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The Characteristics of Nanoscale Sunscreen Particles

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ABSTRACT

The Characteristics of Nanoscale Sunscreen Particles

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This thesis describes the characterization and photochemical behavior of metal oxide nanopigments derived from commercial sunscreens. We investigate their size, morphology, elemental composition, and crystal structure using electron microscopy, x-ray diffraction and energy dispersive x-ray spectroscopy. TiO₂ and ZnO nanoparticles are known to exhibit photochemistry in their pure form. We report the reactivity of derived materials towards Congo red, and spin trap electron paramagnetic resonance spectroscopy.

The results of this work have implications for regulation of nanotechnology in consumer products. Currently nanoscale TiO₂ and ZnO can be legally added to sunscreens without any requirement that their size be disclosed on the product labels. Our results reveal that these sunscreen components can be photoactive, resulting in the degradation of Congo red dye and generation of hydroxyl radicals. These observations warrants a reexamination of the policy that holds nanoscale materials identical to their bulk counterparts for labeling purposes.
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Chapter 1

Introduction

This thesis characterizes the inorganic ingredients present in nine commercial sunscreens. The features of titanium dioxide and zinc oxide nanocrystals such as size, shape, crystal structure and photoactivity can be correlated to the reactivity and ultimately toxicity in both cellular and whole animal studies. Both with diminishing size, and particularly altered phase composition, these substances can become reactive towards biological systems. Whether these observations of "pure" nanocrystalline oxides have any bearing on the properties of metal oxide nanocrystals in sunscreen inorganic particles is an outstanding question. The aim of this work is to examine directly pigments derived from sunscreens. We both fully characterize their physical properties so as to map them onto known trends for these systems. We also evaluate their photochemical behavior in non-biological setting as this can be a highly relevant predictor of subsequent acute and chronic effects.

An introduction to sun protection, and the nature of the blocking agents available for sunscreens is presented in Chapter 2. Ultraviolet radiation from the sun is well known to cause skin cancer in humans; sun protection in the form of topically applied sunscreens is a primary measure for preventing this disease. Consumers have a wide selection of sunscreen formulations to choose from, and these contain both organic and inorganic active ingredients. Recently, more and more sunscreens are using nanoscale inorganic uv-blocking agents because of their small size, transparency and efficacy. An outstanding issue in light of the large consumer market for these products is the potential
for unanticipated health effects arising from the nanoscale size of the pigments. This chapter also provides a brief outline of the debate about this issue as it relates to metal oxides as this motivates the fundamental research that follows.

Chapters 3 and 4 describe the methods used for the measurement of pigment physical and chemical properties, particularly their free radical production and photo-oxidation behavior. The adverse effects of nanoscale materials mostly result from their enhanced chemical activity; for this reason, we evaluated this reactivity using the spin trap electron paramagnetic resonance spectroscopy and the decolorization of Congo red dye. After a discussion of these methods in Chapter 3, we then proceed to describe how we examined the physical measurement of the material structure. Taken together these methods include:

- electron microscopy – to image the samples
- dispersive x-ray spectroscopy – to verify elemental composition
- x-ray diffraction – to get the crystal structure of investigated materials
- decolorization test – to check if analyzed particles are photoactive
- spin trap electron paramagnetic resonance spectroscopy – to test if sunscreen inorganic filters are able to generate hydroxyl radicals.

Chapters 5 and 6 presents the results from all of the experiments mentioned above. Physical properties (size, shape, composition, crystal phase) of nanoscale materials present in investigated sunscreens are revealed and thoroughly described in Chapter 5. The results from tests of chemical photoactivity are found in Chapter 6. And lastly, a summary of the whole research is concluded in Chapter 7, which is the last chapter of this thesis.
Chapter 2

Background

This chapter describes physiological effects on the skin caused by solar ultraviolet radiation and explains how skin is protected when sunscreen is applied. Currently, over-the-counter (OTC) sunscreen drug products are recommended for people by official organizations such as the American Academy of Dermatology and the American Cancer Society in order to prevent unwanted exposure to harmful ultraviolet radiation (UVR) [1, 2]. These electromagnetic waves cause sunburn, premature aging and several types of skin cancer including melanoma, basal, and squamous cell carcinoma [3-5].

2.1 UVR adverse effects

Excessive sun exposure of the skin results in both acute and delayed adverse effects because high energy ultraviolet radiation emitted by the sun is able to penetrate into the skin layers damaging the cells.

Ultraviolet radiation emitted by the sun consists of UVA (400 – 320 nm), UVB (320 – 290 nm) and UVC (290 – 200 nm) wavelengths. Shorter wavelengths are more energetic and thus more dangerous for the skin. Fortunately, UVC is completely absorbed in the atmosphere and does not reach the earth's surface. UVA comprises most (> 95 %) of the incident on the skin ultraviolet radiation, penetrates deeply reaching both the epidermis and the dermis. Only 5% of UVR that reaches the skin is UVB, more energetic than UVA and mostly absorbed by epidermis which is the outer layer of skin.

The skin is one of the most important organs in the human body. It protects the human body and consists of three distinct layers: epidermis, dermis, and subcutaneous
fat. The outer surface of epidermis (stratum corneum) is formed from dead skin cells that are constantly renewed by cells from the living part below. Melanocytes, cells responsible for production of pigment (melanin), are a major component of this layer and the melanin that they contain both regulates the amount of UVR that enters the skin by absorption and imparts the color to the skin. The epidermis is also home to the Langerhans cells which signal the immune system when foreign agents are present. Between the epidermis and subcutaneous tissues is the dermis which is comprised as fibers, mainly collagen and other elastic fibers that impart strength and resiliency to the skin. The dermis also contains nerve endings, sweat and oil glands as well as hair follicles [3]. The last layer – fat – separates the skin from the muscle tissue.

In depth discussion of the adverse effects of UV light on the skin are described in detail by other, for example see [3-5]. The skin responds to UVR exposure by stratum corneum thickening and tanning because of increased melanin synthesis by melanocytes. Overexposure to UV rays causes sunburn, a reaction of the skin to the direct skin cells damage mostly caused by UVB radiation. The skin reddening appears due to increased blood content which is a defensive mechanism against damage caused by UVB. In spite of inflammatory response, damaged cells appear in epidermis and can become cancerous. The shorter UVB wavelengths are directly absorbed by cellular DNA and lead to its lesions [6].

Longer UVA radiation is not absorbed directly by DNA but it can be absorbed by other endogenous molecules leading to production of reactive oxygen species (ROS), which then react with proteins, lipids and DNA [7]. In this way, UVA causes indirect cellular damage. UVA does not cause reddening of the skin, and its effects are not as
rapid. However, it is a silent and slow toxin. It contributes to delayed effects which include a reduction in the skin's strength and elasticity (photoaging) as well as skin cancer. Therefore, cumulative sun exposure over the years causes chronic health effects for multiple reasons.

Sun exposure is not wholly negative for humans. The production of vitamin D, for example, results from the photolysis of 7-dehydrocholesterol in the epidermis and is an essential component of bone health. Vitamin D is responsible for regulation of calcium concentration. Casual sun exposure of 15 minutes per day is enough to produce the body's requirement of vitamin D and with overexposure resulting in negative effects as described above [4]. Lack of sun exposure has also been linked to deficiencies in mental health, and some clinical studies of depression have found improvements after moderate treatment with sun lamps [8, 9]. Therefore, getting a little sunshine is important for feeling good and generating vitamin D. However, too much sun can cause health issues, from sunburns to skin cancer.

As summer approaches, people often go tanning. They think it makes them look better. However, the perfect golden tan – the result of the “baking” in the sun is nothing else like a reaction of the body to photodamage that may result in skin cancer. This form of cancer is the most common of all cancers in the United States [10]. Each year, in US around 1 million diagnosed non-melanoma skin cancer cases (basal cell carcinoma and squamous cell carcinoma) are considered to be sun-related and around 2700 people die yearly from non-melanoma skin cancer. Melomana – the third type of skin cancer is the most dangerous because causes about 8,110 deaths per year from about 59,940 cases (data from 2007) [2, 11]. Thus, it is necessary to protect the skin from sun damage.
Some organizations such as the American Academy of Dermatology, the American Cancer Society or the Centers for Disease Control and Prevention recommend sunscreens with SPF of at least 15 and broad-spectrum coverage as elements of a general strategy for sun protection [1, 2, 10]. The following sections present the rules for sunscreens as well as their UV blocking mechanism.

2.2 Sunscreen regulations

In the United States sunscreens are regulated by Food and Drug Administration (FDA) as over-the-counter (OTC) drugs. The FDA sets the standards for formulating, testing and labeling sunscreen products. It requires that sunscreen formulation yield at least sun protection factor (SPF) of 2 when the dose of 2 mg/cm² of emulsion is applied on the skin [12]. The SPF value is a good measure of the time it takes for sun-exposed skin to redden, and thus it only gives information about UVB protection. The concept of the “sun protection factor” was introduced in 1962 by Franz Greiter and currently, SPF is a worldwide standard for determining the effectiveness of sunscreens [13]. However, in order to achieve the suggested protection and the SPF provided on labels, an average adult should use about 1 oz (29 g) of sunscreen [14]. Whether personal compliance with this application level is high remains an outstanding question [15]. Of particular concern is whether a consumer’s behavior towards sun exposure relaxes after applying too little of a sunscreen. If consumer’s feel a false sense of protection and do not use enough material, they may increase their exposure to UVR.

Currently, there is no measure of UVA protection. Sometimes, producers advertise “broad spectrum protection” which indicates that sunscreen protects from both UVB and UVA rays. Analyzing the protection against UVA rays is much more
complicated than for UVB because longer wavelengths do not cause an apparent sunburn that consumers can identify; instead, its effects are more subtle but still of grave concern (e.g. skin cancer). The FDA has proposed a new labeling system for UVA protection that is based on a scale of one star (low protection) to four stars (the highest protection), although this system is still not mandatory which help better inform consumers [16].

Non-governmental organizations have been highly critical of the policies used by the FDA and other governments to label and inform consumers about sunscreens. The Environmental Working Group (EWG), for example, charges that FDA has failed in its mission to protect consumers from ineffective sunscreens. Its literature review of sunscreen chemicals found that many products contain unstable and toxic agents. Most critical in their analysis was the finding that many high-SPF sunscreens do not protect from UVA radiation [17]. This conclusion derives from the fact that the absorption spectrum of many organic sun blocking agents do not cover UVA radiation; notably inorganic sun blocking agents do offer such coverage.

2.3 Organic and inorganic sunscreens

Sunscreens contain active ingredients that attenuate UVR. Currently, there are 17 active ingredients approved in the United States of which 15 are organic and 2 are inorganic (see Table 2.3-1.) [12, 18, 19]. The sunscreen label must list all active ingredients using the terminology defined by the table: such terms are referred in some cases as the “monograph” for an ingredient. There is no approved term or “monograph” for nanoscale materials which has been one complication for policies that aim to expand the labeling of consumer products.
Table 2.3-1  Sunscreen active ingredients [12, 18, 19].

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Maximum concentration (%)</th>
<th>Protection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aminobenzoic acid (PABA)</td>
<td>15</td>
<td>X</td>
</tr>
<tr>
<td>Avobenzone</td>
<td>3</td>
<td>I</td>
</tr>
<tr>
<td>Cinoxate</td>
<td>3</td>
<td>X</td>
</tr>
<tr>
<td>Dioxybenzone</td>
<td>3</td>
<td>X, II</td>
</tr>
<tr>
<td>Ecamsule</td>
<td>10</td>
<td>X, I and II</td>
</tr>
<tr>
<td>Homosalate</td>
<td>15</td>
<td>X</td>
</tr>
<tr>
<td>Menthyl anthranilate</td>
<td>5</td>
<td>II</td>
</tr>
<tr>
<td>Octocrylene</td>
<td>10</td>
<td>X</td>
</tr>
<tr>
<td>Octyl methoxycinnamate</td>
<td>7.5</td>
<td>X</td>
</tr>
<tr>
<td>Octyl salicylate</td>
<td>5</td>
<td>X</td>
</tr>
<tr>
<td>Oxybenzone</td>
<td>6</td>
<td>X, II</td>
</tr>
<tr>
<td>Padimate O</td>
<td>8</td>
<td>X</td>
</tr>
<tr>
<td>Phenylbenzimidazole sulfonic acid</td>
<td>4</td>
<td>X</td>
</tr>
<tr>
<td>Sulisobenzone</td>
<td>10</td>
<td>X, II</td>
</tr>
<tr>
<td>Titanium dioxide</td>
<td>25</td>
<td>X, I and II</td>
</tr>
<tr>
<td>Trolamine salicylate</td>
<td>12</td>
<td>X</td>
</tr>
<tr>
<td>Zinc oxide</td>
<td>25</td>
<td>X, I and II</td>
</tr>
</tbody>
</table>

X – protects against UVB radiation (290 – 320 nm), I – protects against 340 – 400 nm, II – protects against 320 – 340 nm

2.3.1  Organic sunscreen ingredients

The organic sunscreens contain chemical molecules with specific uv-absorbing elements such as aromatic or benzene rings. Absorbed energy is then released through non-radiative means, for example as heat [20]. Most of these materials protect only against UVB (290 – 320 nm) and/or UVA II (320 – 340 nm) radiation (see table 2.2-1)
and do not absorb UVA I rays (340 – 400 nm). While simple to manufacture and apply, these organic uv-blocking agents suffer from a number of limitations. To achieve broad spectrum coverage as well as high SPF, sunscreen formulations must mix different organic agents. Furthermore, some organic molecules are photo-unstable, including avobenzone [21, 22]. The breakdown of one sunscreen ingredient can cause decomposition of another agent and in consequence not only let UV radiation through to the skin but also create unknown photoreaction products of which, the health effects are unknown. Moreover, some people exhibit allergic reactions to organic sunscreen ingredients which can be worsened by the sun [23]. Therefore, the second type of sunscreen UV filters – inorganic pigments – is gaining in popularity.

2.3.2 Inorganic sunscreen ingredients

Inorganic agents have been used in sunscreens since the 1950s [24]. FDA approved the bulk forms of titanium dioxide (TiO₂) and zinc oxide (ZnO) pigments, referred to as “micronized” powders, because they are nontoxic and effective UVR blockers. Inorganic sunscreens ensure broad spectrum coverage (UVA and UVB) by scattering and absorption; they are also photostable and non-irritating. Conventional TiO₂ and ZnO pigments were fabricated as white pigmented powders with particles as large as a hundred microns (1μm = 10⁻⁶ m). Beside UV filters in cosmetics, micronized particles also find use in commercial paints, plastics and paper.

The first generation of inorganic sunscreens was poorly received by consumers because these products were poorly adhering to the skin and white because of the large particle size. By reducing the particle size from the micro- to nanoscale (1 nm = 10⁻⁹ m), these problems can be largely overcome as the sizes are too small to scatter visible light.
TiO$_2$ and ZnO nanoscale particles have thus become a popular alternative to the organic UV filters.

