CHARACTERIZATION OF SUNSCREEN CONTAINING ZINC OXIDE NANORODS FOR SUNSCREEN APPLICATIONS

by

NUR ATHIRAH BINTI AHMAD ZAKI

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NUR ATHIRAH BINTI AHMAD ZAKI

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LIST OF SYMBOLS

hkl	Miller indices
E_g	Band gap energy
eV	Electronvolt
h	Planck's constant
c	Speed of light
λ	Wavelength
d	Lattice plane distance
n	Integer
θ	Scattering angle
°C	Degree Celsius
g	Grams
Å	Armstrong
mL	Millilitre
nm	Nanometer

LIST OF ABBREVIATION

ZnO	Zinc oxide
TiO_2	Titanium dioxide
UV	Ultraviolet
FDA	Food and Drug Administration
SPF	Sun protection factor
INCI	International Nomenclature of Cosmetic Ingredients
NPs	Nanoparticles
E_g	Band gap energy
CW	Critical wavelength
FESEM	Field emission scanning electron microscope
EDS	Energy dispersive spectroscopy
TEM	Transmission electron microscope
UV-Vis	Ultraviolet-Visible
XRD	X-ray diffraction
Zn	Zinc
O	Oxygen
SEM	Standard error of mean
MED	Minimal erythema dose

PENCIRIAN PELINDUNG MATAHARI YANG MENGANDUNGI NANOROD ZINK OKSIDA UNTUK APLIKASI PELINDUNG MATAHARI

ABSTRAK

Peningkatan penggunaan zink oksida yang sebagai komponen utama dalam produk kosmetik dan pelindung matahari adalah kerana keupayaan mereka menyerap sinar UV. Objektif utama penyelidikan ialah mencirikan pelindung matahari yang mengandungi ZnO nanorod pada kepekatan yang berbeza menggunakan spektroskopi FESEM-EDS, TEM, UV-Vis dan ATR. Bentuk rod adalah struktur dominan di dalam serbuk ZnO yang diperhatikan pada FESEM dan TEM dengan kelebaran 61-70 nm. Saiz purata kristal ZnO nanorod ialah 36.7 nm. Krim asas pelindung matahari diperbuat daripada minyak mineral dan lilin. Serbuk ZnO dicampur untuk mendapat kepekatan 5, 10, 15, 20, 25, 30, 35 dan 40 wt%. Spektra penyerapan UV pelindung matahari dengan kepekatan ZnO nanorod yang tinggi menunjukkan penyerapan UV tertinggi dalam kawasan UV. Pada kepekatan 40 wt% ZnO nanorod, sampel menunjukkan puncak tajam pada 376.3 nm dengan bacaan 3.28 eV. Gelombang kritikal yang mengandungi ZnO nanorod memaparkan perlindungan spektrum luas di kawasan UVA. Spektrum ATR tidak menunjukkan fungsian baru yang antara krim asas dan ZnO nanorod adalah stabil. Kestabilan sampel pelindung matahari diuji melalui pengemperan, pemisahan fasa dan warna selama 35 hari yang disimpan dalam suhu bilik. Sampel yang mengandungi kepekatan 20 hingga 40 wt% ZnO nanorod menunjukkan fasa yang stabil selama 35 hari. Nilai pH menunjukkan bahawa semua pelindung matahari ZnO nanorod mempamerkan sifat berasid sekitar 5.95 hingga 5.61 dan mempunyai nilai pH yang boleh diterima untuk aplikasi kulit manusia. Ringkasnya, pelindung matahari yang mengandungi ZnO nanorod yang disediakan dalam kajian ini mempunyai potensi yang besar sebagai pelindung matahari yang berkesan untuk kulit manusia.

CHARACTERIZATION OF SUNSCREEN CONTAINING ZINC OXIDE NANORODS FOR SUNSCREEN APPLICATIONS

ABSTRACT

The growing applications of zinc oxide (ZnO) material as the main component are found in cosmetic products and sunscreen due to their efficient ability to absorb UV rays in broad spectrum. The main objective in this research work was to characterize sunscreen containing ZnO nanorods at different concentrations using FESEM-EDS, TEM, UV-Visible and FTIR spectroscopy. Rod shape was the dominant structure in the ZnO powder viewed in FESEM and TEM with a width range of 61-70 nm. The average crystallite size of the ZnO nanorods were at 36.7 nm. The base cream of sunscreen was made from mineral oil and beeswax. ZnO powder was added with the concentrations of 5, 10, 15, 20, 25, 30, 35 and 40 wt% into the base cream. The UV-Vis absorbance samples spectra with higher concentration of ZnO nanorods exhibited highest UV absorbance in UV ranges. At 40wt% ZnO nanorods, samples exhibited sharp peak at 376.3 nm with 3.28 eV band energy. The calculated critical wavelength of sunscreen contained ZnO nanorods exhibited a broad-spectrum protection in UVA region. ATR spectra showed no new functional bonds created between base cream and ZnO nanorod particles. The cream/ZnO mixture were stable in that the stability of the sunscreen samples were tested through centrifugation test, color and phase separation for 35 days stored in room temperature. The samples contain 20 to 40 wt% of ZnO nanorods showed stable phase over 35 days storage. The pH determination was determined, and it showed that all concentrations of sunscreen ZnO nanorod exhibited slightly acidic properties (pH 5.95 to 5.61) and had acceptable pH value for human skin application. In summary, the sunscreen contained ZnO nanorods prepared in this work possessed great potential as an effective sunscreen for UVA and UVB protection on human skin.

