

# Sun Protection Factor Analysis of Sunscreens Containing Titanium Dioxide Nanoparticles

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## ABSTRACT

The effect of micronization on sun protection factor (SPF) were tested on two types of titanium dioxide ( $TiO_2$ ) with primary particle sizes of 20 nm and 170 nm. Oil/water creams with 5%, 10%, and 20% concentrations of each type of  $TiO_2$  were prepared, and SPF was measured using both *in vitro* and *in vivo* methods. *In vitro* analysis demonstrated that submicron-sized  $TiO_2$  cream had a lower SPF value than nanosized  $TiO_2$  formulations of the same concentration. *In vivo* experiments confirmed this result, and a strong correlation between *in vitro* and *in vivo* measurements was observed. Furthermore, the SPF values of nanosized  $TiO_2$  sunscreen were concentration-dependent in the range of 5% to 20%. Scanning electron microscopy results indicate that the higher SPF of nanosized  $TiO_2$  formulations may be due to the formation of multilayer agglomerates by small particles at nano-scales, leading to a reduced void space between particles and a more efficient barrier to protect skin from sunlight.

Key words: Sun protection factor (SPF), nanosized  $TiO_2$ , submicron  $TiO_2$

## INTRODUCTION

Exposure to solar radiation has been closely linked with the development of photocarcinogenesis, photoageing, and photosensitivity in humans; thus, it is very important to use sunscreens in order to reduce risk of sun-induced skin cancer<sup>(1-4)</sup>. Ultraviolet radiation (UVR, 200 - 400 nm) is divided into three sections termed UVA, UVB, and UVC<sup>(5)</sup> by wavelength. UVB radiation, ranging from 290 - 320 nm, is the principal cause of sunburn, or solar erythema. Sunscreens containing UVB filters can protect against erythema with a level of performance indicated by the product's sun protection factor (SPF). SPF is an indicator of the efficacy of sunscreen products against UVB radiation and is defined as the time required for irradiation to produce minimal perceptible erythema of sunscreen-protected skin relative to the time required for the same damage to occur to unprotected skin<sup>(6)</sup>. SPF can be determined by *in vivo* or *in vitro* methods<sup>(6,7)</sup>.

Sunscreens are classified as either chemical absorbers or physical blockers depending on their mechanism of action. Physical blockers such as titanium dioxide ( $TiO_2$ ) and zinc oxide contain inert metal particles that reflect and scatter UVR. In addition, they are photostable and

are far less likely than chemical absorbers to cause skin irritation and sensitization<sup>(8)</sup>.  $TiO_2$  absorbs broad-spectrum ultraviolet radiation and has become a frequently used physical UV filter in sunscreen formulations. Micronized  $TiO_2$  has been found particularly protective against harmful UVB rays<sup>(9)</sup>. Metal oxide particle sizes in the range of 200 - 500 nm are optimal for reflecting visible light. However, they form a thick visible pigment layer on the skin. To overcome this drawback and develop a more cosmetically acceptable product, particles ranging from 10-50 nm in size have been recently developed which scatter less visible light and are virtually transparent on the skin<sup>(10)</sup>. These nano-formulations can enhance skin penetration of some additional sunscreen ingredients such as octyl methoxycinnamate, a chemical UVB filter, to further improve skin protection against UV light<sup>(11)</sup>. In contrast, many studies have demonstrated that nanosized  $TiO_2$  particles remain on the skin surface or the outer layers of the stratum corneum with no observable skin or intracellular penetration<sup>(12-16)</sup>. These results indicated that nanosized  $TiO_2$  particles currently used in cosmetic sunscreens present no risk to human health and increase both UV protection and aesthetic appearance when applied to skin. To date, relatively few reports described the effect of  $TiO_2$  particle size on UVB blocking efficiency in cosmetic preparations. Micronized particles are sensitive

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to electrostatic effects and readily form aggregates and agglomerates before, during, and after manufacturing, which may lead to decrease in efficacy<sup>(17)</sup>. However when inorganic sunscreens such as TiO<sub>2</sub> were used in aqueous media, agglomeration into larger particles coincided with higher SPF<sup>(18)</sup>. It remains unclear what mechanism is responsible for this phenomenon. The purpose of this study was to investigate the effect of TiO<sub>2</sub> particle size on UVB protection efficiency measured with SPF values. Two types of TiO<sub>2</sub>, submicron-scale and nanosized-scale, were used to prepare creams containing 5%, 10%, and 20% TiO<sub>2</sub>, corresponding to TiO<sub>2</sub> concentrations in commercially available products. The SPF value of each formulation was determined by *in vitro* and *in vivo* methods.

## MATERIALS AND METHODS

### I. TiO<sub>2</sub> and SPF Reference Formulations

Two different sources of TiO<sub>2</sub>, Kemira AFDA and UV-Titan M212, were purchased from Kemira Pigments OY Company (Finland). Kemira AFDA (> 99.0%) is an uncoated anatase pigment with a primary particle size (PPS) of approximately 170 nm. UV-Titan M212 (> 85%) is an ultrafine rutile pigment coated with alumina and glycerol (PPS ca. 20 nm). An SPF 15 reference sunscreen formulation with the same active ingredients as COLIPA P3 high SPF reference formula was acquired from Cosmetech Laboratories (USA).

