

# Human Skin Penetration of Sunscreen Nanoparticles: In-vitro Assessment of a Novel Micronized Zinc Oxide Formulation

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## Key Words

Nanoparticle safety · Risk assessment · Transdermal delivery

## Abstract

The extent to which topically applied solid nanoparticles can penetrate the stratum corneum and access the underlying viable epidermis and the rest of the body is a great potential safety concern. Therefore, human epidermal penetration of a novel, transparent, nanoparticulate zinc oxide sunscreen formulation was determined using Franz-type diffusion cells, 24-hour exposure and an electron microscopy to verify the location of nanoparticles in exposed membranes. Less than 0.03% of the applied zinc content penetrated the epidermis (not significantly more than the zinc detected in receptor phase following application of a placebo formulation). No particles could be detected in the lower stratum corneum or viable epidermis by electron microscopy, suggesting that minimal nanoparticle penetration occurs through the human epidermis.

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## Introduction

The application of sunscreens to protect against UV-induced skin damage, such as sunburn, cancer, premature aging and photoallergies, is generally recommended. Physical sunscreens that do not undergo any chemical decomposition when they are exposed to UV radiation, such as titanium dioxide (TiO<sub>2</sub>) and zinc oxide (ZnO), have been introduced as supposedly safer alternatives to chemical agents. They can offer a wider spectrum of photoprotection than many chemical sunscreens [1]. These inorganic materials also have proven efficacy against UV-induced skin damage [2, 3]. However, opaque inorganic oxide formulations lack cosmetic acceptability, with a reported trend towards lower and insufficient application rates [4]. Nanoparticles of TiO<sub>2</sub> and ZnO are transparent in formulations spread on the skin surface. This transparency provides the cosmetic acceptability not achievable with larger-particle formulations.

The potential of solid nanoparticles, defined as having at least one dimension of 100 nm or less, to penetrate the stratum corneum and diffuse into underlying structures lies at the centre of the debate concerning their safety for topical use. The European Scientific Committee on Consumer Products has issued a number of opinions on the subject, but still believes that there is currently insufficient information for a proper safety evaluation of micro-

fine ZnO (some information can be found at: [www.europa.eu.int/comm/health/ph\\_risk/committees/sccp/documents](http://www.europa.eu.int/comm/health/ph_risk/committees/sccp/documents)). Central to this issue is the lack of available data demonstrating whether manufactured nanoparticles can gain access to the epidermis after topical application [5]. Dermal administered nanoparticles are known to localize to regional lymph nodes, potentially via skin macrophages and Langerhans cells [6]. The observation that endothelial cells possess a large capacity for the internalization of nano-scale particulate matter has also increased fears of potential pro-inflammatory or cytotoxic potential of nanomaterials compared to their larger-size counterparts [7].

Lademann et al. [8] could not detect any absorption of micronized TiO<sub>2</sub> particles (crystal size approximately 17 nm) into the epidermis following repeated application of an o/w emulsion to the forearms of volunteers over several days; only the upper stratum corneum and hair follicles showed any evidence of particle penetration. This finding, and the recent work of Alvarez-Roman et al. [9], suggests that the dermal penetration of TiO<sub>2</sub> microparticles reported earlier by Tan et al. [10] were in fact associated with hair follicle openings and not due to direct diffusion through the layers of the epidermis. Dussert et al. [11] used electron microscopy to show that there was no evidence of penetration into human epidermis for both TiO<sub>2</sub> and ZnO nanoparticles dispersed in a topically applied o/w emulsion; however, the length of skin exposure of the formulations in this study was not specified.

Although nanoparticles of physical sunscreens do not appear to penetrate into the viable epidermis, there is potential systemic exposure to nanoparticulate inorganic sunscreens which may partially dissolve following topical application [12]. The goal of the present study was to assess the potential epidermal penetration and systemic exposure of a novel ZnO nanoparticulate sunscreen formulation. We determined both the location of ZnO particles within the epidermis and the total amount of zinc penetrating through epidermal membranes *in vitro* over a 24-hour period after topical application of the ZnO nanoparticulate sunscreen formulation.

