfaces available for efficient absorption. Band gap values were calculated for each sample from their corresponding Tauc plots (Figure E.1) and are listed in Table 5.1. The $E_g$ value of 3.30±0.02 eV for the pristine TiO$_2$ (P25) nanoparticles is in reasonable agreement with other reported findings for the commercial product. A slightly lower $E_g$ value was obtained for the pristine CeO$_2$ nanoparticles (3.28±0.01 eV) as compared to the TiO$_2$. As with the extinction coefficients and absorbance efficiencies, the $E_g$ values tended to increase from 2.5–5 at% CeO$_2$, then decreased again at a CeO$_2$ loading of 10 at%. However, the separation between $E_g$ values calculated from the nanocomposite sam-
5.2 Results and Discussion

Samples only vary between 1–3%, which is insubstantial to suggest major modification to the core TiO\(_2\) nanoparticles due to the CeO\(_2\) loading. This could be considered beneficial, in the sense that TiO\(_2\) is already considered a highly effective UVB absorber and so, keeping the \(E_g\) of the composite materials to within this range is beneficial for ensuring suitable UV filtration when employed in sun protecting products.

![Figure 5.7: (left) UV-Vis absorption spectra recorded for the CeO\(_2\)/TiO\(_2\) composites, as well as pristine TiO\(_2\) and CeO\(_2\) nanoparticles for 30 mg L\(^{-1}\) suspensions prepared in ethanol. (right) Corresponding Beer-Lambert plots used to calculate extinction coefficient values.](image)

The photocatalytic activities of the composite samples were evaluated by measuring the degradation of CV dye under UV and solar-simulated light irradiation over a period of 1 and 4 hr, respectively. Figure 5.8 (top) and Table 5.2 highlight the photodegradation efficiencies and rate constants determined for the degradation of CV in the presence of the nanocomposite and pristine powder samples under UV irradiation. Of the samples tested, the pristine TiO\(_2\) (P25) nanoparticles displayed the highest degradation rate (\(k_{app} = 53.8 \pm 2.0 \times 10^{-3} \text{ min}^{-1}\)), nearly completely degrading the CV dye within the 1 hr irradiation time. The photocatalytic degradation of organic dyes in the presence of TiO\(_2\) has been thoroughly studied, and it is well understood that, upon excitation by photons higher in energy than its respective band gap, the formation of photoexcited e\(^-\)/h\(^+\) pairs occurs.\(^{526,529,598}\) These photoexcited species can then reduce/oxidise the dye directly or interact with dissolved O\(_2\) or other oxygen containing species present, such as H\(_2\)O, to produce ROS that cause degradation indirectly. The efficiency of this degradation process

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is strongly dependent on the recombination of these photoexcited species as a percentage of the incident photon rate, known as photonic efficiency. \(^{427}\) TiO\(_2\) has been shown to have a relatively high photonic efficiency in comparison to other semiconducting materials which, combined with its relatively cheap manufacturing, explains its extensive use and study in photocatalysis. Furthermore, the mixed anatase/rutile composition of the TiO\(_2\) (P25) tested has also been previously shown to display photodegradation efficiencies greater than either single phase. \(^{599}\) The 2.5 at\% CeO\(_2\)/TiO\(_2\) nanocomposite exhibited the second highest degradation rate \((k_{app} = 11.7\pm0.6\times10^{-3}\ \text{min}^{-1})\) and reduced the dye concentration to approximately 50%. For the remaining samples, very low degradation rates were observed with \(k_{app}\) values of 1.3±0.2, 0.9±0.1 and 0.5±0.1\times10^{-3}\ \text{min}^{-1} determined for the 5 at\% CeO\(_2\)/TiO\(_2\), 10 at\% CeO\(_2\)/TiO\(_2\) and pristine CeO\(_2\) samples, respectively.

Figure 5.8: Photoactivity assessment of the tested samples, highlighting the (left) relative absorbance behaviour of the CV dye and the (right) degradation kinetics when exposed to (top) UV radiation and (bottom) simulated solar radiation. Data represents the mean ± SeM (n = 3 experiments).
The substantial reduction in photoactivity for the nanocomposite and pristine CeO$_2$ could, in part, be attributed to the lower UV absorbing capabilities of these materials in comparison to TiO$_2$ (P25) (Figure 5.7 (left)), however, one particular nanocomposite stands out from the rest. The near negligible degradation observed in the case of the 5 at% composite does not coincide with its still relatively high UV absorbance properties. Combined with the minimal modification seen in the band gap of this nanocomposite compared to the pristine TiO$_2$ (P25) sample, the low photoactivity observed could be attributed to a reduction in ROS generation (due to increased recombination of charge carriers) or ROS scavenging (due to the presence of CeO$_2$). For the latter case, it could be suggested that the effect is dependent on the loading of CeO$_2$. Despite displaying lower UV absorbance efficiency than the 5 at% composite, the 2.5 at% sample displayed much higher photoactivity under UV irradiation ($k_{app} = 11.7\pm0.6\times10^{-3} \text{ min}^{-1}$ compared to $k_{app} = 1.3\pm0.2\times10^{-3} \text{ min}^{-1}$). This could suggest that at this CeO$_2$ loading ratio, the ability for the CeO$_2$ present to act as an antioxidant is outweighed by the photocatalytic activity of the core TiO$_2$, in spite of the lower absorptive properties. However, as the CeO$_2$ loading is increased, a drastic reduction in degradation is observed as well as a peaking in UV absorbance for the 5 at% loaded sample before decreasing again in the 10 at% loaded sample. It is thus evident that there is a trade-off between obtaining the antioxidant properties of the CeO$_2$ surface loaded nanoparticles with maintaining adequate UV protection afforded mainly by the core TiO$_2$ nanoparticles and is influenced by the CeO$_2$ loading concentration.
5.2 Results and Discussion

Table 5.2: CV dye degradation and rate constants \( (k_{app}) \) calculated from the photocatalytic degradation experiments under UV and solar simulated (AM1.5G) irradiation for the pristine and composite samples. Errors shown correspond to the SeM between three separate experiments.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Dye degradation (%)</th>
<th>Rate constant ( k_{app} \times 10^{-3} )(min(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>UV</td>
<td>AM1.5G</td>
</tr>
<tr>
<td>TiO(_2) (P25)</td>
<td>96±2</td>
<td>86±5</td>
</tr>
<tr>
<td>2.5% CeO(_2)/TiO(_2)</td>
<td>52±7</td>
<td>29±7</td>
</tr>
<tr>
<td>5% CeO(_2)/TiO(_2)</td>
<td>8±5</td>
<td>13±1</td>
</tr>
<tr>
<td>10% CeO(_2)/TiO(_2)</td>
<td>5±4</td>
<td>9±5</td>
</tr>
<tr>
<td>CeO(_2)</td>
<td>3±1</td>
<td>30±3</td>
</tr>
</tbody>
</table>

Figure 5.8 (bottom) highlights the photodegradation results for the samples tested when exposed to solar simulated light. In a similar manner to the UV photodegradation tests, the pristine TiO\(_2\) (P25) displayed vastly superior photocatalytic activity \( (k_{app} = 8.16±0.17 \times 10^{-3} \text{ min}^{-1}) \) as compared to the nanocomposite and pristine CeO\(_2\) samples. Furthermore, the photocatalytic activity of the nanocomposite samples under solar simulated light follows the same trend observed when exposed to only UV radiation, with greater CeO\(_2\) loading leading to a lower perceived activity \( (k_{app} = 1.43±0.03, 0.62±0.04, 0.44±0.04 \times 10^{-3} \text{ min}^{-1}) \) for the 2.5 at%, 5 at% and 10 at% CeO\(_2\)/TiO\(_2\) nanocomposite samples, respectively. Similarly, the reasons for this trend across the nanocomposite samples are likely similar to those outlined previously for the UV photodegradation results since there is little direct absorbance within the visible light region for the nanocomposite samples from which changing the light source can have a major impact.

Notably, the pristine CeO\(_2\) sample when exposed to simulated solar light displayed an enhancement in photoactivity as compared to when exposed purely to UV, but still afforded some protection for the dye itself against decomposition by solar simulated light. A possible explanation as to why the protective effect of CeO\(_2\) in this case is not as pronounced as compared to the nanocomposite samples, where the CeO\(_2\) loading is significantly lower,
could be due to the influence of surface defects and surface defect concentration. The main type of surface defect that occurs with ceramic nanoparticles are oxygen vacancies which, in the case of CeO$_2$, results in the reduction of surface Ce$^{4+}$ to Ce$^{3+}$, so as to compensate for the effects of electrostatic forces. The presence of these surface based Ce$^{3+}$ states suggests the presence of Ce$_2$O$_3$, a phase not observed in XRD analysis of the pristine CeO$_2$ since it is limited to the surface and likely masked by the higher volume loaded CeO2 phase. Ce$_2$O$_3$ enables the absorption of visible light wavelengths and has been reported to have a significantly smaller band gap than CeO$_2$ of 2.40 eV.$^{600,601}$ The reason such absorption features were not evidenced in the absorption spectra of CeO$_2$ could be attributed to the very fact that it is a phenomenon strictly limited to the surface of the CeO$_2$ nanoparticles, whereas absorption spectroscopy considers the entire bulk. Because of the additional limited visible light absorption afforded, the CeO$_2$ scavenging capabilities are also in direct competition with the photocatalytic properties of the material from both UV and visible light excitation. However, the contribution to photocatalysis due to visible light excitation in pristine CeO$_2$ is still not so significant since the dye itself is still afforded some protection over the 4 hr exposure period as compared to the dye degradation in absence of any catalyst. This effect is also further limited in the case of the nanocomposite samples due to the reduced loading of CeO$_2$ in these samples relative to the pristine CeO$_2$ and thus a more pronounced reduction in photocatalytic activity is observed instead.