### II. Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM)

Electron microscopy was conducted using a JEOL JEM-2010 and JSM 7000F microscopes (Japan) for SEM and TEM, respectively. A thin, electron transparent film (Formvar/Carbon film on 200 mesh copper, supplied by Agar Scientific, Essex, England) was used to hold samples in place while in the area of the objective lens of the TEM.

### III. UV-Visible Absorption Measurements

Qualitative UV-visible absorbance spectra from both types of TiO<sub>2</sub> (0.001% w/w, in water) were obtained on a Spectrophotometer Cary 50 UV (Varian, USA) in the wavelength range between 250 and 700 nm.

### IV. Microfine TiO<sub>2</sub> Dispersion in Glycerin

Each TiO<sub>2</sub> pigment (5 g) was homogenously dispersed in glycerin (5 mL) with an ultrasound machine. Both homogeneous dispersion samples were analyzed with a scanning electron microscope (SEM) and an SPF *in vitro* test (as described in Section VII).

### V. Preparation of Sunscreen Creams

An oil/water (O/W) blank cream was prepared as a cream base (Table 1). For the preparation of the cream base, the water phase (hectorite, water, TEA, glycerin) was heated to 75°C and homogenized at 6000 rpm. The water phase was subsequently dispersed slowly into a pre-heated oil phase at 75°C. The cream base was formed and cooled to room temperature. Either submicron or nanosized TiO<sub>2</sub> was added to the water phase before emulsification at a concentration of 5%, 10%, or 20%. Finally, the TiO<sub>2</sub> content in all 6 sunscreen formulations was determined using a colorimetric method<sup>(19)</sup>.

### VI. Photon Correlation Spectroscopy (PCS) and Polydispersity Index (PI)

The particle diameters and polydispersity indices of 6 sunscreen preparations were simultaneously measured using a PCS Submicron Particle Size Analyzer (Beckman Coulter, USA). The samples were diluted with filtered double-distilled water to provide optimal scattering range. Three samples of each preparation were analyzed, and every sample was measured twice.

### VII. In vitro SPF Measurement

The effect of TiO<sub>2</sub> sunscreen preparations on the transmittance of UVA and UVB radiation (290 - 400 nm) through a tape substrate (Transpore<sup>®</sup> tape, 3M GmbH, Germany) was assayed on a UV-1000S transmittance analyzer (Labsphere<sup>®</sup> Co., USA). Approximately 80 mg of each sunscreen was evenly spread over Transpore<sup>®</sup> tape with a finger cot. Fifteen minutes after sunscreen application, 5 areas on each tape were scanned twice. Five samples of each preparation and blank control were analyzed by this method, and the mean SPF and standard deviation across samples was recorded for each preparation.

### VIII. In vivo SPF Measurement

*In vivo* SPF determinations were made according to the International harmonized SPF test Method<sup>(5)</sup>. Eighteen healthy volunteers of Fitzpatrick's skin type I-III were recruited, and all volunteers provided written informed consent prior to entry into the study. A solar simulator with a 150W xenon lamp (Model 601, Solar Light Co. Philadelphia, PA, USA) provided a spectral output in the ultraviolet range. WG-320 and UG-11 filters were used to provide UVA (320 - 400 nm) and UVB (290 - 320 nm) wavelength spectra for a total combined wavelength range of 290 - 400 nm. Test areas were delineated on each subject's back in the region between the scapula and the waist, each approximately 35 cm<sup>2</sup> in size, which were designated for the application of a sunscreen preparation or SPF 15 reference standard, or left unprotected for the determination of minimal erythema dose (MED). Using

**Table 1.** Composition of the cream base

| Ingredient                           | Supplier                                 | Percent by weight |
|--------------------------------------|--|-------------------|
| Cetyl palmitate                      | SASOL GmbH, Germany                      | 2.0               |
| Cetearyl alcohol                     | SASOL GmbH, Germany                      | 2.0               |
| Glyceryl monostearate & Na-stearate  | APS Chemicals, Malaysia                  | 1.2               |
| Glyceryl stearate & PEG-100 stearate | Croda, Singapore                         | 1.5               |
| Polysorbate 60                       | Uniqema International, USA               | 2.0               |
| Stearic acid & palmitic acid         | Akzo Nobel Chemicals GmbH, Malaysia      | 2.0               |
| Isopropyl palmitate                  | Uniqema Sdn. Bhd, Malaysia               | 5.0               |
| PEG-8 beeswax                        | Croda International, Spain               | 1.0               |
| White oil                            | Crompton Corporation, USA                | 4.0               |
| Dimethicon 451/350                   | Toshiba Silicone, Japan                  | 0.2               |
| Isohexadecane                        | Croda International, Netherlands         | 4.0               |
| Methylparaben                        | Ueno Fine Chemicals Industry, Japan      | 0.2               |
| Propylparaben                        | Ueno Fine Chemicals Industry, Japan      | 0.1               |
| 2-phenoxyethanol                     | Akzo Nobel Surface Chemistry AB, England | 0.3               |
| Hectorite                            | Elementis Specialities, England          | 0.5               |
| TEA 99%                              | Opical Chemicals, USA                    | 0.4               |
| Glycerin                             | Palm-Oleo Sdn. Bhd, Malaysia             | 3.0               |
| Water                                |  | qsp 100.0         |