## Materials and Methods

### ZnO Sunscreen Formulation

**Particle Manufacturing.** ZnO nanoparticles were produced using MCPTM technology by Advanced Nanotechnology Limited. MCP technology uses high-energy dry milling to induce chemical reactions during ball collisions. Agglomeration of nanoparticles is

minimized by ensuring that the particles are encapsulated on formation by a solid diluent phase (typically sodium chloride). The diluent phase is subsequently removed by a simple washing process, and the nanoparticles were transferred into the desired oil phases. ZnO particles were coated with polymethylsilsesquioxane using Advanced Nanotechnology's proprietary method. Briefly, the nanoparticles were dispersed in a solvent phase and silicate monomers were mixed into the dispersion. Whilst the dispersion was vigorously stirred, a polymerization initiator was gradually added to form polymethylsilsesquioxane on the particle surface.

**Formulation Preparation.** The following three samples were prepared for testing: (i) a ZnO dispersion made with 60 wt% of silicate-coated ZnO in caprylic capric triglyceride (ZinClear-S\_60CCT), (ii) a typical o/w emulsion sunscreen with 20 wt% ZnO (using ZinClear\_40CCT) and (iii) a blank o/w emulsion sunscreen made without ZnO.

**Particle Size Determination.** Size and size distribution of ZnO nanoparticles were characterized before applying coatings on the particles. Four different measurement techniques, namely, transmission electron microscopy (TEM), specific surface area analysis with the Brunauer-Emmett-Teller nitrogen-gas absorption method (BET), X-ray diffraction (XRD) and photon correlation spectroscopy (PCS), were employed. Powders that were dried at 150°C were used for BET and XRD measurement. The crystallite size and crystal structure of the powder were examined via XRD using a Siemens D5000 X-ray diffraction spectrophotometer with Cu-K $\alpha$  radiation. The mean crystallite size was estimated from the width of diffraction peaks using the Scherrer equation. BET was used at 77 K, using a Micromeritics Gemini 2360 Surface Area Analyser. BET-specific surface area was used to estimate the corresponding spherical particle size using the following equation:

$$d = 6,000/(S \cdot \rho) \quad (1)$$

where  $d$  is the mean particle size (nm),  $S$  is BET-specific surface area (m<sup>2</sup>/g), and  $\rho$  is the density of ZnO particles (5.61 g/cm<sup>3</sup>). The microstructure of the powder was examined via TEM using a JEOL 2000FXII with a beam energy of 80 keV. For TEM studies, the washed powder was dispersed in methanol using an ultrasonic bath and a drop of the suspension was placed on a copper grid coated with holey carbon film. Particle size distribution of an aqueous suspension of nanoparticles (0.01 wt% concentration) was measured using Nicomp 380 ZLS. It should be noted that accurate quantification of particles with dimensions in the lower nanometer range is not fully validated using the above techniques and that some controversy exists regarding accurate sizing below 100 nm.

**Transparency.** Transparency of ZnO nanoparticle suspensions was measured using a Varian Cary 300 Bio UV/Vis spectrophotometer. Specular transmittance values of an aqueous suspension of ZnO nanoparticles (0.01 wt%), as described above, was measured in a quartz cell with an optical path length of 10 mm at wavelengths from 200 to 800 nm.

### *In-vitro Human Epidermal Membrane Penetration*

**Epidermal Membrane Preparation.** Female human skin donated following abdominoplasty was used. Epidermal membranes were prepared from full thickness tissue, which had been cleaned of underlying fat by blunt dissection using the heat-separation technique, which involves immersion in water at 60°C for 1 min and peeling the epidermis and follicle structures off the underlying dermis [13].