While it is possible to reason that nanoscale pigments are present in commercial formulations, it is not possible to know this for a fact from the labels. For this reason, we were motivated to recover the inorganic components of commercial systems to confirm ours and others expectations that the samples were indeed nanoscale. They represent a significant human exposure to engineered nanoparticles, and thus the features of the materials are important to measure and make available to the larger community. However, the sections that follow outline the existing information pieced together from peer-reviewed and other sources as to the size and quality of TiO$_2$ and ZnO particles.

2.4 Nanoscale TiO$_2$ and ZnO particles

Nano is a prefix pertaining to things on a scale of approximately 1 to 100 nanometers, where 1 nm is $10^{-9}$ m (or a billionth of a meter). To better visualize this scale, a human hair is of the order of 60,000 to 120,000 nm and a typical red blood cell is 2000 nm – 5000 nm in diameter [25]. Thus nanoparticles because of their size bridge both the chemical and biological realm of science. These materials can take on a variety of formats, including isolated components or nanoparticles (with lengths in two or three dimensions greater than 1 nm and smaller than about 100 nm) or nanostructures (with at least one dimension smaller than 100 nm) [26]. Nanomaterials have unique physical, chemical, and biological properties owing to their size. Therefore, manufactured nanoparticles of different chemical composition are attractive for commercial development and application. Currently, engineered nanomaterials are incorporated into electronic, cosmetics, automotive and medical products to improve their quality and
performance. The Project on Emerging Nanotechnologies (PEN) reports that, there are currently over 800 manufacturer-identified products that use nanotechnology [27]. The most rapidly growing sector according to PEN’s online inventory is the Health and Fitness with a total of 502 products, including sunscreens. Much of their value in sunscreens derives from the unique size-dependent TiO₂ and ZnO optical properties that render them both transparent at visible wavelengths but strongly absorbing/scattering at UVB and UVA wavelengths.

2.4.1 Crystal structure and optical properties

Crystal structure plays a significant role in determining material properties. TiO₂ is fabricated in two crystal forms, anatase and rutile, but also exist naturally as brookite. Both TiO₂ as well as ZnO particles can be manufactured either through thermal or wet methods [28, 29]. The temperature of the preparation method determines the crystal structure. At higher process temperatures, the bonds between Ti and O atoms break, allowing their rearrangement which leads to a greater diversity of possible phases. Anatase and rutile both have a tetragonal crystal structure where each Ti⁴⁺ ion is octahedrally coordinated to six O²⁻ ions (Figure 2.4-1). The TiO₆ octahedra are distorted and linked differently in anatase and rutile. Anatase and rutile structures differ further in the number of atoms in the unit cell and the lattice constants. A rutile unit cell contains six atoms, with lattice constants a = 4.595 Å and c = 2.959 Å. An anatase unit cell contains twelve atoms per unit cell with lattice parameters a = 3.784 Å and c = 9.515 Å [30, 31]. Anatase is less dense than rutile, and in the bulk transforms to the more stable rutile phase at 915 °C [32]. Interestingly, when crystalline titania is made with nanoscale dimensions the favored phase is anatase [33].
Figure 2.4-1  Structure of tetragonal anatase and rutile TiO$_2$, where O atoms are shown as white and Ti atoms as black spheres [31].

Zinc oxide has a tendency to adopt hexagonal wurtzite structure in which Zn$^{2+}$ ion is surrounded by four tetrahedrally coordinated O$^{2-}$ ions, and vice versa. There are two zinc and two oxygen atoms per unit cell with lattice constants $a = 3.2495$ Å and $c = 5.2069$ Å [34]. The wurtzite structure is most stable and thus most common at ambient conditions. However, zinc oxide can also crystallize in two other forms: cubic zincblende, and the rarely observed cubic rocksalt [35].

a. Refractive index

Titanium dioxide has a superior opacity to ZnO because of its higher refractive index which for rutile crystal phase is 2.7 and for anatase 2.6 [32]. For ZnO at visible wavelengths the refractive index is 2.0 [34]. TiO$_2$ is slightly less appropriate for than ZnO for sunscreens, however, because its higher index leads to a greater potential for scattering and opacity in thicker applications. High refractive index and large particle
size enhance scattering of visible light and consequently they cause the opaque appearance of inorganic sunscreens. As a result, despite their ability to block UV light, sunscreens that contain micronized particles are preferred by consumers. The smaller size of TiO$_2$ and ZnO nanoparticles reduces visible light scattering and creates transparent, aesthetically pleasing sunscreen products.

b. **Scattering**

Scattering is a process based on the redirection of electromagnetic waves due to their interaction with matter; unlike absorption the process is elastic in nature and the intensity and direction of scattered light depends strongly on particle properties (refractive index, particle shape and size) as well as on the wavelength of the incoming light. Tilley et al. and Jahnke et al. give a good basic discussion of scattering and models to describe this scattering are well established [36, 37].

If the incoming electromagnetic wave meets a large particle surface then reflection, refraction and diffraction occur. These events occur for particle diameters much bigger than the wavelength of the incident radiation and can be described by geometrical optics that allows predicting the orientation of redirected light.

If the particle diameter is equal to or much smaller than the wavelength of incident radiation then Mie and Rayleigh scattering take place, respectively. These types of scattering consist of the absorption of electromagnetic radiation by oscillators (electrons or dipoles) and re-emission of the light in unknown directions, different from the incoming wave.

For Mie scattering emitted waves from different parts of the same particle can interfere constructively or destructively with each other. Therefore, the Mie scattering
pattern is complex and is larger in the forward than in the backward direction. This type of scattering is nearly wavelength independent. Hence, both Mie theory and optic geometry effects describe that scattering at all visible wavelengths (400 – 700 nm) is almost equal. It is such scattering of visible light that causes opacity of inorganic particles bigger than 100 nm dispersed in sunscreen emulsion.

When particles are much smaller than the wavelength of the incoming light (d < 100 nm), the Rayleigh scattering appears with a uniform pattern. Rayleigh scatter is mirrored in the forward and backward directions. The intensity of re-directed light, according to Rayleigh theory, decreases as the fourth power of the wavelength of the incident light. Therefore, these small particles do not scatter wavelengths of electromagnetic radiation equally. Moreover, they scatter visible light less vigorously then UVR. So, sunscreen particles smaller than 100 nm appear transparent on the skin and still effectively attenuate harmful ultraviolet radiation.

Popov et al., Robb et al. and Stamatakis et al. studied influence of TiO₂ and ZnO particle diameters on UV rays scattering efficiency using Mie theory. They reported that zinc oxide below 60 nm in diameter effectively attenuates UVA and UVB radiation with no detectable haziness in the emulsions. The efficient UV blocking action for round, well dispersed TiO₂ particles ranges from about 50 to about 120 nm [38-40]. Unfortunately, TiO₂ sizes which are effective from the UVR attenuation point of view are not completely transparent when incorporated into the sunscreen. Therefore, manufacturers must balance cosmetic acceptability and optimum UV protection by careful control of particle size in these systems.
c. Absorption

In addition to scattering, sunscreen particles also attenuate harmful UV rays by absorption. In absorption, these semiconducting materials interact with the incident radiation so that an electron is promoted from the valence band into the conduction band if the energy of the radiation is greater than the band gap of the oxides. TiO₂ and ZnO are semiconductors with energy band gaps that lie in the UVR regime. For zinc oxide ranges the bulk band gap is approximately 3.4 eV (λ = 365 nm) [34]. For titania, the band gap is sensitive to phase composition. Rutile and anatase titania have energy gaps of 3.1 eV (λ = 397 nm) and 3.3 eV (λ = 377 nm), respectively [32]. These gaps can be converted to the wavelength of light needed for absorption through:

\[ E = \frac{\hbar \cdot c}{\lambda}, \]

where: \( \hbar \) is Planck’s constant, \( c \) – speed of light and \( \lambda \) – wavelength. The energy of ultraviolet radiation that reaches the Earth’s surface lies in the regions of 3.10 – 3.87 eV for UVA and 3.87 – 4.28 eV for UVB. Therefore, both metal oxides are able to absorb UVR. Photons with energy lower than the energy gap of sunscreen particles will be attenuated only by scattering. Moreover, absorption for TiO₂ and ZnO is invariant with particle size above ~10 nm [41, 42]. So, titania and zinc oxide particles with diameters smaller than 100 nm are equally effective as ultraviolet absorbers as the same weight of microscale materials.

Absorption is not size-dependent for TiO₂ and ZnO particles bigger than 10 nm; however, for smaller sizes a quantum size effect can be observed. When the dimensions of a crystal are smaller than the bulk exciton Bohr radius (the average distance in an electron-hole pair) the energy levels available for charge carriers becomes quantized and
results in a blue shift of the band gap. For example, ZnO quantum dots (QDs) with sizes below 5 nm are good candidates for short wavelength optoelectronic devices [43, 44]. For the purpose of attenuation of UV light, extremely small nanoparticles (d < 5 nm) may not possess band gaps large enough to ensure adequate blockage of UVA radiation.

In summary, TiO₂ and ZnO nanoscale particles can both scatter and absorb ultraviolet light. As a result, these nanoscale particles are good options for consumers who need both sun protection without any visible scattering. A very important issue for the use of pigments in sunscreens, however, is the extent to which they change their effective size once formulated. Interparticle interactions may drive clustering leading to aggregates and agglomerates as discussed in the following sections.

### Table 2.4-1 Values of parameters of TiO₂ (rutile and anatase) and ZnO [31, 32, 34].

<table>
<thead>
<tr>
<th>Parameter</th>
<th>TiO₂</th>
<th>ZnO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crystal structure</td>
<td>Rutile</td>
<td>Anatase</td>
</tr>
<tr>
<td>Crystal system</td>
<td>tetragonal</td>
<td>tetragonal</td>
</tr>
<tr>
<td>Lattice parameters</td>
<td>a = 4.595 Å</td>
<td>a = 3.784 Å</td>
</tr>
<tr>
<td></td>
<td>c = 2.959 Å</td>
<td>c = 9.515 Å</td>
</tr>
<tr>
<td>Refractive index</td>
<td>RI = 2.7</td>
<td>RI = 2.55</td>
</tr>
<tr>
<td>Band gap</td>
<td>E₉ = 3.1 eV</td>
<td>E₉ = 3.3 eV</td>
</tr>
<tr>
<td></td>
<td>λ = 397 nm</td>
<td>λ = 377 nm</td>
</tr>
<tr>
<td>Density</td>
<td>4.2 – 4.3 g/cm³</td>
<td>3.8 – 3.9 g/cm³</td>
</tr>
</tbody>
</table>

### 2.4.2 Aggregation and agglomeration

According to the standard for nanotechnology terminology from American Society for Testing and Materials [26]:
- An aggregate is a discrete group of particles strongly bonded together and not easily broken apart
- An agglomerate is a group of particles held together by relatively weak forces (for example, Van der Waals or capillary), that may break apart into smaller particles upon processing.

![Diagram of a single crystal nanoparticle, aggregate, and agglomerate](image)

Figure 2.4-2 Representation of change in particle size [45].

Aggregates and agglomerates, presented schematically in Figure 2.4-2, are much bigger than primary fabricated particles. Their dimensions can exceed 100 nm. Therefore, these clusters may no longer behave as nanoparticles with respect to their solution properties, but may still exhibit the optical properties characteristic of a nanoscale material. This is apparent with nanoscale primary particles of titania (d < 15 nm) that are clear in solution, but upon evaporation or agglomeration take on a distinctly white appearance. A well dispersed pigment in sunscreen also improves performance because it ensures even coverage; it is realistic to expect that agglomeration of particles would lead to patches of skin effectively uncovered. Data presented in Figure 2.4-3 illustrates that the scattering of UV light is weaker when particles have diameters > 200 nm and this agrees with experimental results obtained by Stamatakis et al., Robb et al. and Popov et al. [38-40].
Figure 2.4-3   A and B figures present theoretically calculated dependence of scattering efficiency on particle size for ZnO and TiO₂ materials, respectively [45].

There are several options for stabilizing particles against aggregation. Polymer and/or organic surface treatments can make TiO₂ and ZnO particles hydrophobic or hydrophilic. This protects the pigment against strong aggregation, and improves their dispersion in solvents. Additionally, complex processes such as grinding and/or milling can reduce agglomeration and enhance the dispersion of sunscreen particles [28, 46].

2.4.3 Photochemistry

Enhanced chemical reactivity is another unique property that nanoparticles offer which in the specific case of sunscreens can be problematic. Nanoparticles can be more reactive than their bulk counterparts simply because present more surface per gram; often material surfaces are the most reactive part of a system. For isolated spherical particles of radius $r$ and density $\rho$, the general expression for this surface area per unit mass of material is equal to
\[
\frac{S}{V \cdot \rho} = \frac{4 \cdot \Pi \cdot r^2}{\frac{4}{3} \cdot \Pi \cdot r^3 \cdot \rho} = \frac{3}{r \cdot \rho},
\]

where \( S \) is a particle surface and \( V \) is a particle volume [47]. Therefore, specific surface area (SSA) is inversely proportional to the particle size. To the extent that a material has a reactive surface in the bulk, it will result in net more sites for reactivity if the material is constructed with nanoscale dimensions. For some systems, unique chemical properties can emerge when nanoparticles are prepared with small dimensions. In these cases, the surface structures of the inorganic phase are altered as a result of the small size leading to active sites that exhibit particularly notable reactivity.

These general observations about nanoparticle reactivity are exemplified in the case of TiC\(_2\) and ZnO materials. Both systems can act as photocatalysts which after absorption of light (typically in the UVA range) will produce highly reactive oxygen species (ROS) [48-50]. This capability has been exploited in many ways. For example, nanoscale titania can be used in water treatment to destroy bacteria and viruses; for self-cleaning windows; and for the removal of organics from wastewater streams [51-54]. In some cases, ZnO has been reported to be even more efficient than TiC\(_2\) in photodegradation of organic pollutants [55-57]. Therefore, photoactivity under ultraviolet absorption is a concern in a sunscreen product particularly if the effect is catalytic and results in the generation of reactive oxygen species.

There has been relatively little research into the safety of sunscreen particles. It has been reported by Dunford et al. and Hidaka et al. that TiO\(_2\) and ZnO particles extracted from sunscreens damaged DNA [58, 59]. Brezova et al. showed photogeneration of highly reactive radicals by diluted sunscreen emulsions that contained
titanium dioxide [60]. These results raise concerns on the suitability of TiO₂ and ZnO as sunscreen components, but did not associate specifically the properties with the nanoscale features. Some have speculated that contaminants and other species in the native sunscreen emulsions were responsible for the observed effects. Others have attributed the reactivity to ROS generation by the nanoscale semiconductor particles used as inorganic pigments titanium dioxide and zinc oxide.