CHAPTER 1 : INTRODUCTION

1.1 Nanotechnology

Nanotechnology research and the production of nanomaterial have grown steadily worldwide especially in the developed countries [1] and the global socioeconomic value of nanotechnologies is progressively increasing [2]. The perspective of nanotechnology towards application of research-based knowledge which able to develop the nanoscale in terms of sizes and structures dependent properties from associated individual atoms, molecules or a bulk material. Nanotechnology provide innovative applications and offers an advanced approach process of synthesis and modification of nanomaterial to obtain better physical, chemical, electrical, thermal, mechanical, biological properties and functionalities due to their nano size [3] as shown in Fig. 1.1.

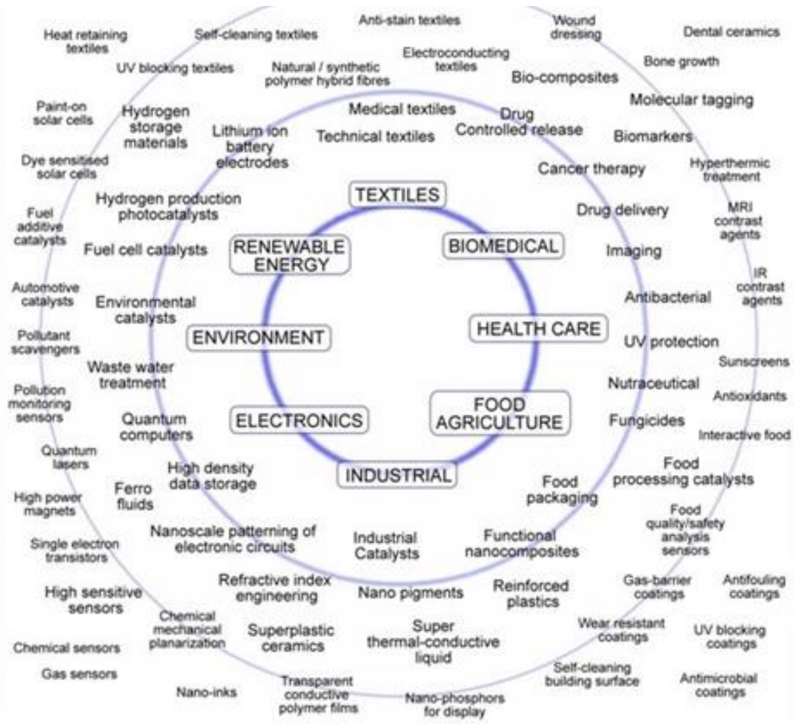

Figure 1.1: Application of nanotechnologies [4].

Nanomaterial is a material with any dimension in the nano scale or having internal structure or surface structure at the nano scale which could exhibit novel characteristics compared to the same material in bulk forms [5]. They can be as spherical, tubular, irregularly shaped and can also exist in fused, aggregated or agglomerated forms. At this nano scale, quantum effects are important in determining the properties and characteristics of the material [6]. For example, sports equipment where nanoparticles are added to the materials to make them stronger whilst often being lighter. They have been applied in tennis racket productions, golf clubs and shoes. Besides that, healthcare production using nanoparticles are used in sunscreen, they offer good protection and can be rubbed in without any white spot.

1.2 Sunscreen

Humans are susceptible to sunlight because of our outdoor activities and the risk of getting a bad effect from sunlight is high such as sunburn, blistering, uneven skin tone, ageing and skin cancer for more severe cases. To avoid theses skin problem, it is recommended to protect the skin from solar ultraviolet radiation (UVR) by seeking shade, avoiding sun exposure around noon, wearing clothes, and applying sunscreen [7] . According to a study examined in Australia, the trend in sun protection behavioural from 2007 to 2012 were observed clearly that the use of sunscreens were predicted to be second higher in reducing the risk of overexposure to UV radiation when outdoors [8] illustrated in Fig. 1.2.

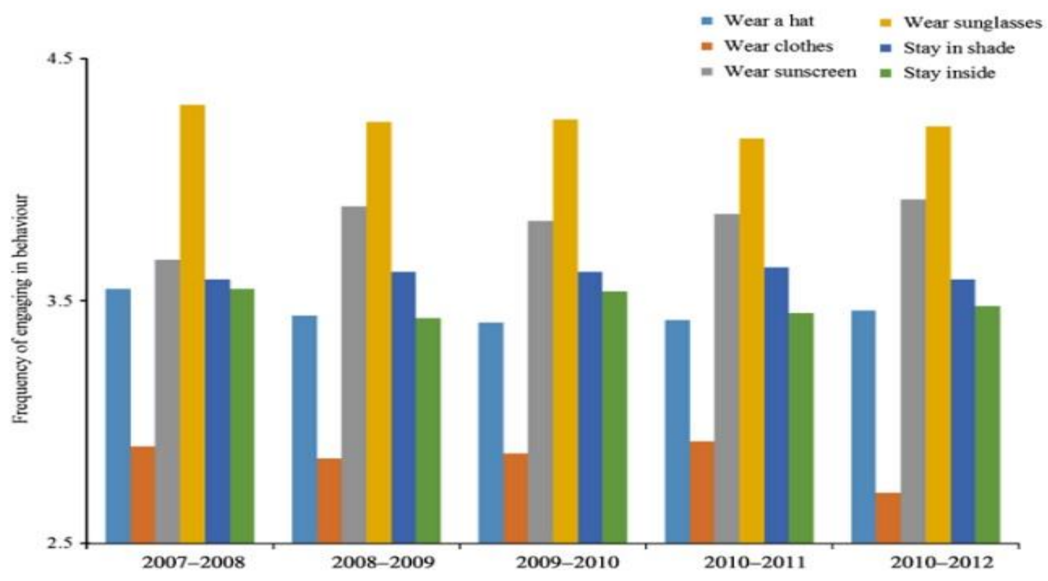

Figure 1.2: Illustration of trends of sun-protection behaviours [8].