the solar simulator, the MED of the skin of mid-back was first determined (between 16 and 24 hours) for each subject by measuring the UV energy required for the development of a faint erythema after exposure. After MED determination, separate areas on the mid-back were used to test the SPF of experimental samples and the SPF 15 reference standard. The sample and the reference standard ( $2.0 \text{ mg/cm}^2 \pm 2.5\%$ ) were applied to the appropriate designated test site and spread evenly using a finger cot. Irradiation of the sites began no less than 15 minutes after application. The SPF value of the test sample and reference standard was calculated from the MED of the protected skin relative to that of the unprotected skin as follows:

$$\text{SPF} = \frac{\text{MED of protected skin (test sample or standard)}}{\text{MED of unprotected skin}}$$

## RESULTS AND DISCUSSION

### I. SEM and TEM Characterization and UV-visible Transmittance of Two Sizes of $\text{TiO}_2$ Particles

SEM and TEM (Figure 1) illustrated the morphological differences in the shape and size distribution of the 2 types of  $\text{TiO}_2$  particles. Submicron-sized  $\text{TiO}_2$  particles observed under SEM were spherical in shape with a diameter range from approximately 80 nm to 220 nm, with a small amount of apparent aggregation (Figure 1A). TEM revealed particles of an oblong shape approximately 178 nm in size (Figure 1B). In contrast, SEM of nanosized  $\text{TiO}_2$  particles revealed highly agglomerated features and a spherical shape (Figure 1C). TEM indicated that these particles were approximately 20 nm in size (Figure 1D).

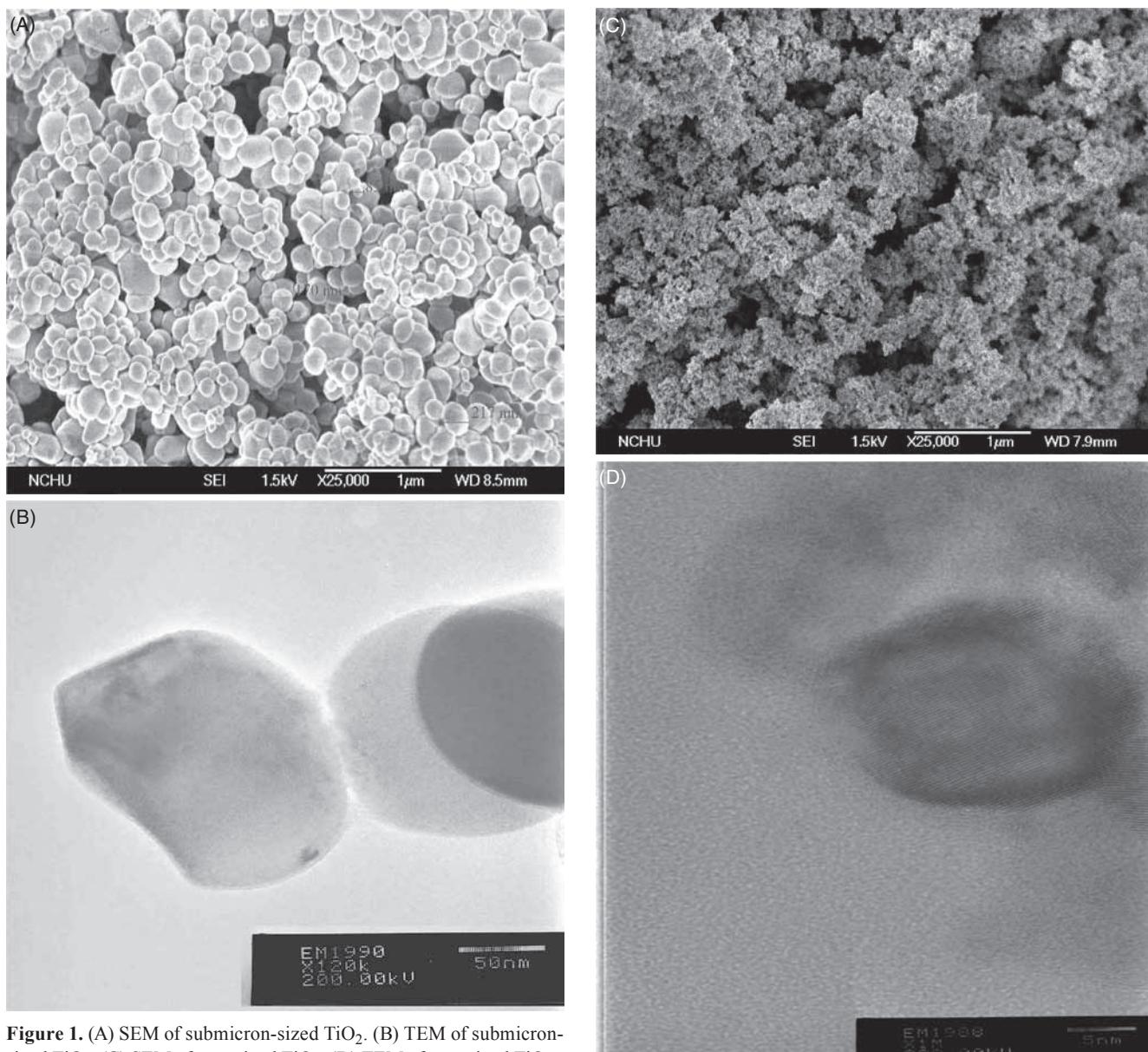
The qualitative UV-visible transmittance plot in the range of 250 nm to 700 nm of both types of  $\text{TiO}_2$  particles at a concentration of 0.001% in water are shown in Figure

