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Original Article

Analysis of titanium dioxide and zinc oxide nanoparticles in cosmetics

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ABSTRACT

There have been rapid increases in consumer products containing nanomaterials, raising concerns over the impact of nanoparticles (NPs) to humankind and the environment, but little information has been published about mineral filters in commercial sunscreens. It is urgent to develop methods to characterize the nanomaterials in products. Titanium dioxide (TiO_2) and zinc oxide (ZnO) NPs in unmodified commercial sunscreens were characterized by laser scanning confocal microscopy, atomic force microscopy, X-ray diffraction (XRD), and transmission electron microscopy (TEM). The results showed that laser scanning confocal microscopy evaluated primary particle aggregates and dispersions but could not size NPs because of the diffraction limited resolution of optical microscopy (200 nm). Atomic force microscopy measurements required a pretreatment of the sunscreens or further calibration in phase analysis, but could not provide their elemental composition of commercial sunscreens. While XRD gave particle size and crystal information without a pretreatment of sunscreen, TEM analysis required dilution and dispersion of the commercial sunscreens before imaging. When coupled with energy-dispersive X-ray spectroscopy, TEM afforded particle size information and compositional analysis. XRD characterization of six commercial sunscreens labeled as *nanoparticles* revealed that three samples contained TiO_2 NPs, among which two listed ZnO and TiO_2, and displayed average particle sizes of 15 nm, 21 nm, and 78 nm. However, no nanosized ZnO particles were found in any of the samples by XRD. In general, TEM can resolve nanomaterials that exhibit one or more dimensions between 1 nm and 100 nm, allowing the identification of ZnO and TiO_2 NPs in all six sunscreens and ZnO/TiO_2 mixtures in two of the samples. Overall, the combination of XRD and TEM was suitable for analyzing ZnO and TiO_2 NPs in commercial sunscreens.

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1. Introduction

The rapid development of nanotechnology has resulted in an increasing number of nanomaterial-based consumer products and industries. Because of their unique physical properties, nanomaterials have dramatically transformed the function and application of commercial products, including wound dressings, cosmetics, detergents, food packaging, drug delivery, biosensors, and antimicrobial coatings [1]. Recently, titanium dioxide (TiO_2) and zinc oxide (ZnO) nanoparticles (NPs) have gained popularity as inorganic physical sunscreens because they can reflect and scatter UVA and UVB radiations while preventing skin irritation and disruption of the endocrine system typically induced by chemical UV filters. Also, these NPs may be transparent and pleasant to touch [1,2]. However, safety concerns regarding their utilization in consumer products have recently emerged. Reports have suggested that sunscreen NPs induce cyto- and genotoxicity through oxidative stress [3]. Zvyagin et al [4] and Tilman et al [5] have shown that TiO_2 and ZnO NPs could not penetrate the deep layers of healthy adult skin. In contrast, Wu et al [6] demonstrated that TiO_2 NPs could enter the deep layers of porcine epidermis as well as hairless mouse skin. Because the impact of NPs on humans is poorly understood, no clear regulation has been implemented for NPs among international authorities.

The International Cooperation on Cosmetic Regulation define a nanomaterial in cosmetics as an insoluble, intentionally manufactured ingredient with one or more dimensions ranging from 1 nm to 100 nm in the final formulation. In addition, the nanomaterial must be sufficiently stable and persistent in biological media to enable potential interactions with biosystems [7]. In 2012, the International Organization for Standardization underlined that the physicochemical characterization of nanomaterials was critical for the identification of test materials before toxicological assessment (ISO/TR13014). Physicochemical parameters include particle size/particle size distribution, aggregation/agglomeration state, shape, surface area, composition, surface chemistry, surface charge, and solubility/dispersibility [8]. A safety guideline on nanomaterials in cosmetics issued by the United States Food and Drug Administration [9] recommended that the product be evaluated by analyzing these physicochemical properties. NPs may aggregate when added to cosmetics, making their characteristics in the final products essential.

Sunscreen formulations are very complex and opaque, hindering NP detection and characterization. Finding appropriate analytical methods to achieve this characterization without product modification and misleading dilution is an important issue. Some studies have investigated single particles in noncomplex matrices [10,11] but few reports have discussed NP characteristics in complex formulations. Tyner et al [12] have evaluated the ability of 20 analytical methods to detect TiO_2 and ZnO NPs in unmodified commercial sunscreens. Variable-pressure scanning electron microscopy, atomic force microscopy (AFM), laser scanning confocal microscopy (LSCM), and X-ray diffraction (XRD) were considered applicable and complementary for NP characterization in sunscreens. Guidelines on the safety assessment of nanomaterials in cosmetics from the Scientific Committee on Consumer Safety suggested the use of at least two methods, of which one should be electron microscopy, preferably high-resolution transmission electron microscopy (TEM), to determine size nanomaterial parameters [13]. Here the size parameters of NPs in six different commercial sunscreens were evaluated by TEM, AFM, LSCM, and XRD. Analytical results were compared to assess the effectiveness of these methods in characterizing NP-based cosmetics.

2. Materials and methods

2.1. *Sunscreen samples and NP controls*

Six commercial sunscreens were selected based on product descriptions and promotion flyers mentioning the presence of inorganic NPs in their formulation. Table 1 lists inorganic ingredients and their amounts in the cosmetics. Among these sunscreens, two contained only TiO_2, two contained only ZnO, and two contained a combination of TiO_2 and ZnO. All sunscreens were obtained without prescription; one product was made in Korea, one in France, and the others were produced in the USA. Standard solutions of TiO_2 (107 nm) and ZnO NPs (76 nm) used as control samples were purchased from Sigma–Aldrich (St Louis, MO, USA). The TiO_2 NP standard solution consisted of anatase and rutile crystals.

2.2. *AFM*

AFM analyses were performed using an Asylum Research MFP-3D system (Goleta, CA, USA) in tapping mode. Maximum scan areas were 90 μm × 90 μm. The cantilever and samples were located using a charge-couple device monitor. Unmodified sunscreens were transferred onto a glass slide, flatted with a glass coverslip, and air-dried. Size-related sample imaging was conducted at 10 μm, 5 μm, 2 μm, and 1.2 μm scan widths. Acquired phase and height images were analyzed using Asylum Research IGOR PRO-based software.

2.3. *Laser scanning confocal microscopy*

Laser scanning confocal microscopy (LSCM) characterizations were conducted using a Zeiss LSM 710 LSCM (Wetzlar, Germany) equipped with a HeNe laser ($\lambda_{HeNe} = 561$ nm) and a 63× objective (NA1.4). A small amount of sunscreen was placed on

Table 1 – Inorganic ingredient contents and sun protection factors (SPF) of analyzed sunscreen products.

Product No.	Origin	Claimed ingredients (%)		SPF
		TiO_2	ZnO	
COM 1	USA	5	10	30+
COM 2	USA	5	10	30+
COM 3	USA	—	20	30+
COM 4	Korea	1.4	—	35
COM 5	France	Not listed	—	50+
COM 6	USA	—	6.8	30+