IJPSR (2015), Vol. 6, Issue 6 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 23 October, 2014; received in revised form, 22 January, 2015; accepted, 21 February, 2015; published 01 June, 2015

EX-VIVO PENETRATION OF NANOMETRIC ZnO and TiO_2 ACROSS ACTINICALLY DAMAGED PORCINE SKIN: DEVELOPMENT OF AN ALBINISTIC SKIN PROTECTION TREATMENT

J. Chifamba *[1], A. Dube [2] and C. C. Maponga[1, 3, 4]

College of Health Sciences [1], University of Zimbabwe, Harare, Zimbabwe
Pharmaceutics Discipline [2], School of Pharmacy, University of the Western Cape, Cape Town, South Africa
School of Pharmacy and Pharmaceutical Sciences [3], University at Buffalo, Buffalo, New York, USA
Translational Pharmacology Research Core [4], NYS Center of Excellence in Bioinformatics and Life Sciences, Buffalo, New York

Keywords:

Titanium dioxide, Zinc oxide, Oculocutaneous albinism, nanoparticles, actinically damaged skin penetration.

Correspondence to Author:
J Chifamba

Lecturer and Research Fellow
Department of Pharmaceutics, School of Pharmacy, College of Health Sciences, University of Zimbabwe
630 Churchill Avenue, Mount Pleasant, P O Box MP167, Harare, Zimbabwe.

E-mail: chifambajoey@gmail.com

ABSTRACT: The skin penetration and safety of nanometric zinc oxide and titanium dioxide incorporated in a cosmeceutical cream, specifically designed to retard skin actinic damage in tropical Oculocutaneous albinism was investigated *ex-vivo* through simulated actinically damaged porcine skin. Franz diffusion cells were used in an experiment modeled in line with related work done by Diembeck *et al* (1999); the OECD guideline, document no 428 and the EU opinion SCCNFP 0750/03. The following experiment was distinguished by its use of simulated actinic damaged skin characteristic in albinism in the tropics. The subsequent analytical studies were modified, since skin stripping was not practical on damaged skin. Analysis for the nanomaterials was conducted on both the Franz cell receptor phase fluid as well as extractions from the entire skin tissue material after the diffusion process. Quantification analysis for the recovered titanium was done by ICP-AES and the zinc was assayed using Flame AAS. The total recoveries of zinc from the skin extracts ranged between101.35-103.20% of the total zinc applied. The amounts of zinc recovered were comparable in treated, untreated skin, and the receptor phase. Mean total recoveries of titanium for all categories ranged between 98.73% and 99.24%, of the total applied titanium. No titanium was found in the receptor phase. The results show that neither nanometric titanium nor zinc ions can penetrate both normal and actinically damaged porcine skin, thus, suggesting minimal systemic exposure when used in treatments for actinically damaged albinistic persons.

INTRODUCTION: Human albinism is a congenital group of pigmentation disorders that manifest as a partial or complete lack of melanin in the skin, hair and eyes [1].

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.6(6).2339-48

Article can be accessed online on: www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.6(6).2339-48

The condition, which has no known cure, affects all vertebrates and organisms afflicted have no natural protection against solar Ultra Violet Radiation (UVR) which has a capacity to cause actinic damage [2]. Albinistic persons are therefore susceptible to actinic damage, giving rise to conditions such as freckles, erythema, solar urticaria, oedema, solar keratoses, premature aging and various skin cancers including squamous and basal cell carcinomas [2]. Due to the dependence of UVR on latitude and altitude and its correlation with the solar zenith angle, the worst forms of

actinic damage in albinism are evident in people living within tropical Africa [3]. The higher UVA and UVB doses and longer hours of sun exposure in tropical Africa has marginally shortened the life expectancy of people living with albinism compared to the rest of the population [3, 4]. Although, albinism affects 1 in 17000 people world-wide, countries like Zimbabwe, Namibia, Tanzania, Kenya and other Sub Saharan populations have incidences of albinism that are as high as 1 in less than 1000 people [4].

This demonstrates that human Albinism has both racial and geographical biases affecting Sub Saharan Africans more than any other group. No specific treatment to retard actinic damage in Albinistic people living within the tropics and elsewhere has been developed [5]. Effective products developed for Albinistic treatments must therefore take account of racial skin type differences as well as geographical impacts on actinic damage and UVR effects [6].

In this investigation, a treatment was specifically developed to retard actinic damage in albinistic individuals living in tropical Africa. The cosmeceutical employed nanometric metallic oxides of zinc and titanium for their broad spectrum sunscreen effects [7, 8]. It also incorporates *Aloe Excelsa, Trichilia Emertica* and *Myrothamnus Flabellifolia*ethnic herbal extracts, which have proven anti-inflammation, wound healing and anti-aging properties respectively [9], in an encompassing treatment to retard actinic damage in albinistic persons that took consideration of variances in skin types and geographical conditions. This study was carried out between 2013 and 2014 in tropical conditions and the experiments were done using actinically damaged porcine skin, whereby actinic damage was induced by an alkaline solution and a xenon arc solar simulator.