TiO₂ and ZnO particles are both semiconductors with band gaps that lie in the ultraviolet radiation regime. Therefore, absorption of photons with energy greater than or equal to the energy gap of the semiconductor particle excites electrons from the valence band to the conduction band creating electron-hole pairs (see Figure 2.4-4). These charge carriers either get trapped at the surface or recombine and radiate energy non-radiatively. Electrons (e⁻) and holes (h⁺) can be captured by any ions, atoms or molecules that exist on the particle surface. Often these trapped carriers are sufficiently long lived such that they can desorb into the solution environment and participate in chemical reactions.

Figure 2.4-4 Schematic representation of redox process at the surface of irradiated TiO₂ (ZnO) particle.
This chemistry can get very active when these surfaces are stabilized in water. In aqueous solution, oxygen (O₂) adsorbs to particles surfaces where it behaves as an electron acceptor (reaction 4). Water molecules (H₂O) or hydroxyl groups (OH⁻) adsorbed on the surface of the TiO₂ or ZnO are specifically reactive with photogenerated holes, forming highly reactive hydroxyl radicals (reaction 1). The photogenerated holes are strong oxidizers and may also oxidize other aqueous phase species (reaction 2) as well as decompose ZnO (reaction 3) – a substance with a low oxidation potential [61]. A detailed description of photocatalysis at the surface of illuminated TiO₂ particle is presented in [31]. Below we summarize these steps:

\[
\text{TiO}_2 (\text{ZnO}) + h\nu \rightarrow e^- + h^+
\]

Oxidation

1. \( h^+ + \text{OH}^- (\text{H}_2\text{O}) \rightarrow \text{OH}^- \)

2. \( h^+ (\text{OH}^-) + \text{adsorbed species} \rightarrow \text{oxidized product} \)

3. \( \text{ZnO} + 2h^+ \rightarrow \text{Zn}^{2+} + \frac{1}{2}\text{O}_2 \)

Reduction

4. \( e^- + \text{O}_2 \rightarrow \text{O}_2^- \)

5. \( \text{O}_2^- + \text{H}^+ \rightarrow \text{HOO}^- + e^- \rightarrow \text{HOO}^- + \text{H}^+ \rightarrow \text{H}_2\text{O}_2 \)

6. \( 2 \text{HOO}^- \rightarrow \text{H}_2\text{O}_2 + \text{O}_2 \)

According to the reactions presented above, in the aqueous phase and in the presence of metal oxide particles, photoinduced reactive radicals such as hydroxyl (OH⁻), superoxide (O₂⁻), hydroperoxy (HOO⁻) radicals are generated. Two radicals: O₂⁻ and
HOO· exist in an equilibrium state in aqueous solutions and both are weak oxidants compared to OH-. Hydroxyl radicals are extremely dangerous species for biological systems because they rapidly react (rate constants > $10^9$ M$^{-1}$s$^{-1}$) with many cell components (DNA, proteins and lipids) leading to cell damage [62-64].

Whether this photochemistry will be relevant in sunscreen formulations is an outstanding question. The overall photocatalytic activity of semiconductor particles strongly depends on many parameters; most critically the surface composition. This feature depends on the different fabrication methods, particle size and morphology, the specific surface area, the catalyst and oxidant concentrations, source and power of irradiation, treatment time, pH values as well as crystal structure in case of TiO$_2$ particles [31, 65, 66]. Anatase TiO$_2$ is a stronger photocatalyst than rutile due to the nature of its surface and parameters including:

- smaller particle size (therefore higher surface area per mass) resulting from the lower temperature of particle fabrication
- enlarged band gap in comparison with rutile which result in higher reduction potential for the conduction band electrons
- higher number of surface hydroxyl groups (hole scavengers) which lower electron-hole recombination rate
- higher capacity to absorb oxygen which is electron scavenger (hence lower recombination rate, too) [67].

In conclusion, TiO$_2$ and ZnO materials are inert in the bulk form as are many wide band-gap metal oxides. However, as many studies now demonstrate when formed of nanoscale dimensions they may photogenerate reactive holes and electrons, hydrogen
peroxide ($\text{H}_2\text{O}_2$) and radicals ($\text{OH}^-$, $\text{O}_2^-$ and $\text{HOO}^-$). These ROS may have harmful effects on the cells as well as may decompose organic compounds which are present in sunscreens [68-71]. Therefore, manufacturers must modify TiO$_2$ and ZnO particles to make them less active and applicable in sunscreen formulations. It has been reported that photocatalytic activity of semiconductor particles can be reduced by coating and doping described in the following section [72, 73].

2.4.4 Photocatalytic inactivation

It is possible to "turn-off" the activity of a nanoparticle by coating its surface with an agent that blocks access to the solution environment, or introduces quenching agents (e.g. defects) in the particle interior. This can be achieved in a number of ways including:

- surface coating with inorganic materials such as silica (SiO$_2$) and alumina (Al$_2$O$_3$) [71, 74]
- doping with manganese or vanadium [72, 75].

Inorganic coatings have been made using a variety of liquid- or vapor-phase deposition methods [76-78]. However, fabricating smooth and homogeneous nanoscale coating layers is complex and expensive. There are several problems with the wet-chemistry coating methods such as difficulty in controlling process conditions or necessary post-treatment procedures, for example filtration. Neither liquid-phase nor chemical vapor deposition method assures uniform coating thickness and homogeneity [77, 79]. On the other hand, it has been reported that photocatalytic inactivation of TiO$_2$ pigments requires at least 4-5 monolayers of silica [80]. If the inorganic barrier is too
thin, photogenerated holes and electrons may tunnel through the coating layer [72]. Moreover, Egerton et al. showed enhanced photoactivity of TiO$_2$ particles toward oxidation of propane for less than 5 wt % coating level for silica. Only high levels of silica, on the order of ~ 15 wt % (0.5 nm) for nanoparticle with ~ 130 m$^2$g$^{-1}$ surface area can render particle surfaces inactive [71]. According to Egerton et al. nanoparticles modified by alumina are more photoactive than pigments coated with the same amount of silica. Therefore Al$_2$O$_3$ is less effective in surface coating than SiO$_2$.

The second inactivation method is based on incorporation of additional element within metal oxide nanoparticles that act as electron/hole traps. Doping of TiO$_2$ (n-type semiconductor) by manganese (p-type ions) speeds up recombination of photogenerated carriers and reduces their lifetime. Under these conditions, photoelectrons and holes are not able to migrate to the particle surface and react with adsorbed species [75]. Therefore, doping of nanoparticles may reduce their photocatalytic activity. In summary, there are many ways to modify nanoscale TiO$_2$ and ZnO particles so that they are less active chemically and more suitable for sunscreen applications.

### 2.4.5 Regulatory policy as related to sunscreens

Currently, there are no legal FDA regulations that require control over the size and photoactivity of inorganic sunscreen ingredients or that require labeling of these systems. As has been pointed out, even if regulation was forthcoming there are no standards for the terminology or measurement of nanoparticle features. As a result, commercial sunscreens may contain coated or uncoated forms of TiO$_2$ and ZnO particles with whatever size and surface coating the manufacturer deems appropriate and commercially viable. Because there is no need to identify nanoscale ingredients on the
labels, consumers have little information about what they are exposed to. Much of the arguments and logic for this decision are captured in the 1999 public commentary period on the challenge to declare a nanoscale form of metal oxides as distinct from the existing monograph of “micronized” particles. FDA did not adopt this change and ruled that nanoscale TiO$_2$ and ZnO materials were appropriately described by the existing terminology of the bulk materials [12]. Manufacturers and regulators have not yet found an avenue to respond to the observations that TiO$_2$ and ZnO nanomaterials may behave chemically different than their bulk counterparts.

Recently, the FDA began requesting public comments on the issues surrounding nanoparticles in sunscreens [16]. A number of organizations, ranging from industry to non-profit groups, have weighed in on what is a contentious debate. Friends of the Earth and Consumers Union have raised concerns about the potential risks of sunscreen ingredients; they requested health and safety regulations for products containing nanoparticles [81-83]. In response, industry organizations such as the Personal Care Products Council countered that we have all the information needed to see that these materials are inert and serve an important function in lowering exposure to UVR [84]. The next section presents a brief review of the existing literature on the toxicology of nanoscale sunscreen pigments and the specific material issues that arise from this analysis.

2.5 Health effects of nano-TiO$_2$ and ZnO materials

Nanotechnology is a new and rapidly developing area; the materials used in its many diverse applications are designed to exhibit new physical and chemical properties. Whether these unique features translate into unanticipated biological properties is
a question that many nanochemists and toxicologists alike are addressing. Not surprisingly, material properties such as diameter, composition, shape and surface composition have all been implicated as key parameters in their toxicological characteristics. Within this larger literature, specific study of ZnO and TiO₂ nanoparticles has focused on their potential for skin penetration, their capacity for ROS generation in biological environments, their diverse effects on cell function, and their impact on whole animals particularly after inhalation exposures.

Most relevant for sunscreens is the potential for nanoparticles to translocate across the skin. For particles with diameters less than 50 nm, there is some expectation that they could cross the stratum corneum and endure into the viable epidermis and dermis [85]. Several studies have been done on penetration of TiO₂ and ZnO particles into the skin layers. These were reviewed for example by Nohynek et al. who concluded that sunscreen nanoparticles remain in the top most layer of the skin (stratum corneum), penetrate deeply into the hair follicle openings but do not penetrate into the living part of the skin (epidermis and dermis) [86].

Absorption of TiO₂ and ZnO particles into the skin was studied in vivo (animal models as well as biopsies of human skin) and in vitro (human and pig skin), by different methods such as: tape stripping, laser scanning microscopy and/or transmission electron microscopy. However, these tests were performed on healthy, intact, immobilized skin samples, far away from the “real world” conditions. Penetration of fluorescence beads and fullerenes was enhanced when skin was flexed and damaged skin is an ineffective barrier for nanoparticles [87, 88]. Mortensen et al. studied influence of UV light on skin penetration by quantum dots (QD diameter ~ 30 nm) using murine model [89]. They
showed the nanoparticles penetrate more readily through uv-damaged skin than non-compromised mouse skin. Moreover, penetration of QDs was also observed into the dermis of intact porcine skin [90].

Sunscreen pigments in commercial formulations have sizes similar to quantum dots tested by Rayman-Rasmussen et al. and Mortensen et al. [89, 90]. However, TiO$_2$ and ZnO nanoparticles have never been tested under UV light and with flexed skin. Introducing additional variables like sun light and skin motion would create “real world” conditions that might better inform decisions about their exposures. Further research is needed to determine if sunscreen particles are able to penetrate through the skin and get into the bloodstream. Also, while skin penetration is important, if people are applying grams of these materials over the bodies every day, then other exposure routes (ocular, ingestion) may be just as relevant.

2.5.1 Interaction with biomolecules

However these nanoparticles reach the cells of the body, once incorporated into biological systems there are many ways in which they could interact with biological systems. A literature review of metal oxide nanoparticle toxicity including TiO$_2$ and ZnO provides a good summary of the current state of the art [91]. Surprisingly, even in the absence UV irradiation these systems have some biological activity [92]. For example, the crystal form of titania (whether it is anatase or rutile) can drastically effect its acute toxicity to cells in culture [69]. It has been reported that uncoated TiO$_2$ nanoparticles under UV light will generate reactive oxygen species (ROS) and cause cell injury. ROS are natural byproducts of cellular metabolism balanced by antioxidants, so increases in ROS levels alone cannot be ascribed solely to the nanocrystal materials. However, in
excess amounts result in oxidative stress and damage to cellular structures [93]. One group has extracted from commercial sunscreens titania and zinc oxide particles in a manner similar to that employed in this work. These materials enhanced the ultraviolet-induced damage of DNA by producing strand breaks on DNA plasmids [58, 59, 94].

In the bloodstream, nanoparticles may be transported and accumulated in a variety of organs and tissues. Based on hairless mice model, it has been shown that intradermally injected QDs (PEG coated with a mean diameter of 37 nm) are transported by the subcutaneous lymphatic system and accumulate into lymph nodes, liver, kidneys and spleen [95]. The important and currently unknown issue is the duration of exposure within the human body. Additionally, the clearance or degradation of nanoparticles may amplify the toxicity. One model anticipates that should sunscreen nanoparticles find their way into the body, they would be marked with proteins as foreign agents and consumed by phagocytes (immune cells). After delivery to the lymph nodes they would be biodegraded into biologically safe components. Phagocytosis is a basic defense mechanism against exogenous substances. However, if particles evade this mechanism, unknown health effect may occur. Some recent data suggests that regardless of the composition, nanoparticles will bind different biomolecules to their surfaces [96, 97]. This may create unexpected complexes between nanoparticles and biomolecules that would lead to both acute and chronic biological effects.

Much research remains to settle questions about whether nanoparticles in sunscreens have negative biological effects. It is well documented that under UV light TiO2 and ZnO particles have greater reactivity and toxicity as compared to their bulk counterparts. While these materials may not easily penetrate the skin, they do lodge deep
into hair follicles and make persist on the skin surface for long periods. In-vitro studies suggest that even in the dark these materials can damage biomolecules, and certainly under ultraviolet exposure they are potent pro-oxidants.
Chapter 3

Photoactivity tests

In this work we use several standard methods to evaluate the chemical activity and ROS generation by nanoscale materials. In our first approach, we consider the decolorization of Congo red dye and monitor the degradation via an optical method. In the second approach we use electron paramagnetic resonance (EPR) spectroscopy using a spin trap. Spin trap EPR results in the formation of detectable, long-lived spin adduct that corresponds quantitatively to hydroxyl radical formation. Together the two methods are complementary and help us interrogate the overall reactivity and kinetics of the photo-oxidative processes.

3.1 Decolorization of Congo red dye

Congo red (CR) is an azo dye resistant to biodegradation and used, for example, in the textile industries. The molecular structure of Congo red dye is presented in Figure 3.1-1. Decolorization of the CR in aqueous suspension of TiO$_2$ and ZnO under UV or solar illumination is a common method to evaluate the photo-activity of nanoparticles. It also has some relevance as removal of dyes generally in textile wastewater is an outstanding problem [51, 98, 99]. This technique leverages the fact that when dyes degrade, it is straightforward to measure the process using ultra-violet visible spectroscopy using the absorbance feature at $\lambda = 496$ nm. Decolorization of the dye molecule can occur in two ways. First, ROS species that are free in solution can attack and oxidize Congo red. Second, Congo red adsorbed to the surface of particles can become directly oxidized by photogenerated holes. This latter method requires that the
dye become adsorbed to the nanoparticle surface. CR decolorization thus measures total system reactivity which includes direct, surface mediated oxidation, and indirect oxidation via ROS.