Sunscreens are product that is often used over the time, which is a desirable in terms of skin protection and skin cancer prevention [9]. Sunscreen protection is stable over time and 8 hours after sunscreen application about half of the received photoprotection is maintained in spite of bathing and physical activity [10]**.** Many types of sunscreen available on the market today for various purposes and lifestyles. They can be in the form of lotions, creams, gels, waxes, butters and sprays [11].

The survey study sought to investigate preventative behaviours regarding sun exposure and skin cancer detection at an international scale results application of sunscreens for sun protection. These studies involved people aged from 15 to 65 from 23 countries [12]. Besides that, a study have been carried out among young Americans came to the conclusion that 83% of children and 36% of teenagers suffered from sunburn during summer linked to sun products not being used correctly [13]. Almost all skin cancer are the effects of over exposure to UV radiation and could be prevented by applying a layer of sunscreen to minimise exposure to the sunlight. However, people often use too little sunscreen, failure to apply it to all exposed skin, reapply it inadequately or view sunscreen as a substitute for other sun-protection practices rather than as an adjunct to them. It needs to be used correctly to provide the expected level of protection [14].

UV rays can damage the skin by sunburn ,premature aging ,photo-allergies [9] and eventually lead to skin cancer in a long term exposure [15]. There are three kind of solar radiation component and only two that can pass through the ozone layer and reach the earth surface which is known as UVB (290-320 nm) and UVA (320-400 nm) radiation [35-36]. Meanwhile, UVC (200-280 nm) radiation can be neglected because it is filtered by the ozone layer.

UVB and UVA rays are able to induce skin cancer and other relative skin problem [18] due to their greater amount of energy. An exposed to the UVB rays for a short term may contribute to sunburn [16] while UVA rays are responsible for skin pigmentation [19] and contribute to melanoma skin cancer. In addition, the penetration of UVA is much deeper than UVB rays up to dermis layer and cause the initial problem of early age-related skin alteration. Thus, sunscreens are applied to the skin as a shielding or protective film providing external protection against the invisible UV radiation [20].

1.3 Zinc Oxide

Zinc oxide (ZnO) powder is an inorganic compound, white powder (refer Fig.1.3) and has long been used in various for thousand years. ZnO can be considered as mature engineering material with annual production approaching one and a half million tons per annum [21].

Figure 1.3: Zinc oxide powder.

In the last decade, huge significant interest in the number of scientific publications involving ZnO in UV/blue LEDs, piezoelectric devices, optoelectronic displays, UV lasing, varistor, ceramics, rubber vulcanization, pharmaceuticals, cosmetics and textiles [23–30]. This metal oxide exhibits a wide potential application because of their unique properties in optical, chemical sensing, semiconducting, electric conductivity, and piezo-electric properties [30]. ZnO is known as II–VI semiconductor because Zn and O are classified into groups two and six in the periodic table [31]. Particles of ZnO exhibit uniform size range and shape with wurtzite structure and possess wide band gap 3.37eV at room temperature and possess a n-type electrical conductivity [32]. As summarized, properties of zinc oxide are tabulated in Table 1.1.

Table 1.1: Physical properties of ZnO [21], [33].

Properties	
Chemical formula	ZnO
Lattice constant (T=300 K)	a_0 0.3296 nm c_0 0.5206 nm
Molar mass	81.408 g/mol
Density	5.606 g/cm^3
Melting point	2248 K
Gap energy	3.37 eV, direct
Intrinsic carrier concentration	<10^6 cm^{-3}
Exciton binding energy	60 meV
Electron effective mass	0.24
Electron mobility (T = 300 K)	200 cm^2/Vs
Hole effective mass	0.59
Hole mobility (T = 300 K)	5-50 cm^2/Vs
Solubility in water	0.16 mg/100 mL
Refractive index	2.004
Stable crystal structure	Wurtzite
Thermal conductivity	0.6-1.1 Wcm^{-1}K^{-1}
Electronic configuration	Zinc: 3d^{10} 4s^2 Oxygen: 2s^2 2p^4
Elastic modulus	149-159 GPa
Hardness	8.5-8.9 GPa
Specific heat capacity	40.3 Jmol^{-1}K^{-1}

The largest production of ZnO in bulk production are produced via two large-scale processes, namely indirect (French) process and direct (American) process and until now the largest amount of ZnO produced by the former [21]. ZnO possesses many industrial grades summarized in Table 1.2 and differentiation between the grades is based on their purity, composition and specific surface area of the powder, and the process through which is it made.

Table 1.2: Industrial grade of zinc oxide [21].