It can be concluded from these photodegradation experiments that the application of CeO$_2$ to the surface of highly photoactive TiO$_2$ nanoparticles can influence the photocatalytic performance. The drastic reduction in photocatalytic activity observed for the nanocomposite samples relative to the pristine TiO$_2$ (P25) sample adds further evidence towards to the potential of CeO$_2$ as new additive coating material for inorganic UV filters.
5.2 Results and Discussion

5.2.3 In Vitro Cytotoxicity in Absence and in the Presence of UV Radiation

Cell cytotoxic and phototoxic assays were performed using the pristine TiO$_2$ and CeO$_2$ nanoparticle samples, as well as the 5 at% CeO$_2$/TiO$_2$ as a result of the low photocatalytic activity and high UV attenuation it displayed, making the ideal sample for testing amongst the different CeO$_2$ loaded samples prepared. The HaCaT cell line was chosen for both cytotoxic and phototoxic assays as it is composed of keratinocytes, the major cell type of the epidermis and the superficial layers of skin in most intimate contact with external contaminants.$^{602,603}$ Figure 5.9 highlights changes in the cell viability of the HaCaT cells when exposed to increasing concentrations of pristine TiO$_2$ (P25), CeO$_2$, the 5 at% CeO$_2$/TiO$_2$ nanocomposite and a known nanoparticulate toxicant, ZnO (Sigma Aldrich, size < 100 nm).$^{604,605}$ Cell viability was reduced significantly after 24 hr incubation in the presence of the tested ZnO nanoparticles at concentrations above 10 mg L$^{-1}$. From the concentration-response curve obtained, the half maximal inhibitory concentration (IC$_{50}$) for ZnO nanoparticles tested was reached and calculated to be 16±1 mg L$^{-1}$. In contrast, cell viability was only partially reduced in the presence of CeO$_2$, TiO$_2$ (P25) or 5 at% CeO$_2$/TiO$_2$ with cell viability significantly greater than that of cells incubated in the presence of corresponding cytotoxic concentrations of ZnO. Unlike ZnO nanoparticles, for the pristine and nanocomposite samples the half maximal inhibitory concentration could not be reached and the final cell viabilities of HaCaT cells at the highest concentration tested (300 mg L$^{-1}$) were only reduced to 87±5%, 79±9% and 70±10% for the CeO$_2$, 5 at% CeO$_2$/TiO$_2$ and TiO$_2$ (P25) samples, respectively. The cell viability reduction observed across all tested concentrations did not vary substantially between samples, suggesting minimal differences in toxicity for the samples tested and a marginal influence of the CeO$_2$ loading on the core TiO$_2$ nanoparticle toxicity in absence of external UV radiation sources.
5.2 Results and Discussion

Figure 5.9: Impact of the pristine TiO$_2$ (P25), CeO$_2$, and nanocomposite CeO$_2$/TiO$_2$ samples on the mitochondrial function of HaCaT human keratinocytes over a 24 hr incubation period. At the end of the incubation period, cell viability was assessed via the MTS assay. Data represents mean ± SeM (n = 3 experiments). One-way ANOVA and Tukey post-hoc tests were performed to assess statistically different data sets. ** refers to $p < 0.01$ for the ZnO NP data set when compared to all other nanoparticle and nanocomposite sample data sets for the corresponding concentrations.

Figure 5.10 depicts the MTS assays performed with the HaCaT cell line and the pristine and nanocomposite samples under UV exposure for 5 and 15 min prior to the 24 hr incubation period. Under both exposure periods, a significant reduction in viable cells relative to the control was observed in absence of the test samples. This is a consequence of the highly cytotoxic and genotoxic effects of UV radiation, which comprises of highly energetic wavelengths capable of inducing DNA lesions and elevating intracellular ROS levels, causing oxidative stress and leading to apoptosis.\textsuperscript{199,606} For the 5 min UV exposure period (Figure 5.10 (left)), the percentage of viable cells incubated with the test nanoparticle samples at 50 and 100 mg L$^{-1}$ did not vary significantly to the viability of the cells incubated in absence of test sample. This coincides with the relatively low toxicity observed from the MTS assays performed in absence of UV light at these concentrations (Figure 5.9). However, each test sample at 100 mg L$^{-1}$ caused a small but statistically significant increase in cell viability compared to cells exposed to UV light in the absence of each corresponding test material. This would suggest that, despite exposure to UV
radiation for the allotted period, some protective effect was afforded by the tested samples. Indeed, as has been shown through UV-Vis absorption spectroscopy (Figure 5.7), each of the tested samples, to a varying degree, display UV absorptive capabilities. However, with this screening and thus absorption of the incident UV by the samples tested, ROS generation was expected to occur, particularly for the TiO$_2$ (P25) sample which was shown to have prominent photocatalytic properties (Figure 5.8). One possible reason as to why this protective effect is more apparent than the potential toxicological effects of ROS production could be due to a lack of cellular internalization of the nanoparticles, leading to insubstantial cellular damage and impairment of metabolic activity. Another possibility is that, for the time period and intensity of UV emitted to the cells and the tested samples, the rate of ROS production was insufficient to induce a state of oxidative stress. Most animal cells contain natural enzymatic antioxidants to counteract ROS and other free radicals produced as by-products of metabolism or, such as in this work, ROS produced indirectly by UV radiation.$^{607,608}$

![Figure 5.10: HaCaT cell viability after 24 hr incubation with TiO$_2$ (P25), 5 at% CeO$_2$/TiO$_2$ and CeO$_2$ when exposed to UV radiation prior for (left) 5 min and (right) 15 min at an intensity of 6 mW cm$^{-2}$. HaCaT cell viability (% of control) refers to the normalized absorbance readings for all nanoparticle, nanocomposite and cell only wells exposed to UV irradiation relative to a control plate in absence of UV exposure for each concentration tested. Data represents mean ± SeM (n = 3 experiments). One-way ANOVA and Tukey post-hoc tests were performed to assess statistically different data sets. * and ** refer to $p < 0.05$ and $p < 0.01$ when compared to the Cell Only data sets for the corresponding concentrations. † and †† refer to $p < 0.05$ and $p < 0.01$ when compared to the TiO$_2$ (P25) data sets for the corresponding concentrations.](image-url)
5.2 Results and Discussion

For the 15 min UV exposure period (Figure 5.10 (right)), an overall decrease in cell viability is observed across all samples and concentrations as compared to the 5 min exposure period, simply as result of the higher dose of UV radiation impacting the cells. In contrast to the 5 min exposure period results (Figure 5.10 (left)), a significant decrease in cell viability was observed when incubated with the TiO₂ (P25) nanoparticles at a concentration of 100 mg L⁻¹ compared to UV exposed cells incubated in the absence of test sample. In this instance, the rate of ROS production may be exceeding the rate at which these species can be scavenged by natural cellular processes, leading to a state of oxidative stress, metabolic impairment and potentially cell death. In addition, the screening effect afforded by the UV absorbing TiO₂ (P25) nanoparticles is also outweighed by its potential free radical production, leading to cell damaging effects akin to the degradation of CV during the photodegradation experiments. In the case of the pristine CeO₂ nanoparticles, cell viability was maintained at 25 mg L⁻¹ whilst an increase in cell viability was observed for CeO₂ nanoparticle concentrations at 50 and 100 mg L⁻¹ compared to UV exposed cells incubated in the absence of test sample. As with the 5 min exposure period tests, the increase in cell viability at these higher test concentrations could be a result of the UV shielding afforded by the absorptive properties of the particles. Contributions from the free radical scavenging ability of the CeO₂ nanoparticles could also be aiding in protecting the cells from photo-induced ROS and in minimizing oxidative damage. A combination of both free radical scavenging and UV shielding by the CeO₂ nanoparticles is likely the cause for the perceived increase in cell viability seen at these higher concentrations, as has been previously shown. It can also be seen that the loading of CeO₂ nanoparticles at the surface of TiO₂ has an impact on the phototoxicity of the core material. Cell viability was maintained across all tested concentrations for the 5 at% CeO₂/TiO₂ sample as compared to UV exposed cells incubated in the absence of test sample. The significant difference in cell viability between the pristine TiO₂ (P25) nanoparticles and the nanocomposite sample, particularly at concentrations of 50 and 100 mg L⁻¹, suggests that the application of CeO₂ at this loading concentration is sufficient in mitigating the potentially phototoxic properties of the core TiO₂. The reason for this...
could impart be due to a reduction in TiO$_2$ surface active sites due to coverage by the CeO$_2$ nanoparticles, as had been previously suggested in explaining its low photocatalytic activity towards the degradation of CV. It is also possible that the biocompatibility of TiO$_2$ in the nanocomposite materials has been improved due to the low toxic and phototoxic effects exerted by the application of the CeO$_2$ nanoparticles and the potential scavenging of photo-produced ROS, as demonstrated by the pristine CeO$_2$ nanoparticles in this work.