While the method obfuscates the mechanism of degradation, it offers information that is quite important for assessing overall chemical activity. Most important, the photoactivity of sunscreen pigments can be quantified as a Congo red half-life time ($t_{1/2}$). This is also simple and fast test and most critically is quite quantitative. In addition, the method is visually compelling and could be used as a screen because active particles result in the disappearance of the red color even without measuring CR absorbance.

![Figure 3.1-1 Molecular structure of Congo red dye.](image)

3.2 Spin trap electron paramagnetic resonance spectroscopy

The second method we use offers less quantitative, but more mechanistic, insight into the processes by which nanocrystalline oxides degrade materials. Hydroxyl radicals are difficult to measure due to their short lifetime and high reactivity. Spin trap electron paramagnetic resonance spectroscopy (EPR) is perhaps the best method to tackle this difficult problem.
Electron Paramagnetic Resonance (EPR) is a technique that allows us to study species possessing electrons with unpaired spins. The following short description of the basic physical concept of EPR theory is based on [100].

The unpaired electron has a spin ($S$) given by two projections $m_s = +\frac{1}{2}$ and $m_s = -\frac{1}{2}$. These two projections in a magnetic field have different energies, as shown in Figure 3.2-1 (Zeeman Effect). The unpaired electron have a lower energy state when its spin moment is aligned with the magnetic field ($m_s = -\frac{1}{2}$) and higher energy state when spin moment is aligned against magnetic field ($m_s = +\frac{1}{2}$). The energy difference between these states is $\Delta E$. In the equation for $\Delta E = g \beta B_0$, the only unknown is the $g$-factor that can be determined by irradiating the sample with an electromagnetic wave of known frequency and sweeping the magnetic field until the resonance condition occurs and the radiation is absorbed. Then $g$ is equal to $\frac{h}{v} \beta B_0$. In EPR, the frequency of the electromagnetic radiation is chosen in GHz, which is the microwave frequency.

![Parameter Description](image)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>$g$</td>
<td>$g$-factor</td>
</tr>
<tr>
<td>$\beta$</td>
<td>Bohr magneton</td>
</tr>
<tr>
<td>$B_0$</td>
<td>Resonance field</td>
</tr>
<tr>
<td>$h$</td>
<td>Planck’s constant</td>
</tr>
<tr>
<td>$v$</td>
<td>Frequency</td>
</tr>
<tr>
<td>$m_e$</td>
<td>Mass of electron</td>
</tr>
<tr>
<td>$e$</td>
<td>Electronic charge</td>
</tr>
<tr>
<td>$2\pi h = h$</td>
<td></td>
</tr>
<tr>
<td>$\beta = \frac{eh}{2m_e} \approx 9.274 \times 10^{-24}$ JT$^{-1}$</td>
<td></td>
</tr>
</tbody>
</table>

Figure 3.2-1 Energy level diagram for the free electron as a function of applied magnetic field $B$, showing EPR absorption [100].
In summary, EPR is the resonance absorption of electromagnetic radiation (microwave) by an unpaired electron in the presence of magnetic field. It measures the energy difference between energy levels for the unpaired electron in the magnetic field and gives a single line spectrum at the energy level $\Delta E$. However, the electron is not free but interacts with atomic nuclei that posses its own nuclear spin ($I$). The Zeeman transition is split into $2I + 1$ lines with a magnitude of the splitting ($A$) called the hyperfine splitting constant. For example, the interaction of an electron with hydrogen nuclei ($I = \frac{1}{2}$) yields EPR spectrum containing two lines (Table 3.2-1 B). If a number of equivalent nuclei in molecule is “n”, then the EPR spectrum consists of $2nI + 1$ lines and can be complicated. Spacing between the EPR spectral lines indicates the degree of interaction between the unpaired electron and the perturbing nuclei. So, to correctly identify the radical, it is necessary to correctly assign EPR spectrum to the investigated species. Different examples of the hyperfine interactions are presented in Table 3.2-1.

In some instances, sample species have lifetimes too short to be directly investigated by EPR. Hydroxyl radicals for example exist only with nanosecond lifetimes and cannot be detected directly. Therefore, it is necessary to extend the existence of short lived species by trapping them with other paramagnetic molecules, creating long-lived radical adducts that can be easily detected by EPR. This technique is called spin trap EPR.
Table 3.2-1  Examples of hyperfine interactions [101].

<table>
<thead>
<tr>
<th>FREE ELECTRON $S = 1/2$</th>
<th>A</th>
</tr>
</thead>
<tbody>
<tr>
<td>HYDROGEN $I = 1/2$</td>
<td>B</td>
</tr>
<tr>
<td>NITROGEN ($^{14}$N) $I = 1$</td>
<td>C</td>
</tr>
</tbody>
</table>

Interaction with nitrogen and hydrogen.
Magnitude of hyperfine splittings:
$A_N \gg \gg A_H$

Interaction with nitrogen and hydrogen.
Magnitude of hyperfine splittings:
$A_N = A_H$

**Spin trap electron paramagnetic resonance spectroscopy** is based on the reaction of a nitrone compound (spin trap) with a reactive short-lived radical (R) to give a relatively long-lived nitroxide product (spin adduct) which can be visualized using EPR.
The unpaired electron in the nitroxide spin adduct is localized in a π-orbital of the nitrogen and oxygen atoms. Therefore, it interacts with nitrogen (I = 1) and oxygen (I = 0) nucleus. Additionally, the unpaired electron interacts with the attached groups R, R₁ and R₂ if they have atoms with nuclear spins less than three bond lengths away [101]. The practical limit of radical detection in aqueous solution by spin trap EPR is about 1μM [102]. Furthermore, the intensity of the EPR signal reflects the concentration of spin adduct at the moment the spectrum is acquired. Hence, the EPR spin trap technique is a powerful method for detection of fairly low concentrated, short-lived paramagnetic species.

The most popular spin traps able to trap hydroxyl radicals are α-(4-Pyridyl 1-oxide)-N-tert-butynitrone (POBN) and 5,5-dimethyl-1-pyrroline-N-oxide (DMPO), which structures are presented in Table 3.2-2. Both POBN and DMPO were successfully used for hydroxyl radical detection from ultraviolet light irradiated TiO₂ aqueous suspensions [49, 50, 103]. DMPO was also used in order to detect radicals in suspensions of ZnO particles [48].
Table 3.2-2 Structures of spin traps [62].

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Name</th>
<th>Structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>POBN</td>
<td>α-(4-Pyridyl-oxide)-N-tert-butylnitrone</td>
<td><img src="image" alt="POBN Structure" /></td>
</tr>
<tr>
<td>DMPO</td>
<td>5,5-dimethyl-1-pyrroline-N-oxide</td>
<td><img src="image" alt="DMPO Structure" /></td>
</tr>
</tbody>
</table>

3.2.1 POBN

POBN is a popular hydroxyl radical spin trap due to a high reaction rate with hydroxyl radical \( \sim 1.9 \times 10^9 \text{ M}^{-1} \text{ s}^{-1} \) [102]. The EPR spectrum of POBN-OH consists of a triplet of doublets (similar to presented in Table 3.2-1 D) because of the interaction of unpaired electron with nitrogen and β-hydrogen nucleus that does not belong to the radical (see the reaction schematic in Figure 3.2-3). Hydrogen from OH· is placed in the γ position and for low concentration of POBN-OH γ-hydrogen splitting does not appear. The magnitude of hyperfine slitting constants recorded, for example, by Janzen et al. were \( A_N = 15.03 \text{ G} \), \( A_H^\beta = 1.67 \text{ G} \), \( A_H^\gamma = 0.32 \text{ G} \) and by Jaeger et al. \( A_N = 15.0 \text{ G} \), \( A_H^\beta = 1.72 \text{ G} \), \( A_H^\gamma = 0.35 \text{ G} \) [103, 104]. Jaeger et al. did not observe the hyperfine splitting due to γ-hydrogen for UV irradiated aqueous solution of TiO₂ particles. They were able to record γ-hydrogen splitting for more intense POBN-OH spectrum created by solutions of platinized TiO₂ particles.
There are many experimental factors, such as pH of the medium, spin trap concentration, concentration of the radicals, as well as instrumental settings e.g. modulation amplitude that impact the magnitude of hyperfine splitting constants which can contribute to slightly varied spectra. These issues complicate the quantitative analysis of the data, but EPR signatures are widely used to confirm ROS species type and relative amounts. Bauettner et al. explained that the polarity of solvent caused an increase in $A_N$ [105]. Hence, analyzing EPR spectrum and comparing with previous results, all experimental parameters should be considered. Moreover, the spin adduct stability depends on the experimental conditions. The POBN-OH decomposes to tert-butyl hydroaminoxyl radical which results in an EPR spectrum composed of four lines with 1:2:2:1 intensities [104]. Janzen et al. reported that at pH = 7.0 half-lifetime ($t_{1/2}$) of hydroxyl spin adduct is only 10 s. However, it increases at pH = 6.0 to 80 s.

POBN is known as a poor spin trap of superoxide radical. In contact with $O_2^{-}$ it forms POBN-OOH adduct that quickly decomposes, leaving no EPR spectra. The nitroxide formed upon reaction with superoxide also consists of triplet of doublets similar to POBN-OH. However, it can be distinguished from POBN-OH thanks to the difference in hyperfine splitting constants which for POBN-OOH are: $A_N = 14.16$ G, $A_H^\beta = 1.75$ G [106].

![Figure 3.2-3](image.png)

Figure 3.2-3   The schematic reaction of POBN with OH$^-$ [62].
3.2.2 DMPO

The other popular nitrone spin trap for OH\(^-\) detection is DMPO having a reaction rate higher than for POBN (~ 3.4 \(\times\) 10\(^9\) M\(^{-1}\) s\(^{-1}\)). The lifetime of DMPO-OH adduct is \(t_{1/2} = 2.6\ h\) at pH 7.4, which is much longer comparing to POBN-OH [102]. The EPR spectrum of DMPO-OH adduct displays four characteristic lines with intensities 1:2:2:1 (similar to presented in Table 3.2-1 E) and hyperfine coupling constants \(A_N = A_H = 14.8\ G\) [106].

![Figure 3.2-4 Schematic illustration of formation of DMPO-OH by reaction of hydroxyl radical with DMPO [62].](image)

DMPO is also able to trap superoxide radical forming DMPO-OOH adduct with \(A_N = 14.3\ G, A_H^\beta = 11.7\ G, A_H^\gamma = 1.25\ G\). DMPO-OOH is unstable (\(t_{1/2} = 2\ min\)), decomposing into DMPO-OH and other silent species [102]. Therefore, it is necessary to confirm if investigated system is producing free hydroxyl radicals or if there are coming from the breakdown of DMPO-OOH.

A well understood method, which helps confirm the presence of OH\(^-\), is the simple addition of a hydroxyl radical scavenger such as ethanol (EtOH). Both, DMPO and EtOH react with hydroxyl radical at approximately equal rates. Therefore, adding an excess of ethanol relative to the spin trap makes OH\(^-\) more likely to react with EtOH and produces \(\alpha\)-hydroxyethyl radical (CH(CH)\(_2\)OH\(^-\)). Then CH(CH)\(_2\)OH\(^-\) is trapped by
DMPO forming DMPO-CH(CH)_3OH adduct which spectrum consists of six lines with \( A_N = 15.8 \text{ G} \) and \( A_H = 22.8 \text{ G} \) [102].

\[
\text{MeCH}_2\text{OH} \rightarrow \overset{\text{OH}^-}{\text{MeC-OH}} \rightarrow \overset{\text{DMPO}}{\text{DMPO-CH(CH)_3OH}}
\]

Figure 3.2-5  Schematic illustration of formation of DMPO-CH(CH)_3OH by reaction of \( \alpha \)-hydroxyethyl radical with DMPO [107].

For this work, both POBN as well as DMPO spin traps were employed in order to verify the presence of generated hydroxyl radicals from particles extracted from sunscreens under UV irradiation. In keeping with the prior discussion, ethanol is also added to the DMPO probes in order to confirm the initial signal results from the generation of free OH·.
Chapter 4

Materials and Methods

This section describes the procedures that were followed in completing the experiments discussed in this thesis, particularly the characterization of nanoscale materials in commercial sunscreens. Nine commercially available sunscreen products were purchased in local drug stores. None of the sunscreen labels provided any information about the form or size of the inorganic material. Therefore, scanning electron microscopy was used to image evaporated sunscreen residues and determine the presence of nanoscale features. Then the composition of nanoscale particles was verified by energy dispersive x-ray analysis. The average size of these inorganic sunscreen ingredients and their shape were investigated using transmission electron microscopy. To get the crystal structure of analyzed materials x-ray diffraction analysis was used.

Furthermore, the nanoscale materials were extracted from commercials sunscreens and photoactivities were tested using a Congo red decolorization test and spin trap electron paramagnetic resonance spectroscopy (EPR). Moreover, generation of hydroxyl radicals using spin trap EPR was tested on TiO$_2$ and ZnO standard samples. These were TiO$_2$ and ZnO particles recommended by manufacturers (Basf and Merck) for sunscreens as well as pure nanoscale particles obtained from Sigma-Aldrich and Evonik Degussa Corporation. The photoactivity of these standard samples was compared with the photoactivity of particles extracted directly from commercial sunscreens.
Chemicals

All chemicals used in experiments described in the following sections were obtained from commercial sources and used without further purification, unless stated otherwise. All water was obtained from a Millipore purification system (18.2 MΩ·cm). Ethanol, chloroform and chemicals used for phosphate buffer preparation were obtained from Fisher Scientific (Pittsburgh, PA). The Congo red dye (85%) was purchased from Aldrich (Milwaukee, WI). The high purity spin traps POBN (>99%, HPLC) and DMPO (>99%, GLC) as well as 4-Hydroxy-tempo known as TEMPO (≥98%, HPLC, EPR) were obtained from Alexis Biochemicals (San Diego, CA) and stored at -20 °C.

4.1 Consumer sunscreen products

Nine over the counter sunscreen products with high (30 and above) sun protection factor were purchased at a Walgreen’s and CVS Pharmacy Drug Stores (Table 4.1-1). Seven of the nine products specified TiO₂ or ZnO as active ingredients. The manufacturer of one sunscreen (sample 03) indicated on the label that in this product titanium dioxide served as inactive ingredient, which is inconsistent with current FDA regulations where TiO₂ and ZnO are both considered to be sunscreen active ingredients. The weight fraction of titanium dioxide and zinc oxide present in the investigated sunscreens ranged from 1.2 – 9.1 wt % (data from ingredient lists) which is within the legal range approved by the FDA.

Only seven sunscreens indicated that they were active at both UVA and UVB wavelengths. Two sunscreens (samples 04, 06) with SPF +45 did not list “wide range protection.” SPF refers only to the UVB radiation, but does not include the hazardous UVA. Therefore, sunscreens 04 and 06 in spite of labeling high SPF +45 may not protect
consumers properly, or their label is incomplete. Two products (samples 00 and 02) recorded also the age group ("baby" and "kids") for which the product was marketed.