ZnO Grade	Nominal purity (%)	Specific surface area (m^2g^{-1})	Production process
Gold seal	99.995	4-7	French process
Pharma grade	99.8-99.9	3-9	French process
White seal	99.8	3-5	French process
Red seal	99.5	3-5	French process
Green seal	99.6-99.7	4-10	French process
American grade	98.5-99.5	Max.3	American process
Active grade	93-98	Min 30	Wet process
Feed grade	90-99	-	Various

U.S. Food and Drug Administration (FDA) has conducted extensive views on safety of ZnO particles for use as color additive in drugs, cosmetics, sunscreen and skin protectant active ingredient. This make ZnO generally known as safe product among the five zinc compounds by the U.S. Food and Drug Administration (21CFR182.8991) [34].

ZnO is widely used in applications and it is an essential element in industrial manufacturing such as paints, toothpastes, beauty products, cosmetics, drug carriers, fillings in medical materials, plastics, rubber, textiles, floor coverings, and industrial coatings [9], [22–25]. Besides that, these oxides has been aggressively studies as an active material for skin protection agent which provide functions such as UV protection, antibacterial activity and self-cleaning [26–28].

1.4 Problem statement

Zinc oxide particles can be found in the ointments, creams and lotions to protect against sunburn and other damage to the human skin which caused by harmful ultra violet light. ZnO is known as the broadest spectrum UVB and UVA absorber which have been approved by the US Food and Drug Administration (FDA) and it is completely photostable and also considered as non-irritating, non-allergenic and non-comedogenic [42]. Nowadays, many commercial sunscreen products label the presence of ZnO and claimed high SPF value on their packaging products to attract the attention of consumer. The use of specific ZnO particles in sunscreen should be seen as important as most cosmetic companies only make ZnO presence as a condition without emphasizing the type, shape, size of the ZnO particles in their sunscreen products [43],[44].

Besides that, high SPF which offered by the commercial sunscreen products only report the level of protection against UVB region (280-320nm) meanwhile some commercial sunscreen products are not concerned about the protection against UVA rays compared to UVB rays. This issue is due to the SPF calculation that does not include UVA region in the SPF equation only UVB region is considered. Many manufacturers produce sunscreen according their standard [45], hence many commercial sunscreen products on the market are not safe and not efficient where they are unable to offer protection from UVA radiation [46]. Thus, further research on these material's efficacy for UVA regions and stability in the sunscreen should be studied to preserve product efficacy and stability.

The motivation of this work was to prepare sunscreens containing ZnO that focused on ZnO nanorod structure and analyse several properties of these sunscreen with respect to their particle morphology, size distribution and optical performance.

The sunscreens were prepared with different concentrations of ZnO and the correlation between concentration and UV absorption was discussed. Besides that, the sun protection factor values, critical wavelength and stability of sunscreen were evaluated in terms of stability and efficacy of sunscreen performance in the UVB and UVA regions. Moreover, one commercial sunscreen containing ZnO was used to benchmark against the performance of the sunscreen samples of this work.

1.5 Objectives of study

The principal objectives of this research work are elaborated in the following points:

1. To investigate the structural properties of ZnO nanorods in powder.
2. To prepare sunscreen containing different concentration of ZnO nanorods powder.
3. To study the optical properties of sunscreen containing ZnO nanorods.
4. To evaluate the properties of sunscreen containing ZnO nanorods by their pH value, separation phase and SPF value.

1.6 Scope of study

The research on ZnO covers a wide area of investigation. A specific and concise scope was made to direct the experimental, results and discussions presented in this research work. Firstly, this work focused on ZnO nanorod in powder as main material. The applications of ZnO nanorods have driven this study to expand the uses of pure ZnO powder. Besides, the respective ZnO samples were synthesized through French process [25].

Secondly, this research work focused on sunscreen containing ZnO nanorods at different concentration to make a comparative assessment of their optical properties in UV range. The characterization of sunscreen was limited to physical, optical and chemical analyses. Besides that, the stability tests are conducted: color, phase separation and centrifuge process as well as pH determination to observe the conditions of sunscreen samples after various time intervals for 35 days storage in room temperature. Besides that, the determination of SPF value of sunscreen contained different concentrations were measured by UV spectrophotometer diluted method and Mansur mathematical equation. One commercially available sunscreen containing ZnO was purchased from drug store and it was used as a benchmark for this work. This characterization and evaluation were compared with the sunscreen of this work.

CHAPTER 2 : LITERATURE REVIEW

2.1 Skin and Sunscreen

Skin is the largest organ in mammals which consists of three main layer epidermis, dermis and deeper subcutaneous tissue illustrated in Fig. 2.1 [47]. The skin are able to serve as a protective barrier at the interface between the human body and the surrounding environment [48]. Exposure to ultraviolet (UV) radiation has been linked to a number of human skin problems, including sunburn, skin cancer, premature aging of the skin, cataracts, and immune suppression [49-51]. There are possibly two processes may occur during radiation (photons) enter the skin.

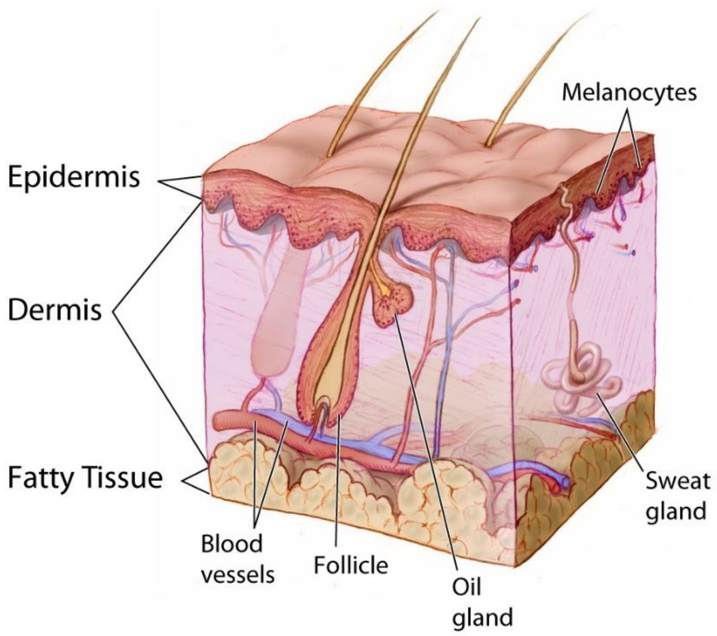

Figure 2.1: Skin structure [47].

