



Efficacy of a Model Nano-TiO₂ Sunscreen Preparation as a Function of Ingredients Concentration and Ultrasonication Treatment

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ABSTRACT

Background: TiO₂ nanoparticles (NPs) which are used in sunscreen formulations are able to block ultraviolet (UV) radiation with a higher efficiency compared with micro-scale particles. The concentration of corresponding particles is an important factor in UV attenuation effects as well as cell toxicity profiles. Herein, TiO₂ NPs were dispersed using ultrasonication treatment and a sunscreen cream was prepared using TiO₂ NPs.

Methods: The effect of TiO₂ concentration (i.e. a physical barrier) and octocrylene (i.e. a chemical UV filter) as well as sonication time (i.e. aggregation preventer) were studied on UVB blocking efficiency of the preparation by measurement of sun protection factor (SPF). Response surface methodology was employed to investigate the effect of the inputs (independent parameters) on the output (dependent parameter).

Results: Findings indicated that maximum amount of nano-TiO₂ and octocrylene make the preparation most effective. The effect of ultrasonication in breaking the agglomerates was however dominated by the effect of concentration of octocrylene, possibly due to hydrophobic interactions between NPs and octocrylene. Also, TiO₂ NPs showed a significant increase in cytotoxicity profile of the preparation.

Conclusion: In conclusion, introduction of the nanoparticles, as the dominant factor, to the sunscreen product increased both efficacy and cytotoxicity of the product.

Introduction

Exposing skin to sunlight for a long time usually is damaging, which makes the skin look dry and leathery.¹ It is currently believed that most skin cancers can be avoided by preventing sun damages.² Sunscreens help protect skin from sunlight, especially in humans with sensitive skin.³ Performance of sunscreens is determined by sun protection factor (SPF) which is an indicator of the fraction of sunburn-producing UV rays that reach the skin. Sunscreens with higher SPF values could provide a better UV protection for the skin. The value of SPF depends on the type and concentration of UV absorbing agent which is used in preparation of sunscreens.^{4,5} Sunscreens designed for people with sensitive skin, are often based on TiO₂ and/or ZnO since these mineral UV blockers make less skin damage compared with chemical UV absorbers.⁴

Chemical absorbers may also cause DNA damage by producing free radicals. Also, they could be allergic when used as a UV filter.⁶

TiO₂ particles are widely used as a white pigment to create opacity and whiteness in products such as toothpastes due to its brightness, high reflective index and resistance to discoloration under UV light. These properties make TiO₂ a stable and capable substance to protect the skin from UV light when they are used in sunscreens. Interestingly, opacity and reflective index of TiO₂ particles depend on the size of particles.⁷ Due to lack of visible light scattered by nano-sized TiO₂ particles, these nanoparticles (NPs) have been introduced as interesting alternatives to conventional ones in sunscreen products to provide UV protection without leaving a white appearance on the skin.⁸ It has been shown that using TiO₂/ZnO NPs -instead

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of conventional TiO₂ and ZnO powders- increases SPF of the sunscreen. For instance, SPF of 3.65 for ZnO and 4.93 for TiO₂ NPs have been obtained in comparison with SPF of 2.90 and 1.29 for conventional ZnO and TiO₂ particles, respectively.⁹

Reviewing the literature, a direct relation is suggested between SPF of sunscreen and concentration of TiO₂ NPs.¹⁰ TiO₂ NPs are able to increase SPF in both UVA¹⁰ and UVB.¹¹ Size of the nanoparticles also appears to be an important factor affecting SPF in sunscreen products.^{11,12} On the other hand, cytotoxic effects of nanoparticles is an important concern nowadays. Parameters such as concentration, administration method, crystalline form, size of particles and time of exposure have been shown to strongly affect the toxicity of such nanoparticles.¹³⁻¹⁶ It is believed that adverse effects from the nanoparticles in general are related to the capability of producing free radicals (e.g. hydroxyl radicals) as a function of sunlight exposure.¹⁷ Photo-activity of TiO₂ particles depends on their size. By decreasing the particle size to values less than 100 nm, a substantial increase in photo-activity is observed, which may cause production of relatively high hydroxyl radicals. Therefore, TiO₂ NPs, theoretically, could be more toxic than microparticles.¹⁸