This *ex-vivo* safety investigation of the dermato-pharmacokinetics of nanomaterials on actinically damaged skin, aimed at determining whether nanoparticles of TiO_2(CAS number 13463-67-7) and ZnO (CAS number 1314-13-2) can penetrate damaged and compromised skin when formulated in actinic damage retardation treatments for use by albinistic persons leading to systemic exposure. We utilized vertical static Franz diffusion cells using15 different porcine skin dermatomes. The effects of formulation concentration and extent of permeation on trans-epidermal nanometric Titanium (Ti) and Zinc (Zn) was therefore determined.

MATERIALS AND METHODS:

Materials:

A mixture of rutile and anatase nanoparticles of titanium IV oxide dispersion, 43-47% w/w in xylene, (<100nm DLS particle size), Lot number MKBN8669V and zinc oxide nanoparticles dispersion 50% w/w in H_2O,(<35nm APS), Lot number MKBQ0692Vwere obtained from Sigma Aldrich, Germany. Cetyl alcohol, stearic acid, glycerol monostearate (GMS) and ceto stearyl alcohol were sourced from Savanna Fine Chemicals South Africa. Liquid paraffin and petrolatum were supplied by Engen, South Africa. Tween 20, triethanolamine and potassium dihydrogen orthophosphate were sourced from Merck Chemicals, South Africa. 96% ethanol was obtained from Astra Chemicals Zimbabwe. *Myrothamnus Flabellifolia* was extracted using steam distillation, *Trichilia emetica* was obtained through cold expeller extraction and *Aloe excelsa* gel was obtained through physical extraction of the leaf sap from the plants by the researchers during this study. Sodium PCA, EDTA, Carbopol 940®, methyl and propyl parabens, castor oil, cocoa butter, lanolin, sodium hydroxide, deionized water and all consumables were availed by the University of Zimbabwe, College of Health Sciences, School of Pharmacy, pharmaceutics laboratory.

Full thickness porcine skin samples were excised from visually intact skin from the abdomen of three 6 months old domestic landrace porkers obtained from Barnstone enterprises abattoir, which were dermatomed and used in the study through facilitation by the University of Zimbabwe Veterinary Science Department. Animal ethics approval was granted through license L610 by the Animal Ethics Research Committee.

Methods:

The *ex vivo* laboratory experiments in this investigation were conducted with reference to related studies by Diembeck *et al*(1999)[10], relevant

excerpts from the OECD guideline number 428 (2004a) [11] and the subsequent guideline document 28 (2004b) [12] as well as the EU SCCNFP opinion number SCCNFP O750/03 (2003) [13].

Formulation of cream O/W emulsion dosage form:

All oil soluble starting materials (stearic acid, ceto stearyl; alcohol, GMS, cocoa butter, liquid paraffin, petrolatum, fixed oil herbs, castor oil, propyl paraben) were weighed into a thermal jacketed stainless steel vessel and heated to 80^{o}C. This was referred to as the oil phase. All water soluble ingredients (deionized water, caustic potash, EDTA, carbopol 940®, colorant, methyl paraben) formed the water phase and were weighed into a separate but similar vessel and heated to 85^{o}C.The oil phase ingredients were slowly added to the water phase ingredients while continuously agitating using a variable speed emulsifying mixer at 2200rpm.

Nanoparticles of TiO_2 and ZnO were added in at this stage. After the emulsion had formed, triethanolamine was added to buffer the pH and facilitate optimum thickening for carbopol 940® and the resultant cream was allowed to naturally cool down by removal of heating sources. Once the cream had cooled to 50^{o}C, the volatile components including essential oils (*Myrothamnus flabellifolia*) and fragrances were added. Degradable herbal extracts (*Aloe excelsa*) were also added to the cream at this stage.

Oil in water cream emulsions with 5% TiO2 and ZnO were formulated and optimized. Standard opaque creams containing 5% normal $TiO_2$and 5% ZnO were also formulated.18 formulation samples were thus used in the study: 6 with nanomaterials; 6 without any metallic oxides and 6 with normal $TiO_2$and ZnO. The starting materials and all formulations with the nanomaterials, without any metallic oxides and those with the normal metallic oxides were identical.

Preparation of phosphate buffer solution at pH 7.4:

In the study, Phosphate Buffer Saline (PBS) solution was used in the receptor phase of the Franz diffusion cells.PBS solution was prepared by adding 27.8g KH_2PO_4 together with 6.30g NaOH. NaOH was diluted to 1560ml with deionized water and the KH_2PO_4 was diluted to 1000ml with deionized water. The two solutions were mixed together to make the phosphate buffer solution and the pH was measured with a Shimadzu pH meter. 10% Orthophosphoric acid and 10% sodium hydroxide was used to adjust the pH to 7.4. The solution was used in this study for the aqueous solubility determination, standard preparations and as the receptor phase during the Franz cell diffusion studies.

Aqueous solubility determination of nanometric TiO_2 and ZnO:

An excess of TiO_2 and ZnO was added to 50ml of the PBS solution (pH 7.4). The temperature was maintained at 32 °Celsius and the super-saturated solution was constantly stirred by a magnetic stirrer using a water-bath. After 24 hours, the supersaturated sample was filtered, and analyzed by atomic absorption spectrum. This was conducted in duplicate.

Preparation of skin for the skin penetration experiments:

The *ex-vivo* skin diffusion studies made use of abdominal porcine skin obtained from three 6 months old landrace Porkers. Landrace breed porkers weighing between 30 and 40 kg which were being commercially slaughtered were used. The skin was excised from the pig abdomen 48 hours after inducing actinic damage on the area to be excised (using the method outlined below) and within minutes of slaughter, the hair of test animals was carefully removed by depilatories and the full thickness skin was removed from the abdominal region.

