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Characterizing titanium dioxide and zinc oxide nanoparticles in sunscreen spray

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Synopsis

OBJECTIVE: Numerous commercial products contain titanium dioxide (TiO_2) and zinc oxide (ZnO) nanoparticles (NPs); however, many of these are not labelled as containing NPs. This study sought to develop an effective means of characterizing TiO_2 and ZnO NPs in sunscreen sprays, including the size, shape and composition of the particles as well as their aggregation/agglomeration characteristics.

METHODS: Transmission electron microscopy (TEM) coupled with a window-type microchip K-kit/copper grid and X-ray diffraction (XRD) was used to characterize the oxide NPs.

RESULTS: TME pre-treatment was performed using two approaches: using a conventional copper grid (requiring dilution) and using a K-kit (not requiring dilution). The use of K-kit in conjunction with XRD makes it possible to obtain direct measurements from samples that have not undergone pre-treatment, which could otherwise alter the nature of the samples, such as the degree of agglomeration. XRD was used to obtain information related to particle size and crystal structure. A strong correlation was observed between XRD and TEM measurements.

CONCLUSION: The proposed measurement methods were shown to be highly effective in the characterization of oxide NPs in sunscreen sprays, providing consistent information related to NPs and their interactions in the formulations.

Résumé

OBJECTIF: De nombreux produits commerciaux contiennent des nanoparticules (NP) du dioxyde de titane (TiO2) et de l'oxyde de zinc (ZnO). Toutefois, beaucoup de ces produits ne sont pas étiquetés comme contenant des NP. Cette étude a cherché à développer un moyen efficace de caractériser les NP de TiO2 et ZnO dans les aérosols de protection solaire, y compris la taille, la forme et la composition des particules ainsi que leurs caractéristiques d'agrégation/agglomération.

MÉTHODES: La microscopie électronique à transmission (MET) couplée avec une grille cuivre de type à fenêtre micropuce K-kit et la diffraction des rayons X (XRD) ont été utilisées pour caractériser les NP d'oxyde.

RESULTATS: Le pré-traitement TME a été effectuée en utilisant deux approches: l'utilisation d'une grille de cuivre conventionnel (nécessitant une dilution) et en utilisant une K-kit (ne nécessitant pas de dilution). L'utilisation de K-kit conjointement avec diffraction des rayons X permet d'obtenir des mesures directes à partir d'échantillons qui n'ont pas subi un prétraitement qui, sinon, pourrait modifier la nature des échantillons, tels que le degré d'agglomération. DRX a été utilisé pour obtenir des informations relatives à la taille des particules et la structure cristalline. Une forte corrélation a été observée entre les mesures XRD et TEM.

CONCLUSION: Les méthodes de mesure proposées ont été présentées pour être très efficace dans la caractérisation des NP d'oxyde dans les sprays de protection solaire, et de fournir des informations cohérentes liées aux NP et leurs interactions dans les formulations.

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Introduction

Numerous consumer products are based on nanomaterials; however, few of these specify the size, shape and composition of the constituent materials [1]. According to statistics compiled by the Project on Emerging Nanotechnologies (PEN), 1628 commercial products containing nanomaterials (including 150 cosmetic products) were sold between 2005 and 2013 [2]. A survey conducted by the International Cooperation on Cosmetic Regulations (ICCR) reported the widespread use of TiO_2 and ZnO NPs for the absorption of UVA/UVB radiation in personal skincare products. These materials can help to prevent skin irritation as well as disruption of the endocrine system typically induced by chemical UV filters [3]. These NPs may also be transparent and pleasant to the touch [4]. The fact that consumers are increasingly being exposing to nanomaterials is driving the development methods for characterizing the materials in inorganic physical sunscreens.