5.3 Conclusion

Commercially used TiO$_2$ nanoparticles in sunscreen products have the potential to generate free-radical species such as ROS when exposed to UV radiation. Such free radical species have been shown to cause oxidative damage to other active sunscreen ingredients, leading to a loss in protection, as well cause cytotoxic and genotoxic effects to human cell lines, particularly when exposed to UV radiation. Thus, modification of the photocatalytic activity of these particles whilst maintaining adequate UV attenuation is essential for their continued safe use in such products. The addition of free radical scavenging CeO$_2$ nanodots through a simple precipitation method to the surface of highly photoactive commercial TiO$_2$ nanoparticles was employed to demonstrate an alternative to classic silica and alumina based coatings. It was shown that an optimal CeO$_2$ nanodot loading of 5 at% was required for drastically reducing the photocatalytic activity of the core TiO$_2$ whilst also maintaining excellent UV absorptive properties. Furthermore, the phototoxic properties of the core commercial TiO$_2$ nanoparticles towards HaCaT cells were shown to be diminished in the nanocomposite sample due to the potential biomimetic antioxidant behaviour of CeO$_2$. Thus in this chapter, we have demonstrated the potential for CeO$_2$ nanodots as an additive to commercial sunscreen active TiO$_2$ that can help improve biocompatibility, provide UV protection and minimize formulation degradation.
Chapter 6

Hydrothermal Synthesis of Rutile TiO$_2$ Nanorods and their Decoration with CeO$_2$ Nanoparticles as Low-Photocatalytic Active Ingredients in UV Filtering Applications

The following chapter describes and discusses the research reported in an article submitted to the *Journal of Materials Science.* Abbreviations used throughout this chapter have been previously outlined in Section 3.1.3.

6.1 Introduction

TiO$_2$ has long been used as an inorganic based UV filtering ingredient in many sunscreen products. Modern formulations often contain TiO$_2$ in the form of nanoparticles due to the enhanced absorption provided across the UVA (320 – 400 nm) and UVB (290 – 320 nm) wavelength bands. Moreover, with increased transparency, when well dispersed, in the visible light region (400 – 700 nm), significant cosmetic advantage is afforded. However, there is concern associated with the enhanced photocatalytic activity of this material at this size range and their role in the formation of ROS such as the highly reactive OH$^-$ radical. TiO$_2$ nanoparticles have also been shown to induce genotoxic and cytotoxic
effects on human cell lines, particularly after exposure to UV radiation, which leads to the production of ROS. ROS production can also affect other active ingredients present in sunscreen formulations. Degradation of these ingredients can lead to a loss of sunscreen efficacy and lowering of the labelled SPF. The extent of ROS production and the photocatalytic activity of TiO$_2$ can be modified by manipulating material parameters such as the crystal phase, particle size and the surface coating.

The two main crystal phases linked with TiO$_2$ use in photocatalysis are anatase and rutile. Often, the anatase phase is associated with higher photocatalytic activity, however, there is also substantial evidence to suggest certain compositions of anatase-rutile mixtures can outperform either single phase. One such proprietary mixture, AEROXIDE® TiO$_2$ P25 (also known as Degussa P25), is a popular IUPAC reference material used in photocatalysis research, and is a TiO$_2$ nanopowder with an anatase-rutile ratio of 4:1. This same ratio of anatase-rutile has also been found in certain sunscreen products containing TiO$_2$ which were shown to cause accelerated damage to organic surface coatings used in outdoor roofing applications. As such, an essential parameter for improving the safety of nanoparticulate TiO$_2$ in sunscreens is to ensure the use of rutile TiO$_2$. Although lower in activity, the rutile crystal phase can still exhibit substantial photocatalytic properties, leading to the need for additional modification. Surface coatings have been utilized as a means of mitigating the photocatalytic effect of nanoparticulate TiO$_2$. Different types of coating materials can be used and are often based upon Si or Al oxides, hydroxides or polymers. The principle mechanism behind this process is that the photo-inactive coating helps promote recombination of photo-excited e$^-$/h$^+$ pairs in the core TiO$_2$ material, by presenting an insulating layer with an increased band gap, thus reducing the probability of ROS production. However, such methods are not entirely foolproof as evidenced by the incorporation of additional antioxidant compounds in many sunscreen formulations to counteract remnant ROS produced. Complex coating materials can also require lengthy synthesis processes and hence increase the price of production. Our groups has previously investigated the surface modification of TiO$_2$ nanoparticles with bismuth sub-carbonate ((BiO)$_2$CO$_3$) and achieved a product with lower photocatalytic activity, relative
6.1 Introduction

to bare TiO$_2$, whilst still maintaining adequate UV protection.$^{602}$ The last chapter two chapters of this thesis work have also dealt with surface modifications of TiO$_2$ nanoparticles using chitosan (Chapter 4) and CeO$_2$ (Chapter 5), respectively. However, the core TiO$_2$ nanoparticles used were the aforementioned highly photoactive P25 TiO$_2$, and so, the composite produced is not directly suitable for UV applications. As outlined in the previous chapter, CeO$_2$ is a wide-band gap semiconducting material that has been previously investigated as an alternative coating material due to its ability to absorb UV radiation and mediate ROS production by cycling of surface Ce sites through the 3+/4+ oxidation states.$^{62}$ Combined with in vitro and in vivo evidence of its superoxide dismutase mimetic activity in human cells exposed to radiation, as well as the improved biocompatibility demonstrated in Chapter 5, CeO$_2$ nanoparticles could be the solution to countering the photocatalytic activity of TiO$_2$ used in sunscreens.$^{615-617}$ In addition to the reduced photocatalytic activity, certain criteria outlined by governing health and cosmetic regulatory organisations, such as the European Union SCCS need to be considered when developing cosmetically used TiO$_2$. $^{67}$ These include the purity, crystal phase composition, aspect ratio and surface area of the core TiO$_2$ nanoparticles and the stability of the coating material.

In this Chapter, we describe the preparation of rutile TiO$_2$ nanorods and a CeO$_2$/rutile TiO$_2$ nanocomposite material, by facile hydrothermal and precipitation routes and describe the potential application of these nanoparticles for use in UV filtering applications, with an emphasis on controlled TiO$_2$ particle morphology and reduced ROS generation in the nanocomposite material. Rutile TiO$_2$ nanorods were prepared by treating an amorphous TiO$_2$ precursor under mild hydrothermal conditions. Subsequently, the TiO$_2$ nanorods were decorated with CeO$_2$ nanoparticles through a simple chemical precipitation method. An investigation into the optical and morphological properties of the materials was carried out. Furthermore, the photocatalytic activity of the composite and pristine materials were assessed through the irradiation of the water soluble dye, crystal violet (CV) with UV radiation and solar simulated light. The performance of these synthesized materials were also compared to two commercial TiO$_2$ products, namely,
AEROXIDE® TiO\textsubscript{2} P25 (Evonik, DP25) and rutile TiO\textsubscript{2} nanoparticles (Sigma Aldrich, SR). Finally, the new materials are benchmarked against the SCCS criteria mentioned above and the suitability of these materials for application as inorganic UV absorbers are also assessed.

### 6.2 Results and Discussion

#### 6.2.1 Establishment of Synthesis Conditions for Obtaining the Rutile TiO\textsubscript{2} Phase

**Materials Characterisation**

The initial conditions for preparing the rutile TiO\textsubscript{2} nanoparticles were established through a series of hydrothermal experiments, cycling through various HNO\textsubscript{3} concentrations and adjusting the autoclaving temperature. Figure 6.1 highlights the XRD patterns obtained for the samples prepared under differing acid and temperature conditions. The amorphous nature of the precipitated powder obtained prior to hydrothermal treatment is depicted in Figure F.1, which suggests that any induced crystallinity seen post-hydrothermal treatment is a result of the treatment process. In Figure 6.1 (*left*), modification of the HNO\textsubscript{3} concentration resulted in a progressive transition in crystal phase, starting from a mixture of the anatase (PDF card 96-101-943), rutile (PDF card 96-900-7532) and brookite (PDF card 96-900-4138) crystal phases before transitioning to purely the rutile phase at higher concentrations of acid. The presence of brookite in the samples prepared in 3M and 6M HNO\textsubscript{3} (H3M and H6M) coincides with previously reported findings when preparing TiO\textsubscript{2} nanoparticles through precipitation in acidic media and low temperatures.\textsuperscript{618,619}

It has also been suggested that brookite nuclei may play a major role in facilitating the phase transformation of the initial precursor powder to the rutile phase during hydrothermal treatment.\textsuperscript{620}
6.2 Results and Discussion

Figure 6.1: Variation of the crystal phase of the synthesized TiO$_2$ as influenced by the (left) concentration of HNO$_3$ (when treated at 180$^\circ$C) and (middle) autoclaving temperature (when treated with 16M HNO$_3$).