The epidermis was prepared surgically by the heat separation technique, which involved soaking the entire abdominal skin in water at 60 °C for 45 sec, followed by careful removal of the epidermis[10].The skin was prepared and then frozen within 24 hours of the slaughter. The skin samples were prepared using a surgical blade at a thickness of 1000µm. This thickness included the epidermis and part of the dermis. The dermatomed skin was placed on top of Whatman® filter paper and circles with a diameter of approximately 50 mm were punched

into the skin. It was ensured that each circle of skin on the filter paper was big enough to cover the Franz cells diffusion area [17]. The skin circles were wrapped in foil and quick frozen until needed.

Simulation of actinic damage in porcine skin:
A 49 cm^2 (7cm x 7cm) area of the pig's abdominal section was chosen, the area was cleanly shaven through depilatories. 20ml of a 10% sodium hydroxide solution was applied onto 2g hospital grade cotton wool, the drenched cotton wool was evenly spread with tongs over the selected area demarcated by a stencil and bandaged over by sticky bandages for 30 minutes while the pig was sedated. The high density poly ethylene (HDPE) stencil was left secured over the affected area for the entire 30 minutes. The affected area was then exposed to a xenon arc solar simulator for 30 minutes at maximum intensity while the pig was still sedated.

***In vitro* skin permeation studies in Franz diffusion cells:**
The *in vitro* skin permeation studies were carried out using static vertical Franz diffusion cells[14] with a diffusional area of 3.2 cm^2. Porcine abdominal skin was mounted between the compartments of the diffusion cell with stratum corneum facing the donor compartment. The receiver phase was PBS pH 7.4, stirred at 300 rpm by a magnetic stirrer. Six Franz cells were used for each experiment, 4 with the test formulation and two control cells. The buffer (pH 7.4) was pre-warmed to 37^oC in a water bath an hour before the experiment commenced. The donor phase formulation was placed in at 32^oC.

The donor and receptor compartments of each Franz cell were greased with commercial grade vacuum grease. The two compartments of the Franz cell were placed together, sealed with vacuum grease to prevent leakage and secured together with a horseshoe clamp. 50ml of the prepared PBS solution pH 7.4 was added to the receptor compartment and 4g of the cream formulation under investigation was placed into the donor phase compartment. The donor compartment was covered to avoid the loss of constituents by evaporation. The assembled Franz cells were placed on a Franz cell stand and placed in a water bath with a magnetic stirrer (mixed receptor phase contents to maintain homogeneity) ensuring that only the receptor compartment of the Franz cell was immersed. This was recorded as time 0.0h. The entire receptor buffer phase was removed at predetermined time intervals and replaced with fresh buffer to maintain sink conditions [14].

The extracted samples were analyzed for Zn and Ti by ICP and Atomic Absorption spectroscopy. Titanium samples were analyzed by Inductively Coupled Plasma - atomic emission spectroscopy (ICP-AES Varian; Wavelength 336nm, external calibration, linear range 0.05-10mg/l). Flame atomic absorption spectrometry (Varian AAS, Spektr AA220) wavelength 220.5 nm, linear range 0.02 -1mgZN/l) was used for Zn analysis. The receptor phase was extracted every hour for six hours, giving extraction times of 1.0; 2.0; 3.0; 4.0; 5.0 and 6.0 hours. Twelve hour skin diffusion studies were done and the amount of TiO_2 and ZnO that diffused through the skin *ex-vivo* was determined.

It was observed during other studies that very low amounts of TiO_2 and ZnO permeate through the skin for the greater duration of the experiments, leading to problems with accurate detection and quantification. So, it was decided to do a single extraction after 12 hours, thus analyzing only the total amount of nanomaterials that diffused through the porcine skin to the receptor phase. The unabsorbed cream base remaining in the donor compartment was also analyzed to quantify the unabsorbed metallic oxides. After the diffusion process the mounted skin membrane was dismounted and crushed. The crushed material was acid treated and subsequently analyzed to quantify epidermal Ti.

Statistical Techniques:
Statistical analysis was done on the results of the permeation test but the results may be unreliable because there were only three observations per group. Descriptive statistics for each group were obtained; these include the mean for normal data, median for non-normal data and standard deviation. It was decided that the data had a sufficiently normal distribution for the performance of robust tests so omnibus tests were performed which

included the one-way analysis of variance (ANOVA).

RESULTS:

Cosmeceutical cream:

Only Cosmetic Ingredient Review (CIR) listed ingredients were used in the formulation and they showed good compatibility with the nanomaterials and the herbal extracts. TiO_2 was incorporated at 5 % m/m which corresponds to 3% m/m Ti in the formulation. ZnO was also incorporated at 5 % corresponding to 4%m/m Zn (**Table 1**). The formulations gave an emulsion cream which was aesthetic on application. The following formulation was used as the base cream for the studies.