2. Compared to submicron-sized  $\text{TiO}_2$ , nanosized  $\text{TiO}_2$  demonstrated lower transmittance in the range of 290 nm to 320 nm and higher transmittance in the visible wavelength range of 500 nm to 700 nm. Decreasing particle size to micronized form resulted in less scattering of visible light and effectively attenuated UVB (290 - 320 nm), leading to a more cosmetically acceptable product. In the UV spectrum, micronization may shift the protective spectrum, via its property as an absorbing agent, toward shorter UVB wavelengths<sup>(17)</sup>. This result suggests that nanosized  $\text{TiO}_2$  may be superior to submicron-sized  $\text{TiO}_2$  at the blocking of UVB radiation.

## II. Effect of $\text{TiO}_2$ Particle Size on SPF in Glycerin Dispersion

To determine whether reduced particle size was

responsible for enhanced SPF, glycerin dispersions of both  $\text{TiO}_2$  formulations were prepared. SEM analysis of the glycerin dispersion samples demonstrated significantly different particle size and shape (Figure 3). Submicron-sized  $\text{TiO}_2$  formed few aggregates or agglomerates. Nanosized  $\text{TiO}_2$  particles formed large, rounded agglomerates consisting of many smaller spherical particles. Nanosized particles overlapping in aggregates may lead to a lower void fraction, and multilayer agglomerates spread to form a thick film may block UVB more effectively than submicron-sized  $\text{TiO}_2$  particles. In an *in vitro* test of the SPF of these glycerin-dispersed samples, we found that nanosized  $\text{TiO}_2$  had a higher SPF value ( $17.1 \pm 0.9$ ,  $n = 5$ ) than submicron-sized  $\text{TiO}_2$  ( $2.8 \pm 0.1$ ,  $n = 5$ ), confirming our SEM observations.



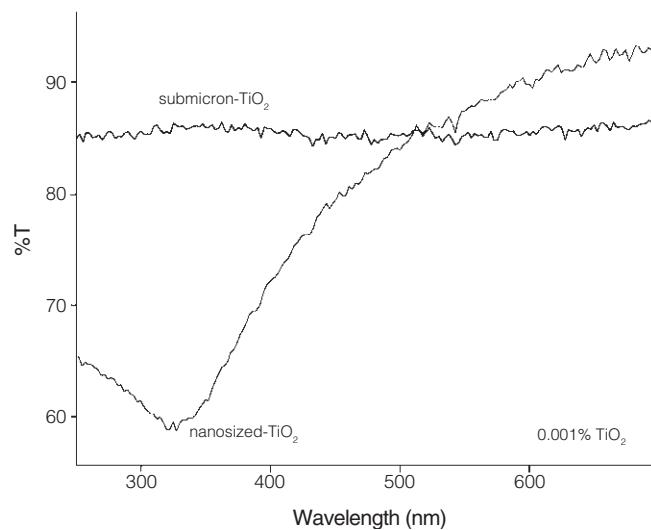
**Figure 1.** (A) SEM of submicron-sized  $\text{TiO}_2$ . (B) TEM of submicron-sized  $\text{TiO}_2$ . (C) SEM of nanosized  $\text{TiO}_2$ . (D) TEM of nanosized  $\text{TiO}_2$ .