The distinction between the mixed phase and pure phase samples is also evident when comparing their respective Raman spectra (Figure 6.2). A number of Raman active peaks featured for the H3M and H6M samples whilst four distinct features were seen for both samples prepared in 16M HNO$_3$ but at differing treatment temperatures (H16M and HTIO2, respectively). From experimental evidence and computational studies, the number of Raman active modes typically seen for the common TiO$_2$ crystal phases are 4, 6 and 36 corresponding to the rutile, anatase and brookite phases.$^{621-624}$ Brookite bands present for the H3M sample were assigned in accordance with reported peak positions and are attributed to the $A_{1g}$ (126, 152, 194, 247, 413, 452, 544 and 636 cm$^{-1}$), $B_{1g}$ (213, 286, 322, 501 cm$^{-1}$), $B_{2g}$ (366, 460 and 583 cm$^{-1}$) and $B_{3g}$ (171 cm$^{-1}$) symmetries.$^{623,625}$ Similarly for the H6M sample, brookite peak assignments are given for the $A_{1g}$ (126, 152, 195, 247 and 545 cm$^{-1}$), $B_{1g}$ (214, 284, 320 and 500 cm$^{-1}$), $B_{2g}$ (367 cm$^{-1}$) and $B_{3g}$ (172 cm$^{-1}$) symmetries. It is also possible that some of these peak assignments could be due anatase vibrational modes as there is substantial overlap between certain anatase/brookite Raman active transitions. Such modes include the anatase $E_g$ (152 and 172 cm$^{-1}$) and $A_{1g}$ (500 cm$^{-1}$) vibrational modes. The four major peaks observed at approximately 143, 446 and 609 cm$^{-1}$ for both the H16M and HTIO2 samples are assigned to the $B_{1g}$, $E_g$ and $A_{1g}$ Raman active modes for the rutile crystal phase. These also appear prominently
in the H6M sample spectrum, corroborating with the higher rutile phase content observed from XRD. The broad feature centred at 235 cm$^{-1}$ has been previously attributed to crystal lattice disorder or second-order scattering.$^{626,627}$ No evidence of brookite or anatase Raman active modes for the two 16M HNO$_3$ synthesized samples again corroborates with the XRD data, highlighting the rutile phase purity of the samples.

**Figure 6.2:** Raman spectra for the H3M, H6M, H16M and HTIO2 samples.

Figure 6.3 highlights SEM and TEM images obtained for the H3M, H6M and H16M samples prepared at 180°C and the HTIO2 sample prepared at 150°C. A mixture of particle morphologies were observed for both the H3M and H6M samples, varying from smaller spheroidal particles to larger, elongated rod-like particles which can be attributed to the mixed crystal phase composition for these samples. These differences in morphology for particles in the same sample are thought to arise due the mixed crystal phase composition of the sample and was further investigated through EELS. Figure 6.4 compares the EELS profiles for the Ti L$_{2,3}$ edges taken from different particles observed through TEM.
6.2 Results and Discussion

Analysis of the H6M sample. The line profile obtained for the spectra labelled rutile was collected from a rod-like particle (Figure F.2), similar to those present in the H16M and HTIO2 samples. The splitting and shape of the L$_3$ edge peak centered at 459.6 eV is in agreement with previously reported findings for the rutile TiO$_2$ crystal phase and is attributed to electron transitions from the $2p_{3/2}$ state to $e_g$ state of the Ti 3$d$ orbital produced by crystal field splitting.$^{518,628}$ Variation in shape of this transition between TiO$_2$ crystal phases is due to the differences in the coordination of oxygen around titanium and can be used as a method for studying the crystal structure of individual particles. As can be seen, the line shape of this peak varies when obtained from the more spheroidal particles, giving shapes consistent with previously reported EELS spectra for the anatase and brookite crystal phases.$^{629,630}$ The identification of all three main TiO$_2$ crystal phases is also consistent with the XRD and Raman data obtained. Both the H16M and HTIO2 samples displayed elongated particles of varying length. The rod-like morphology formed is indicative of rutile particle growth along the [001] orientation and has been previously ascribed to rapid rutile chain growth along the $c$ axis of the TiO$_6$ octahedra due to corner sharing on opposite ends in the (001) plane.$^{513,631}$ Employing an acid-based solvent during hydrothermal synthesis of TiO$_2$ nanoparticles has also been previously shown to influence the crystal phase formed.$^{554,632,633}$ A high concentration of NO$_3^-$ has been suggested to facilitate and promote corner shared bonding, as in the case of the rutile crystal phase, and could explain the crystal phase transformation observed at higher concentrations of HNO$_3$ treatment.$^{572,634}$ There is also a difference in the length and size of the rod-like particles formed when hydrothermally treated at different temperatures. Particles obtained at 180$^\circ$C (H16M $\bar{x}$(width) = 50±10 nm) are notably larger than those obtained at 150$^\circ$C (HTIO2 $\bar{x}$(width) = 16±3 nm) and treated for the same period of time (24 hr). This is expected considering particle growth is strongly governed and facilitated by the temperatures and pressures employed during synthesis.
6.2 Results and Discussion

Figure 6.3: SEM and TEM (inset) micrographs of the hydrothermally synthesized TiO$_2$ samples.

One of the proponents for a commercially “acceptable” UV filter, and subsequent sunscreen formulation, is transparency. Inorganic based UV filters, such as TiO$_2$, have long suffered issues with this due to their inherently high refractive index and large particle size (aggregates in the µm range), contributing to substantial visible light scattering and opaqueness. Reducing the primary particle size can help improve the ‘transparency’ of such particulate filters by enhancing UV absorption mediated by a higher percentage of surface atoms compared to bulk TiO$_2$ and a reduction in visible light scattering governed by Mie theory. It is also an important criterion outlined by the SCCS to ensure that the particle size fits with the number size distribution of 30 – 100 nm. As such, for the remainder of this particular study, the hydrothermally synthesized rutile TiO$_2$ discussed is that prepared at 150°C using 16M HNO$_3$ owing to its smaller particle size relative to the 180°C treated sample. Furthermore, in accordance with SCCS criteria for cosmetic TiO$_2$ nanoparticles, the 150°C, 16M HNO$_3$ hydrothermally prepared sample addresses the crystal phase criteria by being composed solely of the rutile crystal phase. In addition, the morphology and aspect ratio of these particles are in line with variants included in the criteria ie being of lanceolate/needle shape and having an aspect ratio between 1.0 to
6.2 Results and Discussion

4.5 (calculated aspect ratio of 6±2 falls within this range based upon length and width measurements as shown in Table 6.1).

![Figure 6.4: EELS line profiles obtained for sample H6M. EELS profiling location shown in Figure F.1.](image)

6.2.2 Comparative Performance of Hydrothermally Synthesized Rutile TiO$_2$ and Nanocomposite CeO$_2$/TiO$_2$ Compared to Commercial Products as a Potential UV Filter

Materials Characterisation

Figure 6.5 depicts the XRD patterns for the commercial TiO$_2$ powders, DP25 and SR, as well as the as-prepared HTIO2 and CTIO2 nanoparticles. Of the samples tested, the SR, HTIO2 and CTIO2 samples displayed single phase reflections, indexed to the rutile crystal phase as expected. DP25 exhibited a mixed phase composition consisting of approximately 80% anatase to 20% rutile, which corroborates with previously published findings for the material.$^{635,636}$ The lack of reflections due to a CeO$_2$ impurity phase in the CTIO2 sample could be a result of a lack of crystallinity but also due to the very low loading of CeO$_2$ expected. In fact, the weight loading percentage (wt%) of Ce relative to Ti was determined to be 7±4 wt% as calculated through EDS (Figure F.4 and Table F.1). The associated mean crystallite sizes and BET specific surface area values are listed in
Table 6.1: Experimental results obtained relating to crystallite/particle size and surface area. The TEM particle size data represents mean ± standard deviation (SD) (count = 100). Errors for the crystallite size and surface area were generated by the specific software used for measurement. * Mean size for the CeO$_2$ nanoparticles.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mean Crystallite Size (XRD) (nm)</th>
<th>Mean Particle Size (TEM) (nm)</th>
<th>Surface Area (m$^2$ g$^{-1}$)</th>
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</thead>
<tbody>
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<td>DP25</td>
<td>37±4</td>
<td>40±20</td>
<td>59.2±2.1</td>
</tr>
<tr>
<td>HTIO2</td>
<td>25±3</td>
<td>L: 90±20</td>
<td>44.0±0.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>W: 16±3</td>
<td></td>
</tr>
<tr>
<td>SR</td>
<td>54±5</td>
<td>60±20</td>
<td>22.4±0.3</td>
</tr>
<tr>
<td>CTIO2</td>
<td>-</td>
<td>1.8±0.4*</td>
<td>74.6±1.9</td>
</tr>
</tbody>
</table>

**Figure 6.5:** XRD patterns for the commercial TiO$_2$ and hydrothermally synthesized powders tested.

A comparison of the particle morphologies and sizes between the synthesised and commercial samples is shown in Figure 6.6. The DP25 sample consisted of a relatively inhomogeneous mixture of spherical, ellipsoidal and cubic particles with a mean particle size of 40±20 nm. Similarly, the other commercial product, SR, also displayed particles
of varying morphology albeit with a larger mean particle size of 60±20 nm. The larger particle size of SR is also consistent with the smaller specific surface area calculated as compared to DP25 (22.4±0.3 compared to 59±2 m² g⁻¹). HTIO2 and CTIO2 both consist primarily of the hydrothermally synthesized rutile TiO₂ nanorods as shown previously in Figure 6.3. The mean widths and lengths for these rod-like particles were determined to be 16±3 and 90±20 nm, respectively. The specific surface area for HTIO2 was calculated to be 44.0±0.8 m² g⁻¹, lower than that of DP25, which could be again attributed to differences in particle dimensions, but also the synthesis and treatment methods involved in preparing either sample.