**Table 1.** Results of ZnO particle size analysis

Method of production	TEM (size)	BET (based on specific surface area)	XRD (based on crystallite size)	PCS (based on dynamic light scattering)
MCP ZnO	15–40 nm	30 nm	26 nm	30 nm

*In-vitro Diffusion Studies.* Epidermal membranes were mounted in static, horizontal Franz-type diffusion cells with an exposed surface area of approximately 1.3 cm<sup>2</sup> and receptor phase volume of roughly 3.5 ml. Membranes were allowed to equilibrate over phosphate-buffered saline (PBS) for 30 min and membrane integrity determined using electrical resistance (>20 kΩ) prior to dosing with ZnO formulation or control. PBS at pH 7.4, containing the mild surfactant Mackanate DC-30 (2%) to facilitate suspension and dispersion of any particles penetrating the membrane, was used as receptor phase throughout the study. Receptor phase was continuously stirred with magnetic fleas and maintained in a water bath at 35°C in order to give a membrane surface temperature of approximately 32°C. Membrane treatments consisted of 10 μl/cm<sup>2</sup> of either (i) ZinClear-S\_60CCT (n = 8), (ii) 20% ZinClear o/w emulsion sunscreen formulation (n = 8), (iii) placebo cream base containing no ZnO nanoparticles (n = 3) and (iv) no treatment, with the surface of the epidermal membrane left clean (n = 3). Following application of the formulations or control and spreading across the surface of the epidermis, 500-μl samples of receptor phase were removed and replaced with fresh solution at 12 and 24 h.

*Analysis of Zinc.* Zinc concentrations in receptor phase samples were determined using inductively coupled plasma-mass spectrometry by Queensland Health Pathology and Scientific Services, Coopers Plains, Queensland, Australia. Briefly, samples were acidified (1:9) with a reference element mixture and mixed. Standards were prepared by spiking 1 ml of blank buffer solution with various concentrations of the analyte elements and diluting (1:9) with acidified reference element mixture and mixing. Solutions were introduced into an argon plasma where ions were produced which then passed through a series of focussing lenses into a quadrupole where the ions were separated according to their mass/charge (m/v) ratio. Values for individual isotopes are then converted into concentrations from calibration parameters via the system software.

#### Data Analysis

The cumulative amount of zinc penetrating the epidermis was estimated from the zinc concentration in receptor phase samples, the receptor phase volume and accounting for samples removed at each time point. The difference in epidermal penetration of zinc under each test condition was determined by ANOVA with significance taken at  $p < 0.05$  and differences between groups identified using Tukey's post-hoc test.

#### Electron Microscopy

Separate samples of skin tissue, dosed in an identical manner to the epidermal membranes used in the penetration studies, were processed for TEM by conventional methods and embedded in

araldite. Semi-thin sections (1 μm) were stained with 1% toluidine blue in 5% sodium tetraborate and examined with a Zeiss light microscope. Thin sections (70 nm) were prepared on an LKB Nova ultramicrotome and examined in a Philips 410LS TEM at an accelerating voltage of 80 kV.

## Results

*Formulation Characterization.* The micronization processing used in the preparation of these particles produced nanoparticles of 26–30 nm, as determined by TEM, surface area analysis (BET), XRD and PCS (table 1). Figure 1a shows a TEM image of the commercial ZnO nanoparticles used in this study. It is evident that the particles had a significantly low degree of agglomeration. Figure 1b shows the particle size distribution determined by PCS. A narrow particle size distribution is evident. Due to their small size, low degree of agglomeration and narrow size distribution, the ZnO nanoparticles used in this study were more transparent than other ZnO particles, as shown in their increased light transmittance in the visible light range (approx. 400–700 nm) (fig. 1c).

*In-vitro Human Epidermal Membrane Penetration.* Penetration of zinc into the receptor phase from untreated epidermal membranes over 24 h was  $0.09 \pm 0.04 \mu\text{g}/\text{cm}^2$  and that from placebo-treated epidermis increased to  $0.22 \pm 0.12 \mu\text{g}/\text{cm}^2$ . There was no significant difference in the amount of Zn penetrating through the epidermal membrane following application of the two sunscreen formulations and the placebo formulation or untreated membranes over the 24-hour period (fig. 2a). There was a trend towards higher receptor phase levels following application of the two sunscreen formulations versus controls, which was probably due to dissolved Zn; however, the total amount absorbed was found to be less than 0.03% of the applied amount of material (fig. 2b).

*Electron Microscopy.* Penetration of ZnO nanoparticles was found to be limited to the outer surface of the stratum corneum and loose, desquamating cells of the upper stratum corneum only (fig. 3). No particles could be detected in the lower stratum corneum layers or viable epidermis.