### III. The SPF of Sunscreens

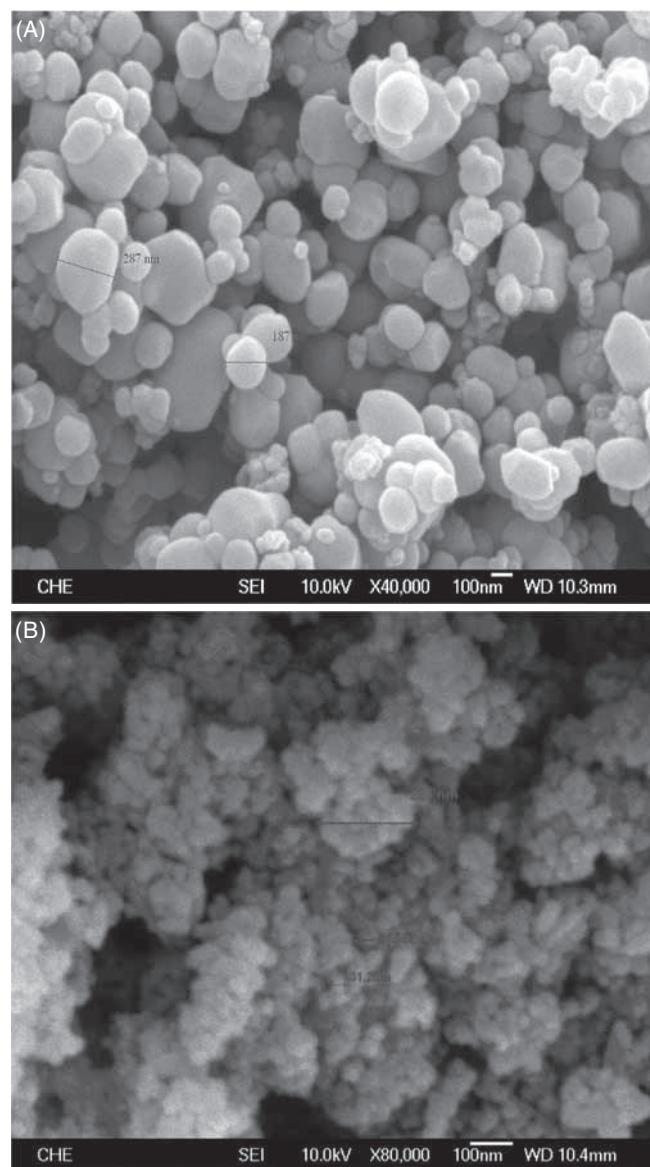
In order to determine the influence of  $\text{TiO}_2$  particle size on SPF value, sunscreen bases were prepared containing two different formulations of  $\text{TiO}_2$  pigment, submicron-sized and nanosized, in concentrations of 5%, 10%, and 20%. Before *in vitro* or *in vivo* SPF analysis, the exact  $\text{TiO}_2$  content in each formulation was determined (Table 2).

#### (I) *In vitro* SPF Determination

Figure 4 shows the *in vitro* SPF values of submicron-sized and nanosized  $\text{TiO}_2$  creams in the concentration of 5%, 10%, and 20%. The SPF value of the filterless cream base was low ( $1.07 \pm 0.01$ ,  $n = 5$ ). The SPF values of submicron-sized  $\text{TiO}_2$  creams were in the range of  $2.1 \pm 0.1$  to  $3.0 \pm 0.2$ , with the maximum SPF value measured in cream containing 10%  $\text{TiO}_2$ . SPF values of nanosized



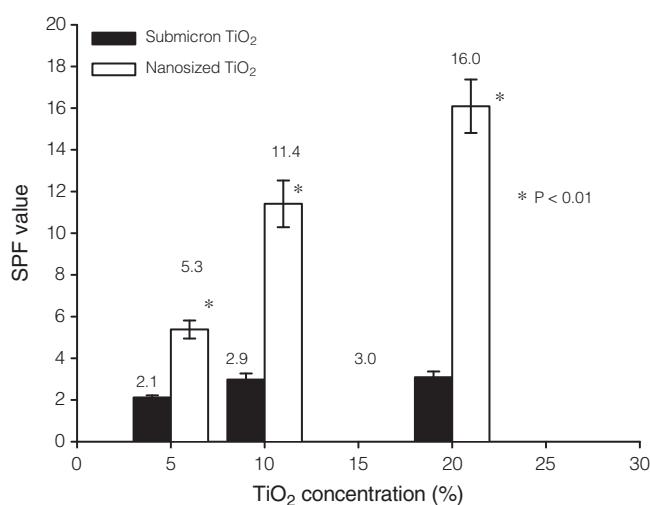
**Figure 2.** UV-visible transmittance spectra of submicron-sized  $\text{TiO}_2$  and nanosized  $\text{TiO}_2$  at 0.001% concentration in water.



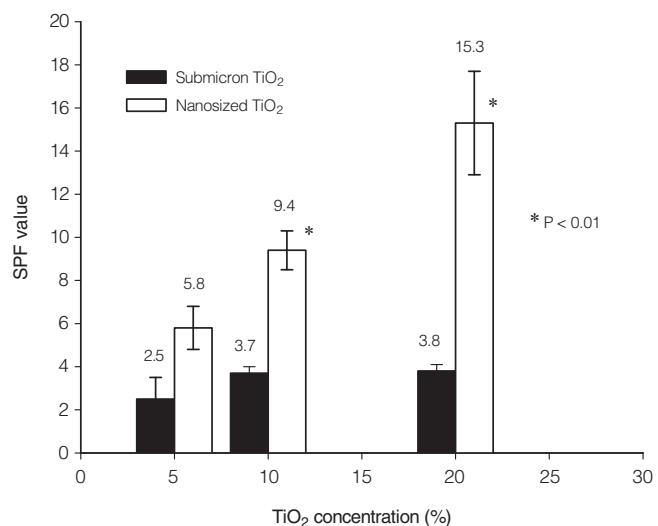
**Figure 3.** (A) SEM of submicron-sized  $\text{TiO}_2$  in glycerin dispersion. (B) SEM of nanosized  $\text{TiO}_2$  in glycerin dispersion.