![SEM and TEM micrographs of the DP25, HTIO2, SR and CTIO2 samples.](image)

**Figure 6.6**: SEM and TEM (inset) micrographs of the DP25, HTIO2, SR and CTIO2 samples.

Notably, the CTIO2 sample was found to have a specific surface area of 75±2 m² g⁻¹, approximately 21% larger than that of DP25, despite being primarily based upon the same rutile TiO₂ as those in the HTIO2 sample. A possible reason for the increased surface area could be due to the presence of extremely fine CeO₂ nanoparticles at the surface of the rutile rods in CTIO2. As depicted in Figure 6.7 (**left**), the CeO₂ nanoparticles appear deposited, not as a uniform coating of complete coverage, but as small aggregates or even as individual particles along the surface of the core TiO₂ rods (Figure 6.6 (**middle**)) and
6.2 Results and Discussion

(right)). The same precipitation behaviour atop of TiO$_2$ surfaces was depicted also in Chapter 5. The addition of these extremely fine particles ($\bar{x} = 1.8\pm0.4$ nm) along the surface of the TiO$_2$ rods could be providing additional sites for gas sorption, leading to an overall increase in the specific surface area. High-angle annular dark-field (HAADF) images of these fine CeO$_2$ nanoparticles (Figure 6.7 (right)) suggest these particles are crystalline as evidenced by the uniformity of lattice fringes, which further suggests that the lack of a CeO$_2$ impurity phase from XRD of CTIO2 is due to the low loading of CeO$_2$ relative to the core TiO$_2$.

![Figure 6.7:](left) High-angle annular dark-field (HAADF) image of the CTIO2 composite sample. (middle) EELS map detailing the distribution of Ti and Ce for the particles shown (left) in the form of heat map. (right) High resolution HAADF of the particles shown in (left), highlighting the presence of a CeO$_2$ nanoparticle at the surface of the rutile TiO$_2$.](image)

**Optical Properties and Photocatalytic Activity**

The ultraviolet filtering properties of the commercial and synthesized samples were assessed through dilute UV-Vis spectroscopy. Figure 6.8 highlights the absorption spectra obtained for each sample in EtOH. DP25 displayed the highest absorbance, with peak absorbance occurring in the UVB wavelength region, coinciding with its use as a UVB filtering agent in sunscreening products. The $E_g$ calculated for DP25 has been calculated to be $3.30\pm0.02$ eV, which is in close agreement with previously reported findings. The commercial rutile sample, SR, showed significantly less absorbance, with peak absorbance centred within the UVA region. Band gap values of $3.04\pm0.05$, $2.94\pm0.05$ and $2.95\pm0.03$ eV were calculated for the HTIO2, SR and CTIO2 samples. The narrowing of these band gap values is a reflection of the rutile crystal phase composition of these
6.2 Results and Discussion

samples relative to DP25, which consists of a mixed anatase/rutile crystal composition. Although the absorbance properties of the HTIO2 and CTIO2 samples are less than that of DP25, substantial absorbance across both the UVA and UVB wavelengths regions highlights their suitability as UV protective agents.

![UV-Vis absorption spectra](image)

**Figure 6.8:** UV-Vis absorption spectra recorded for the commercial and as-prepared TiO\(_2\) samples for 30 mg L\(^{-1}\) suspensions prepared in ethanol. The absorbance spectra for a sample of CeO\(_2\) nanoparticles (30 mg L\(^{-1}\)) prepared through the same precipitation process used for the CTIO2 nanocomposite is also shown for reference.

The photocatalytic activities were evaluated through the photo-mediated catalytic decomposition of CV dye. Figure 6.9 displays the relative decrease in CV absorbance over time and the respective kinetics plots when exposed to UV radiation (Figure 6.9 (top)) and solar simulated light (Figure 6.9 (bottom)). The apparent rate constants, \(k_{app}\), for each tested sample were calculated and are listed in Table 6.2. For both light sources, DP25 exhibited the highest performance of degradation with \(k\) values of \(40 \pm 1\) and \(12.3 \pm 0.4 \times 10^{-3}\) min\(^{-1}\). This was expected since, not only is it manufactured and used as a reference photocatalyst, but also when considering the materials properties. Coupled with its already very high UV absorbance, relative to the other samples, DP25 is also a mixture of the anatase and rutile crystal phases. The anatase TiO\(_2\) crystal phase is often cited as having greater photocatalytic activity than the rutile phase, particularly in relation to the degra-
6.2 Results and Discussion

It has also been reported that mixed phase TiO$_2$ displays even greater photocatalytic activity relative to either of the single phases, depending on the composition. Sunscreen products in the past that have used micronized TiO$_2$ previously have been shown to contain particles of a similar crystal phase composition to that of DP25. In fact, a study investigating the discolouration of coated steel panels linked the usage of sunscreen products containing DP25-like TiO$_2$ by workers installing the panels to the early onset of degradation. The reason for this discolouration was attributed to the photocatalysed production of ROS or, more specifically, OH$^*$. As such, it is desirable to modify sunscreen based TiO$_2$ in a manner that mitigates this free radical production whilst also maintaining adequate protection from UV radiation.

**Table 6.2:** Optical band gap ($E_g$) values and rate constants ($k_{app}$) determined for the samples under UV and solar simulated irradiation.

<table>
<thead>
<tr>
<th>Sample</th>
<th>$E_g$ (eV)</th>
<th>Rate Constant $k_{app}$ ($\times 10^{-3}$)(min$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>UV</td>
</tr>
<tr>
<td>DP25</td>
<td>3.30±0.02</td>
<td>40.4±1.1</td>
</tr>
<tr>
<td>HTIO2</td>
<td>3.04±0.05</td>
<td>10.1±0.3</td>
</tr>
<tr>
<td>SR</td>
<td>2.94±0.05</td>
<td>7.4±0.1</td>
</tr>
<tr>
<td>CTIO2</td>
<td>2.95±0.03</td>
<td>0.9±0.1</td>
</tr>
</tbody>
</table>

Samples HTIO2 and SR both displayed reduced UV and solar simulated light photocatalytic activities, as compared to DP25. A number of factors may be in play to explain the observed results. To begin, both HTIO2 and SR are purely rutile which, as previously mentioned, is often found to be less active than that of the anatase crystal phase. Another factor involved, is the reduced absorbance by these samples across the UVA and UVB bands relative to DP25. This means that the production of ROS will likely be reduced due to the decreased excitation of the catalysing material through UV photon absorption. Yet another factor to consider is the lower specific surface areas of HTIO2 and SR as
6.2 Results and Discussion

compared to DP25. In this instance, the reduced surface area means that there are fewer surface active sites for the CV dye and free water based species (H$_2$O, OH$^-$, H$_3$O$^+$) to adsorb to. This impairs the ability for the catalysing material to directly degrade the dye or indirectly degrade it through the production of ROS, thus leading to a decrease in photocatalytic activity. Although lower in UV absorbance performance, taking into account the reduced photocatalytic activity seen and the physical parameters in line with SCCS criteria, the HTIO2 sample also, on its own, appears an ideal platform for conducting future investigations into the surface modifications of sunscreen based TiO$_2$ UV filters. In the case of the CTIO2 sample, yet a further reduction in photocatalytic activity whilst under either UV or solar simulated light was observed. As with HTIO2 and SR, CTIO2 also has weaker absorbance across the UV region as compared to DP25, however, the surface area calculated for CTIO2 is much larger, which would suggest some other factor is involved. Furthermore, a comparison of the UV absorptive properties of the CTIO2 nanocomposite as compared to pristine CeO$_2$ nanoparticles (Figure 6.8) at the same concentration (30 mg L$^{-1}$) reveals that the CeO$_2$ imparts minimal additional UV absorbance benefits, which was demonstrated also in Chapter 5. This is particularly apparent as the actual loading of CeO$_2$ in the CTIO2 nanocomposite (7±4 wt%) is significantly lower than the amount of CeO$_2$ present in the UV Vis absorbance measurements of the CeO$_2$ nanoparticles. As such, the contribution of the CeO$_2$ nanoparticles in the CTIO2 sample towards the decreased photocatalytic activity observed due to UV 'blocking' is relatively small. Instead, deposition of CeO$_2$ nanoparticles on the surface of the rutile TiO$_2$ rods in CTIO2 could be providing a means of inhibiting free radical production or scavenging free radicals before degradation may occur. Indeed, CeO$_2$ nanoparticles have been reported to behave as an antioxidant as a result of a large number of surface defect sites. These defect sites enable the reversible oxidation/reduction of the cerium cation by interaction with surface adsorbed molecules, enabling scavenging of free radical species.$^{500}$

It has also been suggested that the size of the CeO$_2$ nanoparticles can impact this free-radical scavenging ability, whereby, as the particle size decreases, the antioxidant activity increases.$^{62,596}$ The presence of CeO$_2$ in CTIO2 is thus enabling free radical scavenging
of photogenerated ROS whilst also blocking surface active sites on the core rutile TiO$_2$ particles for adsorption of other molecules. This scavenging and blocking interplay is not perfect however, as evidenced by the small degradation that still occurs under both light sources, but is certainly much improved compared to DP25, HTIO2 and SR. Combined with its absorbance across the UVA and UVB regions, the material shows great potential as a new active sunscreening ingredient. The very low photocatalytic activity observed for the CTIO2 sample also addresses another important SCCS criteria in relation to TiO$_2$ nanoparticle cosmetic use. Ideally, new TiO$_2$ based UV filters should have no photocatalytic activity, however, the SCCS considers up to 10% activity relative to a standard or corresponding un-coated/un-doped reference to be acceptable. In this instance, CTIO2 displays up to 2% (UV light) and 4% (solar simulated light) of the photocatalytic activity of DP25, a material with a crystal phase composition exact to that of a previously used commercial TiO$_2$ UV filter (these percentages are based upon the calculated rate constants listed in Table 6.2).\textsuperscript{53} Compared to the uncoated form, HTIO2, the composite sample is also substantially low in activity (9% and 15% under UV and solar simulated light), further emphasizing its applicability as a potential UV filter in sunscreening products.
6.3 Conclusion