The aim of this study is to concurrently evaluate the factors affecting efficacy and cytotoxicity of a formulation containing octocrylene (i.e. a chemical UV absorber) and naked TiO₂ NPs (as physical UV blockers). Thus, the effect of two formulation parameters (i.e. concentration of octocrylene and TiO₂ NPs) as well as ultrasonication time (i.e. a processing parameter, to minimize the aggregation

of nanoparticles) on the SPF of the preparation were investigated. We then determined the cytotoxicity of the preparation to find the effect of TiO₂ NPs as possible toxic agents.

Preparation of sunscreen formulation using TiO₂ NPs

The sunscreen cream base was prepared based on the formulation by Croda (Germany). Oil phase (i.e. cream base) contained sorbitan stearate (Croda Chemicals, UK, 2.4% W/W), polysorbate 60 (Croda Chemicals, UK, 3.6% W/W), cetostearyl alcohol (Croda Chemicals, UK, 3.6% W/W) and medium chain triglycerides (Croda Chemicals, UK, 9.6% W/W) were used in the preparation. Water phase was prepared by addition of glycerol (Croda Chemicals, UK, 2.4% W/W) to distilled water. The mixtures were heated separately to 65-70° C and mixed together by pouring water phase slowly into oil phase under stirring. Pure powder of Nanoparticulate TiO₂ (P25, Degussa, Germany) and octocrylene (Kuoching Chemical Co., China) were added to the mixture under continuous stirring at 300 rpm after cooling formulations at room temperature using the values given in Table 1. Prepared samples were then ultrasonicated (ultrasound homogenizer Bandelin, Germany, 70% power) for 0, 5 and 10 min according to Table 1.

Experimental design

We used response surface methodology (RSM) as a statistical method to fit our experimentally obtained data into a mathematical model. RSM may be used to study the relationships and interactions between the independent parameters and the output.¹⁹

Table 1. Box–Behnken experimental design and obtained responses.

Run No.	Independent variables			Dependent variable
	TiO ₂ content (% W)	Octocrylene content (% W)	Sonication duration (min)	Sun Protection Factor (SPF)
1	5	5	5	4.82
2	5	10	10	8.50
3	5	5	5	3.80
4	10	5	0	4.44
5	5	0	0	1.13
6	10	10	5	10.27
7	10	5	10	6.59
8	5	5	5	5.05
9	5	10	0	8.65
10	0	10	5	6.56
11	0	5	10	3.38
12	5	0	10	2.81
13	5	5	5	5.53
14	0	5	0	3.39
15	10	0	5	4.51
16	5	5	5	5.73
17	0	0	5	0.00