Table 4.1-1 Identification of investigated sunscreens.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Brand</th>
<th>Inorganic Active Ingredient [wt %]</th>
<th>UV protection range</th>
<th>SPF</th>
</tr>
</thead>
<tbody>
<tr>
<td>00</td>
<td>Banana Boat Baby</td>
<td>TiO₂ (2.4)</td>
<td>UVA and UVB</td>
<td>50</td>
</tr>
<tr>
<td>01</td>
<td>Neutrogena</td>
<td>TiO₂ (9.1)</td>
<td>UVA and UVB</td>
<td>30</td>
</tr>
<tr>
<td>02</td>
<td>Banana Boat Kid</td>
<td>TiO₂ (1.2)</td>
<td>UVA and UVB</td>
<td>30</td>
</tr>
<tr>
<td>03</td>
<td>Banana Boat Sport</td>
<td>TiO₂*</td>
<td>UVA and UVB</td>
<td>50</td>
</tr>
<tr>
<td>04</td>
<td>Walgreens</td>
<td>ZnO (6.86)</td>
<td>—</td>
<td>+45</td>
</tr>
<tr>
<td>05</td>
<td>Olay</td>
<td>ZnO (6.0)</td>
<td>UVA and UVB</td>
<td>30</td>
</tr>
<tr>
<td>06</td>
<td>CVS</td>
<td>ZnO (6.86)</td>
<td>—</td>
<td>+45</td>
</tr>
<tr>
<td>07</td>
<td>Blue Lizard</td>
<td>ZnO (10.0), TiO₂ (5.0)</td>
<td>UVA and UVB</td>
<td>+30</td>
</tr>
<tr>
<td>08</td>
<td>Aveeno</td>
<td>NA</td>
<td>UVA and UVB</td>
<td>30</td>
</tr>
</tbody>
</table>

* listed TiO₂ as an inactive ingredient

4.2 Nanoscale materials extracted from sunscreens

From seven sunscreens (samples 00-02 and 04-07) the inorganic materials were extracted and used in photoactivity tests. The particles were unable to be extracted from an oily emulsion for sample 03. Samples of the extracted particles were shipped to Galbraith Laboratories Incorporation (Knoxville, TN) to verify they elemental composition.

In order to extract particles, first, 5 g of the emulsion containing the active agents were treated with 30 ml chloroform. Then, the sunscreen solution was centrifuged at 4500 rotations per minute (rpm) for 5 minutes using Marathon 22k Centrifuge. Dilution and centrifugation procedures were repeated three times. Next, the sunscreen extract was
dried on a hot plate in a laboratory hood. Finally, particles were collected and stored in the dark at room temperature.

Next, 200 mg from each of 7 sunscreen powder samples (samples 00-02 and 04-07) was transferred into small glass bottles and shipped to Galbraith Laboratories – a commercial analytical firm that can analyze bulk samples for metal content. Each sample was analyzed for 5 elements: carbon, titanium, zinc, silicon and aluminum. Carbon was determined according to the ASTM D5373/D5291 procedure and all other elements were measured by Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES).

4.3 Nanoscale materials obtained from companies

Five standard samples (P25, A-ZnO, Z-COTE, T-ECO, T-AVO) were obtained from companies. Aeroxide P25 from Evonik Degussa Corporation (Parsippany, NJ) was used as a reference sample for photocatalytic tests because these particles are recommended by manufacturers as standard photocatalysts. Aeroxide P25 are titanium dioxide particles with an average size of about 21 nm [108]. A-ZnO from Sigma-Aldrich (Milwaukee, WI) is pure, uncoated zinc oxide with particles smaller than 100 nm [109]. Photoactivity of both P25 and A-ZnO samples was compared to extracted from sunscreen particles as well as standard nanoscale particles recommended by manufacturers for sunscreens.

Three standard samples of ZnO and TiO₂ were obtained from the following companies: ZnO called Z-COTE from Basf (Florham Park, NJ), TiO₂ called Eusolex T-AVO and Eusolex T-ECO from Merck KGaA (Darmstadt, Germany). Delivered by Basf Corporation, the uncoated zinc oxide particles (Z-COTE) have mean particle size smaller
than 200 nm [110]. Eusolex T-AVO and Eusolex T-ECO are titanium dioxide particles coated with silica (SiO₂) and alumina (Al₂O₃) plus simethicone, respectively. The Eusolex particle dimensions are reported to be about 100 nm [111, 112].

Table 4.3-1 Identification of inorganic materials obtained from companies.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Company</th>
<th>Elemental Composition</th>
<th>Coating</th>
<th>Average Particle size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Z-COTE</td>
<td>Basf</td>
<td>ZnO</td>
<td>uncoated</td>
<td>&lt; 200 nm</td>
</tr>
<tr>
<td>T-AVO</td>
<td>Merck</td>
<td>TiO₂</td>
<td>SiO₂</td>
<td>&lt; 100 nm</td>
</tr>
<tr>
<td>T-ECO</td>
<td>Merck</td>
<td>TiO₂</td>
<td>Al₂O₃, Simethicone</td>
<td>~ 100 nm</td>
</tr>
<tr>
<td>P25</td>
<td>Evonik Degussa</td>
<td>TiO₂</td>
<td>uncoated</td>
<td>21 nm</td>
</tr>
<tr>
<td>A-ZnO</td>
<td>Aldrich</td>
<td>ZnO</td>
<td>uncoated</td>
<td>&lt; 100 nm</td>
</tr>
</tbody>
</table>

4.4 Scanning electron microscopy

Scanning electron microscopy (SEM) was used to determine presence of nanoscale materials in commercial sunscreens. This technique provides a topographical image of the analyzed sample.

The first type of SEM samples was prepared by spreading a commercial sunscreen directly on carbon specimen mount stub from Electron Microscopy Science (Hatfield, PA, US). First, 5 mg of cream was introduced onto the stub using a needle. Then, a glass cover slide was placed on the stub and gently pressed to spread the cream; the cover slide was removed from the surface to leave a thin white film with approximately 3 mg of cream remaining (Figure 4.4.1). The samples were then dried at room temperature in a laboratory hood for 24 hours and imaged without further surface treatment.
The second type of SEM samples was prepared by diluting 0.3 g of sunscreen in 8 ml of solvent, either water (for samples 03 and 08) or ethanol (all other samples). The solutions were shaken by hand for approximately 30 seconds. Then 50 μl was placed onto the carbon specimen mount stub. The sample was then dried at room temperature for 24 hours and analyzed without further treatment.

All SEM images were obtained on an FEI Quanta 400F Field Emission scope equipped with secondary electron (SE) detector and a liquid nitrogen cooled energy dispersive x-ray spectroscopy (EDS) detector (Genesis system from EDAX Incorporation) with a working distance of 10 mm and a spot size setting of 3. Secondary electrons generated by the electron beam scanned across the surface of the sample showed the topography of the surface features. Therefore, beam voltage was varied as necessary to obtain the best image, with 20 kV usually being sufficient to provide good signal in High Vacuum mode. SEM instrumentation was computer controlled using a PC running Windows XP and images were saved as digital files that were downloaded for further analysis.

In a typical imaging experiment, evaporated sunscreen residues were imaged at randomly selected different locations at magnification levels 1,000X, 5,000X, 10,000X,
and then three images were collected at 50,000X magnification level. Images at higher magnification level were collected only for diluted sunscreens (three images at 100,000X) because charging effects were worse at higher magnification level for sunscreen residues and in consequence produced bad quality images. For poorly conductive material such as the layer of the evaporated sunscreen the energy from the primary electrons is retained by the sample instead of being shed to an electrical ground. In consequence, the excess surface charge causes image artifacts such as for example bright spots as well as worsened resolution. SEM images obtained for diluted sunscreens were better quality than for sunscreen residues.

4.5 Dispersive x-ray analysis spectroscopy

As the electron beam of the SEM is scanned across the sample surface, it generates secondary electrons as well as x-rays. The energy of each x-rays is characteristic of the element which produced the radiation. Therefore, scanning electron microscope equipped with the energy dispersive x-ray analysis spectroscopy (EDS) detector allows analyzing elemental composition of features in the SEM image.

The same sunscreen residues were used for both SEM imaging and EDS elemental analysis. The presence or absence of Zn or Ti elements was confidently assess at concentrations of 1 wt % or higher. Semi-quantitative analysis was also performed on these samples, using ZAF correction factor performed automatically by the computer software. ZAF correction considers the effects such as Z – atomic number, A – x-ray absorption and F – secondary x-ray fluorescence that influence the intensity of x-rays for the analyzed element.
4.6 Transmission electron microscopy

Two separate experiments were performed using transmission electron microscopy (TEM). The goal of the first experiment was to examine the size and shape of the titanium dioxide and zinc oxide materials present in commercial sunscreens. The second experiment was done in order to image the surface coating of nanoscale particles recommended by manufacturers for sunscreens (Z-COTE, T-AVO and T-ECO).

The advantage of TEM analysis is the possibility of physical observation of the particles with higher resolution than SEM. However, TEM requires ultra thin specimen because the image is formed from the interaction of the electrons transmitted through the specimen. Therefore, to examine the size and shape of the TiO₂ and ZnO particles, analyzed sunscreens were first diluted, then deposited on TEM grids and finally imaged.

A 25 µl solution was created from each sunscreen by diluting 50 mg of sunscreen in 8 ml of water (sample 03) or ethanol (all other samples) and shaking. The solutions were deposited directly on 300 mesh copper grids covered with carbon-coated film from Ted Pella Incorporation (Redding, CA). The samples were then dried at room temperature in a laboratory hood for 24 hours.

TEM images of diluted sunscreens (2048 pixels × 2048 pixels in size) were obtained on a JEOL 1230 high contrast transmission electron microscope (HC-TEM), employing an accelerating voltage of 120 kV. All samples were imaged at randomly selected different locations at a magnification level 30,000X. For each sample, images were collected to obtain one hundred particles for size analysis. Assuming a normal distribution, and measuring one hundred particles there should be an error of less than 10% in the average size [113]. However, the accuracy of the size distribution for sample
populations of one hundred is poor. This sample size results from the time-consuming nature of the measurement. Obtained TEM images were unsuitable for automatic software analysis. Therefore, if clear edges of the particle were seen in the image, the dimensions were defined manually and recorded with both a length and width measurement. In Figure 4.6-1 is presented TEM image with sized objects (red circles) that length and width are marked with white lines.

Moreover, not all objects observed in TEM image were counted (see green squares in Figure 4.6-1). The aggregates were excluded from the analysis of particle size; hence, the measured size is the primary particle size. Formation of aggregates can result from the TEM sample preparation during the drying of the sample. They are not necessarily present in the original suspension of the sunscreen emulsion. Because of the mentioned above weaknesses of TEM analysis, the particle-size distribution is not reported in this thesis. However, measured particles are classified into two groups as “nanoparticle” or “nanostructured” materials. These terms are consistent with the British Standards Institution [114] and the American Society for Testing and Materials [26].

If images taken from a single sample yield at least 30 objects that had all dimensions smaller than 100 nm, then the sample was coded as containing nanoparticle material. Nanostructured material is less restrictive and reserved for samples for which 30 objects were measured that had at least one dimension smaller than 100 nm.
Figure 4.6-1  TEM image obtained for sample 05 with sized particles in the red circles and excluded from the analysis features in the green squares.

Three samples (Z-COTE, T-AVO and T-ECO) were imaged using JEOL transmission electron microscope, model JEM 2100 F equipped with a field emission gun, operated at 200 kV in order to see their surface characteristics. These samples were prepared by diluting in ethanol (by shaking) particles obtained from Merck and Basf companies, to get a final concentration of 5 mg/ml. Then, the particle solution (25 µl) was deposited on TEM grid, dried at room temperature in a laboratory hood for 24 hours and imaged.
4.7 X-ray diffraction

X-ray diffraction (XRD) was used to reveal the crystal structure of sunscreen particles. Films created by the evaporation of 0.5 g of commercial sunscreen directly on 75 x 25 mm glass microscope slide under ambient conditions provided sufficient diffraction for phase composition analysis. XRD analysis was carried out on a Rigaku D/Max Ultima II Powder Diffractometer equipped with Cu Kα radiation, employing a scanning rate of 0.08°s⁻¹ and 2θ ranges from 20° to 80°. Each spectrum was taken using a voltage of 40 kV and a current of 40 mA.

4.8 Photoactivity tests

TiO₂ and ZnO sunscreen particles were tested for photoactivity, using two methods: Congo red decolorization test and spin trap electron paramagnetic resonance spectroscopy.

4.8.1 Decolorization of Congo red dye

Decolorization of Congo red (CR) dye was used to check photoactivity of TiO₂ and ZnO particles extracted from consumer sunscreen products. Samples for the dye decolorization test were prepared by dispersing 10 mg of extracted from sunscreen powder into 10 ml of water. In order to disperse agglomerates, nanoparticles were sonicated for 10 min using Fisher Scientific sonicator (FS6, power 40W). Then 2.5 ml of Congo red stock solution was added to nanoparticle dispersion to achieve the final concentration of azo dye 30 μM L⁻¹.
The photocatalytic reactions were placed inside a Luzchem 4-V photoreactor (30 cm wide, 30 cm deep and 20 cm high) equipped with a magnetic stirrer and 14 UVA lamps with an emission maximum at $\lambda = 350$ nm. Lamps were arranged in the following way: 6 lamps at the top and 4 on each side. The intensity of the UV light was examined with a digital light meter (model SLM-110) from A.W. Sperry Instruments Incorporation (Hauppauge, NY) and it was 3.6 mW cm$^2$. First, the reaction dispersion was magnetically stirred in the dark for 1 hour to establish the adsorption/desorption equilibrium of the dye on the catalyst surface. Then, it was placed inside the photoreactor. At given irradiation time intervals, 0.8 ml of the solution was taken and centrifuged (speed: 14.5 rpm) for 3 min using Eppendorf MiniSpin Centrifuge to separate the particles. The disappearance of Congo red was monitored using the absorbance feature at $\lambda = 496$ nm as a function of irradiation time. Carry 5000 UV-Vis-NIR Spectrophotometer from Varian Incorporation (Walnut Creek, CA) was used for absorption measurement. Beside consumer sunscreen samples of TiO$_2$ and ZnO particles, a TiO$_2$ reference sample – P25 was examined. The photoactivity of tested samples was quantified as Congo red half-lifetime expressed in a unit mass basis ($t_{1/2m}$). This is the amount of time required for degradation of 50 % of the dye in a presence of 10 mg of sunscreen extract. The experiment was repeated twice to determine error of measurements.
4.8.2 Spin trap electron paramagnetic resonance spectroscopy

Twelve samples were examined for generation of hydroxyl radicals using spin trap electron paramagnetic resonance spectroscopy (EPR). These were extracted from sunscreens TiO$_2$ and ZnO particles, the same which were used for CR decolorization test as well as described in section 4.3 standard samples (P25, A-ZnO, Z-COTE, T-AVO, T-ECO).