In this Chapter, a low photocatalytic nanocomposite material based upon rutile TiO$_2$ nanorods decorated with CeO$_2$ nanoparticles for use as an ‘active’ ingredient in sunscreen products was developed. The rutile TiO$_2$ nanorods with controlled morphology were produced using a facile hydrothermal method and exhibited physical characteristics in line with cosmetic regulatory guidelines for use of TiO$_2$ in cosmetic products. We demonstrated that deposition of a small CeO$_2$ loading (Ce/Ti weight percentage equal to 7±4 wt%) at the surface of the TiO$_2$ nanorods can greatly reduce photocatalytic activity of the bare material as well as other commercial variants of TiO$_2$ nanoparticles under both UV and solar simulated light exposure. The reduction in photocatalytic activity and maintaining of the UV filtering properties of this nanocomposite material highlights its potential
6.3 Conclusion

for application in sunscreen products.
Chapter 7

Conclusion and Future Work

Ultraviolet radiation exposure is a known carcinogen and, with the ever increasing number of incidences of skin cancers occurring each year, the application of sunscreen products containing ultraviolet filters has become an important part of minimising and preventing skin-related diseases. There is also an never-ending need to develop and implement new ultraviolet filters that provide improved protection and long-term stability which, although important for all general consumers, is particularly important for populaces in countries that experience above-average yearly doses of ultraviolet radiation such as Australia. Recently, concerns amongst consumers and the scientific community have been raised over the application of inorganic ultraviolet filtering nanoparticles in these products. Studies investigating the potential penetration of these nanoparticles through human skin after topical application have suggested that the particles are unlikely to reach viable skin cells, however, concerns over the free radical generating capabilities of these nanoparticles requires attention. In particular, sunscreen nanoparticles based on certain compositions of TiO$_2$ have been shown to exert significant oxidative potential through the photo-induced generation of reactive oxygen species. This oxidative potential has also been shown to have an impact on viable mammalian cells, inducing states of oxidative stress and apoptosis. To counteract this photocatalytic activity, manufacturers may coat sunscreen based titanium dioxide nanoparticles with inert materials, however this can also be detrimental to the ultraviolet filtering properties of the resultant nanoparticles. As such, the purpose of this thesis was to address this issue and attempt to develop titanium dioxide based
nanocomposite materials with excellent ultraviolet filtering capabilities and diminished photocatalytic potential.

Manufacturers will commonly employ coating materials based upon silicon and aluminium oxides, hydroxides and stearates. The use of polymeric coatings have also been explored in literature, with one such promising candidate being chitosan, and forms the basis for the first major chapter of this thesis work. Through a spray-drying technique, chitosan particles and chitosan/TiO$_2$ nanocomposite particles were successfully produced. Using a commercial-grade photocatalysis TiO$_2$ powder for the core nanoparticles, the thermal, optical and photocatalytic properties were assessed. The resulting nanocomposite particles obtained featured TiO$_2$-load dependent encapsulation efficiency, with excessive loading leading to the presence of excess TiO$_2$ nanoparticles at the surface of the chitosan shell. As such, a more optimal TiO$_2$ loading was found when the weight ratio of TiO$_2$ to chitosan was 1:1. No modification to the TiO$_2$ crystal phase was observed as a result of the chitosan encapsulation, whilst Fourier-transform infrared spectroscopy revealed characteristic absorption peaks attributed to chitosan and TiO$_2$ vibrational modes. A decrease in the activation energy for thermal degradation of chitosan in the nanocomposite samples, as compared to the pristine chitosan particles, suggested a decrease in thermal stability in the nanocomposite samples. Examination of the UV filtering properties of the nanocomposite samples through diffuse reflectance revealed a slight red-shift in major absorbance into the UVA region, which was significant considering the primarily UVB absorbing properties of pristine TiO$_2$ nanoparticles. However, the chitosan encapsulation also introduced increased visible light absorption, leading to significant colouration of the powders obtained. Assessment of the photocatalytic activity of the nanocomposite particles compared to both pristine chitosan and TiO$_2$ revealed a major reduction in activity. As such, the work demonstrated the potential for an organic polymer, namely chitosan, to be employed as an encapsulating agent for sunscreen based TiO$_2$ nanoparticles. Further examination of the UV protective ability of these nanocomposite particles requires addressing however, with incorporation into a sunscreen formulation and the subsequent sun protection factor evaluated. In addition, emulsion characteristics such as
suspension stability, chemical stability and interaction with other formulation ingredients must be assessed, particularly under prolonged exposure to ultraviolet radiation.

The next chapter of this thesis work involved investigation of CeO\(_2\) nanoparticles as a coating/partial coating of TiO\(_2\) nanoparticles. Literature reports of CeO\(_2\) nanoparticles and their influence on viable mammalian cell lines have demonstrated its potential biomimetic antioxidant activity. CeO\(_2\) nanoparticles have also been demonstrated to display UV filtering properties and thus could play a dual-role as a coating material for sunscreen based TiO\(_2\). Commercial-grade photocatalysis TiO\(_2\) nanoparticles were decorated with chemically precipitated CeO\(_2\) nanodots at different atomic concentrations of Ce to Ti. Increased loading of the CeO\(_2\) nanodots resulted in the increased presence of CeO\(_2\) aggregates atop the core TiO\(_2\) nanoparticles, with the CeO\(_2\) particle size increasing up to 5 nm in diameter. Although the UV absorptive properties of the nanocomposite samples were decreased as compared to the pristine TiO\(_2\) nanoparticles, substantial UV absorbance was still observed. In addition, the potential free radical scavenging properties of the CeO\(_2\) nanodots were demonstrated through the ultraviolet and solar-simulated light driven photochemical degradation of crystal violet dye. A significant reduction in the photocatalytic potential of the nanocomposite samples compared to pristine TiO\(_2\) was observed and did not coincide with the still significant UV absorbance measured. This could suggest that some other UV blocking mechanism is at effect and could be due to scavenging of reactive species generated by the core TiO\(_2\) nanoparticles by the decorating CeO\(_2\) nanodots. The potential cytotoxic and phototoxic properties under ultraviolet illumination of the nanocomposite and the pristine components were evaluated towards the HaCaT human skin cell line. It was found that, for both the nanocomposite and pristine CeO\(_2\) samples that toxicity was minimal and that the changes in cell viability were insignificant as compared to the control. The potent phototoxid potential of pristine TiO\(_2\) nanoparticles was shown through the reduced cell viability measured and was significantly different as compared to the control and the nanocomposite and pristine CeO\(_2\) samples. Thus, the work performed demonstrates that the application of CeO\(_2\) nanodots to TiO\(_2\) can have a substantially beneficial effect in improving biocompatibility of sunscreen based TiO\(_2\)
nanoparticles through the reduction of photocatalytic activity. Translation of this diminished photocatalytic activity to specific crystal phase compositions of TiO$_2$ needs to be addressed to ensure applicability of the nanocomposite as an active ingredient in sunscreen products and is the focus of the last major chapter of this thesis work.

The crystal phase composition, particle morphology, ultraviolet filtering and photocatalytic properties of sunscreen based TiO$_2$ nanoparticles are key properties with specific criteria outlined by various governmental regulating bodies. To address these criteria, a hydrothermal synthesis method was employed to synthesize rutile TiO$_2$ nanoparticles of elongated shape, substantial ultraviolet absorbance and reduced photocatalytic activity as compared to other TiO$_2$ crystal phases/compositions. Employing nitric acid as a digesting and coordinating agent, the optimal conditions for obtaining rutile TiO$_2$ were determined to be with 16 M nitric acid at 150°C for 24 hr. Other synthesis conditions either yielded mixed phases of TiO$_2$ consisting of the rutile, anatase and brookite crystal phases or produced particles of sizes larger than 100 nm in all directions. Subsequently, these rutile nanoparticles were decorated with CeO$_2$ nanodots in a similar manner to those prepared in the prior chapter and the ultraviolet filtering and photocatalytic properties assessed. The CeO$_2$/rutile TiO$_2$ nanocomposite displayed excellent ultraviolet absorption albeit, to a lesser extent as compared to the pristine rutile TiO$_2$ and commercial-grade photocatalysis TiO$_2$ nanoparticles. However, the significantly reduced photocatalytic activity of the nanocomposite under both ultraviolet and solar-simulated light irradiation, out of line with its ultraviolet absorption properties, further highlights the potential free radical scavenging properties of CeO$_2$ and its applicability as a coating for sunscreen based TiO$_2$ nanoparticles. To further complement the work performed, incorporation of the nanocomposite into sunscreen emulsions must be performed to assess the sun protective and ultraviolet A protective factors. Furthermore, to appease consumer concerns over the use of nanoparticles in such cosmetic and therapeutic sunscreen products and to further demonstrate the biocompatibility of the nanocomposite material produced, both in vivo and ex vivo dermal penetration experiments must be performed. Finally, a deeper understanding of the mechanism behind the reduction in photocatalytic potential imparted by
the CeO$_2$ nanoparticles must be investigated. Studies employing electron paramagnetic resonance could be employed to directly observed and demonstrate the free radical scavenging properties of CeO$_2$ and add further weight to its potential application in sunscreen products as an active coating ingredient and antioxidant compound.
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Appendix A