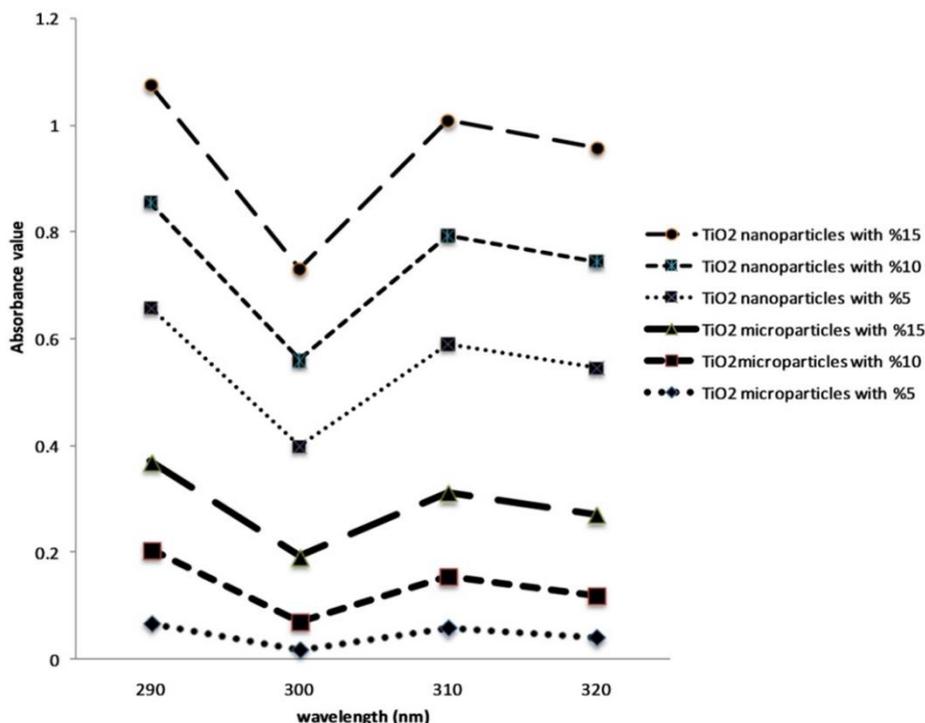


Figure 1. UV absorbance of micro and NPs of TiO₂ as a function of TiO₂ concentration (%) and wavelength (nm).

Box-Behnken design was employed in the work as a designing tool which needs only three levels. Thus, lesser experiments are required to be performed compared with central composite design which needs five levels. Seventeen formulations were obtained using Design-Expert (version 7.0.0, Stat Ease, USA) and a second order polynomial function model was used to predict the values of responses (equation 1). The relationships between 3 parameters (i.e. content of TiO₂ NPs, octocrylene concentration and duration of ultrasonication) as independent variables (inputs) and SPF as dependent response (output) were studied by response surfaces generated by the software.²⁰

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{33} X_3^2 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3 \quad \text{Eq.(1)}$$

Where Y is predicted response (i.e. SPF), β_0 is intercept, β_1 , β_2 and β_3 are linear coefficients, β_{11} , β_{22} and β_{33} are squared coefficients, β_{12} , β_{13} and β_{23} are the interaction coefficients of the equation and X_1 , X_2 and X_3 are the independent variables.

Contour plots and 3D graphs were employed to show the interactions between the independent variables and the dependent ones.

UV absorbance and sun protection factor (SPF)

Each sample was diluted 100 times with ethanol and ultrasonicated for 5 min with power output of 200 W and frequency of 20 kHz. A further 50 times dilution with ethanol was performed and UV-Visible

absorption was measured from 290 to 320 nm (which has been divided into UVB region), against ethanol as blank. SPF value was calculated by Mansur equation (equation 2).²¹

$$\text{SPF} = \text{CF} \times \sum_{290}^{320} \text{EE}(\lambda) \times I(\lambda) \times \text{Abs}(\lambda) \quad \text{Eq.(2)}$$

Where EE (λ) is an erythemal effect spectrum, I (λ) is solar intensity spectrum, Abs (λ) is Absorbance of sunscreen product and CF is correction factor (=10).²²

Morphology of TiO₂ NPs

Size and morphology of TiO₂ NPs were studied by Scanning Electron Microscopy (SEM, Philips XL30, Netherlands) after gold sputtering. Ultrasonicated samples containing TiO₂ NPs or octocrylene or both, were dried for 15 h at room temperature under vacuum and studied by SEM.

MTT assay for examination of TiO₂ NPs toxicity

Fibroblasts for toxicity study were isolated based on a procedure reported before.²³ Briefly, skin specimens were obtained by human skin biopsy and incubated at 0.2% trypsin (Sigma-Aldrich, USA), collagenase I (Gibco, USA) and collagenase II (Gibco, USA) under sterilized conditions. Then, the isolated cells were cultured in DMEM high glucose (Gibco, USA) with 10% FBS (Gibco, USA) for 5 days. Afterwards, the cultured cells were exposed to 1:100 dilution of ultrasonicated sunscreen samples contained 5% and 10% of TiO₂ NPs, then, incubated overnight at 37° C in a 5% CO₂ humidified

incubator. MTT, (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide) (Sigma-Aldrich, USA) was used to evaluate the cell metabolic activity after exposure. All treated samples were rinsed 3 times by PBS and incubated with MTT. After 3.5 h, formazan crystals production was quantified by measuring absorbance at 570 nm by adding HCl/isopropyl alcohol.