The management of cosmetics containing nanomaterials varies from country to country. Following the top-down policy of the EU, the European parliament announced regulation (EC) No 1223/2009 in 2009. These regulations outline rules for the labelling of nanomaterial in cosmetics. Specifically, after 11 July 2013, commercial products containing nanomaterials must be registered at least 6 months prior to release on the market. Nanomaterial refers to an insoluble or biopersistant material that is intentionally manufactured with one or more external dimensions or an internal structure on the scale of 1–100 nm. Notification of intent to manufacture nanomaterials must include the name of the chemical (IUPAC) as well as the sizes, physicochemical information and toxicity [5]. In contrast, the FDA in the United States takes a bottom-up position, foregoing regulations related to the inclusion of nanomaterials in cosmetic products, in

favour of recommended guidelines, as outlined in Guidance for Industry Safety of Nanomaterials in Cosmetic Products (2014). Concern regarding nanobased products is driving the need to characterize nanomaterials to facilitate management.

In 2012, the International Organization for Standardization (ISO) emphasized that the physicochemical characterization of engineered nanoscale materials is important for the identification of test materials prior to 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 [6]. Safety guidelines on nanomaterials in cosmetics issued by the US FDA [7] underline the importance of a number of factors, such as physicochemical properties and aggregation/agglomeration of nanoparticles in the final product. Characterizing NPs in the final product would be an ideal solution; however, cosmetics formulations are too complex and opaque to allow such examination. Most instruments used for the characterization of these materials require pre-treatment, which can alter the viscosity, aggregation/agglomeration and pH of the final products. Clearly, there is a need for methods capable of evaluating products without diluting or otherwise modifying them.

Guidelines presented by the Scientific Committee on Consumer Safety (SCCS) related to the safety assessment of nanomaterials in cosmetics suggests that at least two methods be employed, including electron microscopy, preferably high-resolution transmission electron microscopy (TEM), to determine the size of nanomaterials [8]. This study employed TEM coupled with a window-type microchip K-kit/EDS and X-ray diffraction (XRD) to determine the particle size, particle size distribution, shape and aggregation/agglomeration of NPs in commercial sunscreen sprays.

Materials and methods

Sunscreens samples and standardized nanoparticle controls

This study selected three commercial sunscreen sprays, which mention the inclusion of inorganic NPs in their formulation without using the term 'nano'. One of the samples contains only TiO_2, one contains only ZnO, and one contains a combination of TiO_2 and ZnO, as shown in Table I. All sunscreens can be obtained without prescription. One product was made in Korea, and the other two were produced in Taiwan. Standard TiO_2 powder, including anatase and rutile crystals used for crystal structure analysis, was purchased from Alfa Aesar (Ward Hill, MA, U.S.A.) Standard solutions of ZnO NPs (76 nm), also used for crystal structure analysis, were purchased from Sigma-Aldrich (Saint Louis, Missouri, U.S.A.). NIST standard reference material 1898 (TiO_2 NPs in powder form) was used as a size control for the verification of testing methods. This material consisted of anatase and rutile crystals with an average size of 19 ± 2 and 37 ± 6, as determined by XRD. The characteristic reflections were as follows: anatase [200] and rutile [111] phase.

Table I Summary of commercial sunscreen sprays tested in this study

Product no.	Product name	Origin	Ingredients		SPF
			TiO_2	ZnO	
1	Double effect cooling UV sun spray	Korea	?*	2.25%	50
2	Sun protector spray	Taiwan	?*	–	50
3	Sun protector spray	Taiwan	?*	–	50

*Indicates inclusion of ingredient.
'—'indicates that the substance is not present.

X-ray diffraction

Diffraction patterns were collected using a PaNalytical Pro X'pert Pro X-ray diffractometer (Netherlands) using Cu K_α irradiation. Prior to evaluation, diffraction patterns from NIST standard reference material 1976b were recorded and used to plot a graph of full widths at half maximum (FWHM) vs. 2θ to determine the instrumental profile parameter for all angles (20–135°).