Chapter 1 Supplementary Information

Figure A.1: Spectral data used in the calculation of UV indices as well as in vitro sun protection factor (SPF) measurements for sunscreen products. a) Spectral irradiance of the ‘standard sun’ as a function of the wavelength-dependent erythemal effectiveness of UV radiation. b) The product of the spectral irradiance and erythemal effectiveness curves seen in a). Figure reproduced from Heinrich et al (2004).
## Appendix B

### Chapter 2 Supplementary Information

Table B.1: TGA approved UV filtering ingredients for use in therapeutic sunscreens in Australia.\(^6\)

<table>
<thead>
<tr>
<th>Ingredient Name</th>
<th>Chemical/Trade Names</th>
<th>Max Allowed Concentration (% w/w)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bemotrizinol</td>
<td>Bis-ethylhexyloxyphenol methoxyphenol triazine, Bemotrizinolum, Escalol S, Tinosorb S</td>
<td>10</td>
</tr>
<tr>
<td>Benzylidene camphor sulfonic acid</td>
<td>(\alpha-(2\text{-oxoborn-3-ylidene})\text{toluene-4-sulphonic acid, Meroxyl SL} )</td>
<td>6</td>
</tr>
<tr>
<td>Butyl methoxy dibenzoylmethane</td>
<td>4-tert-butyl-4-methoxy dibenzoylmethane, Avobenzone</td>
<td>5</td>
</tr>
<tr>
<td>Camphor benzalkonium methosulfate</td>
<td>N,N,N-trimethyl-4-(oxoborn-3-ylidenemethyl)anilinium methyl sulfate, Meroxy SO</td>
<td>5</td>
</tr>
<tr>
<td>Cinoxate</td>
<td>2-ethoxyethyl (para)-methoxycinnamate</td>
<td>6</td>
</tr>
<tr>
<td>Ingredient</td>
<td>Description</td>
<td>Concentration</td>
</tr>
<tr>
<td>----------------------------------------------------------------------------</td>
<td>-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------</td>
<td>---------------</td>
</tr>
<tr>
<td>Diethylamino hydroxybenzoyl hexyl benzoate</td>
<td>2-[4-(diethylamino)-2-hydroxybenzoyl]-hexyl ester, Uvinul A Plus</td>
<td>10</td>
</tr>
<tr>
<td>Dioxybenzone</td>
<td>Benzophenone 8</td>
<td>3</td>
</tr>
<tr>
<td>Disodium phenyl dibenzimidazole tetrasulfonate</td>
<td>1H-benzimidazole-4,6-disulfonic acid, 2,2-(1,4-phenylene)bis, disodium salt, Bisimidazylate, Neo Heliopan AP</td>
<td>10</td>
</tr>
<tr>
<td>Drometrizole trisiloxane</td>
<td>2-(2H-benzotriazol-2-yl)-4-methyl-6[2-methyl-3-[1,3,3,3-tetramethyl-1-[(trimethylsilyloxy]-disiloxanyl]-propyl-phenol, Silatrizole, Mexoryl XL</td>
<td>15</td>
</tr>
<tr>
<td>Ecamsule</td>
<td>Terephthalylidene dicamphor sulfonylic acid, Meroryl SX</td>
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</tr>
<tr>
<td>Homosalate</td>
<td>Homomenthyl salicylate, 3,3,5-trimethylcyclohexyl 2-hydroxybenzoate</td>
<td>15</td>
</tr>
<tr>
<td>Isoamyl methoxycinnamate</td>
<td>Isoamyl para-methoxycinnamate, Amiloxate</td>
<td>10</td>
</tr>
<tr>
<td>4-methylbenzylidene camphor</td>
<td>3-(4-methylbenzylidene)-camphor, Enzacamene</td>
<td>4</td>
</tr>
<tr>
<td>Mentyl anthranilate</td>
<td>5-methyl-2-(1-methylethyl) cyclohexanol-2-aminobenzoate, Meradimate</td>
<td>5</td>
</tr>
<tr>
<td>Methylene bis-benzotriazolyl-tetramethylbutylphenol</td>
<td>Bisotrizole, Tinosorb M</td>
<td>10</td>
</tr>
<tr>
<td>Octocrylene</td>
<td>Octocrilene, 2-cyano-3,3-diphenyl acrylic acid, 2-ethylhexyl ester, 2-ethylhexyl-2-cyano-3,3-diphenylacrylate, Uvinul N</td>
<td>10</td>
</tr>
</tbody>
</table>

* Concentration values are typically expressed in percentage by weight.
<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Description</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Octyl methoxycinnamate</td>
<td>Ethylhexyl methoxycinnamate, Octinoxate, Univul MC</td>
<td>10</td>
</tr>
<tr>
<td>Octyl salicylate</td>
<td>Ethylhexyl salicylate, 2-ethylhexyl salicylate, Octisalate</td>
<td>5</td>
</tr>
<tr>
<td>Octyl triazone</td>
<td>Ethylhexyl triazone, 2,4,6-trianalino-(para-carbo-2’-ethylhexyl-1’-oxy)-1,3,5-triazine, Uvinul T</td>
<td>5</td>
</tr>
<tr>
<td>Oxybenzone</td>
<td>Benzophenone-3, 2-benzoyl-5-methoxyphenol, Univul M</td>
<td>10</td>
</tr>
<tr>
<td>Padimate O</td>
<td>Ethylhexyl dimethyl PABA, 2-ethylhexyl 4-dimethylaminobenzoate, octyl dimethyl PABA</td>
<td>8</td>
</tr>
<tr>
<td>PEG-25 PABA</td>
<td>Ethoxylated ethyl 4-aminobenzoate, PEG-25 PABA, Uvinul P</td>
<td>10</td>
</tr>
<tr>
<td>Phenylbenzimidazole sulfonic acid</td>
<td>2-phenylbenzimidazole-5sulfonic acid, 2-phenyl-5-sulfobenzimidazole, Ensulizole</td>
<td>4</td>
</tr>
<tr>
<td>Polysilicone-15</td>
<td>Dimethicodiethylbenzalmalonate, Parsol SLX</td>
<td>10</td>
</tr>
<tr>
<td>Sulisobenzone</td>
<td>Benzophenone 4, 5-benzoyl-4-hydroxy-2-methoxybenzene sulphonic acid, Uvinul MS</td>
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<tr>
<td>Sulisobenzone sodium</td>
<td>Benzophenone 5, 5-benzoyl-4-hydroxy-2-methoxybenzene sulphonic acid, sodium salt</td>
<td>10</td>
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<tr>
<td>Titanium dioxide</td>
<td>E171</td>
<td>25</td>
</tr>
<tr>
<td>Chemical</td>
<td>EWG hazard score</td>
<td>Skin penetration</td>
</tr>
<tr>
<td>--------------------------------</td>
<td>------------------</td>
<td>------------------</td>
</tr>
<tr>
<td>Triethanolamine salicylate</td>
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<tr>
<td>Tris-biphenyl triazine</td>
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<td></td>
</tr>
<tr>
<td>Zinc oxide</td>
<td></td>
<td>Pigment white 4</td>
</tr>
</tbody>
</table>

Table B.2: EWG hazard scores for selected UV filters commonly found in sunscreen products. Ratings drawn from various factors outlined in literature reports pertaining to the UV filters listed\textsuperscript{230, 273, 299, 359, 404, 641–659}

<table>
<thead>
<tr>
<th>Chemical</th>
<th>EWG hazard score</th>
<th>Skin penetration</th>
<th>Hormone disruption</th>
<th>Skin allergy</th>
<th>Other concerns</th>
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</thead>
<tbody>
<tr>
<td>Oxybenzone</td>
<td>8</td>
<td>Detected in breast milk; 1% to 9% skin penetration in in vitro studies</td>
<td>Weak estrogen, moderate anti-androgen; associated with altered birth weight in human studies</td>
<td>Relatively high rates of skin allergy</td>
<td></td>
</tr>
<tr>
<td>Octyl methoxy-cinnamate</td>
<td>6</td>
<td>Detected in breast milk; &lt;1% skin penetration in in vitro and in vivo studies</td>
<td>Hormone-like activity; reproductive system, thyroid and behavioral alterations in in vivo studies</td>
<td>Moderate rates of skin allergy</td>
<td></td>
</tr>
<tr>
<td>Ingredient</td>
<td>Rating</td>
<td>Skin Penetration</td>
<td>Hormone Disruption</td>
<td>Toxic Breakdown Products</td>
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<tr>
<td>----------------------</td>
<td>--------</td>
<td>------------------</td>
<td>--------------------</td>
<td>-------------------------</td>
<td></td>
</tr>
<tr>
<td>Homosalate</td>
<td>4</td>
<td>Detected in breast milk; &lt;1% skin penetration in in vitro and in vivo studies</td>
<td>Disrupts estrogen, androgen and progesterone</td>
<td></td>
<td>Toxic breakdown products</td>
</tr>
<tr>
<td>Octisalate</td>
<td>4</td>
<td>Skin penetration in in vitro studies</td>
<td></td>
<td>Rarely reported cases of skin allergy</td>
<td></td>
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<tr>
<td>Octocrylene</td>
<td>3</td>
<td>Detected in breast milk; skin penetration in in vitro studies</td>
<td></td>
<td>Relatively high rates of skin allergy</td>
<td></td>
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<tr>
<td>Titanium dioxide</td>
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<td>No conclusive evidence of skin penetration</td>
<td>No evidence of hormone disruption</td>
<td>None</td>
<td>Inhalation concerns</td>
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<tr>
<td>Zinc Oxide</td>
<td>2</td>
<td>&lt;0.01% skin penetration in human volunteers</td>
<td>No evidence of hormone disruption</td>
<td>None</td>
<td>Inhalation concerns</td>
</tr>
<tr>
<td>Avobenzone</td>
<td>2</td>
<td>Very limited skin penetration</td>
<td>No evidence of hormone disruption</td>
<td>Breakdown products causes relatively high rates of skin allergy</td>
<td>Unstable in sunshine, must be mixed with stabilizers</td>
</tr>
</tbody>
</table>
Mexoryl SX 2 <0.16% skin penetration in human volunteers No evidence of hormone disruption Skin allergy is rare