Results

UV attenuation of micro- and nano-TiO₂

Figure 1 shows the UV absorbing ability of micro- and nano- TiO₂. From the Figure, nanosized TiO₂ has higher absorption compared with micro-TiO₂ samples. Also, by increasing the concentration of micro- or nano-particles, the absorbance increases.

Box-Behnken design

Experimental design was used to model the effects of independent variables including concentration of octocrylene (as a chemical absorber) and TiO₂ NPs (as a physical barrier) and ultrasonication duration on the efficacy the prepared sunscreen. The data were fitted with full quadratic second-order polynomial equation. The lack of fit F-values of 0.85 was obtained for the model, which indicates insignificance relative to the pure error. Additionally, the model F-value was 33.96 that shows significant obtained model (i.e. p-value < 0.05). Table 2 gives the results of analysis of variance for the models.

The response surfaces were generated by the software and used to investigate the impact of independent variables on responses follows. In each plot, interaction of two parameters was studied while the third one was in its mid-level value. From Figure 2, value of SPF increases due to higher amount of either octocrylene or nano-TiO₂. Additionally, Figure 2b indicates that ultrasonication does not affect the SPF value when TiO₂ content is zero. However, increase of ultrasonication time can lead to a slight increase in SPF value in samples which have high concentration of NPs. On the contrary, when octocrylene concentration is high (see Figure 2a), ultrasonication time is not changing the SPF. While at lower octocrylene contents, higher ultrasonication makes the SPF higher. Additionally, from the equation 3, no synergistic effect may be obtained between the concentration of chemical and physical blocker on the SPF value. Our findings also show that higher percentage of octocrylene increases the SPF value sharply, while TiO₂ NPs possess less important impact on SPF value.

The equation fitted to the data was as:

$$\text{SPF} = 0.176 + 0.204 \times \text{TiO}_2 + 0.507 \times \text{Octocrylene} + 0.075 \times \text{ultrasonication} + 0.022 \times \text{TiO}_2 \times \text{ultrasonication} - 0.018 \times \text{Octocrylene} \times \text{ultrasonication} + 0.022 \times \text{Octocrylene}^2 \quad \text{Eq.(3)}$$

Table 2. Summary of ANOVA results.

Source	Sum of Squares	Degree of freedom	Mean square	F value	p-value
Model	105.9378	6	17.6563	33.95643	< 0.0001
Residual	5.199693	10	0.519969		
Lack of fit	2.911973	6	0.485329	0.848581	0.5925
Pure error	2.28772	4	0.57193		
Cor. total	111.1375	16			
R ²	0.95				
Adjusted R ²	0.93				
Predicted R ²	0.86				