**Table 2.** Mean particle size (PS  $\pm$  SD) vs. polydispersity index (PI  $\pm$  SD) and *in vitro* SPF value (mean  $\pm$  SD) of  $\text{TiO}_2$  in the cream formulations

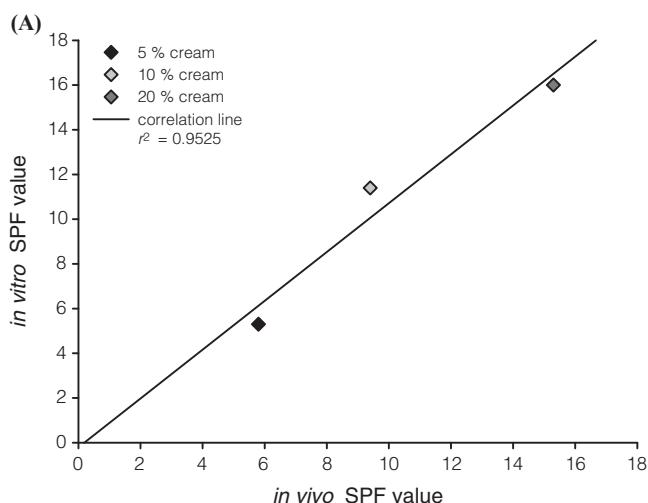
| Titanium Dioxide               | Added (%) | Determined (%) | PS (nm)          | PI                | SPF ( <i>in vitro</i> ) |
|--------------------------------|-----------|----------------|------------------|-------------------|-------------------------|
| None                           | 0         | 0              | —                | —                 | $1.07 \pm 0.01$         |
| Submicron-sized $\text{TiO}_2$ | 5         | 5.3            | $186.9 \pm 4.0$  | $0.261 \pm 0.041$ | $2.11 \pm 0.10$         |
|                                | 10        | 9.8            | $280.6 \pm 10.7$ | $0.348 \pm 0.064$ | $2.97 \pm 0.28$         |
|                                | 20        | 21.1           | $320.8 \pm 14.7$ | $0.311 \pm 0.093$ | $3.09 \pm 0.27$         |
| Nanosized $\text{TiO}_2$       | 5         | 5.6            | $219.5 \pm 27.8$ | $0.307 \pm 0.096$ | $5.38 \pm 0.43$         |
|                                | 10        | 10.4           | $309.2 \pm 9.4$  | $0.364 \pm 0.054$ | $11.41 \pm 1.12$        |
|                                | 20        | 20.9           | $532.5 \pm 44.2$ | $0.394 \pm 0.122$ | $16.08 \pm 1.28$        |



**Figure 4.** *In vitro* SPF measurements of submicron-sized TiO<sub>2</sub> and nanosized TiO<sub>2</sub> cream.



**Figure 5.** *In vivo* SPF measurements of submicron-sized TiO<sub>2</sub> and nanosized TiO<sub>2</sub> cream.



**Figure 6.** (A) Correlation of nanosized TiO<sub>2</sub> SPF values measured using *in vivo* and *in vitro* methods. (B) Correlation of submicron-sized TiO<sub>2</sub> SPF values measured using *in vivo* and *in vitro* methods.

TiO<sub>2</sub> creams increased with TiO<sub>2</sub> concentration from 5% to 20%, with values ranging from  $5.3 \pm 0.4$  to  $16.0 \pm 1.2$ . SPF values were significantly different between the 2 types of particles ( $p^* < 0.01$ , *t*-test).