Instrument broadening was calculated as follows:

$$\mathrm{FWHM}_{\mathrm{observe}} = \mathrm{FWHM}_{\mathrm{instrument}} + \mathrm{FWHM}_{\mathrm{size+strain}} \quad (1)$$

Unmodified sunscreen samples and NIST standard reference material 1898 were transferred directly into a metal holder for TEM analysis using a step size of 0.03 and a scanning speed of 0.07 steps s^{-1} over a 2θ range from 20° to 90°. Data were matched for crystal-phase identification and smoothed for the background using X' Pert High Score Plot software (PANalytical, Almelo, The Netherlands). Anatase and rutile forms of TiO_2 have been applied in cosmetic products, especially rutile TiO_2. The FWHM of reflections was calculated using Origin 8 (OriginLap, Northampton, MA, U.S.A.). Reflections including 011 (at 25.2° 2θ) for anatase TiO_2, 110 (at 27.4°2θ) for rutile TiO_2 and 010 (at 31.7°2θ) for ZnO were selected for size analysis. Grain sizes were estimated using the Scherrer equation, as follows:

$$D = (0.94 \cdot \lambda)/(\mathrm{FWHM}_{\mathrm{size}} \cdot \cos\theta), \quad (2)$$

where D is the grain size, λ is X-ray wavelength (λ = 1.54051 Å), and θ is the Bragg angle.

Conventional transmission electron microscopy

Prior to assessment, the magnification and scale marker of TEM instruments (JEOL JEM-2100F, Akishima, Tokyo, Japan) were calibrated to ensure smooth instrument operation and to validate all measurement procedures. After shaking the sunscreen samples, aliquots of the products were obtained from the bottles and diluted using ethanol. NIST standard reference material 1898 was similarly diluted with ethanol. A single drop (10 μL) of the resulting dispersions was deposited on a carbon-coated copper grid, wicked using filter paper and air-dried at room temperature. The size and shape of particles were analysed at an acceleration voltage of 200 kV and magnification of 80 000–100 000×. Element composition was determined using energy-dispersive spectroscopy (EDS; EDAX phoenix, Mahwah, NJ, U.S.A.). The size of the oxide NPs was measured using ImageJ software (developed at the National Institutes of Health, U.S.A.) based on the average of at least 200 particles. The average size, size distribution and aspect ratio were calculated using Microsoft Excel software (Microsoft, Redmond, Washington, U.S.A.).

Transmission electron microscopy with window-type microchip K-kit

A window-type microchip K-kit developed by MA-tek for TEM was used for the observation of NPs under wet conditions [9]. The K-kit (length of 1.3 mm and height of 1.3 mm) provides a narrow chamber with a width suitable for the sorting of NPs and preventing aggregation during the drying process. Untreated aliquots of shaken sunscreen were loaded directly into a chamber between the top and bottom substrates of the K-kit using a pipette. The chamber (with a height of 2 μm) was then sealed. Drying the wet samples left the precipitated NPs in the K-kit for TEM analysis. We then imaged the NOAAs (nano-objects and their aggregates and agglomerates) of NPs in the final products and calculated the physical state of NOAAs using the TEM images.

Results and discussion

X-ray diffraction analysis

X-ray diffraction patterns were used to determine the crystal structure and grain size of particles in untreated commercial sunscreen sprays. Diffraction peaks at increasing scattering angles (2θ) are related to specific lattice planes, d_{hkl} [10]. Particle size is associated with peak intensities and widths, wherein peak broadening is generally assumed to occur below 200 nm; that is, the broader the diffraction is, the smaller the size of the crystals [11]. The mean grain size of particles in the samples was estimated using the Scherrer equation (Eq (2)), assuming the absence of strain. XRD reflections of the sunscreen samples are presented in Fig. 1. The grain sizes of the NIST standard reference material 1898 diffraction [(101) and (200) for anatase, (110) and (111) for rutile] were 22.267 ± 0.115, 21.095 ± 0.223, 35.163 ± 0.536 and 40.671 ± 3.830, respectively ($n = 5$). The size of the reflections [(200) for anatase and (111) for rutile] was consistent with standards outlined in NIST 1898, thereby confirming the XRD results. The strongest diffraction peak of anatase phase at $2\theta = 25.2^\circ$ corresponds to reflections from the 011 plane, and the strongest line from the rutile phase corresponds to reflections from the 110 plane at $2\theta = 27.4^\circ$. For the analysis of size, we selected reflection 010, which corresponds to the peak at 31.7° associated with ZnO. We also calculated the size of particles in the sunscreen samples. ZnO NPs of 24 nm were obtained in COM 1. In COM 1–COM 3, the sharp reflections correspond to TiO_2 particles with crystallite sizes below 100 nm, as shown in Table I.