Table B.3: List of TiO$_2$ nanoparticle samples and selected physicochemical properties. Data reproduced from Tang et al, (2018).$^{378}$

<table>
<thead>
<tr>
<th>Sample</th>
<th>Crystalline Phase</th>
<th>Primary Particle Size (nm)</th>
<th>Coating Material</th>
<th>Purity (wt%)</th>
<th>SSA (m$^2$ g$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P25</td>
<td>80% anatase, 20% rutile</td>
<td>31±8</td>
<td>-</td>
<td>99.5</td>
<td>61.7</td>
</tr>
<tr>
<td>AR52</td>
<td>78% anatase, 22% rutile</td>
<td>52±9</td>
<td>Silica/Alumina</td>
<td>≥98</td>
<td>34.0</td>
</tr>
<tr>
<td>AR23</td>
<td>Anatase</td>
<td>23±8</td>
<td>Silane</td>
<td>99.8</td>
<td>283.7</td>
</tr>
</tbody>
</table>
Appendix C

Chapter 3 Supplementary Information

Figure C.1: Light emission profile for the OSRAM Ultra-Vitalux 300 W sunlamp. Figure reproduced from Deka et al, 2008.

Table C.1 displays the calculated seeding numbers used for selectively seeding and growing the HaCaT cells for a particular day at approximately 90% confluency. These seeding numbers were calculated using Equation 3.16 and adjusting the doubling time (DT) based on the first few cell counts after bringing up the cells from frozen storage.
Table C.1: Seeding Numbers ($S_N$) calculated using Equation 3.16 for the HaCaT cells for different doubling times ($DT$).

<table>
<thead>
<tr>
<th>Incubation Days ($N_{Days}$)</th>
<th>DT = 22 hr</th>
<th>DT = 23 hr</th>
<th>DT = 24 hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>7.04</td>
<td>7.28</td>
<td>7.50</td>
</tr>
<tr>
<td>2</td>
<td>3.30</td>
<td>3.53</td>
<td>3.75</td>
</tr>
<tr>
<td>3</td>
<td>1.55</td>
<td>1.71</td>
<td>1.88</td>
</tr>
<tr>
<td>4</td>
<td>0.73</td>
<td>0.83</td>
<td>0.94</td>
</tr>
<tr>
<td>5</td>
<td>0.34</td>
<td>0.40</td>
<td>0.47</td>
</tr>
<tr>
<td>6</td>
<td>0.16</td>
<td>0.20</td>
<td>0.23</td>
</tr>
<tr>
<td>7</td>
<td>0.08</td>
<td>0.09</td>
<td>0.12</td>
</tr>
</tbody>
</table>

Due to the light absorbing nature of the phenol red medium used for culturing the HaCaT cells, a different medium or solvent was required to prevent the cells from drying out without absorbing the incident visible and UV light during the phototoxic assays. Figure C.2 displays the absorption plots measured for phenol red free medium and DPBS, demonstrating the reason for using DPBS due to its lack of UV or visible light absorption.

Figure C.2: Absorbance profiles for the phenol red free media (DMEM/F12) and DPBS.
Appendix D

Chapter 4 Supplementary Information

Figure D.1: Chemical structures of chitosan and chitin monomers.
**Figure D.2:** Particle size distribution and histogram plots for the (top-left) CHI, (top-right) 1:1 CHI/TiO$_2$ and (bottom) 2:1 CHI/TiO$_2$ samples (count = 400 per sample).

Figures D.3 and D.4 highlight the TGA and DTA curves obtained during thermal analysis of the chitosan/TiO$_2$ nanocomposite and pristine particles. The data obtained at varying heating rates were used in calculating the degradation energy ($E_a$) for each sample through application of the Kissinger model for polymer thermal degradation (Equation 4.1).
Figure D.3: TGA curves for the a) CHI, b) 2:1 CHI/TiO$_2$ and c) 1:1 CHI/TiO$_2$ samples treated at various heating rates.
Figure D.4: Derivative curves obtained from TGA for the a) CHI, b) 2:1 CHI/TiO$_2$ and c) 1:1 CHI/TiO$_2$ samples treated at various heating rates.

Figure D.5: Kinetics plots for the degradation of crystal violet dye as ascribed by the Langmuir-Hinshelwood relationship in the presence of the spray-dried and commercial materials.
Appendix E

Chapter 5 Supplementary Information

Figure E.1 displays the individual Tauc plots used for calculating the optical band gap ($E_g$) values for the samples tested. As can be seen, plotting of the absorption coefficient to an exponent value correlating to the type of electronic transition that occurs between conduction band and valence band of a semiconductor against the wavelength energy yields a plot with an absorption edge. Extrapolation of this linear absorption edge to the x-axis yields an approximation of $E_g$. 
Figure E.1: Tauc plots for the a) pristine TiO$_2$ nanoparticles, b) 2.5 at%, c) 5%, d) 10 at% CeO$_2$/TiO$_2$ nanocomposites and e) pristine CeO$_2$ nanoparticles.
Figure E.2: UV-Vis absorption plots and corresponding Beer-Lambert relationship plots for the a) TiO$_2$ (P25), b) 2.5 at%, c) 5 at%, d) 10 at% and e) CeO$_2$ samples prepared.
Figure F.1 shows the XRD pattern obtained for the titanium precursor powder obtained from direct precipitation of TBT using concentrated NH$_4$OH in water. After drying and crushing the precipitant obtained into a fine powder, the XRD pattern was collected to yield a plot devoid of any major features. This would suggest that the powder obtained is amorphous in nature and lacks and characteristic peaks associated with the common TiO$_2$ crystal phases. As such, any subsequent patterns obtained following hydrothermal treatment of this precursor powder could be solely contributed to the high pressure, low temperature autoclaving process in HNO$_3$. 
EELS was used to distinguish between different crystal phases of TiO$_2$. Although chemically similar in composition, slight differences in the structure of the rutile, anatase and brookite crystal phases results in differences in the fine structure of their EELS profiles. Thus for mixed phase samples, differentiation of these crystal phases may be achieved. As observed in Figure F.2, this technique was employed to investigate the crystal phase composition of particles of different morphology in sample H6M. Individual spectra per pixel of the scanned areas (Figure F.2 b)) were averaged to produce the line profiles shown in Figure F.2 a), demonstrating the presence of all three common TiO$_2$ crystal phases and supporting the XRD data obtained for the sample.
**Figure F.2:** a) EELS profiles obtained across the pixels numbered in c), which is the region of interest outlined in b). The sample examined here is the 6M HNO$_3$ 180°C treated sample.

**Figure F.3:** Particle size distribution and histogram plots for the (top-left) DP25, (top-right) SR, (bottom-left) HTIO$_2$ and (bottom-right) CTIO$_2$ samples (count = 100 per sample). The particle sizes measured for the HTIO$_2$ and CTIO$_2$ samples correspond to the nanorod width and the CeO$_2$ nanodot sizes for these samples, respectively.
Figure F.4: Example EDS spectrum collected from the CTIO2 sample prepared on holey carbon copper grid during TEM analysis.

Table F.1: EDS results obtained on the CTIO2 composite sample detailing the relative Ce/Ti atomic and weight percentages.

<table>
<thead>
<tr>
<th>Element</th>
<th>Ti</th>
<th>Ce</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Wt.%</td>
<td>At.%</td>
</tr>
<tr>
<td>Measurement</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>94.34</td>
<td>97.99</td>
</tr>
<tr>
<td>2</td>
<td>87.97</td>
<td>95.54</td>
</tr>
<tr>
<td>3</td>
<td>93.81</td>
<td>97.79</td>
</tr>
<tr>
<td>4</td>
<td>97.13</td>
<td>99.00</td>
</tr>
</tbody>
</table>

Mean (±SD) 93±4 98±1 7±4 2±1
Figure F.5: Nitrogen gas adsorption isotherm plots for the DP25, SR, HTIO2 and CTIO2 samples.
Figure F.6: UV-Vis absorption plots and corresponding Beer-Lambert relationship plots for the a) DP25, b) HTIO2, c) SR and d) CTIO2 samples prepared.

Figure F.7 highlights the Tauc plots for the samples examined in Chapter 6. The $E_g$ for the samples were calculated as previously outlined through extrapolation of the linear portion of the plots to the x-axis.
Figure E7: Tauc plots obtained from diffuse reflectance for the (top-left) DP25, (top-right) SR, (bottom-left) HTIO2 and (bottom-right) CTIO2 nanoparticle and nanocomposite samples.