The first process will take place where the absorption of UV and visible photons into the skin and initiates chemical changes in the cells. While, second process to occur is by scattering, which means the depth of penetration of radiation into the skin is dependent on the wavelength of the photon [52]. The human skin pigmentation via the formation of melanin helps to protect against solar radiation. Melanin able to prevents the mutagenesis of cellular DNA by blocking the penetration of UV radiation and the degree of protection depends on the skin photo-type and age. Children and young people tend to be more exposed to UV radiation [20]. UVB can penetrate through the epidermis to the upper dermis. Erythema and sunburn are mainly caused by UVB radiation, while UVA and visible light can also cause skin erythema but they are requiring much higher doses than UVB rays [53].

According to Hoffmann-Dorr et al. [54], the generation of micronuclei associated with the induce of oxidative DNA damage by visible light (>395 nm) in melanoma cells. However, the effects of longwave UVA and visible light on human skin have not been comprehensively investigated. As the best approach outer protections, the applications of sunscreen are very helpful to human being. The application of sunscreen products is crucial way as photoprotection strategy [55].

Sunscreen is classified by FDA as an ultraviolet blocking agents to attenuate damaging radiation before it can induced deep into the skin [56-58]. There are two types of sunscreen that can be classified in the market. They are chemical sunscreen or physical sunscreen. Their types are differentiated by the ingredient contained in the sunscreen. The active molecules that act as active UV filters in the sunscreen divided into two groups: chemical (organic) and physical (inorganic) [59]. Table 2.1 shows the list of UV filters that available in sunscreen ingredient.

Table 2.1: List of UV filters [60].

Organic		Inorganic
UVA INCI/Chemical substances	UVB INCI/Chemical substances	UVA/UVB INCI/Chemical substances
Avobenzone Menthyl Anthranilate Benzophenone Oxybenzone Terephathalylidene Dicamphor sulfonic acid	Aminobenzoic acid [PABA] Dioxybenzone Cinoxate Phenylbenzimidazole sulfonic acid Homosalate Octocrylene Octyl methoxycinnamate Octyl Salicylate Oxybenzone	Zinc oxide Titanium Dioxide

Both inorganic and organic UV filters are able to protect skin from UVA and UVB. Some organic UV filters can be classified as either UVA or UVB filters according to their functional groups and their specific absorption characteristics. However, the probability of some combination between organic compound in the sunscreen would become instable and could not perform a good protection as sunscreen [61].

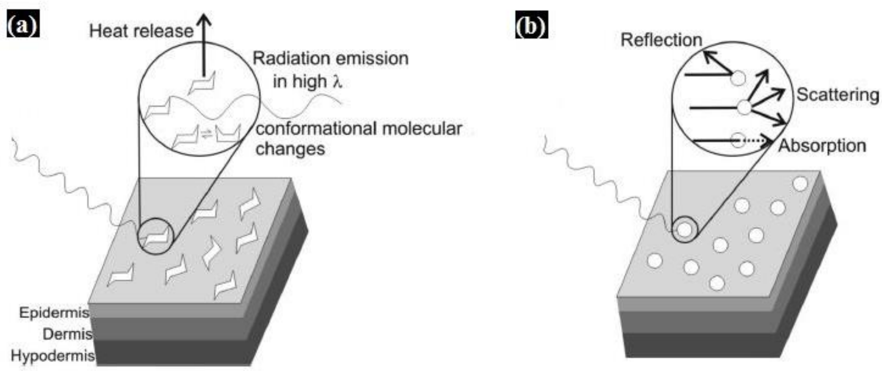

Figure 2.2: Schematic diagram of (a) organic; (b) inorganic UV filters [19].

In Fig. 2.2(a), the organic UV filters also known as chemical filters because their mode of action is related to chemical changes in their molecules that prevent UV radiation reaching the skin. Meanwhile, the inorganic UV filters be called physical due to their physical action such scattering, reflection of UV radiations [17] shown in Fig.2.2(b). There are so many types of sunscreen formulation available in the market and many dermatologists prefer that mineral based sunscreens such as those contained ZnO and TiO_2 are the real way to prevent skin cancer, skin damage and premature aging [62].

2.2 Incorporation ZnO in sunscreen

When addressing the application of ZnO in the sunscreens, it is important to consider the physiochemical properties of ZnO. Zinc oxide occurs naturally in the Earth's crust and exists in two crystalline forms wurtzite and zinc-blade. However, the wurtzite structure is the most common and stable form. In addition, ZnO possesses refractive index in values between 2.3 and 2.0 which is the whitening effect of ZnO is lesser than TiO_2 [63]. The use of zinc oxide in sunscreen is preferred over titanium dioxide because ZnO is a much safer and more effective in the sunscreen than TiO_2 based on physical chemistry and biological effects [64]. Some significant differences between ZnO and TiO_2 is ZnO UV absorption spectrum covers across shorter wavelength UVB 280nm to longer wavelength UVA meanwhile TiO_2 only covers UVB rays from 280-320nm [65]. The stability and safety of ZnO as sunscreen active ingredient certified by FDA [34] approved for use on babies under 6 months of age and children which illustrates it is high degree of safety. Compared to TiO_2, this metal oxide tends to create free radicals that do oxidative damage to body and skin cells and increase aging processes [66].