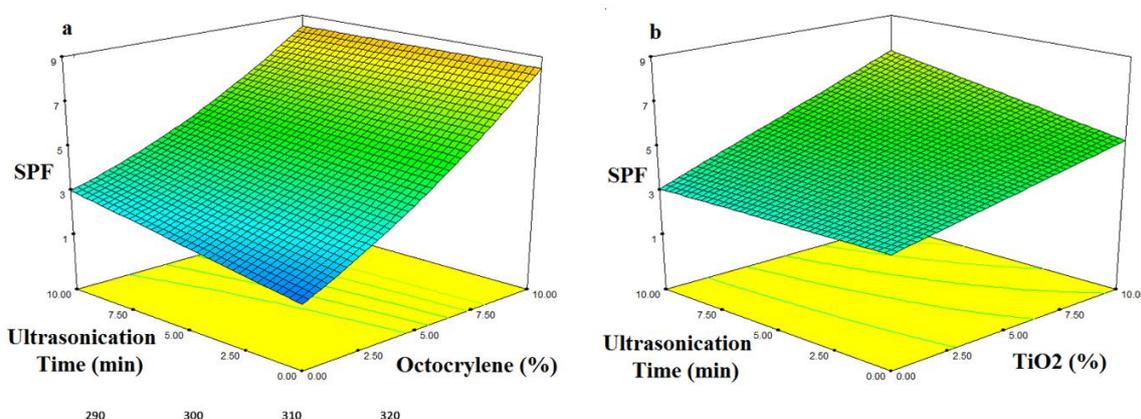


Figure 2. Plot of response surfaces showing the effect of independent variables on SPF of the preparation.

Morphology of nanosized TiO₂

TiO₂ NPs and octocrylene were characterized using SEM. As the Figure 3 illustrates, TiO₂ NPs show a form of aggregation. Figures 3a and 3b show 10% TiO₂ NPs with 0% and 10% octocrylene, respectively. Both samples were ultrasonicated for 5 min. Comparing the details in the Figure, it is clear that introducing octocrylene makes accumulation of particles, thus, larger particles have been obtained. At higher ultrasonication time (i.e. 10 min) and in presence of 5% octocrylene (see Figure 3c), still larger particles are observed which confirms the significant role of octocrylene on agglomeration level.

Cellular viability under TiO₂ NPs treatment

MTT assay was performed for measurement of cellular viability and results were compared with the control group in Figure 4. It should be noticed that MTT assays were performed without UV exposure to study the toxic effect of sunscreen ingredients, rather than studying their photo-catalytic properties. As Figure 4 shows, both TiO₂ groups studied showed a significant decrease in cell viability, compared with the control group. However, no

significant relation was observed between the samples containing 5% and 10% of TiO₂ NPs.

Discussion

In our work, sunscreen formulations were composed of octocrylene and nano-TiO₂ up to 10% of each. The use of octocrylene has been limited by Food and Drug Administration (FDA) to 10%.^{24,25} The findings of this work indicate that the UV absorbance ability of nano-TiO₂ is more than micro-TiO₂. This finding is in good agreement with other studies showing that SPF of TiO₂ NPs is higher than micro-TiO₂.⁹ This is due to ability of very small particles in scattering shorter wavelengths of light.²⁶ Furthermore, as previous studies shows,¹⁰ increasing the concentration of nano-TiO₂ made the UV absorbance value higher. Currently, there is no work on possible relationships between concentration and possible agglomeration in TiO₂ NPs. It is however, arguable that although some aggregates are formed at high concentration values, the overall effect of concentration overcomes the effect of agglomeration.

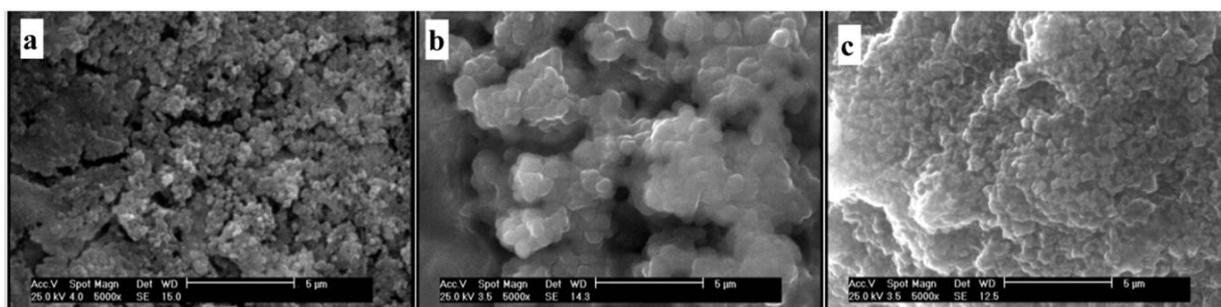


Figure 3. a) The solution contained 10% concentration of TiO₂ NPs and 0% octocrylene which was ultrasonicated for 5 min, b) The solution with 10% concentration of TiO₂ NPs and 10% octocrylene was ultrasonicated for 5 min, c) The solution contained 10% concentration of TiO₂ NPs and 5% octocrylene was ultrasonicated for 10 min.