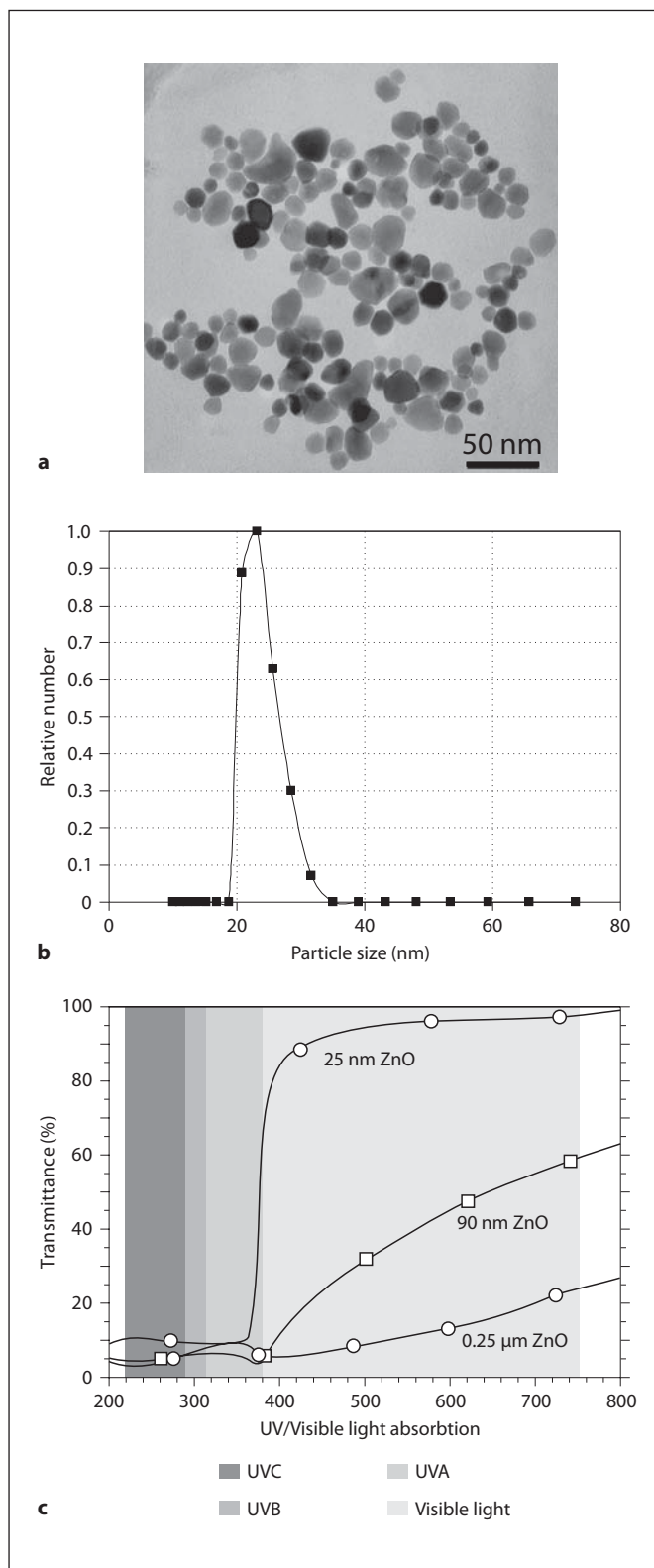
## Discussion

We achieved a narrow distribution of particle size by using the coated ZnO particle technology and manufacturing process described above (fig. 1a). Transparency,

whiteness and UV absorption are measures of quality for a nanomaterial formulation to be used as a clear topical sunscreen. In this study, other types of ZnO particles were shown to have low light transmittance, leaving an opaque sheen on the skin's surface. The use of uniform 25- to 30-nm particles achieves transmission levels of over 70–90% in the visible light regions (400–700 nm) (fig. 1b), resulting in a formulation that looks clear to the human eye.