The EPR experiment was based on the procedure described in [50]. The photogeneration of hydroxyl radicals by TiO$_2$ and ZnO samples was investigated in suspensions prepared by dispersing 10 mg of particles in 1 ml of phosphate buffer (pH = 6.0, concentration = 0.05 M, ionic strength = 0.14) and POBN or DMPO spin trap stock solution (0.2 M, prepared on the same day of experiment). The final concentration of the spin trap in the EPR sample was 0.01 M. These sample suspensions were prepared freshly just before irradiation with UV light. When the experiment with ethanol was performed, then its concentration was 0.82 M. Before use, the pH of the buffer was measured at room temperature using Accumet AB15 pH Meter. All experiments were performed in phosphate buffer with pH value 6.0 that is close to pH of human skin (5.4 - 5.9) [115].

The reactions were conducted in a 4 ml glass vials cooled with cold water as shown schematically in Figure 4.8-1. The vial was placed on the magnetic stirring device under the ultraviolet lamp (Blak-Ray Model B 100 AP/R). The sample volume in the vial was kept constant at 1 ml. Following irradiation with UV light samples were immediately transferred to glass capillary (75 μl, from Fisher Scientific, Pittsburgh, PA) and placed in the cavity of an EPR spectrometer. The EPR spectra were collected with a Varian E-6
The UV lamp intensity at 365 nm was 15 mW/cm² measured with UV meter (model 70260) from Oriel Instruments USA equipped with UV detector (model 70282). The lamp emitted UV radiation of similar energy to sunlight which can be seen in Figure 4.8-2 where the reference spectrum used as a standard within the photovoltaic industry to test new solar cells (ASTM G173-03) and spectrum of the UV lamp Blak-Ray Model B 100 AP/R are presented [116, 117].
The EPR experiment was repeated twice for samples 05, 07, Z-COTE and P25 in order to disclose the error of measurements. For all samples that EPR signals were above limit of quantification (LOQ = 10σ) the concentration of DMPO-OH adduct was calculated. The calculation procedure of DMPO-OH concentration included double integration of EPR spectra using Origin 6.0 software program (see Figure 4-8.3) and verification of spin adduct concentration based on the calibration curve obtained for TEMPOL (Figure 4.8-4) [118]. For the LOQ set at 10σ, the relative uncertainty would be ± 20 % (at the 95 % confidence level) about the true value [119]. The limit of detection (LOD) was defined as an EPR signal that is three times the standard deviation (3σ) of the baseline spectra [120]. The baseline spectra were the EPR spectra that fell outside of the peak sections. Signals below 3σ were reported as “not detected” (ND).
Figure 4.8-3  EPR spectrum of TEMPOL before (A) and after (B) integration.

Figure 4.8-4  The calibration curve obtained for TEMPOL.
Chapter 5

Physical properties – results and discussion

The goals of this work were threefold. First, for a set of commercial sunscreens widely available to consumers, we wanted to determine whether nanoscale materials were present in the products. Of particular interest was to confirm that inorganic sunscreen active ingredients were nanoscale titania and zinc oxide. Second, in the cases where products did contain nanomaterials, we wanted to measure the elemental composition, physical dimensions, shape and crystal structure of these substances. These data provide useful information for researchers who study the human exposure to nanoscale materials broadly. Finally, we wanted to determine whether inorganic pigments present in sunscreens would be photoreactive; though nanoscale titania and zinc oxide can be reactive materials, if appropriately coated they are inert.

5.1 Presence of nanoscale materials in sunscreens

The imaging of sunscreen residues (untreated sunscreens) with scanning electron microscopy provided a clear indication of nanoscale materials in the majority of commercial sunscreens that we tested. One challenge in these measurements was the approximation of the "real world" conditions of sunscreen application and residue formation on skin. Significant washing and centrifugation methods could lead to particle agglomeration or even dissolution; thus we preferred where possible to use more gentle methods for recovering inorganic residues. For instance, one of our SEM sample preparation methods consisted of the direct application of neat sunscreen to SEM specimen holders. Evaporation of these residues under ambient conditions led to thin
film formation, and subsequent imaging identified all non-volatile components. For some sunscreens we first diluted the system with water before mounting it for SEM analysis. In both approaches, the sunscreens were not subject to treatments much different than those found when consumers apply the systems topically.

Nanoscale materials were found in eight of the nine examined samples (samples 00 – 07). They were loosely aggregated in the residues of evaporated sunscreens prepared both with water dilution and neat. The SEM images obtained for sample 08 at 50,000X magnification level showed a smooth and featureless surface indicating that this sample contained no particles. In Figure 5.1-1, from left to right are presented representative images of sunscreens containing the nanoscale materials (A and B) and sunscreen without particles (C).

Figure 5.1-1  SEM images of evaporated sunscreen residues – undiluted samples, all obtained at 50 000X magnification level: (A) sample 01, (B) sample 05 and (C) sample 08. Nanoscale features are presented in Figures A and B and some are marked with the red circles. Sunscreen without nanoscale particles is presented in Figure C.
To improve the images, the sunscreen emulsions were diluted in solvents – either water or ethanol. This treatment made it possible to examine the materials with higher resolution and made quantitative sizing possible as the edges of the material become resolved. Images of representative sunscreen solutions (sample 01 and sample 05) are presented in Figure 5.1-2. In these images the nanoscale materials could be observed with better edge discrimination as compared to SEM images of undiluted samples. We attribute this to the lower organic non-volatile residues found in the diluted samples.

Figure 5.1-2  SEM images of (A) sample 01 and (B) sample 05 diluted in ethanol. The nanoscale materials are well seen and in the best case isolated entities with well defined edges can be observed.

In conclusion, nanoscale materials were identified in eight (samples 00 – 07) of the nine analyzed samples (see Table 5.2-1). These sunscreens listed titanium dioxide and zinc oxide as active ingredients and this data reveals the materials are indeed nanoparticulate in nature. To confirm the composition of these particles, which we
anticipated from the labels to consist of titanium and zinc oxide, we used SEM as an analytical tool.

5.2 Elemental composition of sunscreen nanoscale materials

For samples containing features with nanoscale dimension, EDS analysis pointed towards TiO₂ and ZnO composition. In Figure 5.2-1 are presented representative EDS data obtained for sample 01 (upper, red line) and sample 05 (lower, blue line) that clearly show the chemical composition of analyzed samples.

![Figure 5.2-1 EDS data of sample 01 (upper, red line) and sample 05 (lower, blue line).](image)

Table 5.2.-1 reports whether low (x < 5%), medium (15 % < x < 5%) or high (x > 15%) levels of Ti and/or Zn elements were found in all of the analyzed residues. Only the relative amounts were reported in this thesis because some organic fraction of the sunscreen was evaporated before analysis. Because of this, the inorganic content we found from EDS was more concentrated than in the initial formulation but still the relative observations are of some value. Typically EDS metal concentrations were 3.2 – 42.2 wt % in the residues, as compared to 1.2 – 9.1 wt % TiO₂ and ZnO
concentrations from ingredient lists. However, taking into account this limitation, the correlation between the elemental composition measured by EDS data and that expected based on the reported ingredient lists of sunscreens was positive. In summary, all sunscreens with titanium dioxide and zinc oxide on their labels had nanoscale pigments of these substances. We used transmission electron microscopy (TEM) to get more detailed information about their morphology and size.

Table 5.2-1 Presence of nanoscale particles and their elemental composition in analyzed sunscreen residues.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Nanoscale Pigment?</th>
<th>Elemental Composition</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Ti</td>
</tr>
<tr>
<td>00</td>
<td>Yes</td>
<td>Medium</td>
</tr>
<tr>
<td>01</td>
<td>Yes</td>
<td>High</td>
</tr>
<tr>
<td>02</td>
<td>Yes</td>
<td>Law</td>
</tr>
<tr>
<td>03</td>
<td>Yes</td>
<td>Law</td>
</tr>
<tr>
<td>04</td>
<td>Yes</td>
<td>None</td>
</tr>
<tr>
<td>05</td>
<td>Yes</td>
<td>None</td>
</tr>
<tr>
<td>06</td>
<td>Yes</td>
<td>None</td>
</tr>
<tr>
<td>07</td>
<td>Yes</td>
<td>High</td>
</tr>
<tr>
<td>08</td>
<td>No</td>
<td>NA</td>
</tr>
</tbody>
</table>

Note: "None" here means less than 1 wt % by EDS, "Low" – less than 5 wt %, "Medium" – between 5 wt % and 15 wt %, "High" – more than 15 wt %.
5.3 The average size and shape of sunscreen particles

The small size of sunscreen particles makes transmission electron microscopy essential for the analysis of these materials. In order to fully characterize sunscreen active agents and measure their size and shape, TEM images were obtained for each sample. Figure 5.3-1 shows representative TEM image of sample 01 that contained TiO$_2$ particles (A) and sample 05 with ZnO nanoscale materials (B).

![Figure 5.3-1 TEM images of sunscreens diluted in ethanol with (A) TiO$_2$ nanoparticles (sample 01) and (B) ZnO nanoparticles and nanostructures (sample 05).](image)

The average particle dimensions (length and width) were calculated from the measurement of 100 particles in each sample. Based on these data the “nanoparticles” and “nanostructures” could be distinguished. The term “nanoparticle” means that the particle is smaller than 100 nm in all dimensions; “nanostructure” is a particle with at least one dimension smaller than 100 nm. Therefore, if analyzed sample contained at least 30 objects that had length and width smaller than 100 nm, then we classified its materials as nanoparticles. If the sample had at least 30 objects with only one shorter
dimension (width) under 100 nanometers then it was classified as containing nanostructures. Examples of histograms obtained for samples that contained only nanoparticles (sample 01) and contained both nanoparticles and nanostructures (sample 05) are presented in Figure 5.3-2 A and B, respectively. Classification of nanoscale materials for all samples is included in Table 5.3-1.

Figure 5.3-2 Examples of histograms obtained for samples that (A) contained only nanoparticles (sample 01) and (B) contained both nanoparticles and nanostructures (sample 05).

The TEM analysis revealed that most of the commercial sunscreens contained both nanoparticles and nanostructures (samples 03, 04, 05, 06, 07). These were titania (sample 03), zinc oxide (samples 04, 05, 06) and mixed titania and zinc oxide sunscreens (sample 07). The average length of nanoscale materials for samples 04, 05, 06, which contained only ZnO, was 81 nm, while the average width was 38 nm. Particles of ZnO in some cases had long dimensions that exceeded 100 nanometers. These had a rod-like shape with aspect ratio ~2 (see Figure 5.3-1 B).
The average sizes of nanoscale titania were much smaller than ZnO and for samples 00, 01, 02 the length was 25 nm and average width 10 nm. These were nanoparticles, but sample 03 contained TiO₂ nanoparticles as well as much bigger nanostructures. The average length for these particles was 84 nm and width 65 nm. We note that on their label the manufacturer classified these as inactive sunscreen ingredients.

We also found that sample 07 contained two populations of nanoscale materials: needle-shaped and rod-like. Based on the morphology found for the other systems, we expect that these are respectively TiO₂ and ZnO. The average length of the nanoscale particles for this sample was 65 nm while the shorter dimension was 27 nm. A summary of all sizing data is included in Table 5.3-1.

Table 5.3-1  Average size and classification of sunscreen nanoscale materials.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Nanoscale pigment’s morphology</th>
<th>Average Length [nm]</th>
<th>Average Width [nm]</th>
<th>Aspect ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>00</td>
<td>Nanoparticle</td>
<td>25</td>
<td>16</td>
<td>1.6</td>
</tr>
<tr>
<td>01</td>
<td>Nanoparticle</td>
<td>25</td>
<td>7</td>
<td>3.6</td>
</tr>
<tr>
<td>02</td>
<td>Nanoparticle</td>
<td>22</td>
<td>11</td>
<td>2.0</td>
</tr>
<tr>
<td>03</td>
<td>Nanostructured and Nanoparticle</td>
<td>84</td>
<td>65</td>
<td>1.3</td>
</tr>
<tr>
<td>04</td>
<td>Nanostructured and Nanoparticle</td>
<td>81</td>
<td>41</td>
<td>2.0</td>
</tr>
<tr>
<td>05</td>
<td>Nanostructured and Nanoparticle</td>
<td>82</td>
<td>39</td>
<td>2.1</td>
</tr>
<tr>
<td>06</td>
<td>Nanostructured and Nanoparticle</td>
<td>81</td>
<td>34</td>
<td>2.4</td>
</tr>
<tr>
<td>07</td>
<td>Nanostructured and Nanoparticle</td>
<td>65</td>
<td>26</td>
<td>2.5</td>
</tr>
<tr>
<td>08</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>
5.4 Summary of the electron microscopy analysis

To conclude, for the sunscreens examined for this work, a dense coating of inorganic nanoparticles and nanostructures formed upon residue evaporation. All of these materials were nanosized in some fashion, ranging from nanoparticles with average dimensions below 100 nm to nanoscale rods whose lengths could be slightly more than 100 nm. The rationale for these sizes is well established as they are large enough to efficiently scatter and absorb UV radiation, but not so large as to result in significant optical scatter at visible wavelengths. ZnO materials were usually larger than TiO$_2$ particles (the exception was sample 03). To formulate TiO$_2$ transparent sunscreen requires smaller than ZnO particles, because titania has a larger refractive index (above 2.5) at relevant wavelengths then zinc oxide – 2.0 and scatters visible wavelengths, responsible for opaque appearance stronger [32, 34].

In the literature, the reported sizes that effectively attenuate UVA and UVB radiation (below 60 nm for ZnO and 50 – 120 nm for TiO$_2$) are larger than what was detected by us in samples 00, 01, 02. It may be that by using smaller titania, manufacturer’s improved the feel of the sunscreen or perhaps ensured the sunscreen has a clear appearance even prior to application. In either case, the sizes we measured are smaller than what is required for UVA attenuation.

Besides the presence of nanoscale materials, the interesting shape of zinc oxide and titanium dioxide is also notable. ZnO adopts a wurtzite hexagonal structure, which has an oblong unit cell that will often be reflected in the crystal growth habit [121]. Smaller TiO$_2$ nanoparticles varied in shape. They appeared as needles (sample 01) with an average aspect ratio over 3 (see Figure 5.3-1 A), or as ellipsoids (sample 00) with an
aspect ratio less than 2. Sample 02 contained both needle as well as spherical nanoparticles. TiO$_2$ nanoparticles can be generated with different crystal phases (rutile and anatase) and shapes (spherical and needle shape) depending on the details of the synthetic process [29, 122]. Not only the particle size, but also its shape is a fundamental property that plays an important role in the UV light attenuation efficiency. The scattering efficiency of needle shaped nanoparticles in sample 01 can differ dramatically from the elliptical particles present in sample 00. However, there are no available data about efficiency of UV light scattering by nonspherical nanoscale particles dispersed in sunscreen emulsion.
5.5 The crystal structure of sunscreen particles

X-ray diffraction was used to determine if sunscreen residues contained the more chemically active anatase form of TiO$_2$ particles. Diffractograms for all samples are presented in Figure 5.5-1.