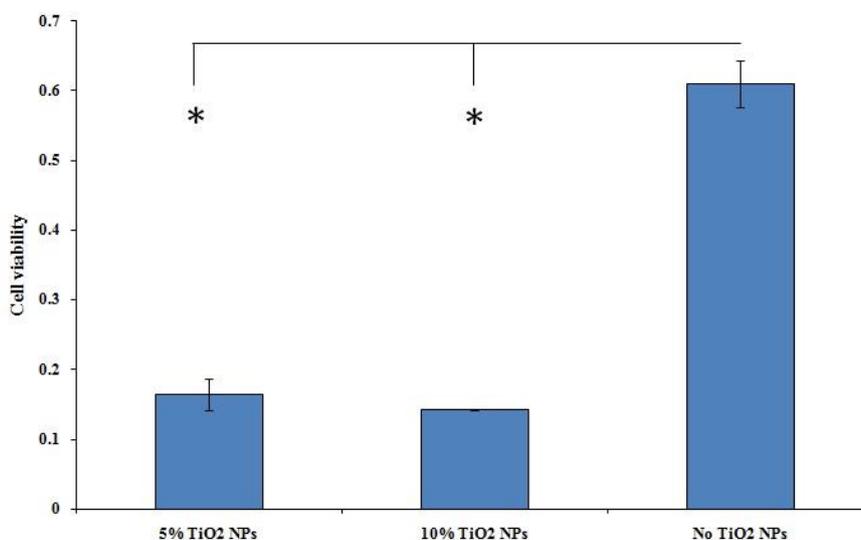


Figure 4. The MTT assay results showing the cellular viability posttreatment by 1:100 dilution of ultrasonicated samples containing 5% and 10% of TiO₂ NPs.

The obtained data showed that increasing the concentration of either TiO₂ or octocrylene makes SPF larger. A finding which is already expected.^{5,10} However, ultrasonication time plays an interesting role here: When TiO₂ content is high, ultrasonication is required to break the agglomerates and make smaller particles (i.e. make SPF higher). On the other hand, at Figure 2a, when octocrylene content is zero, a 5% TiO₂ is available in the preparation which renders a small SPF value to the preparation (i.e. from 1 to 3). Applying ultrasound waves makes these NPs dispersed and smaller, thus, makes SPF higher, whereas at high octocrylene concentration, this effect is believed to be masked by the dominant effect of octocrylene and does not appear in the figure.

Comparing the details of SEM images also shows that apparently, introducing the octocrylene makes larger agglomerated particles due to the hydrophobic interactions between TiO₂ NPs and octocrylene. On the other hand, due to the additive role of octocrylene to light attenuation of TiO₂ NPs, the higher concentration of corresponding NPs can be safer beside a suitable value of UV absorbance activity. Thus, the agglomerated particles of 10% by weight which are dispersed by employment of sonication, can be optimized for optimum UV absorbance in combination of octocrylene.

It should be noted that in our work, to effectively disperse the nanoparticles, ultrasonic treatment has been employed as a potent and green approach in preparing reproducible and monodispersed nano-formulations. Employing sunscreen formulations with coated nanoparticles (i.e. possibly different toxicity and efficacy profiles) is however a common practice in large-scale productions. Therefore, extending the findings of this report to other sunscreen preparation should be done with caution.

Conclusion

This study aimed to determine the effect of concentration of ingredients of a model sunscreen preparation as well as sonication treatment on efficacy of the formulation. The results showed that SPF of the preparation was mainly affected by concentration rather than sonication treatment (i.e. breaking the agglomerates). Introduction of TiO₂ nanoparticles also made a significant increase in cytotoxicity of the preparation.

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Conflict of interests

The authors claim that there is no conflict of interest.

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