TABLE 1: COMPONENTS OF THE OPTIMIZED O/W EMULSIONCREAM CONTAINING NANOMETRIC TiO_2 AND ZnO

Material	Usage %
Stearic acid	4
Cetyl alcohol	3
Glycerol monostearate	2
Peg 400	1
Cocoa butter	1
Trichilia emetica	2
Liquid paraffin (heavy)	1
Petrolatum	1
Glycerin	3
Monopropylene glycol	2
Sodium PCA	1.5
Carbopol 940	0.07
Methyl hydroxybenzoate	0.2
Propyl hydroxybenzoate	0.2
Caustic Potash	0.4
Triethanolamine	0.4
Nanometric TiO_2	5
Nanometric ZnO	5
Watermelon fragrance	0.03
Approved FDC Colorant	0.0055
Trace *Trichilia emetica*	0.5
Aloe Excelsa extract	2
Myrothamnus Flabellifolia extract	2
EDTA	0.4
Distilled Water	qs

Aqueous solubility:

The aqueous solubility of ZnO was found to be 1.6 mg/ml. $TiO_2$was insoluble in water. The results agree with literature [15].

Porcine Skin Preparation and induction of actinic damage:

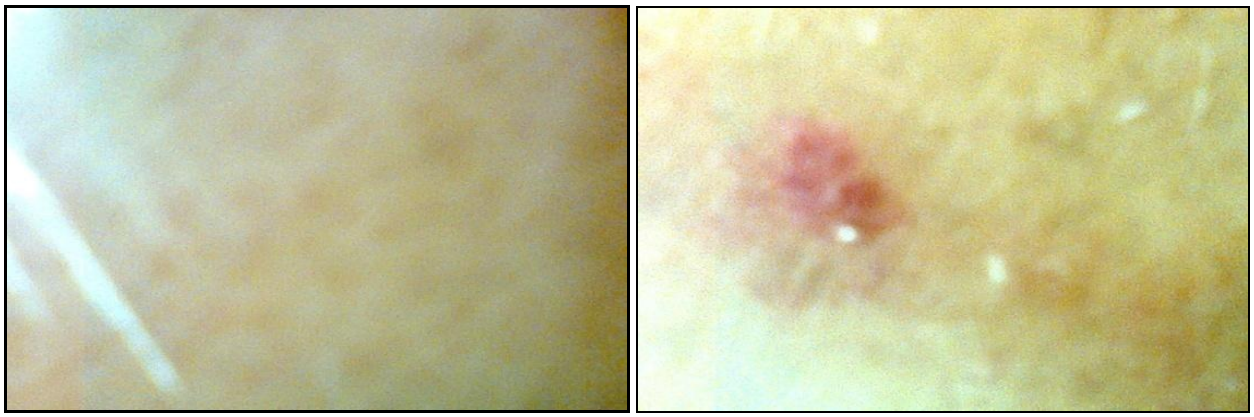
Skin irritations were successfully induced on three animals by 5 % caustic soda and the solar simulator to induce actinic damage on porcine skin. The actinic damage induced compared very well with that which is observed in Albinistic people living within the tropics. Tropical albinistic skin is characterised by inflammation, erytherma as well as various primary lesions which include papules, macules, patches, nodules wrinkles and fissures [2].

Skin penetration studies:

The total Ti recoveries from the dermatomes were in the range 97.83-99.49%, the mean was 98.90± 0.51% for the samples analyzed. Almost all the Ti applied was recovered from the skin surface in both sets of skin types. A small quantity of Ti was found in the upper layers of the skin epidermis. The average Ti recovered from skin membranes ranged from 0.02- 5.18%, the mean was 1.91±1.91%. Epidermal recoveries were marginally higher in actinically damaged skin than intact skin. No Ti was detected in the receptor phase, (**Table 2** and **3**). There was no significant difference in the total recovered Ti from nanomaterials and that from macromolecules of Ti for both damaged and intact skin. The significant difference noted was in the distribution of total recovered Ti between the

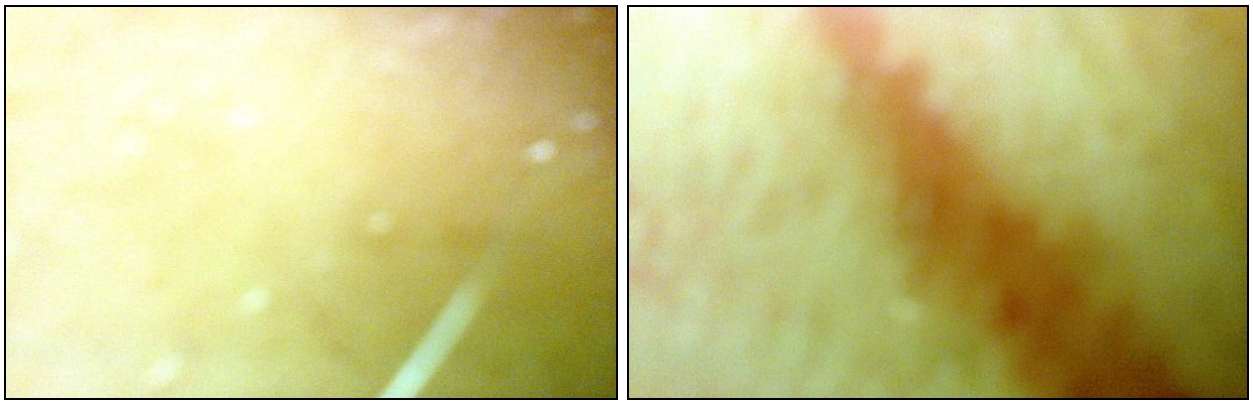
epidermal extracts and the unabsorbed base, **Fig.4** and **5**).