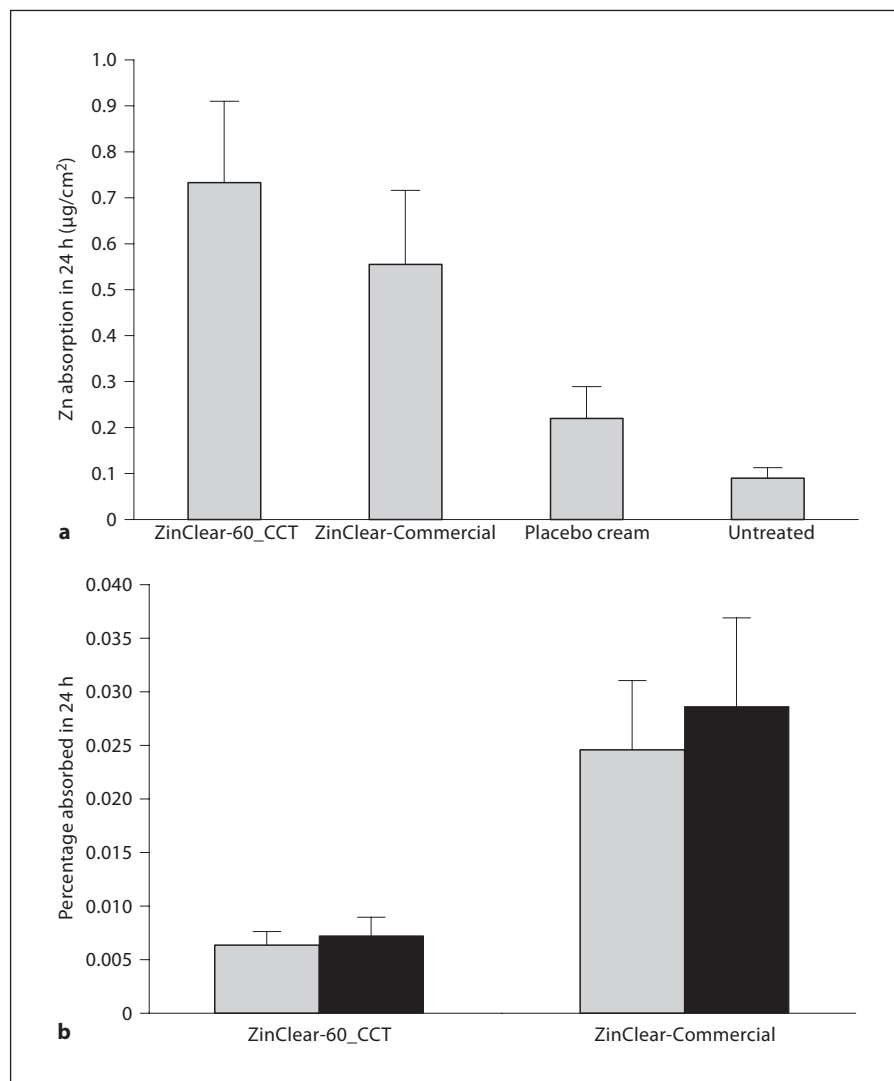
The study demonstrated that the epidermal penetration of zinc was negligible following topical application of this nanoparticulate formulation to human epidermis *in vitro*. These data are consistent with the findings of Lademann et al. [8], who discovered 17-nm TiO<sub>2</sub> particles only in the upper stratum corneum and hair follicles following application to volunteers, and with the results of Dussert et al. [11], who found no sign of penetration of both TiO<sub>2</sub> and ZnO nanoparticles using electron microscopy. Alvarez-Roman et al. [9] suggested that 20- and 200-nm polystyrene nanoparticles preferentially accumulated in follicle openings. More recent studies using pig skin exposed to microfine ZnO (mean primary particle size 80 nm) and TiO<sub>2</sub> (needle-like particles 30–60 × 10 nm) sunscreen formulations suggested that neither particle types were able to penetrate porcine stratum corneum [14].