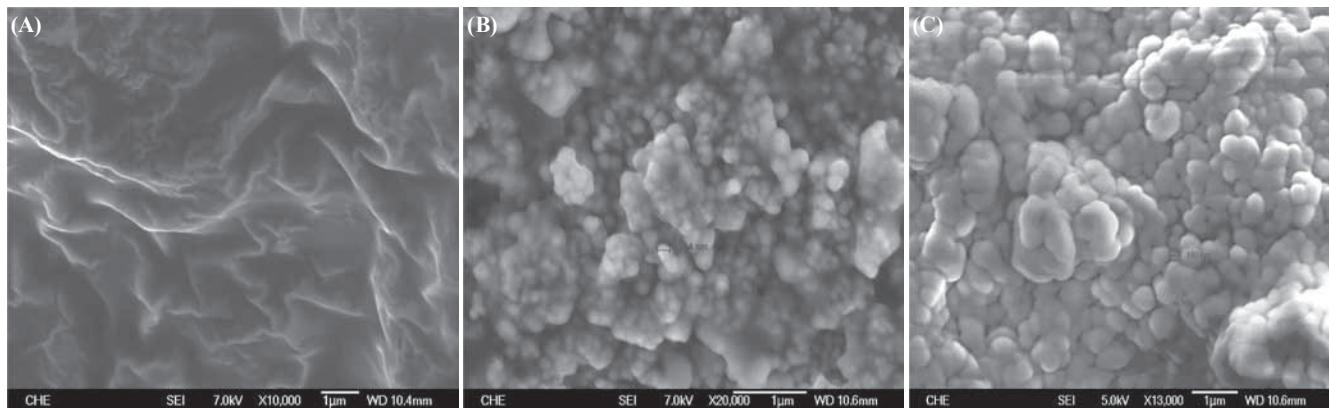
## (II) *In vivo* SPF Determination

Figure 5 shows the *in vivo* SPF values of submicron-sized and nanosized TiO<sub>2</sub> creams in the concentrations of 5%, 10%, and 20%. The results were similar to *in vitro* data. The *in vivo* SPF values of submicron-sized TiO<sub>2</sub> cream did not increase proportionally with the concentration of TiO<sub>2</sub>. In contrast, SPF values of nanosized TiO<sub>2</sub> creams showed a concentration-dependence from 5% to 20% TiO<sub>2</sub>. Moreover, there were significantly different

SPF values between creams of 2 types of TiO<sub>2</sub>, with higher SPF values ( $p^* < 0.01$ , *t*-test) for nanosized TiO<sub>2</sub> creams.

## (III) *In vitro-In vivo* Correlation

Figure 6 illustrates the correlation of SPF values of nanosized TiO<sub>2</sub> and submicron-sized TiO<sub>2</sub> creams measured by either *in vitro* or *in vivo* methods. The *in vivo* SPF values of nanosized TiO<sub>2</sub> creams were consistent with the *in vitro* results, and showed a strong *in vitro-in vivo* correlation ( $r^2 = 0.9525$ ). SPF measurements of submicron-sized TiO<sub>2</sub> creams also showed a strong *in vitro-in vivo* correlation was good ( $r^2 = 0.9989$ ); however, these creams did not show a concentration-dependence in SPF value.



**Figure 7.** (A) SEM of cream base. (B) SEM of cream containing 20% submicron-sized  $\text{TiO}_2$ . (C) SEM of cream containing 20% nanosized  $\text{TiO}_2$ .

#### (IV) Effect of Particle Size and Polydispersity Indices on the SPF in Sunscreens Containing 2 Sizes of $\text{TiO}_2$ Particles

The mean particle sizes and the polydispersity indices (PI) of submicron-sized  $\text{TiO}_2$  and nanosized  $\text{TiO}_2$  creams with 5%, 10% and 20%  $\text{TiO}_2$  concentrations are listed in Table 2. On the whole, the PI values varied in the range of 0.2 to 0.3. Nanosized  $\text{TiO}_2$  creams had higher PI values with higher concentration of  $\text{TiO}_2$  used.

The particle sizes of submicron-sized  $\text{TiO}_2$  creams increased as  $\text{TiO}_2$  concentration, and larger particles ranged from 186 nm to 320 nm, approximately 1 to 2 times the primary particle size of the pigment (170 nm). However, the SPF value of submicron- $\text{TiO}_2$  cream was low and failed to increase progressively with  $\text{TiO}_2$  concentration. This result suggests that aggregates formed between large-sized particles, making it difficult to reduce the void space between particles and leading to incomplete coverage of the skin. Broad particle size distribution and UVB attenuation with particles of larger size are important factors regulating the efficacy of sunscreens. In contrast, the particle sizes of nanosized  $\text{TiO}_2$  enlarge significantly from the primary particle size of the pigment (20 nm). Their values were measured to be between 219 and 532 nm, more than 20 times of the primary particles. This result is consistent with the previous observation that very fine particles at nanometric scales have a tendency to agglomerate to form large particles<sup>(17)</sup>. Furthermore, it was also observed that the greater the  $\text{TiO}_2$  pigment load, the larger the resulting particle size. For SPF measurements, a progressive, concentration-dependent increase in SPF was observed in creams containing nanosized  $\text{TiO}_2$ , which may be explained by the tendency of agglomerated small particles to overlap and a reduced void between particles. The capacity of these creams to completely cover the skin and prevent transmittance of UVB is superior to that of creams containing submicron-sized  $\text{TiO}_2$ .

#### (V) SEM Characterization of Sunscreens

The morphological characterization of submicron-sized  $\text{TiO}_2$  and nanosized  $\text{TiO}_2$  creams containing 20%  $\text{TiO}_2$  and cream base under SEM is illustrated in Figure 7. There were no obvious particles observed in the cream base. Typical aggregates and agglomerates observed in preparations containing submicron-sized and nanosized  $\text{TiO}_2$  are demonstrated in Figures 7B and 7C, respectively. These measurements confirmed the data obtained on mean particle size listed in Table 2. In Figure 7B, agglomerates consisting of many large particles range in size from approximately 150 nm to 300 nm. In Figure 7C, the same agglomerates were observed, with overlap and close contact between particles resulting in a clearly reduced void space.