2.2.1 UV protection of physical sunscreen

ZnO has been widely recommended as the safest UV filters in sunscreen products. Despite these benefits older sunscreens containing ZnO microparticles were limited in popularity by poor cosmetic appearance [67]. Due to the bulk particle size distribution and poor dispersive qualities of the ZnO particles, these sunscreens left a white or opaque film, as well as grainy-residue on the skin. The diminished aesthetics of these sunscreens hindered wide acceptance by the public. This problem was met with a solution in nanotechnology.

Nanotechnology involves the design, production, and application of materials in the size range of 1–100 nm. As existing materials are reduced to this size, a new set of physical, chemical, mechanical, and electrical properties are revealed [68]. Sunscreen with ZnO nanoparticles are easier to spread on the skin surface and are transparent, thus providing better acceptance and cosmetic compliance [69].

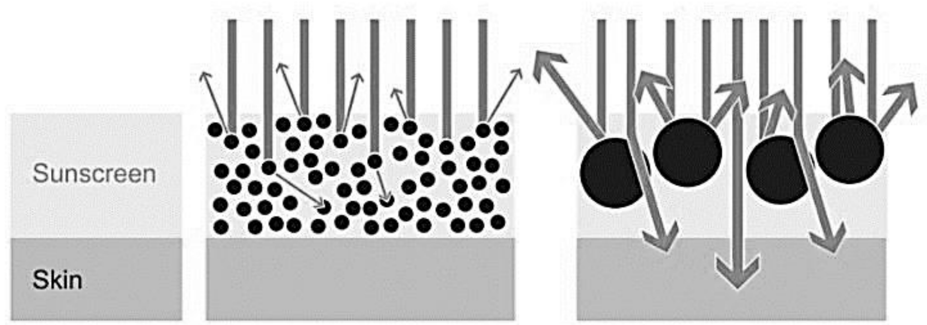

Figure 2.3: UV protection properties according to particles size [70].

Fig. 2.3 showed sunscreen that contained nanoparticles (NPs), it will provides a combination of more efficient UV absorption and high visible transparency when dispersed in a layer compared to sunscreen contained micro size particles [71]. When particles become smaller than 100 nm in size, novel optical characteristics emerge. In semiconducting nanoparticles of sizes comparable to the exciton Bohr's radius, the electronic energy levels are interpreted as discrete energy levels. When ZnO particles become smaller than the optimal light scattering size which is approximately half of wavelength, the visible light is transmitted, and the particles appear transparent. By decreasing particles size, UV protection shifts towards protection against shorter UV wavelength. ZnO nanoparticles able to attenuate shorter wavelength of UV radiation from 280 to 400 nm and offer high UVB and UVA coverage.

Goh et al. [72] reported the absorbance spectra increases with increasing size of ZnO. The results are evaluated in terms of intrinsic particle absorption and the number concentration of particles. The ZnO particles exhibited cubical shapes and a narrow size distribution. At larger sizes, the absorbance decreased with increasing size due to the decrease in number of particles. From the present measurements, the particle size for optimum UV absorbance is 40 nm. Consistent with wider band gap ZnO at 3.22 eV, ZnO are effectively scattered and reflected the visible light while absorption occurs in the UV-range. The valence band possesses many densely packed electron states that allow many absorption possibilities, where the energy absorption exceeds the band gap width [73].

As recommended by the United States Food and Drug Administration (FDA), the protection factor against UVA should be at least one-third of the overall sun protection factor. Apart from size-related optical properties of NPs in skin, sunscreen formulations can influence the sun protecting efficacy of the NPs. However, UV radiation reaching living epidermal cells could be diminished by particle sizes that minimize transmission and maximize absorption and scattering of UV rays [74].

2.2.2 Safety of sunscreen contains ZnO NPs

Concern has been raised about their increased reactivity of NPs due to their small size and their higher specific surface area [75]. The function of the skin, generally attributed to the stratum corneum (SC), prevents the entrance of foreign molecules and pathogens from the environment as well as the loss of endogenous substances. The potential of ZnO NPs to penetrate the SC and diffuse into skin structures become an issue of their safe topical use in cosmetic products. However, no toxicity has been reported for topically applied nanoparticles used in sunscreen [76].

In vivo human studies showed that the penetration of topically applied ZnO NPs was limited only to the superficial layers of the SC and no nanoparticles was detected in the viable epidermis. Regarding Cross et al.[77], while showing no penetration of ZnO NPs through human skin, did show a trend to high zinc ion penetration for topically applied ZnO NPs relative to controls. This is also in agreement reported by Gulson et al.[78] who detected the presence of ^{68}Zn in blood and urine of human volunteers after topical application of sunscreens that contained scale 19 nm ZnO particles enriched with the stable isotope ^{68}Zn under occupied conditions.