The appearance of some zinc in the receptor phase of the present study was not unexpected, due to the amount of Zn<sup>2+</sup> normally present in human skin. The endogenous content of Zn<sup>2+</sup> in human epidermis has been estimated at 60 µg/g dry weight, reducing to approximately 40 µg/g dry weight in the upper dermis [15]. Our observation of 0.09 ± 0.04 µg/cm<sup>2</sup> Zn<sup>2+</sup> accumulation in the receptor solution of untreated skin over a 24-hour period is consistent with release from this epidermal reservoir. Application of the placebo cream base showed a slight but nonsignificant increase in this level, which could have been due to either one or a combination of two effects: (i) occlusion of the surface of the epidermis by formulation excipients, leading to hydration and increased diffusion of solutes within and from the epidermal membrane into the receptor phase [16], and (ii) penetration of trace levels of zinc present in the cream formulation not associated with the ZnO nanoparticles later added in the sunscreen formulations. A further means of assessing the contribution of the skin's own reservoir of endogenous zinc to the amount recovered in the receptor phase would have been to apply much larger zinc particles (in the micrometer size range), known to be too large to pass through the epidermis. However, the potential for solubilization, if



**Fig. 1.** **a** TEM picture of coated ZnO particles. **b** PCS size distribution of micronized ZnO particles. **c** Spectral transmittance of ZnO particles in aqueous solution.

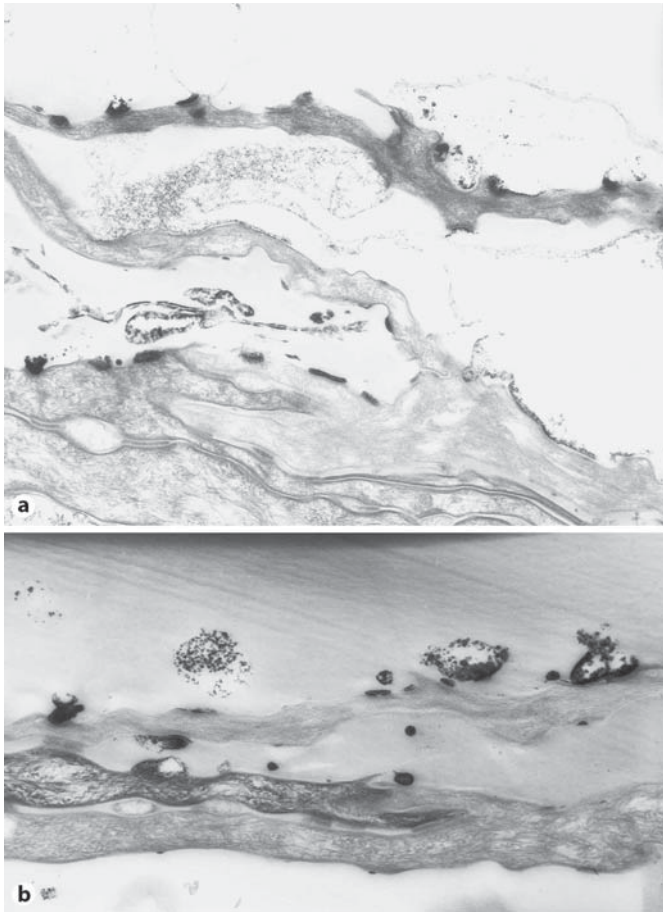
**Fig. 2.** Receptor phase penetration of zinc through human epidermal membrane as amount in microgramme per centimetre square for all treatments (**a**) and as percent of applied amount from the two ZnO-containing treatments (**b**). Columns and bars represent means and standard deviation. Grey column = 12 h, black column = 24 h; n = 8 treatments and n = 3 controls.



only a small fraction, of some of these larger particles and release of small amounts of free zinc that could penetrate the epidermis could not be entirely discounted.

The lack of observation of ZnO particles in the lower stratum corneum and epidermis (fig. 3) together with the trend towards higher Zn levels in the receptor phase of epidermal membranes treated with ZnO nanoparticle-containing formulations (fig. 2) suggests that a small fraction of the particles are dissolving and diffusing through the membrane as elemental zinc. Consistent with this observation, the solubilization and oxidation of nickel particles on the surface of the skin has previously been reported as the mechanism by which stratum corneum-diffusible compounds are generated and able to penetrate intact stratum corneum, presumably by the in-

tercellular route [17]. The penetration of Zn<sup>2+</sup> and ZnO into and through the skin has been studied by many groups. The flux of zinc sulphate through human skin, dermatomed to 410 µm thickness, was estimated to be approximately 2% of applied amount at 24 h from both a hydrogel and petrolatum vehicle base and approximately 3.5% for zinc chloride [18]. The data from the present study are approximately 100-fold below these absorption rates. A second study by the same group looking at the absorption of zinc salts (zinc 2-pyrrolidone 5-carboxylate, ZnO and zinc sulphate), applied as various combinations in emulsion and ointment formulations to human dermatomed skin [19], showed receptor fluid absorption rates at 24 h between 0.09 and 1.19%. The only formulation containing ZnO alone (ointment) showed penetra-