## CONCLUSIONS

The reduced particle size of nanosized  $\text{TiO}_2$  is responsible for its capacity for enhanced SPF. In this study, we demonstrate a strong correlation between *in vivo* and *in vitro* measurements of SPF in sunscreen preparations containing nanosized  $\text{TiO}_2$ , providing evidence of adequate protection of skin from damage induced by UVB radiation. Our results demonstrate that the use of a  $\text{TiO}_2$  particle size less than 50 nm results in sunscreens with higher SPF values than those containing  $\text{TiO}_2$  with a particle size larger than 100 nm. Using SEM, we found that nanosized  $\text{TiO}_2$  agglomerates to form large particles, and increased SPF values correlate with larger particle sizes at the nano-scale. The obtained higher SPFs value suggests that through agglomeration small particles overlap, thereby reducing the void between particles. Consequently, the formation of a multilayer film on skin may be a factor leading to increased efficiency in sunscreens.

## REFERENCES

1. Damian, D. L., Halliday, G. M. and Barnetson, R. St. C. 1997. Broad-spectrum sunscreens provide greater protection against ultraviolet-radiation-induced suppression of contact hypersensitivity to a recall antigen in humans. *J. Invest. Dermatol.* 109: 146-151.
2. Mark, R. 1999. Photoprotection and prevention of melanoma. *Eur. J. Dermatol.* 9: 406-412.
3. Nohynek, G. J. and Schaefer, H. 2001. Benefit and risk of organic ultraviolet filters. *Regul. Toxicol. Pharmacol.* 33: 285-299.
4. Robinson, J. K., Rigel, D. S. and Amonette, R. A. 2000. Summertime sun protection used by adults for their children. *J. Am. Acad. Dermatol.* 42: 746-753.
5. Rai, R. and Srinivas, C.R. 2007. Photoprotection. *Indian J. Dermatol. Venereol. Leprol.* 73: 73-79.
6. COLIPA. 2006. COLIPA Guidelines: International Sun Protection Factor (SPF) Test Method.
7. Diffey, B. L., Tanner, P. R., Matts, P. J. and Nash, J.F. 2000. *In vitro* assessment of the broad-spectrum ultraviolet protection of sunscreen products. *J. Am. Acad. Dermatol.* 43: 1024-1035.
8. Pinnell, S. R., Fairhurst, D., Gillies, R., Mitchnick, M. A. and Kollias, N. 2000. Microfine zinc oxide is a superior sunscreen ingredient to microfine titanium dioxide. *Dermatol. Surg.* 26: 309-313.
9. Van der Molen, R. G., Hurks, H. M. H., Out-Luiting, C., Spies, F., Van't Noordende, J. M., Koerten, H. K. and Mommaas, A.M. 1998. Efficacy of micronized titanium dioxide-containing compounds in protection against UVB-induced immunosuppression in humans *in vivo*. *J. Photochem. Photobiol. B: Biology.* 44: 143-150.
10. More, B.D. 2007. Physical sunscreens: On the comeback trail. *Indian J. Dermatol. Venereol. Leprol.* 73: 80-85.
11. Alvarez-Román, R., Nail, A., Kalia, Y. N., Guy, R. H. and Fessi, H. 2004. Enhancement of topical delivery from biodegradable nanoparticles. *Pharm. Res.* 21: 1818-1825.
12. Dussert, A. -S. and Gooris, E. 1997. Characterization of the mineral content of a physical sunscreen emulsion and its distribution onto human stratum corneum. *Int. J. Cosmet. Sci.* 19: 119-129.
13. Bennat, C. and Müller-Goymann, C. C. 2000. Skin penetration and stabilization of formulations containing microfine titanium dioxide as physical UV filter. *Int. J. Cosmet. Sci.* 22: 271-283.
14. Schulz, J., Hohenberg, H., Pflücker, F., Gärtner, E., Will, T., Pfeiffer, S., Wepf, R., Wendel, V., Gers-Barlag, H. and Wittern, K. -P. 2002. Distribution of sunscreens on skin. *Adv. Drug Deliv. Rev.* 54(suppl.1): S157-S163.
15. Gamer, A. O., Leibold, E. and Van Ravenzwaay, B. 2006. The *in vitro* absorption of microfine zinc oxide and titanium dioxide through porcine skin. *Toxicol. In vitro*, 20: 301-307.
16. Nohynek, G. J., Lademann, J., Ribaud, C. and Roberts, M. S., 2007. Grey goo on the skin? Nanotechnology, cosmetic and sunscreen safety. *Critical Rev. Toxicol.* 37: 251-277.
17. Lautenschlager, S., Wulf, H. C. and Pittelkow, M. R. 2007. Photoprotection. *Lancet*, 370: 528-537.
18. Villalobos-Hernández, J. R. and Müller-Goymann, C. C. 2005. Novel nanoparticulate carrier systems based on carnauba wax and decyl oleate for the dispersion of inorganic sunscreens in aqueous media. *Eur. J. Pharm. Biopharm.* 60: 113-122.
19. El-Sheikh, A. H. and Sweileh, J. A. 2007. A rapid and simple microwave-assisted digestion procedure for spectrophotometric determination of titanium dioxide photocatalyst on activated carbon. *Talanta*, 71: 1867-1872.