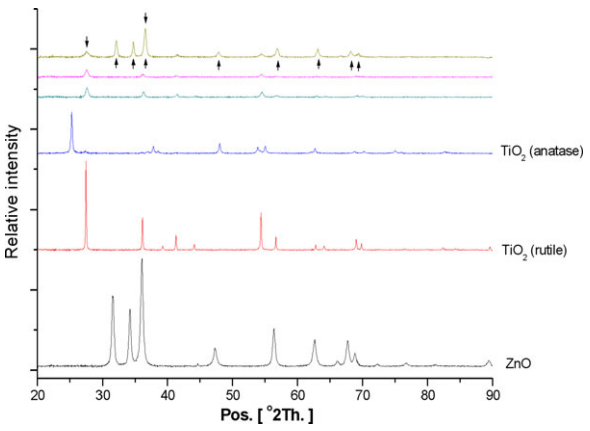

Figure 1 X-ray diffraction patterns of commercial sunscreen spray COM 1–COM 3 compared with anatase TiO_2, rutile TiO_2 and ZnO. Downward arrows indicate the 2θ positions for reflections from the rutile phase of TiO_2, and upward arrows indicate 2θ positions for reflections from ZnO wurtzite structures: (A) COM 1, (B) COM 2 and (C) COM 3.

The Scientific Committee on Consumer Safety (SCCS) recommends against the use of TiO_2 and ZnO nanoparticles in sprayable applications. The extremely small size of inhaled nanoparticles would enable them to penetrate deeply into the lung and even into the circulatory system by passing the pulmonary epithelial barrier. A small fraction of the inhaled particles in the nose could also reach the brain directly via the olfactory bulb [12]. TiO_2 NPs are classified as anatase, rutile or brookite crystalline structures [13]. Rutile TiO_2 is a stable and abundant pigment commonly used in sunscreens due to its higher UV absorption and lower photoreactivity than that of anatase [11]. XRD patterns revealed the existence of rutile TiO_2 in COM 1–COM 3. The SCCS reported that 10 of 15 of the TiO2 they collected displayed rutile phase (crystalline form). The same phase was identified in three of the sprays in this study. The fact that the rutile phase TiO_2 is widely applied in commercial products means that XRD is ideally suited to the characterization of crystal structure and the determination of mean grain size without modifying sunscreen sprays [14].

Conventional transmission electron microscopy

The size, shape and composition of particles in commercial sunscreen sprays were determined by a combination of TEM with EDS, which necessitated dilution prior to imaging. The resolution was unaffected by the other constituents in the samples, such that the electron micrographs reveal the NPs very clearly (Fig. 2). The sizes of TiO_2 NPs in COM 1–COM3 were 27.9 ± 7.0, 24.9 ± 9.1 and 23.9 ± 6.3, respectively. Many countries and organizations have reached a common consensus for the definition of nanomaterials. The EU defines nanomaterials as an insoluble or biopersistant material, which is intentionally manufactured material with one or more external dimensions, or an internal structure, on the scale from 1 to 100 nm [5]. The International Cooperation on Cosmetic Regulations (ICCR) defines a nanomaterial in cosmetics as an insoluble, intentionally manufactured ingredient with one or more dimensions ranging from 1 to 100 nm in the final formulation. A nanomaterial must also be sufficiently stable and persistent in biological media to enable potential interactions with biosystems [15]. All three of the samples in this study contained TiO_2 and ZnO particles exhibiting at least one dimension smaller than 100 nm, thereby qualifying as nanomaterials according to the criteria outlined by the EU and ICCR.