**Fig. 3.** Electron micrographs of human skin showing ZnO nanoparticle mineral components present on the surface of the skin and around desquamating corneocytes (a and b). No penetration into the underlying intact stratum corneum was observed.

tion of 0.29% of the applied zinc into the receptor phase after 24 h. Our current data is approximately 40- and 10-fold lower this rate for the ZinClear-60\_CCT and ZinClear commercial (40\_CCT) sunscreen formulations, respectively. This comparison supports the idea that nanoparticle formation decreases the absorption of ZnO across the skin, probably due to a reduction in the amount of available solubilized zinc on the skin's surface.

Micronization has been used in transdermal formulations for a number of years; however, its ability to increase or decrease skin penetration of encapsulated drugs is debatable. Solid lipid nanoparticles and nanostructured lipid carriers, specifically developed as alternatives to emulsions and liposomes to encapsulate and modulate the uptake of topically applied drugs, have been shown to produce increased skin deposition of agents such as co-

enzyme Q10 and retinol following topical application [20]. More recently, encapsulation of the sunscreen agent octyl methoxycinnamate (OMC) in solid lipid nanoparticles (250 nm) similarly showed increased availability of OMC within porcine skin [21]. However, in this latter study it was recognized that simple formulation thermodynamics resulting in increased partitioning into the stratum corneum contributed to this effect rather than as a direct result of nanoencapsulation itself. Solid lipid nanoparticles have also been associated with increased levels of penetration due to their direct effect on skin hydration, causing increased water retention in the stratum corneum as a result of film formation on the skin surface and effectively occlusion of the application site [22]. Conversely, incorporation of OMC into poly( $\epsilon$ -caprolactone) nanocapsules (374 nm) was recently reported to decrease skin accumulation, attributed to slow release of OMC from the nanocapsules which limits availability of OMC to the stratum corneum whilst maintaining photoprotection [21]. These studies suggest that micronization technology itself may not increase the penetration of solutes into the stratum corneum; however, the size of particles used in the above studies are technically above the 100-nm cut-off for the definition of a 'nanoparticle'. Additionally, it should be borne in mind that application of any topical sunscreen inevitably results in 'some' systemic exposure due to oral and nasal uptake secondary to application to the lips and mouth area, around the nose and via contact of contaminated hands with food [12].

Data from this study have shown that human skin absorption of nanoparticulate coated ZnO was relatively low compared with published absorption rates for chemical sunscreens. Absorption levels, expressed as percentage of applied amount, through human epidermal membranes *in vitro* from a mineral oil vehicle of approximately 5–7% for oxybenzone, 0.03–0.14% for octocrylene, 0.07–0.17% for ethylhexyl methoxycinnamate and 0.09–0.2% for ethylhexyldimethyl PABA were previously reported from the same laboratory in which the current studies were undertaken [23]. A limitation of this study is that it has been conducted in static Franz cells over 24 h using human epidermal membranes, so accumulation in follicular openings was not directly assessed. In addition, flexing human epidermis has also been suggested to lead to the increased translocation of small fluorescent particles into the membrane [24]. The extent to which flexing may affect the penetration of the nanoparticles studied here is not known.

In conclusion, there is obviously need for further research on the fate of other types of nanoparticles,

<100 nm, purposely or accidentally exposed to human skin to increase our appreciation of whether the lack of penetration we have observed with nanoparticulate ZnO is applicable to other chemical species. However, whilst the debate on general nanoparticle safety continues, the case for safe, non-penetrating, transparent, topical ZnO sunscreen formulations appears to be strengthening.

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