In the permeation experiments, the total recoveries for the Zn ions ranged between 101.35and 103.20%. The average recovery was 102.23± 0.54 %(**Table 5** and **6).** It was noted that in either intact skin or actinically damaged skin, virtually, all the total applied Zn was recovered in the donor compartment. Zn was detected in the skin membranes and the receptor phase which was comparable to untreated vehicle used as standard. The total recovered Ti and Zn from all treated samples showed corresponding dependence on skin integrity (**Fig. 6**)



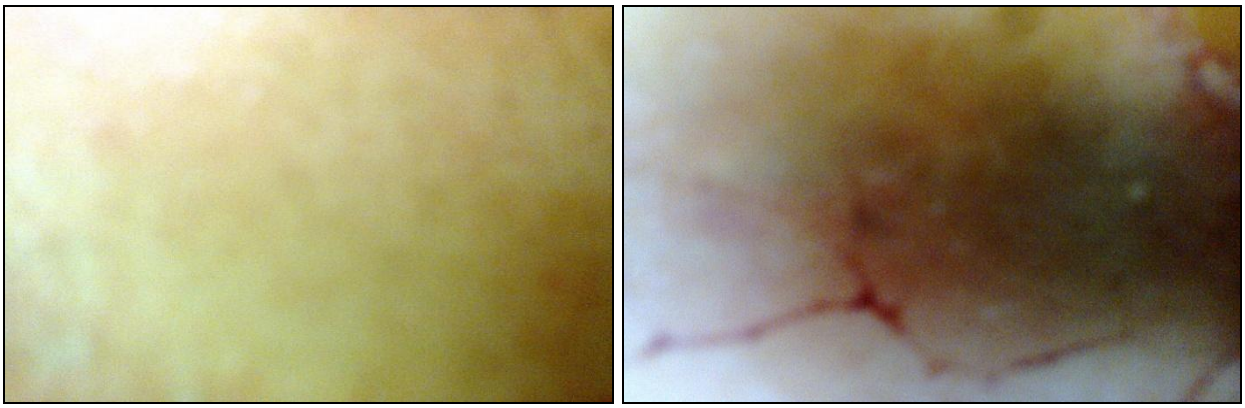
(a) **(b)**

FIG. 1: PORCINE SKIN 1 BEFORE (a) AND AFTER (b) SIMULATEDACTINIC DAMAGE USING 5 % CAUSTIC SODA AND THE SOLAR SIMULATOR



(a) **(b)**

FIG. 2: PORCINE SKIN 2 BEFORE (a) AND AFTER (b) SIMULATEDACTINIC DAMAGE 5 % CAUSTIC SODA AND THE SOLAR SIMULATOR



(a) **(b)**

FIG.3: PORCINE SKIN 3 BEFORE (a) AND AFTER (b) SIMULATED ACTINIC DAMAGE 5 % CAUSTIC SODA AND THE SOLAR SIMULATOR

TABLE 2: NANOMETRIC Ti RECOVERY FROM DAMAGED AND NON-DAMAGED PORCINE SKIN

Formulation	**5%TiO_2 cream (Nano)**					**5% TiO_2 cream (Nano)**				
Skin Type	**induced actinic damage**					**No actinic damage**				
Animal	animal 1	animal 2	animal 3	Average	StDev.S	animal 1	animal 2	animal 3	Average	StDev.S
Total recovery %	97.83	98.39	98.96	98.39	0.57	98.86	99.23	99.02	99.04	0.19
Unabsorbed base %	94.54	93.21	94.32	94.02	0.71	98.2	98.45	98.78	98.48	0.29
Epidermal extraction %	3.29	5.18	4.64	4.37	0.97	0.66	0.78	0.24	0.56	0.28
Receptor fluid %	0	0	0	0	0	0	0	0	0	0

TABLE 3: NORMAL Ti RECOVERY FROM DAMAGED AND NON- DAMAGED PORCINE SKIN

Formulation	**5% TiO_2 cream, (normal)**					**5% TiO_2 cream (Normal)**				
Skin Type	**Induced actinic damage**					**No actinic damage**				
Animal	animal 1	animal 2	animal 3	Average	StDev.S	animal 1	animal 2	animal 3	Average	StDev.S
Total recovery %	99.15	99.49	98.27	98.97	0.63	99.33	99.48	98.9	99.24	0.3
unabsorbed base %	97.52	95.63	95.82	96.32	1.04	99.27	99.36	98.88	99.17	0.26
Epidermal extraction %	1.63	3.86	2.45	2.65	1.13	0.06	0.12	0.02	0.07	0.05
Receptor fluid %	0	0	0	0	0	0	0	0	0	0

TABLE 4: AVERAGE Ti RECOVERY FROM 12 PORCINE PERMEATION STUDIES

Formulation	**5 % TiO_2 Nano Cream**	**5 % TiO_2 nano cream**	**5% TiO_2 Normal Cream**	**5% TiO_2 Normal cream**		
Skin Type	**Actinic damage**	**No Damage**	**Actinic Damage**	**No Actinic damage**		
Code	Actinic Damage	Intact skin	Actinic Damage	intact skin		
	Animals 1,2,3 - % Average Ti Recovery from Table 4				Average	StDev.S
Total recovery %	98.73	99.1	98.97	99.24	99.01	0.19
unabsorbed base %	94.02	98.48	96.32	99.17	97	2.02
epidermal extraction %	4.7	0.63	2.65	0.07	2.01	1.82
Receptor fluid %	0	0	0	0	0	0