Differences in the shape of particles were detected among the various samples. Shape is used to describe the geometry of NPs, wherein aspect ratio (a shape parameter) can be used to express the relationship between width and height. The smallest possible aspect ratio of a circle is 1 : 1. Needle-shaped TiO_2 particles were observed in COM 1, whereas roundish TiO_2 particles were observed in COM 2 and COM 3. TiO_2 NPs with an aspect ratio of 4.09 ± 1.04, 1.72 ± 0.62 and 1.70 ± 0.54 were observed in COM 1–COM 3, respectively (Table II). Roundish ZnO particles with an aspect ratio of 1.35 ± 0.36 were also found in COM 1. The shapes of the TiO_2 structures were close to those of circles, needles and lances [14]. The shapes of the ZnO structures included rod-like

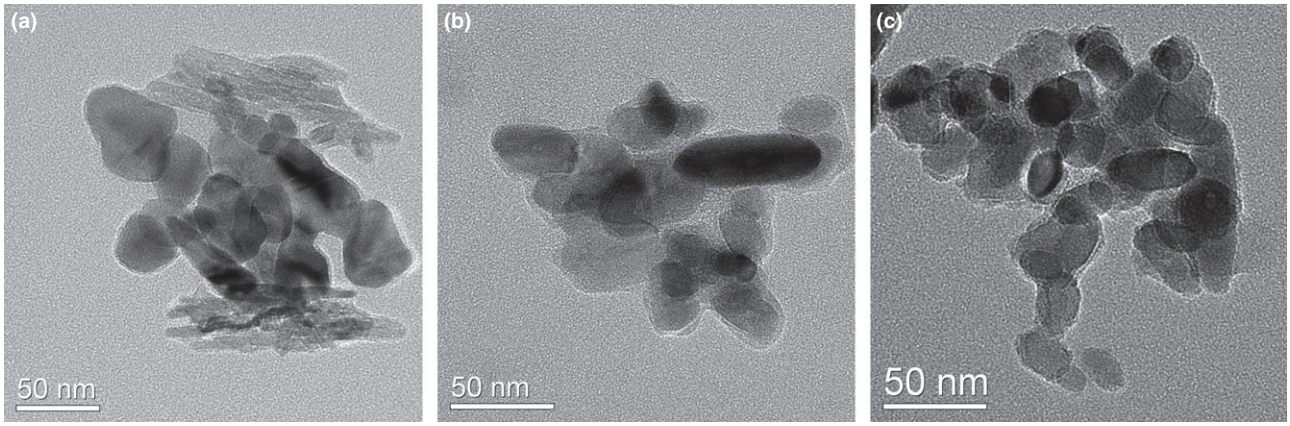

Figure 2 Images of commercial sunscreen spray containing inorganic NPs obtained using transmission electron microscopy with copper grids: (A) COM 1, (B) COM 2 and (C) COM 3. Images were acquired at a beam intensity of 200 kV and a magnification of 80 000–100 000 × . The scale bar is 50 nm.

Table II Results of XRD and TEM analysis of commercial sunscreen spray samples

	XRD			TEM				
Product no.	**Particle**	**Phase**	**PPS (nm)**	**Particle size (nm)**	**Aspect ratio**	**Particle shape**	**Calculated number**	**Elements Detected by EDS**
1	TiO_2	Rutile	19	27.9 ± 7.0	4.09 ± 1.04	Needle shaped	214	Ti, C, O, (Cu)
	ZnO	Rutile	24	27.9 ± 8.8	1.35 ± 0.36	Round-like	223	Zn, C, O, (Cu)
2	TiO_2	Rutile	21	24.9 ± 9.1	1.72 ± 0.62	Round-like	223	Ti, C, O, Si, (Cu)
3	TiO_2	Rutile	25	23.9 ± 6.3	1.70 ± 0.54	Round-like	220	Ti, C, O, Si, (Cu)
N-1898	TiO_2	Anatase	22.267 ± 0.115	23.5 ± 10.0	1.15 ± 0.15	Round-like	301 301	Ti, C, O, (Cu)
		Rutile	35.163 ± 0.536					

XRD: X-ray diffraction; PPS: Primary particle size; TEM: Transmission electron microscopy; N-1898: NIST standard reference material 1898, the mean size vales obtained from the analysis of five randomly selected (n = 5).