![Figure 5.5-1](image_url)

Figure 5.5-1 X-ray diffractograms of (A) TiO$_2$ particles (samples 00, 01, 02, 03) and (B) ZnO particles (samples 04, 05, 06) as well as both ZnO and TiO$_2$ particles (sample 07). Red-contains titania only; Blue-contains zinc only; Green-mixture of titania and zinc.

This analysis revealed that anatase TiO$_2$ nanoscale particles were present in sample 03, the same which labeled TiO$_2$ as an inactive ingredient. Other TiO$_2$ nanoparticles were rutile (sample 00, 01, 02). The observed diffraction pattern for ZnO particles matched well to the hexagonal wurtzite phase (samples 04, 05, 06). Sample 07 contained both rutile TiO$_2$ and wurtzite ZnO particles. XRD analysis confirmed EDS data about elemental composition of analyzed particles.
In summary, nine sunscreen products, commercially available in the United States, were examined for the presence of nanoscale titania and zinc oxide particles. Electron microscopy images identified major populations of nanoparticles and nanostructured materials in eight of the nine samples. These particles were titania and zinc oxide materials, and x-ray diffraction confirmed their crystallinity and identified their phase composition. The next section discusses the chemical activity of these materials in aqueous, non-biological suspensions.
Chapter 6

Photoactivity - results and discussion

It is important to investigate the photoactivity of \( \text{TiO}_2 \) and \( \text{ZnO} \) as sunscreen ingredients in sunscreens as there are currently no regulation for these materials in topical applications. Nanoscale inorganic materials can be used in consumer products without testing their chemical activity. Therefore, there may be unaddressed safety/risk issues associated with the use of these materials. In this thesis, in addition to providing characterization analyses of nanoscale sunscreen particles such as average size, shape, elemental composition and crystal structure, the photoactivity of sunscreen extracts is also investigated and described.

6.1 Decolorization of organic dye by sunscreen extracts

Photoactivity of sunscreen extracts was tested with Congo red dye (CR) which in water gives a red solution with a UV/Vis absorption spectrum at 496 nm. Congo red solution is stable under the UV light (see Figure 6.1-1). However, the dye is decolorized in presence of the sunscreen extracts (Figure 6.1-2). The progress of decolorization was monitored over time by measuring CR absorbance at \( \lambda = 496 \) nm as presented in Figure 6.1-2. The disappearance of the color was accompanied by the photoactivity of \( \text{TiO}_2 \) and \( \text{ZnO} \) particles.

Degradation of Congo red can be caused either by the positive holes which are strong oxidants or by hydroxyl radicals. Such holes are created when electrons of the semiconductor such as \( \text{TiO}_2 \) and \( \text{ZnO} \) are excited to the conduction band, leaving positive holes in the valence band. Excitation occurs upon absorption of light energy equal to or
greater than the band gap energy of nanoparticle. The positive holes besides directly oxidizing CR, can also react with electron donors like water or hydroxide ions to form hydroxyl radicals which are also potential CR oxidants.

Figure 6.1-1 Congo red dye aqueous solution before (A) and after 60 min (B) of UV irradiation as well as its UV/Vis spectra monitored during 60 min irradiation time.

Figure 6.1-2 Sample 05 and Congo red dye aqueous suspension before (A) and after 60 min (B) of UV irradiation as well as its UV/Vis spectra monitored during 240 min irradiation time.
The effect of irradiation time on CR decolorization for all analyzed samples is shown in Figure 6.1-3.

Figure 6.1-3 Experimental data of degradation of Congo red catalyzed by sunscreen extracts and P25, where Co is initial dye concentration and C is the concentration at irradiation time. Note that irradiation of Congo red solution (CR) without nanoscale materials did not change its concentration. Red-contains titania only; Blue-contains zinc only; Green-mixture of titania and zinc.

It was observed for all samples that decolorization increased over irradiation time. The chemical activity of sunscreen extracts was quantified using half life-time ($t_{1/2}$) for 30 $\mu$M L$^{-1}$ Congo red solution (see Table 6.1-1). The value of $t_{1/2}$ was expressed on mass basis ($t_{1/2m}$) because sunscreen extracts beside active ingredients contained also organic impurities.

It is well known that lower concentration of TiO$_2$ and ZnO influence the results of photodegradation of azo dye experiments. For example, Erdemoglu et al. showed that
degradation of Congo red increased linearly with increasing the concentration of TiO$_2$ in 0.001 g/ml – 0.003 g/ml range [98]. In our experiment, concentrations of P25 as well as TiO$_2$ and ZnO materials derived from sunscreens were present at a concentration about 0.001 g/ml. However, to precisely determine concentration of active materials in sunscreen extracts we had all samples (00, 01, 02, 04, 05, 06, 07) analyzed for elemental composition by Galbraith Laboratory Incorporation (see Table 6.1-1). All extracts that contained titanium (00, 01, 02) exhibited at least ten times higher amount of carbon than samples contained mostly zinc (04, 05, 06, 07). Measurement of carbon gives an estimate of the organic matter in sunscreen extracts. Titania particles were much smaller than ZnO particles which could be part of the reason why TiO$_2$ particles were more difficult to wash to remove oily, sunscreen emulsions from their surface.

The weight percentage of TiO$_2$ and ZnO in sunscreen extracts was calculated using the following formula:

$$W_{active} = \frac{X_{extract}}{X_{pure}} \cdot 100\%,$$

where $X_{extract}$ is percent composition of Ti (or Zn) element for titanium dioxide (zinc oxide) material derived directly from sunscreen (data from Galbraith Inc.) and $X_{pure}$ is percent composition of Ti (or Zn) element for pure TiO$_2$ (ZnO) material (see Table 6.1-1).
Table 6.1-1  Elemental composition of derived from sunscreens materials, calculated weight percentage of active inorganic ingredient in sunscreen extracts and half lifetime of Congo red dye expressed on mass basis.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Ti [wt %]</th>
<th>Zn [wt %]</th>
<th>C [wt %]</th>
<th>Al [wt %]</th>
<th>Si [wt %]</th>
<th>(W_{\text{active}}) [wt %]</th>
<th>(t_{1/2m} \times 10^{-2}) [min g]</th>
</tr>
</thead>
<tbody>
<tr>
<td>00</td>
<td>45.0</td>
<td>0</td>
<td>11.4</td>
<td>2.8</td>
<td>0.5</td>
<td>75.1</td>
<td>104</td>
</tr>
<tr>
<td>01</td>
<td>32.7</td>
<td>0</td>
<td>20.7</td>
<td>2.5</td>
<td>0</td>
<td>54.6</td>
<td>106</td>
</tr>
<tr>
<td>02</td>
<td>38.0</td>
<td>0</td>
<td>12.6</td>
<td>3.1</td>
<td>2.2</td>
<td>63.4</td>
<td>15</td>
</tr>
<tr>
<td>04</td>
<td>0</td>
<td>78.0</td>
<td>1.2</td>
<td>0</td>
<td>0.2</td>
<td>92.2</td>
<td>18</td>
</tr>
<tr>
<td>05</td>
<td>0</td>
<td>77.5</td>
<td>1.3</td>
<td>0</td>
<td>0.9</td>
<td>96.5</td>
<td>21</td>
</tr>
<tr>
<td>06</td>
<td>0</td>
<td>78.5</td>
<td>1.3</td>
<td>0</td>
<td>0.2</td>
<td>97.8</td>
<td>20</td>
</tr>
<tr>
<td>07</td>
<td>1.5</td>
<td>76.0</td>
<td>1.2</td>
<td>0.1</td>
<td>0.1</td>
<td>97.1</td>
<td>16</td>
</tr>
<tr>
<td>P25</td>
<td>NA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>100.0</td>
<td>5</td>
</tr>
</tbody>
</table>

From the decolorization test, results indicate the most photoactive sample was P25 TiO\(_2\) \((t_{1/2m} = 5 \times 10^{-2} \text{ min g})\) and none of the examined sunscreens showed the same exact reactivity. However, it is well known that P25 is one of the most effective commercial photocatalyst [123, 124]. Erdemoglu et al. showed that P25 completely degraded Congo red after irradiation for 30 min and our results were consistent with that study [98].

The order of the CR degradation by tested samples was as follows: P25 > Sample 02 > Sample 07 > Sample 04 > Sample 06 > Sample 05. The most photoactive sunscreen powder toward degradation of Congo red dye was extracted from sample 02 that contained TiO\(_2\) pigments. For this sample the Congo Red half lifetime was
Sample 07 that contained both pigments TiO$_2$ and ZnO was the second most active sunscreen sample with $t_{1/2m} = 16 \times 10^{-2}$ min g. It was discovered also that the ZnO pigments (04, 05 and 06) imparted a faster degradation to the analyzed dye than samples 00 and 01 that contained TiO$_2$ nanoparticles. All the ZnO pigments caused considerable destruction of Congo Red ($t_{1/2m} \sim 20 \times 10^{-2}$ min g) while sunscreens 01 and 00 were much less photoactive and their $t_{1/2m}$ was $\sim 105 \times 10^{-2}$ min g.

In summary, ZnO and TiO$_2$ nanoscale particles extracted from commercial sunscreens are photoactive toward decolorization of the Congo red dye. To further delineate whether this reaction proceeds by surface mediated oxidation or via photogenerated reactive oxygen species a spin trap EPR method was used.

6.2 Detection of hydroxyl radicals using spin trap EPR

This section describes results obtained from EPR experiments performed to verify the ability of the TiO$_2$ and ZnO sunscreen particles to photogenerate hydroxyl radicals. Two spin traps POBN and DMPO were used to trap these species. Ethanol was used as an efficient hydroxyl radical scavenger to confirm the origin of DMPO-OH. Next, the concentration of DMPO-OH adduct was calculated for all sunscreen extracts that generated EPR signals with intensities greater than limit of quantification ($10\sigma$). The normalized (per mass of active ingredient) rate of hydroxyl radical generation was calculated from the time profiles of DMPO-OH formation. Two types of samples were tested: sunscreen extracts and standard samples such as P25 titanium dioxide, A-ZnO (zinc oxide nanopowder from Sigma-Aldrich Inc.) and commercial nanoparticles marketed as sunscreen ingredients (Z-COTE, T-AVO, T-ECO).
6.2.1 Sunscreen extracts

The spin traps (POBN and DMPO) were delivered without impurities. No EPR signals were detected when buffered solutions of spin traps were irradiated for 10 min as well as no EPR signals were found when nanoparticles were irradiated without spin traps. Spectra were only observed when the same experiments were performed in the presence of sunscreen extracts and spin traps. As an illustration of typical data, EPR spectra of spin trapped radicals obtained by irradiation (5 min) of sample 05 dispersion containing POBN (A), DMPO (B), and DMPO/EtOH (C) are presented in Figure 6.2-1.

![EPR spectra](image)

Figure 6.2-1 The EPR spectra of spin trapped radicals obtained by irradiation (5 min) of sample 05 dispersion containing POBN (A), DMPO (B), and DMPO/EtOH (C). The EPR settings were: gain $1.25 \times 10^4$ and modulation amplitude 1.6 G.
Spin trapping with POBN

Spin trapping experiment with POBN resulted in two EPR spectra. The first one was triplet of doublets with hfsc $A_N = 14.8$ G, $A_H = 1.8$ G related to POBN-OH (Figure 6.2-1 A). This spectrum was observed for all analyzed sunscreen extracts except sample 00 and sample 01 which had no detectable signals. The second spectrum consisted of quartet with line intensities 1:2:2:1 and hyperfine splitting constants (hfsc) $A_N = A_H = 14.5$ G. This radical is a decomposition product of POBN-OH adduct as well as POBN spin trap. It appears when carbon-nitrogen bond of POBN is disturbed. Then POBN splits into a silent product (benzaldehyde) and the 2-methyl-2-nitrosopropane which when protonated yields a tert-butyl hydroaminoxyl radical with a characteristic four line EPR signal [104]. The data confirms that a POBN spin trap when mixed with pigments derived from sunscreens reacts in ways consistent with the presence of hydroxyl radicals (samples 02, 04, 05, 06 and 07).

The intensity of the POBN-OH signal was lower for sample 02 as compared to samples 04, 05, 06 and 07. This trend corresponds to a greater signal for samples that contain more zinc oxide – namely samples 04, 05, 06 and 07. The signal was detectable, but small, for sample 02 which only contains TiO$_2$. No POBN-OH signal could be detected for sample 00 and 01. However, elemental analysis revealed that TiO$_2$ samples (00, 01, 02) exhibited lower amount of active ingredient and higher amount of organic matter comparing to zinc samples (04, 05, 06, 07). It is well known that the generation of radicals depends on the concentration of semiconductor particles [50]. Therefore, in order to compare activity of analyzed materials, the amount of generated spin adduct present in a sample was quantified and normalized per mass of TiO$_2$ or ZnO.
We used DMPO spin trap to quantify the amount of hydroxyl radicals present in a sample and to confirm results obtained from EPR analysis with POBN. DMPO characterizes higher reaction rate with OH⁻ (3.4 x 10⁹ M⁻¹ s⁻¹) and longer lifetime of DMPO-OH adduct (t₁/₂ = 2.6 h at pH 7.4) comparing to POBN-OH [102].

Detection of OH⁻ radicals using DMPO

EPR experiment with DMPO spin traps confirm the results obtained for POBN: samples which contain more ZnO particles (samples 04, 05, 06, 07) have more intense EPR signals upon illumination. Moreover, DMPO is a more efficient trap for OH⁻ as compared to POBN and revealed EPR peaks for sample 00 and 01 above the limit of detection (LOD = 3σ). Beside these two sunscreen extracts (00 and 01), the intensities of the EPR peaks for other samples were above 1, which is the limit of quantification for our system (LOQ = 10σ). The intensity of EPR signal for DMPO-OH at limit of quantification corresponds to spin adduct concentration ~0.4 μM.

Recorded EPR signals in the presence of DMPO spin trap were composed of four peaks with intensities 1:2:2:1 and hfsc A₀ = A₁ = 14.8 G that were assigned to the DMPO-OH adduct (see Figure 6.2-1 B). However, in order to confirm that DMPO-OH originated from trapping OH⁻ radicals rather than from decomposition of DMPO-OOH, ethanol was added as a hydroxyl radical scavenger. Trapped hydroxyl radicals would result in a disappearance of the DMPO-OH spectrum and an appearance of a new six-line DMPO-CH(CH)₃OH spectrum. This is exactly what was observed (Figure 6.2-1 C). Therefore, the experiments with ethanol confirmed that DMPO-OH originated from trapping of OH⁻.
The times profiles of OH· production by sunscreen extracts shown in Figure 6.2-2 match well (R² > 0.97) to exponential function \( f(t) = A \cdot [1 - \exp(-B \cdot t)] \), where A and B are constants.