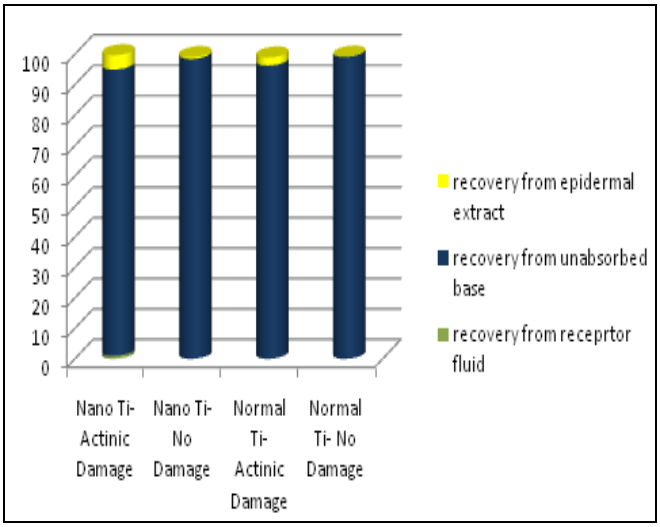


FIG. 4: TOTAL Ti RECOVERIES AND DISTRIBUTION IN DERMATOMES BY ICP-AES

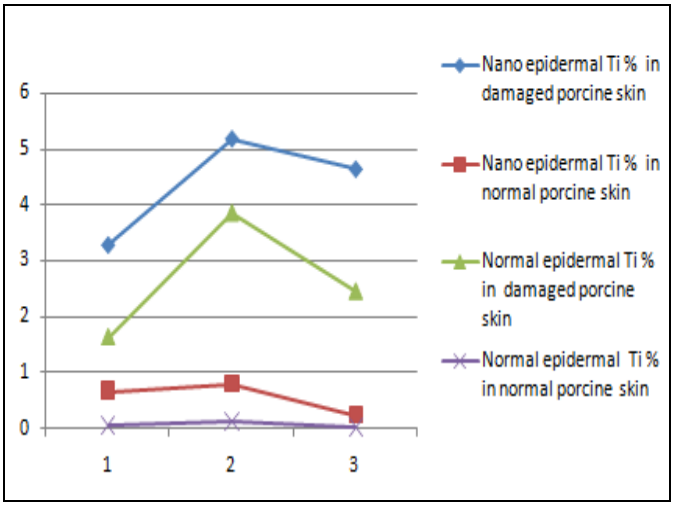


FIG. 5: PERCENTAGE NANOMETRIC AND NORMAL TI RECOVERED FROM EPIDERMAL TISSUE OF 12 PORCINE DERMATOMES BY ICP-AES

TABLE 5: NANOMETRIC Zn RECOVERY FROM DAMAGED AND NON-DAMAGED PORCINE SKIN

Formulation	5% ZnO cream (Nano)					5% ZnO cream (Nano)				
Skin Type	Induced actinic damage					No actinic damage				
Animal	animal 1	animal 2	animal 3	Average	StDev.S	animal 1	animal 2	animal 3	Average	StDev.S
Total recovery %	101.75	101.95	101.96	101.89	0.12	101.35	102.3	102.04	101.90	0.49

TABLE 6: NORMAL Zn RECOVERY FROM DAMAGED AND NON-DAMAGED PORCINE SKIN

Formulation	5% ZnO cream (normal)					5% ZnO cream (Normal)				
Skin Type	Induced actinic damage					No actinic damage				
Animal	animal 1	animal 2	animal 3	Average	StDev.S	animal 1	animal 2	animal 3	Average	StDev.S
Total recovery %	103.2	101.35	103.2	101.88	1.07	102.4	102.86	101.96	102.41	0.45

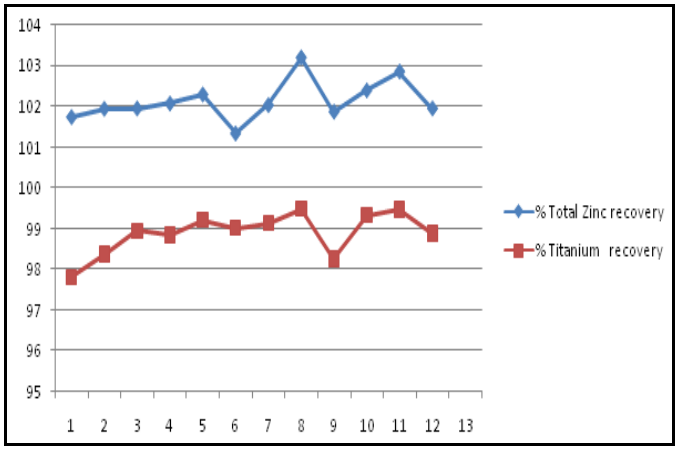


FIG. 6: TOTAL PERCENTAGE TI RECOVERED FROM 12 PORCINE DERMATOMES BY ICP-AES ANALYSIS AND ZN RECOVERIES FROM 12 PORCINE DERMATOMES BY FLAME AAS ANALYSIS.