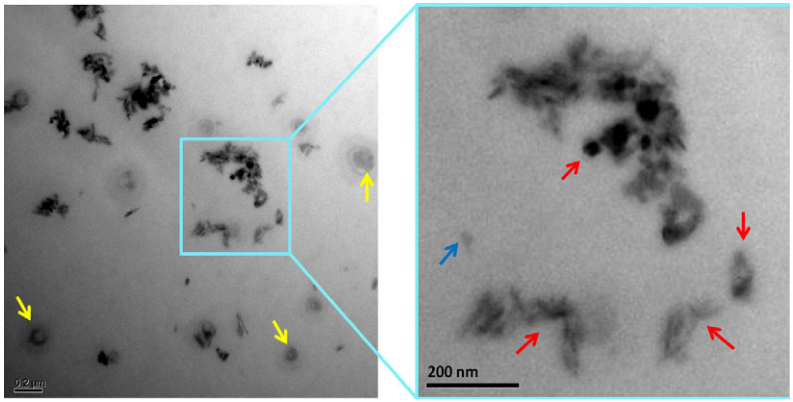

Figure 3 Characterization of NOAAs of TiO_2 and ZnO NPs in the unmodified sunscreen spray COM 1, as determined by transmission electron microscopy with a K-kit. Yellow arrows indicate organic material in the sample. The left picture is an enlargement. Blue arrows indicate the primary particles of inorganic NPs, and red arrows indicate the aggregation/agglomeration state of inorganic NPs. The scale bar is 0. 2 μm.

Table III Characterization of TiO_2 and ZnO NPs according to NOAAs in unmodified sunscreen spray COM 1 using TEM with a K-kit/copper grid

Particle type	Size (nm)		Calculated number (%)	Calculated number	TEM images
	Average	Standard deviation			
Constituted particles					
Particle -1 (Ti/O/Al/Si)	27.9	7.0	–	214	Copper grid
Particle -2 (Zn/O/Si)	27.9	8.8	–	223	
NOAAs in products (TiO_2 + ZnO)					
Nano-objects (NOs)	39.5	15.6	15.7	32	K-kit
Aggregates/agglomerates (AAs)	153.8	107.8	84.3	171	
NOAAs	135.8	107.6	100	204	

NOAA: Nano-objects and their aggregates and agglomerates.

and isometrically shaped particles [16]. Compositional analysis of inorganic residues by EDS revealed the presence of Si in the TiO_2 of COM 2 and COM 3. One previous study reported the modification of TiO_2 NP surfaces using silicon dioxide (SiO_2) and aluminium oxide (Al_2O_3) to reduce photoreactivity and minimize the formation of reactive oxygen species [17]. The nature of the coating on the outer surface of a given nanomaterial determines its stability to degradation/aggregation in a given medium and affects its interaction with biological systems [18]. Cu detected by TEM in this study can be attributed to the copper grids.

X-ray diffraction and TEM size values are in good agreement and provide complementary evidence pertaining to the characteristics of the nanomaterials (Table II). XRD size is usually equal to or smaller than that obtained by TEM [19]. The International Standards Organization (ISO) [20] has released technical specifications related to the average crystallite size and primary particle size (as evaluated by XRD and TEM) for the designation of nanoscale TiO_2 in powder form. The TEM results indicated the number of particles, which made it possible to determine the average size, and the standard deviation of measurement results. The TEM and XRD results in this study were in agreement with the technical specification outlined by the ISO. We can therefore conclude that XRD and TEM are suitable for size-related analysis of particles used in sunscreens.

Transmission electron microscopy with a window-type microchip K-kit

Safety guidelines for nanomaterials in cosmetics issued by the US FDA [7] identify the aggregation/agglomeration of NPs in the final product as an important physicochemical property. Unfortunately, image-based analysis of agglomeration or aggregation is difficult to achieve. TEM is a powerful tool for sizing NPs; how-

