Issues relating to the use of Micro and Nanoparticle Zinc Oxide in Personal Care Products based on Skin Penetration Studies

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ABSTRACT
Many aspects of current data on bulk materials is inadequate (and possibly irrelevant) for extrapolation to their nanoparticulate counterparts especially in relation to the stated applications. Receiving particular attention is the use of nanoparticles such as titania and zinc oxide in personal care products. The current study assessed and compared the in vivo penetration behaviour of nano and micro sized ZnO particulates into human stratum corneum (SC) after topical sunscreen application using a method of sequential removal of layers of stratum corneum by adhesive tapes known as tape stripping. The study revealed that a large proportion of the ZnO applied remained on the surface or at least in the uppermost layers of the SC and the mean percentage of recovery of ZnO after 18 tape strips varied between ~ 70 and 100%. Although the study suggested that there was little apparent difference in the penetration behaviour of either particle size into the stratum corneum, it raised several potentially significant issues relating to the efficacy of using nanosized additives in personal care applications.

KEYWORDS*:* nanoparticle, zinc oxide, stratum corneum, sunscreen, penetration

INTRODUCTION
Nano-particles (ZnO,TiO_2) are increasingly being used as UV absorbers in personal care products (e.g. sunscreens) predominantly because their absorbance efficiency and transparency to visible light increases with decreasing particle size. There is community and scientific recognition that many aspects of current data on bulk materials is inadequate (and possibly irrelevant) for extrapolation to their nano- counterparts. Receiving particular attention are nanoparticles in personal care products where the main concern is associated with their phototoxicology which increases at decreased particle sizes. There may be a negative effect on skin cells due to this. Scant attention has been placed on other behavioural aspects in relation to size such as the fate of these materials including their penetration into and possibly through the skin.

The purpose of the current study was to assess and compare the in vivo penetration behaviour of nano and micro sized ZnO into human stratum corneum (SC) after topical sunscreen application. ZnO particulates of larger particle size in sunscreen are believed not to penetrate the skin but to be retained on its surface after application. Several publications, however, have suggested that skin structure can potentially provide channels for nanoparticle penetration leading to dermal absorption. Of concern with topically applied nanoparticles is whether they can penetrate into human skin and consequently increase their toxicity potential and/or significantly reduce their protective role [1,2].

The scope of the present research is *not* to investigate the potential of transdermal delivery of nanoparticulates, but to assess and compare the in vivo penetration behaviour of nano and micro sized ZnO after topical application. There are few studies in the open literature that report on this.

EXPERIMENTAL
Materials
Sunscreen and base formulations used in this in-vivo study were supplied by Ellis and Associates (Australia). Nano ZnO sunscreen contained 15.31 wt % of 38.4 nm ZnO (primary crystallite size) while the micro ZnO sunscreen contained 15.7 wt % of 398 nm ZnO. Tape used for stripping was polyester electrical tape (3M Product No. 5E1-7385, Australia).
Methodology
The method used involved applying the recommended dose of sunscreen (FDA recommendation for Sun Protection Factor (SPF) determination; 2 mg/cm^2) onto

a cleaned, marked area of the volar forearm of ten healthy female subjects. All subjects received two test treatments (nano ZnO sunscreen (N) and micro ZnO sunscreen (M)) acting as their own control (receiving a base formulation containing no ZnO). Approximately 12 mg of sunscreen was applied across a marked area of 6 cm^2 (1.5 cm x 4 cm) thus delivering the recommended dose.

Individual tapes [2.4 x 5 cm (area; 12 cm^2)] were prepared, equilibrated under ambient conditions (2hrs), weighed to five decimal places (Mettler microbalance AT261 Delta Range) before stripping experiments were performed. Tapes were applied at constant pressure (100 g/cm^2) for 5 seconds prior to slow removal. The treated area was repeatedly stripped 18 times with different tapes. Each tape was reweighed after stripping to determine the mass of SC removed. Subsequent to weighing, tapes were immersed in 20 mL of 35.4% HCl to dissolve ZnO and solution samples analysed for elemental Zn by Inductively Coupled Plasma–Atomic Emission Spectroscopy (ICP-AES) to quantify the amount of ZnO removed.

Treated areas on the left forearm were stripped immediately after sunscreen application (t_0) while the treated areas on the right forearm were stripped at two hours (t_{2h}). A limited selection of tape strips were also analysed spectroscopically using a scanning electron microscopy (SEM) to determine the presence of ZnO on the first and later tape strips.