DISCUSSION: According to Naik *et al*. (2009), skin penetration will only occur if an Active Pharmaceutical Ingredient (API) has an aqueous solubility of at least 1 mg/ml; a molecular weight below 500 Da; a melting point below 200 °C and a partition coefficient (Log P) between 1 and 3.9 [16]. Such parameters and other physical properties of normal macromolecular Ti and Zn do not lie within the required range to facilitate skin penetration and therefore do not pose any threat to systemic exposure when used in sunscreens. Normal Zn and Ti are therefore excellent safe, broad spectrum sun blocks that remain on the skin periphery with no risk of systemic exposure.

The drawback however is the opaqueness in formulations which make preparations unsuitable for all day wear. This problem has recently been circumvented by introduction of nanometric TiO_2 and ZnO, which can be incorporated in clear aesthetic sunscreens [7, 8]. However, there is debate on whether these safe physico-chemical properties of the normal molecules are affected or changed in nano-molecular analogues [8]. For practical product development purposes, it is not fully possible to predict skin diffusion by the use of a few physico-chemical properties from literature, especially when considering that Albinistic skin in the tropics is almost always actinically damaged. Dermatological studies only tend to focus on drug permeation through normal healthy skin. Various researchers have carried out comparative studies on the diffusion of nanomaterials across human and porcine skin through using healthy uncompromised skin and its equivalents [8]. All these studies done before are therefore not relevant to commercial actinic damage retarding treatments product development for people living with Albinism and living in the tropics.

This study therefore carried out tests on simulated actinic damage induced porcine skin that exhibited most symptoms exhibited by Albinistic persons except for open wounds which would obviously compromise accuracy of results. Literature on hand tentatively predicts that nanomaterials might not permeate normal healthy skin that is not compromised in any way[7, 8, 9]. No published studies are available on investigations using damaged skin exhibiting symptoms prevalent in Oculocutaneous Albinism. This experimental study hopes to contribute to the debate on safety of nanomaterials in practical commercial treatments by focusing on actinically damaged skin in tropical conditions and set ups.

Diffusion through the (nonliving) stratum corneum peripheral layer of skin is the rate limiting step for

percutaneous absorption[17]. The permeability properties of the stratum corneum remain unchanged with removal from the body and therefore *ex-vivo* experiments like this one, is appropriate and offer significant advantages over whole animal or human volunteer experiments[18, 19]. We have therefore chosen to assess the health risks posed by nanometric titanium and zinc oxides in compromised skin, through absorption across excised porcine skin mounted on static vertical Franz diffusion cells. Porcine skin is an excellent choice for this study because various studies have validated in numerous investigations that it is similar in structure to that of human skin[18].

More so, the studies were practical in that we determined the dermato- pharmacokinetics of the nanomaterials in actual commercial grade aesthetic formulations which did not exclude the possible effects of other typical cosmeceutical starting materials. The formulation contained no trans-epidermal vehicle with a molecular weight below 500da and no parameter that could promote skin absorption. The induced actinic damage was certified by a practicing specialist dermatologist as a comparable replicate of actinic damage characteristics in Oculocutaneous albinism afflicted people living within the tropics.

Employing the various techniques and methods mentioned above, our investigations showed that irrespective of typical albinistic actinic damage characteristics, there is no evidence of penetration through the skin of Zn and Ti particles regardless of the shape and particle size of the metallic oxides. The nanomaterials and the normal metallic oxides were mostly deposited on the stratum corneum periphery; the small quantities recovered from the epidermal extractions were most likely to be from epidermal furrows wrinkles and hair ducts as evidenced by the wide standard deviations of epidermal recovered materials from the mean in actinically damaged porcine skins as compared to intact skin. There are no marginal differences in distribution of the nanomaterials in both intact and damaged skin from that of the normal molecules. The slight discrepancies observed in the results are most likely to be from the higher probability of nanosized materials being trapped in hair ducts, skin furrows and ridges than any significant active penetration.

In all the 4 study categories documented above involving 12 animal dermatomes, for skin penetration of both nanometric and normal Ti, the average total recoveries for each category were very closely related and ranged from 98.73 to 99.24% with a standard deviation of 0.19. Virtually no titanium was found in the receptor phase for all investigations. What differed was the distribution of the recovered materials. On average 98.83% Ti was recovered from the unabsorbed base in the 2 categories with intact skin compared to an average 95.17% recovery from the 2 investigations with actinically induced damage. Damaged skin retained an average of 3.70% of both normal and nanometric Ti compared to 0.35% for normal intact skin. This was most likely due to accentuation of wrinkles and furrows as a result of actinic damage to the upper layers of the stratum corneum rather than any technical penetration. The standard deviation of epidermal recoveries was very wide at 1.82 implying that the nature and extend of actinic damage and alteration of skin profiles is related to extend of penetration.

In the experiments with zinc oxide, Recoveries of the more than 100% of applied zinc were observed. It was noted that the untreated skin samples and those treated with a base without the sunscreens already had up to 3 µg of zinc which corresponds to at least 2 % concentration of the applied dose in the donor compartment.

The amounts of recovered zinc from the skin samples epidermal extractions and receptor phase were comparable for all skin samples treated and untreated implying that there were no significant contributions from donor phase formulation zinc. The virtual recovery of Zn from untreated samples also indicates that the instrumental techniques and analytical methods were adequate for the purposes of this study. The instrument sensitivity of the analytical tests was also sufficiently high.