According to Leite-Silva et al.[79], they demonstrated topically applied the formulation of ZnO NPs ranges size <30 nm only accumulated within the SC and mainly localized at the furrow-cellular border in vivo human skin. Nevertheless, mostly investigations involving ZnO NPs have been carried out in vitro studies because in vitro result would give the result as the in vivo as closely as possible [78-80].

2.2.3 ZnO nanorods as UV filter

In general, ZnO absorbs UV rays ranging from 280 to 400 nm which makes it an ideal component in sunscreen and cosmetic formulations that aim to block both UVA and UVB radiation [55]. Structure of ZnO particles attracted considerable research activity because of its great potential in fundamental studies of the roles of physical dimension and size as well as applications in optoelectronic devices and functional materials [83]. Most studies conducted on commercial sunscreen found that ZnO structure can influence the effectiveness of UV absorption where the UV protection properties depend on their size and morphology [84]. However, most manufacturers do not conduct specialized studies on the ZnO morphology they use for their sunscreen product [85]. According to Lu et al. [86], a studies conducted on selected sunscreen products from USA, Japan and Taiwan found that most of the ZnO particles contained in the sunscreen contained in the sunscreen were in the form of varied shape and roundish [86].

Recently, the use of ZnO nanorod has increased through many scientific studies [91–96] that have been performed for higher UV protection properties which interest many cosmetic companies. ZnO nanorod has higher surface area and quantum effects, which influence their physical and chemical properties compared to bulk ZnO [93]. Besides that, ZnO nanorod exhibits unique characteristic in optical, electronic and catalytic properties which significantly different from other nanostructure [94].

Table 2.2: Absorbance peak of ZnO according their size and morphology.

Structure of particles	Absorption peak (nm)	Average crystallite sizes (nm)	References
Spherical	352	75	[95]
Irregular shape	360	34	[96]
Rods	373	71	[97]
Plate	372	39.37	[98]
Nanocrystalline	375	44.5	[28]
Nanoparticles	360	26-30	[77]

The synthesis of ZnO nanorod is simple, low cost, high purity and high yield making it having wide potential applications in cosmetic industries and medical area [99]. According to Gopikrishnan et al. [94], ZnO nanorod might be a safe nanomaterial for cosmetics as well as biological applications. Generally, ZnO nanorods potentially can absorb light with energy of hv that matches or exceeds their band gap energy (E_g).

Rusdi et al. [100] had shown the values obtained for the band gap energies of the ZnO nanorods (3.29 eV) wider than spherical nanostructures (3.25eV), corresponding to the violet-blue region of the electromagnetic spectrum. Thus, the ZnO nanorods has higher UV absorption properties where the electrons taking part in the transition from the valence band to the conduction band need greater energy to execute the jump. Hence, ZnO nanorods incorporated sunscreen as an active UV filter can enhance the UV protection properties of the sunscreen and promises comprehensive protection in the broad spectrum.

2.3 Sun Protection Factor

Sun protection factor (SPF) is an index of the protection potential of the product from UVB rays. It was globally adopted and is today written on the labels of all sunscreen products (refer Fig 2.4), including the cosmetic lines against skin photoaging [101].

Figure 2.4: SPF value labelled on the sunscreen packaging.

According to Commission Recommendation [102], the SPF value must be written on the packaging as well as the relevant protection level. It is important to underline that the minimum suggested labelled SPF must be at least 6 and above. The beginning of official and internationally accepted method for sunscreen tests is through in vivo sun protection factor (SPF) method based on the COLIPA standard [103]. Validation of in vivo SPF method is made through an artificial source of UVR on subjects such as human and animal. In Europe, at least 10 subjects must undergo the determination of SPF and can be acceptance [103]. The in vivo SPF was calculated as equation as described in the ISO 24444:2010 standard [20]. There are many conditions required in the in vivo SPF determinations such as are selection of persons for testing, number of subjects, age, skin type, level of sunscreen applied, the source of irradiation, the exposure time and evaluation of the redness of the skin [104]. Due to many considerations need to consider, this method has several drawbacks. This evaluation method is expensive in terms of money and time. It also raises several ethical issues concerning the potential damage to skin volunteers besides make it difficult to adopt in routine quality control [105].

However, in vitro SPF test method would be advantageous because it could generate results faster and cheaper and it can avoid the ethical concerns associated with in vivo testing. At the present, there are several in vitro methods, all used just for screening or developing purposes. One of the in vitro method was introduced and proposed by Sayre et al. [106] in 1979 and is still the most accredited reference [96-108]. The measurement involving absorption of the sunscreen product based on dilute solutions method and in 1986, Mansur et al [120] have been developed a simple mathematical equation which substitutes the in vitro method proposed by Sayre et al [106]. By using Mansur mathematical equation 2.1, SPF value of chemical and physical sunscreen can be determined [116].

$$SPF = CF \times \left[\int_{290}^{320} EE\ (\lambda) \times I\ (\lambda) \times Abs\ (\lambda)\right] \quad (2.1)$$

where: EE (λ) is erythemal effect spectrum; I (λ) is solar intensity spectrum; Abs (λ) is absorbance of sunscreen product; CF is correction factor (=10).

The values of EE x I are constants since they were determined by Sayre et al. cited from [111], and the values are tabulated in Table 2.3. The in vitro SPF methodology is useful as a rapid quality control method. It can be applied during the production process, in the analysis of the final product, and can give valuable information before proceeding to the in vivo tests.

Table 2.3: Product function used in the calculation of SPF.