![Figure 6.2-2](image)

Figure 6.2-2 The DMPO-OH concentration as a function of irradiation time. Data shown for sample 05 and sample 07 are from EPR experiments repeated two times. Error bars indicate mean ± σ. The experimental time dependencies (dots) are fitted to the function \( f(t) = A \cdot [1 - \exp(-B \cdot t)] \), where A and B are constants. Red-contains titania only; Blue-contains zinc only; Green-mixture of titania and zinc.

The rate of DMPO-OH formation (K) was derived from the fitted time dependencies according to the equation \( K = \frac{d[DMPO-OH]}{dt} \bigg|_{t=0} = A \cdot B \) and normalized per mass of TiO₂ and/or ZnO (Kn). The results of all these calculations are shown in Table 6.2-1.
Table 6.2-1  Normalized rate of DMPO-OH generation ($K_n$) for all analyzed samples as well as the fitting data to $f(t) = A \cdot [1 - \exp(-B \cdot t)]$ curve.

<table>
<thead>
<tr>
<th>Sample</th>
<th>$K$ [µM min$^{-1}$]</th>
<th>$A$ [µM]</th>
<th>$B$ [min$^{-1}$]</th>
<th>$R^2$</th>
<th>$K_n \times 10^2$ [µM min$^{-1}$ g$^{-1}$]</th>
</tr>
</thead>
<tbody>
<tr>
<td>00</td>
<td>NQ</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>01</td>
<td>NQ</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>02</td>
<td>0.20</td>
<td>0.90 ± 0.00</td>
<td>0.22 ± 0.00</td>
<td>1.00</td>
<td>0.31</td>
</tr>
<tr>
<td>04</td>
<td>0.65</td>
<td>2.97 ± 0.15</td>
<td>0.22 ± 0.03</td>
<td>1.00</td>
<td>0.71</td>
</tr>
<tr>
<td>05</td>
<td>0.34</td>
<td>3.46 ± 0.00</td>
<td>0.10 ± 0.00</td>
<td>0.99</td>
<td>0.35</td>
</tr>
<tr>
<td>06</td>
<td>0.59</td>
<td>3.27 ± 0.09</td>
<td>0.18 ± 0.00</td>
<td>0.99</td>
<td>0.60</td>
</tr>
<tr>
<td>07</td>
<td>1.01</td>
<td>3.17 ± 0.00</td>
<td>0.32 ± 0.03</td>
<td>0.99</td>
<td>1.04</td>
</tr>
<tr>
<td>P25</td>
<td>21.76</td>
<td>9.76 ± 0.00</td>
<td>2.23 ± 0.03</td>
<td>1.00</td>
<td>21.76</td>
</tr>
<tr>
<td>A-ZnO</td>
<td>3.65</td>
<td>2.45 ± 0.04</td>
<td>1.49 ± 0.10</td>
<td>1.00</td>
<td>3.65</td>
</tr>
<tr>
<td>Z-COTE</td>
<td>8.44</td>
<td>2.85 ± 0.00</td>
<td>2.96 ± 0.00</td>
<td>1.00</td>
<td>8.44</td>
</tr>
<tr>
<td>T-AVO</td>
<td>0.71</td>
<td>0.55 ± 0.04</td>
<td>1.29 ± 0.37</td>
<td>0.98</td>
<td>0.71</td>
</tr>
<tr>
<td>T-ECO</td>
<td>ND</td>
<td></td>
<td></td>
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$K$ – rate of DMPO-OH generation, $R^2$ – coefficient of determination, NQ – not quantified, ND – not detected

The order of the OH• generation ($K_n$) by sunscreen extracts was as follows: Sample 07 > Sample 04 > Sample 06 > Sample 05 > Sample 02. The most photoactive sample 07 contained both TiO$_2$ and ZnO active agents and photogenerated OH• radicals at rate of $1.04 \times 10^2$ µM min$^{-1}$ g$^{-1}$. The EPR signals for 00 and 01 samples that contained titanium dioxide were detectable but their intensities were below limit of quantification, which for our conditions was lower than 0.4 µM of DMPO-OH. Similar behavior was observed for CR experiment, where these two TiO$_2$ samples demonstrated the longest half-lifetime of CR. Sample 07 in both experiments (Congo red degradation and spin trap
EPR) demonstrated the highest activity. It is interesting to note that sample 02 decolorized Congo red quickly ($t_{1/2} = 15 \times 10^2 \text{min g}$) while the generation rate of hydroxyl radicals for this sample was slow ($0.31 \times 10^2 \mu\text{M min}^{-1} \text{g}^{-1}$). Therefore, we can assume other reaction routes may be involved including direct oxidation due to positive holes.

In order to examine photoactivity of pure nanoscale particles, on a per weight basis, we studied five known standard nanocrystals for comparison.

6.2.2 Standard samples

The photoactivity of standard samples such as optimized photocatalyst (P25), zinc oxide nanopowder A-ZnO and sunscreen agents (Z-COTE, T-ECO, T-AVO) was examined using the same procedure as described above for materials extracted from consumer products. Generation of hydroxyl radicals was tested with POBN and DMPO as well as DMPO/EtOH. The time dependencies of DMPO-OH formation for standard samples were fitted to the same exponential function as sunscreen samples described above. The results of the fit and the rate of DMPO-OH generation are presented in Table 6.2-1. In the figure below (6.2-3) are shown the kinetic characteristics of DMPO-OH generation for all samples analyzed in this study.
Figure 6.2-3  Normalized DMPO-OH concentration per mass of active ingredient as a function of irradiation time for all analyzed samples. Data shown for samples 05, 07, P25 and Z-COTE are from EPR experiments repeated two times. Error bars indicate mean ± σ. The experimental time dependencies (dots) are fitted to the function $f(t) = A \cdot [1 - \exp(-B \cdot t)]$, where $A \cdot B = K_n$ (normalized generation rate constant). Red lines contains titania only; Blue contains zinc only; Green mixture of titania and zinc; Solid lines – sunscreen samples; Dotted lines – standard samples.

As expected, out of all materials analyzed in this study, the most active was P25 titanium dioxide photocatalyst ($K_n = 21.76 \times 10^2 \mu M \text{ min}^{-1} \text{ g}^{-1}$). The high rate of hydroxyl radicals generation caused oxidation of spin trap and in consequence extinction of spin adduct formation with longer than 1 min irradiation times. Similar behavior for P25 in the presence of POBN was observed by Brezova et al. [50]. We observed decrease of DMPO-OH formation after 1 min of UV irradiation also for Z-COTE, which was the
second most active sample ($K_n = 8.44 \times 10^2 \text{ mM min}^{-1} \text{ g}^{-1}$). To predict the generation rate constants for these two samples (P25 and Z-COTE) we measured concentrations of DMPO-OH only for the 0.5 min and 1 min of irradiation periods.

Surprisingly, A-ZnO (pure ZnO nanopowder from Sigma-Aldrich) presented lower amount of OH· ($3.65 \times 10^2 \text{ mM min}^{-1} \text{ g}^{-1}$) than sunscreen UV filter Z-COTE. We also examined two TiO$_2$ materials recommended by manufacturers as sunscreen UV filters. These were T-AVO and T-ECO. The first pigments were photoactive and generated $0.71 \times 10^2 \text{ mM min}^{-1} \text{ g}^{-1}$ of DMPO-OH, while the second TiO$_2$ sample was inactive. We did not detect any EPR signal for T-ECO.

To compare photoactivity of pure samples and these derived from consumer products we used normalized (per mass of active ingredients) DMPO-OH formation rates ($K_n$). We observed that sunscreen extracts were less active toward generation of hydroxyl radicals than pure P25 titanium dioxide and pure ZnO (A-ZnO, Z-COTE) materials. The generation rate for the most active from all derived from sunscreens powders (sample 07) was 3.5 times lower than for pure ZnO particles from Sigma-Aldrich (sample A-ZnO) and 20.9 times lower than for P25 photocatalyst. However, it is noted that materials derived from sunscreens powders beside pure inorganic materials contained organic matter that could block the adsorption process of the OH· groups on the particle surface and reduce generation of hydroxyl radicals. Thus, the effect of impurities could affect the spin trap experiment. We demonstrate that Z-COTE, nanoparticles recommended by manufacturer as physical sunscreen agent had hydroxyl radical rate constant 2.3 times greater than A-ZnO nanopowder obtained from Sigma-Aldrich chemical company which is recommended as an active chemical product rather than an ingredient of sunscreens.
Generation rate of hydroxyl radicals for Z-COTE was only 2.6 times lower than optimized catalyst P25. It is entirely possible that incorporated into the consumer sunscreen products ZnO nanoscale materials behave like Z-COTE. If sunscreen extracts were 100% ZnO nanoparticles it is probable they also could generate OH· radicals at similar to Z-COTE rate ($8.44 \times 10^2$ μM min$^{-1}$ g$^{-1}$).

Moreover, when we compare the amounts of hydroxyl radicals generated by sunscreen samples after 10 min of UV irradiation we observed (see Figure 6.2-3 and Figure 6.2-4) that all samples containing ZnO nanoscale materials, both derived from sunscreens (04, 05, 06, 07) as well as pure from Sigma-Aldrich (A-ZnO) generated considerable amounts of OH· ($\sim 2.5 \times 10^2$ μM g$^{-1}$). From this, we can conclude that ZnO sunscreen agents (04, 05, 06, 07, Z-COTE) were more active toward OH· generation than TiO$_2$ (00, 01, 02, T-AVO and T-ECO).

Titanium dioxide extracts 00 and 01 after 10 min of UV irradiation formed detectable EPR signals but with intensities below limit of quantification. T-AVO, during the same period of time generated slightly more radicals ($0.6 \times 10^2$ μM g$^{-1}$) than above mentioned samples 00 and 01. The EPR signal for T-ECO was flat over time; hence this sample was photoinactive under experimental conditions. The most active titanium dioxide sample, excluding P25 was 02 sunscreen extract which after 10 min of irradiation generated $\sim 1.3 \times 10^2$ μM g$^{-1}$ DMPO-OH.
Figure 6.2-4 Concentration of DMPO-OH adduct per mass of active ingredient (TiO$_2$ or ZnO) generated during 1 min, 5 min and 10 min of UV irradiation. The red description on X axis is related to samples that contained only titanium dioxide, blue – zinc oxide and green – both pigments.

Tested titanium dioxide particles recommended for sunscreen application were coated with SiO$_2$ (T-AVO) and Al$_2$O$_3$ (T-ECO). Coating plays an important role in reducing photoactivity of semiconductor particles. A thin coating layer of silica and alumina, such as that used in commercial T-AVO and T-ECO materials, reduces their ability to generate hydroxyl radicals [72, 73]. We confirmed the presence of surface coatings on T-AVO and T-ECO in Figure 6.2-5 A and B, respectively. We note these observations are consistent with some commercial sunscreen formulations, suggesting that in some products containing nanocrystalline titania manufacturer's select to use coated materials. These sunscreen extracts contained either more than 2.5 wt % of Al (samples 00, 01) or larger quantities of both Si and Al (sample 02). In contrast, for
samples containing only ZnO neither Si nor Al was higher than 1 wt % (see Table 6.1-3). Moreover, ZnO particles derived directly from commercial sunscreens after 10 min of UV irradiation showed photoactivity comparable to the pure, uncoated ZnO nanoparticles (A-ZnO). We also confirmed that Z-COTE that generation rate of OH· was only 2.6 times lower than P25 was uncoated as presented in Figure 6.2-5 C.

Figure 6.2-5 TEM images of (A) T-AVO, (B) T-ECO and (C) Z-COTE.

EPR experiments confirm that titanium dioxide nanoparticles with surface coatings have generally lower photoactivity. The coating layer of silica attached to the surface of TiO₂ particles (T-AVO) was able to reduce generation of hydroxyl radicals. However, it was not able to completely prevent generation of free radicals. T-ECO gave the best results in “deactivation” of titania surface. These particles were coated with Al₂O₃ and dimethicone [112]. All samples: photoinactive (T-ECO), with reduced activity (T-AVO) as well as nanoscale particles able to generate high amount of OH· (Z-COTE) are recommended by manufacturers for sunscreens. This is of note as there are currently no regulations in the field of testing sunscreen pigments which, based on our results, vary widely with regard to photoactivity relating to radical production.
Chapter 7

Conclusion

Nine commercial sunscreen products available in the United States were examined for the presence of nanoscale titania and zinc oxide pigments. Electron microscopy images identified major populations of nanoparticles and nanostructured materials in eight of the nine samples. These particles were titania and zinc oxide crystallites; x-ray diffraction confirmed their phase composition. None of the examined sunscreens had labels that specified whether their titania or zinc oxide ingredients were microscale or nanoscale in form.

Moreover, photoactivity of inorganic materials, extracted from seven sunscreens was tested using Congo red dye and spin trap electron paramagnetic resonance. Both methods revealed photoactivity of derived directly from commercial products nanoscale materials. Furthermore, sunscreen extracts showed photoactivity comparable to pure particles obtained from Sigma-Aldrich and Merck companies. ZnO nanoscale particles recommended by BASF Incorporation as sunscreen UV filters (Z-COTE) generated hydroxyl radicals under UV illumination at rate only 2.6 times lower than optimized photocatalyst (P25). The results of EPR experiment showed also that coated with alumina and simethicone titanium dioxide (T-ECO) was inactive toward photogeneration of radicals. Elemental analysis of sunscreen extracts as well as EPR tests showed that coatings are never generally applied to ZnO particles.

It is currently unknown if exposure to sunscreen products that contain TiO$_2$ and/or ZnO nanoscale particles cause adverse health effects. The penetration of sunscreen nanoparticles through the skin has not been conclusively researched. ROS production by
ZnO and TiO₂ nanoparticles has been proven but, there is not yet enough available information about the biological effects. No one knows how they behave in the bloodstream. It is even more difficult to answer all of these questions as the current state of nanoscale materials, in general, is unregulated. Manufacturers use nanomaterials to effectively attenuate UV radiation and make sunscreen transparent on the skin, but they are not currently required to report safety data on these types of ingredients. They treat nanoscale materials as inert if the bulk material is safe. However, it is know that in nanoscale TiO₂ and ZnO under UV light are chemically active. Therefore, perhaps new safety regulations are necessary to manage nano-engineered particles that differ from bulk material, along with requirements for proper consumer labeling/information regarding the reactivity of these materials. Such regulations can inform consumers and help researchers identify the benefits and potential health risks of nanomaterials, which ultimately is necessary to sustain the emerging nanotechnology industry.
References