CONCLUSION: These investigations therefore showed that irrespective of particle size or formulation characteristics, there is no indication that nanoparticles of Ti and Zn can penetrate both

intact and actinically damaged skins causing internal exposure. The recovered materials from the epidermal extractions are more likely to be trappings in skin folds, wrinkles crevices and hair follicles. The dependence of this, on skin profilometry is evidenced by the increases in epidermal extractions for actinically damaged skin due to the change in profile.

Ti and Zn were solely deposited on the surface and upper layers of the epidermis where they are needed to refract UV energy and protect the underlying dermal membranes. These results show for the first time that neither Zn nor Ti can penetrate actinic damage induced porcine stratum corneum.

In summary these results show:

1. Metallic ions both Nano and macromolecules remain on the skin periphery in actinic damage treatment and retarding formulations where they are needed to refract UVA and UVB on both actinically compromised and non-compromised skin.

2. There is direct evidence that there is no dermal penetration of Nanometric Zn and Ti of damaged and non-damaged porcine skin.

3. Based on this absence of internal exposure, it is concluded that, the use of nanometric metallic oxides of Ti and Zn in actinic damage treatments for Oculocutaneous albinism afflicted persons living in the tropics does not pose a health risk.

ACKNOWLEDGEMENTS: The authors are grateful for provision of equipment, facilities and/or materials by the following organizations: University of Zimbabwe School of Pharmacy, Harare Institute of technology, University of Zimbabwe Mining Research Institute, Kutsaga Research Station Zimbabwe, University of Zimbabwe School of Veterinary Sciences, Unilever South Africa, Croda South Africa, Minema Chemicals South Africa and Silchem Pharmaceuticals. We also want to acknowledge with thanks the ICF fund for sponsoring the research.

REFERENCES:

1. Breskin M et al McGraw Hill Medical dictionary for Allied health (2007)1st Ed ISBN-10: 0073510963
2. Gronskov K, Ek J, Brondum-Nielsen K. Oculocutaneous Albinism. Orphanet Journal of Rare Diseases 2007; 2: 43.
3. Carden SM, Boissy R E, Schoettker P J, Good W V Albinism Mordden Molecular diagnosis. The British Journal of Ophthalmology1998; 82 (2): 189–95.
4. De Gruijl *et al*. Photo carcinogenesis: UVA vs. UVB Radiation, Skin Pharmacol. Applied Skin Physiol 2002; 15 (5) .3416-320
5. Machipisa lewis: Rights-Zimbabwe, the last minority group to find a voice. Interpress, news Agency. (February 2005)
6. Marissa D Newman *et a*l. The safety of nanosized particles in TiO2 and ZnO based sunscreens. Journal of the American Academy of Dermatology 2009; 61 (4): 685-691.
7. A.O Gamer *et al*: The invitro absorption of micro fine ZnO and TiO2 through porcine skin. Toxicology in vitro 20 2006; 301-307.
8. Van Wyk B.E Gerickle N ,Van Oudtshoorn B , Medicinal plants of South Africa ,2nd edition , Briza Publication 2009; pp 296-297 ISBN 078-1-875093-37-3
9. Diembeck *et al* Test guidelines for in vitro assessment of dermal absorption, percutaneous absorption of cosmetic ingredients. Food chem. Toxicology 1999; 37 191-205.
10. OECD, 2004a, OECD Guideline for testing of chemicals, method no 428, Skin absorption in vitro Method, adopted April 13 2004.
11. OECD 2004b OECD Guideline document for the conduct of skin absorption studies. OECD
12. SCCNFP O750/03, Basic Criteria for the in vitro assessment of dermal absorption of cosmetic ingredients , 0ctober 2003
13. Franz T., Percutaneous absorption, on the relevance of in vitro data J. Invest, dermatol, 1975; 64 .190-195
14. IUPAC Handbook 2002-3 , IUPAC ,0-9678550-3-9, 2002
15. Naik A , Kaila N Y ,Guy R H, Transdermal drug delivery, overcoming the skin's barrier function, Pharmaceutical Science and technology today, 2000; 3(9):318-326.
16. Dugard, P.H Scott R.C. Absorption through skin, in: Baden H.p (Ed) Chemotherapy of Psoriasis, Pergamon press Oxford 1984; 125-144.
17. Van Ravenzwaay *et al*. The significance of in vitro rat skin absorption studies to human risk assessment. Toxicol, *in vitro* 2004; 18. 219-225.
18. Sheree E Cross *et al*. Human skin penetration of sunscreen nanoparticles. Skin Pharmacology and physiology journal 200; 20: 148-154 series on testing and assessment no 28
19. Kaidi, Z and Jagdish, S. *In vitro* percutaneous absorption enhancement of propanol hydrochloride through porcine epidermis by terpenes/ethanol.1999; 62: 359-366.

How to cite this article:
Chifamba J, Dube A and Maponga CC: *Ex-Vivo* Penetration of Nanometric Zno and Tio_2 across Actinically Damaged Porcine Skin: Development of an Albinistic Skin Protection Treatment. Int J Pharm Sci Res 2015; 6(6): 2339-48.doi: 10.13040/IJPSR.0975-8232.6 (6).2339-48.