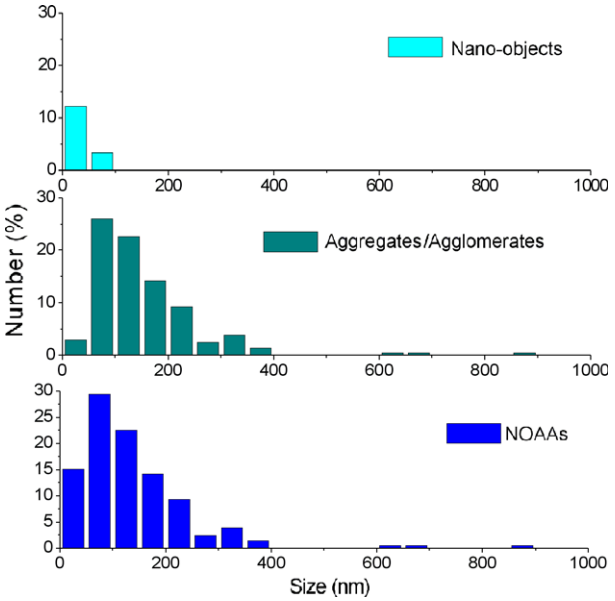

Figure 4 Size distribution of NPs, based on the average of the major and minor axes of 204 TiO_2 and ZnO NPs in unmodified sunscreen spray COM, as determined by transmission electron microscopy with K-kit.

ever, copper grids often produce an uneven spatial distribution of particles and are seldom used in the analysis of aqueous solutions. A K-kit can help to overcome these shortcomings by providing a narrow chamber in which to load liquid, which helps to prevent the aggregation of particles during the drying process [9]. Figure 3 presents the NOAAs of TiO_2 and ZnO NPs in the unmodified sunscreen spray (COM 1) as determined using transmission electron microscopy with a K-kit. The clear visualization of native NOAAs of oxide NPs makes it possible to conduct quantitative analysis when applied to 200 particles. Aggregates/agglomerates of oxide NPs were identified in 171 of the 204 particles studies (84.3%) with an average size of 153.8 nm (Table III and Fig. 4). Among the 204 NPs in Table III, 32 NPs (15.7%) displayed no indication of agglomeration and had an average particle size of 39.5 nm. Most of the aggregates/agglomerates were a mixture of TiO_2 and ZnO NPs.

The results obtained using TEM with copper grids covered with a carbon film were compared with those obtained using the K-kit. The size of oxide NPs in TEM specimens dried on copper grids was slightly smaller than those observed using the K-kit (Table III). The difference may be attributed to the pre-treatment of the copper grids leaving artefacts. Agglomerates that are weakly bonded are more likely to fragment than are aggregates that are more strongly bonded. This means that the toxic properties of these materials may differ considerably from those of the as-received samples. Samples with an agglomeration or aggregation of particles may differ in their effects on ADME behaviour [6]. Our experiments confirm that the K-kit in conjunction with TEM is a convenient sampling device for the analysis of native aggregates/agglomerates of oxide NPs in sunscreen sprays.

Conclusions

This study applied various techniques to measure the physicochemical parameters of TiO_2 and ZnO NPs. XRD was used to illustrate the crystal structure and mean particle size in unmodified sunscreen sprays. TEM in conjunction with copper grids or K-kits was used to determine particle size, size distribution, shape, composition and native aggregation/agglomeration status. The primary particle size was smaller than 100 nm, whereas aggregation/agglomeration exceeded 100 nm in sample COM 1. Good correlation was observed between XRD and TEM measurements; however, some limitations were apparent. XRD is unable to reveal NPs in formulations or provide reliable size analysis above *ca.* 200 nm. TEM combined with EDS can be used to determine the composition of NPs on copper grids; however, it is unable to provide information from TEM with K-kits. The energy of the X-rays emitted from specimens through the upper substrate of a K-kit cannot be measured using an EDS detector because the detector is not located directly above the K-kit. Thus, we determined the composition of target NPs using TEM with a copper grid prior to determining the aggregation/agglomeration status using TEM with a K-kit. Despite the limitations associated with TEM (using copper grids or a K-kit) and XRD, considerable progress has been made. Nonetheless, alternative analytical methods are being sought to determine other size-related properties of unmodified cosmetic products to provide a reference for health authorities and cosmetics manufacturers.

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