RESULTS

Figure 1 presents a summary of results from tape stripping experiments where the cumulative amount of ZnO recovered after 18 tape strips at different sampling times (t_0 and t_{2h}). The mean cumulative recoveries of nano and micro ZnO (nine subjects, 18 TS) were: N_0; 99.04% ± 4.46%, M_0; 107.47% ± 6.22%, N_{2hrs}; 68.16% ± 5.77% and M_{2hrs}; 84.17% ± 5.83%. The differences in the mean recoveries of N_0 and M_0 were respectively compared to N_{2hrs} and M_{2hrs} and were found to be not significantly different.

Figure 2 presents the ratio of ZnO recovered:SC removed versus tape strip number. The proportions of ZnO removed from the $N_{O,}$ M_2 and M_O treatments range between 26-29% whilst that of in N_{2hr} was lower at 23-25%.

DISCUSSION

The current study encompasses two foci relating to the concern associated with the use of topically applied

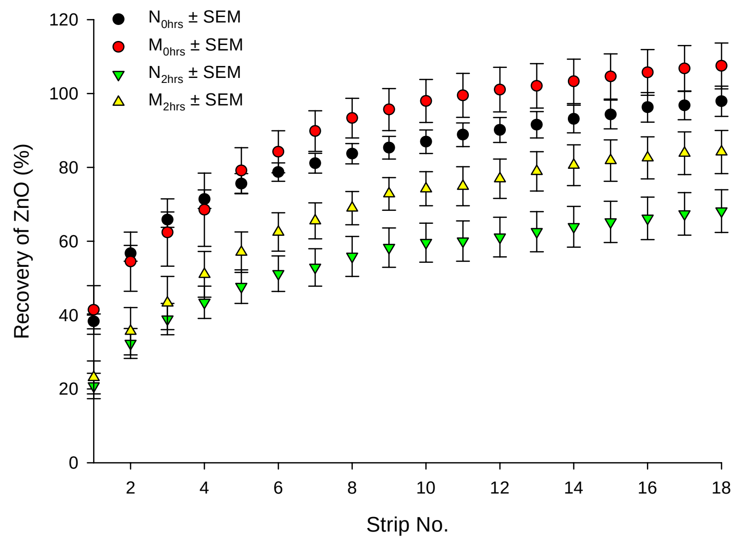

Figure 1. The cumulative amount (mean ± EM†, n = 9) of ZnO recovered (after 18 Tape Strips) at t_0 and $t_{2hrs.}$

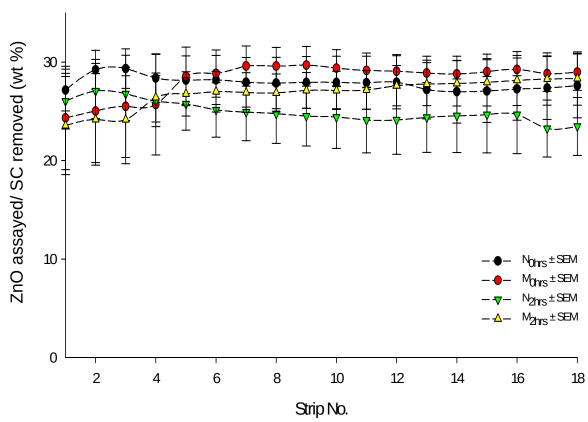

Figure 2. The ratio of ZnO recovered:SC removed by each tape strip. Data are expressed as mean percentage ± SEM†, n = 9.

nanoparticles. These are to (i) verify that a large proportion of ZnO in sunscreens will remain on the surface or in the upper layers of the SC and (ii) determine whether there is a difference between nano and micro ZnO distribution in/on the skin when used in sunscreen applications.

Results from this study indicate a high recovery of both nano and micro ZnO (70-100% of that applied) suggesting that a large proportion of topically applied ZnO remains on the surface or at least in the upper layers of the SC. The highest recoveries were from nano and micro ZnO at t_0 (100%), the lowest from nano ZnO at t_{2h} (68%) with micro ZnO at t_{2h} (84%) intermediate. The majority of the applied sunscreen was removed by the first three strips (Figure 1). Skin stripping studies done in the absence of sunscreens have reported that 20-30 tape strips can remove most of the SC [3]. Where sunscreen is applied removal of the SC is only possible after removing the sunscreen layer. Consequently, the amount of SC removed here was likely to be less than that predicted from the literature.

Recoveries of ZnO at t_0 were expected to be near 100% and this was observed. Presumably, most or all of the applied sunscreen remains on the skin surface due to limited elapsed time for any possible transport processes to occur. Conversely, expected recoveries at longer time intervals (t_{2hr}) were unknown. If the elapsed time was long enough for transport processes to progress then lower recoveries would be anticipated. This is what was observed (68% *cf* 100% for nano ZnO at t_{2h} and t_0). The lower recovery observed may be due to absorption or loss from the surface of the skin. Prima facie, it appears that reducing particle size alone does not result in significantly different penetration behaviour. There seems, however, to be time dependant differences that are more pronounced at smaller particle sizes ($N_{2h,}$ M_{2h} *cf* $N_{to\,,}M_{to}$).

Time dependant factors that may contribute to lower recoveries include loss via skin shedding or desquamation, localisation of particles in macroscopic furrows of the skin or hair follicle (HF) orifices and time-dependent penetration resulting in residual minor portions in deeper layers of the SC.

In vivo studies on the rate of desquamation and regional variation have shown that the forearm has the highest rate of corneocyte loss (mean of 1309 corneocyte/cm2/hr) and so ZnO adhered to corneocytes may be lost from the surface of the skin due to skin shedding [4]. This corneocyte loss however accounts for only ~ 3-5% of the corneocytes within the treated area and whilst contributing to overall loss would not account for the diminished recoveries observed here.

Deeper penetration is unlikely to occur rapidly via tortuous penetration routes and/or transfollicular pathways. ZnO particles may localize in the orifice of HF but in this study this loss would be minimal as the mean percentage area occupied by HF on the volar forearm is as low as 0.09% ± 0.04% [5]. Although unlikely, ZnO particles may penetrate at longer times through the tortuous permeation routes. The vehicle used in sunscreen preparation may have altered the skin condition and enhanced penetration by hydrating the SC [6]. In normal human skin, the SC holds between 15-20% (dry weight) water and further hydration of the SC can lead to profound changes in its barrier properties. This increased hydration can induce swelling of the corneocytes and expand the intercellular lipid junction between them leading to increased particle penetration.

Theoretically, the ratio of ZnO recovered:SC removed should exponentially decrease with strip number. This trend was not observed (see Figure 2). A possible explanation is that ZnO particles are localised in the macroscopic furrows and/or HF of the skin. Progressive skin stripping would remove a thin layer of the applied ZnO as well as deposits of ZnO around the orifice of these inlets. The lower proportion of ZnO assayed in N_{2h} may be due to nano ZnO gaining access to the macroscopic furrows or HF of the skin and remain in these deeper inlets without subsequent removal even after 18 tape strips.

A key relationship to be determined is the amount of ZnO recovered as a function of the depth or thickness of the SC. The data was replotted to represent this relationship and is presented in Figure 3. Analysis of variance (ANOVA) tests determined there was no significant difference if tape stripping commenced immediately after application (i.e. N_0 and M_0) while differences in recoveries between treatments with varied sampling times (i.e. N_0, N_{2h} and M_{2h}) were significantly different.

Determination of the thickness of the SC through skin stripping techniques assumes that the density of the SC is constant throughout its depth and that the distribution of SC on skin-stripped tape is uniform. The general relationship between the number of tape strips and the corresponding amount of SC removed is valid only for skin not treated with a topical product. In the present study, sunscreens were applied and hence the use of weight difference of the tape strips to determine the thickness of SC removed by each tape strip could be misleading. Consequently, the cumulative thickness of SC removed by 18 TS is only an approximation of the true thickness of SC removed.

To further investigate the distribution of ZnO across the treated area, tape strip Nos. 1, 18 and 30 of both nano and micro ZnO at t_{2hrs} were compared by SEM (see Figure 4). The white areas were analysed for elemental content and were confirmed to be ZnO, the grey area shows the corneocytes being removed by the TS. Through Tape # 1 to #30 the grey areas are being replaced by darker areas, suggesting TS no 1 was removing significantly more corneocytes than TS 18 & 30

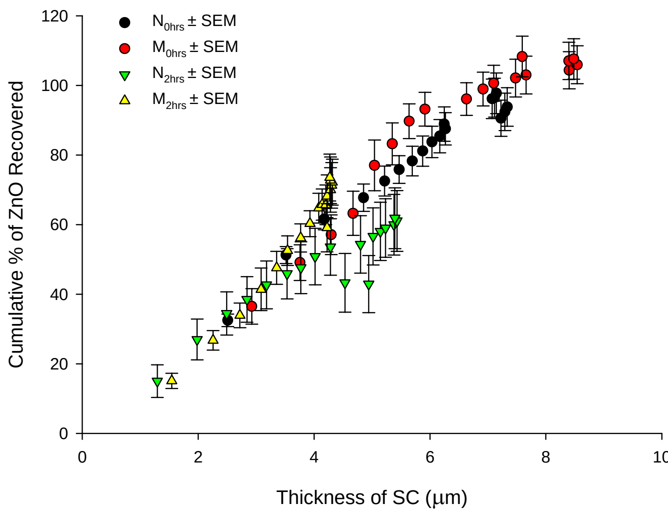

Figure 3. Cumulative percentage of ZnO (expressed as mean percent dose applied ± SEM†, n = 7) recovered versus approximate thickness of SC (18 TS).

The increasing empty spaces indicate that the amount of SC removed decreases with strip number. TS no 1 shows a distribution of ZnO across the tape area while TS 18 and 30 show aggregates of ZnO and supports the explanation that the TS was not evenly removing layers of the applied ZnO but in fact islands of it, possibly resulting from ZnO depositing in macroscopic furrows or HF of the skin

ISSUES

There are several issues regarding the use of nanoparticles in sunscreens or indeed any topologically applied personal care product that are raised by this preliminary study. The key question, as yet not fully answered, is what is the ultimate fate of nano ZnO at even longer times than studied here and how does this behaviour affect the intended UV protective behaviour? If further penetration of the stratum corneum occurs are nanoparticles absorbed through the skin, it is undetermined whether they enter the blood stream as unsolubilised entities or merely accumulate in the deeper layers of the SC. As sunscreen is normally applied repeatedly is the behaviour reported here does enhanced or inhibited? If there is no further penetration into the skin are nanoparticles merely lost via skin shedding or are there radial transportation processes that occur on the skin surface.

Whatever the case are there implications for the efficacy of the recommended doses for achieving satisfactory SPF rating, should these recommended doses be reconsidered and will this vary with the age of the subject. These are key questions that need to be

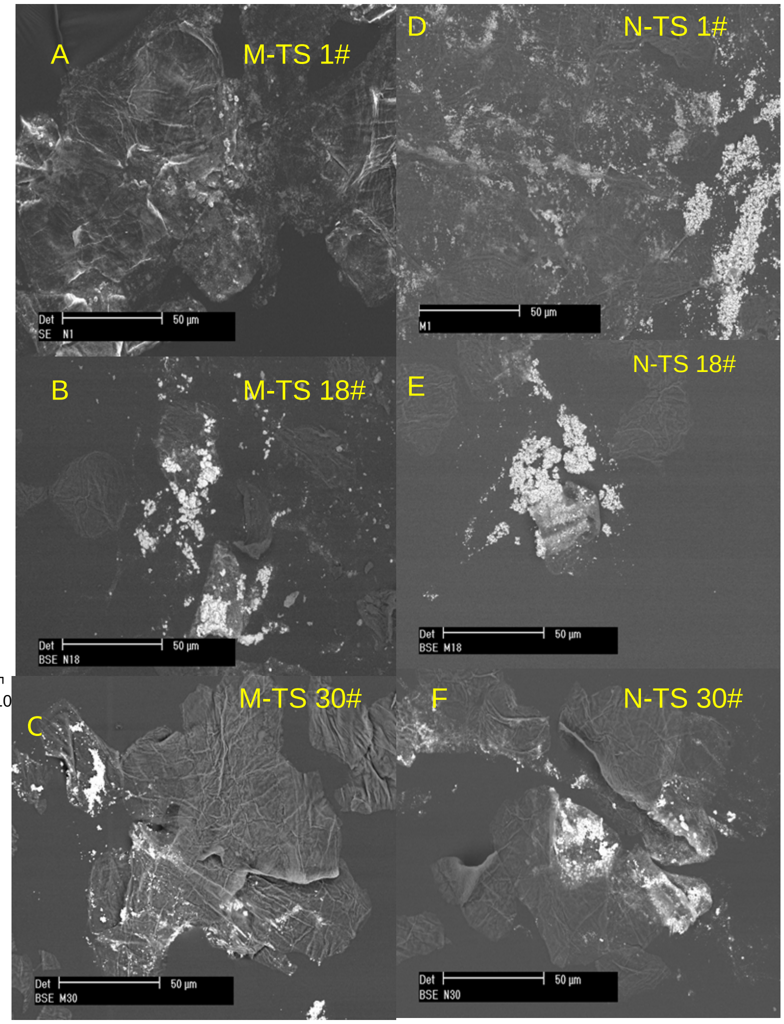

Figure 4 SEM Micrographs (Backscattered) of TS # 1, 18, 30 for nano and micro ZnO at t_{2h}

considered by users, producers and regulators of sunscreens where nanoparticles are employed as functional additives.

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