Wavelength (nm)	EE × I
290	0.0150
295	0.0817
300	0.2874
305	0.3278
310	0.1864
315	0.0839
320	0.0180

Dutra et al. [111] were applied Mansur equation to determine the SPF values of ten different commercially available sunscreens emulsions containing chemical (e.g. octyl methoxycinnamate, octyl salicylate, benzophenone-3) and physical (e.g. ZnO and TiO_2) filters. As a result, the SPF value measurement are in close agreement with the labelled SPF. According to Garoli et al. [121], they were reported in vitro SPF method data used to SPF evaluation of commercial sunscreen showed a good correlation between the in vitro SPF values and the SPF reported by the manufacturer.

2.4 Critical wavelength

The US Food and Drug Administration (FDA) issued a final ruling on the labelling and effectiveness testing of sunscreen products in year 2012. One of the ruling, the FDA adopted the in vitro critical wavelength (CW) as a measure of assessing UVA or broad-spectrum protection [122]. The CW method documents under FDA were provided in the Appendix B. Critical wavelength (CW) is defined as the wavelength greater than 370 nm where 90% of the total area under the absorbance curve with the absorption measures across the 290 to 400 nm.

Specifically, the FDA has ruled that only products with CW ≥370 nm can be labelled as "broad-spectrum" sunscreen [123].

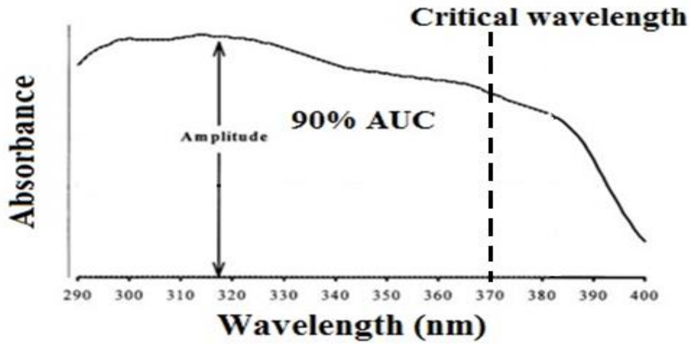

Figure 2.5: The critical wavelength which greater than 370 nm [124].

In vitro CW method is based on the absorption spectrum of a sunscreen product, illustrated in Fig 2.5, which is obtained from UV spectrophotometry and imply the equation 2.2. However, CW method is based on the shape of the absorbance curve and not their amplitude or absorbance intensity.

$$\int_{290}^{\lambda} A_\lambda \, d_\lambda = 0.9 \int_{290}^{400} A_\lambda \, d_\lambda \qquad (2.2)$$

where A_λ is absorbance; λ is critical wavelength of sunscreen. For each absorption spectrum, the trapezoidal integration represent the area under the product absorbance curve [124].

Wang et al. [123] found that within same SPF value can be a various CW measurements for the tested products. Diffey et al, [124] reported only 6 out of 59 commercially sunscreen product marketed in the United States had a critical wavelength of 370 nm or more. Most of the products that achieved a critical wavelength of 370 nm contained a recognized UVA1 (340-400 nm) filter such zinc oxide, titanium dioxide, or avobenzone. However, not all "broad spectrum" sunscreen will offer the same degree of UVA protections. In connection, sunscreen manufacturers can produce better sunscreen with higher UVA protection by access to new or combination of UVA filters and higher permissible levels of UVA filters.

2.5 Stability of sunscreen

Stability of the sunscreen product towards environmental factors is important to remain good lasting performance. Stability of products can be affected by environmental factors such as temperature, light, air, and movements which can cause severe damages on the constituents of the product [125]. Several tests under stability test such as centrifugation test, liquefaction, color and phase separation. Each test would offer a vary quality of the sunscreen product and the information lead to setting the life shelf of cosmetic and pharmaceutical products to be in the market as well [126].

Majority of cosmetic and pharmaceutical creams will change their state over the time. Usually they tend to split into two distant phases. This process is called "phase separation" and this instability could be manifested at different time rates and through a few of thermodynamically destabilizing processes under normal condition [127]. However, the separated phase can be creaming or sedimentation where attributed to density differences between the two phases under the influence of gravity [128]. This is based on acceleration technique by centrifugation. This process will give a force to the formulation to breakage [129]. Creaming is the upward movement of dispersed droplets while sedimentation is the downward movement of particles relative to the continuous phase [130].

Smaoui et al. [116] was reported phase separation and centrifugation were identified their three formulated sunscreen cream stability under different temperature of storage period. All the formulation samples were stable in all storage conditions and no phase separation after centrifuged except samples stored at 40°C, significant decrease due to high temperature contributes to the destabilization of the emulsion by hydrolysis [131]. The formulation of efficient sunscreens is a real challenge for the formulators to sustain the stability of sunscreens obtained performances over the period. The shelf life assessment between sunscreen cream base and active ingredients remains one of the most time consuming and difficult issues for industrial scientists [127]. Besides phase separation, color of sunscreen is also affects the stability of sunscreen [132]. Some combination of UV filter especially organic compounds would have a chemical reaction and may contributed to observed color changes [116]. Organic UV filters are almost used in combination because there is no single active agent that provide broad spectrum protection able to be used at levels allowed by the FDA [133]. Due to their possible harmful interactions between these agents, the restrictions from FDA's Federal Register Administration Regulatory Affairs [134] have limited the choice of suitable combinations of UVB/UVA chemical organic UV filters.

Meanwhile, a sunscreen incorporated ZnO NPs showed greater color stability by promoting maintenance of the actual sunscreen color [135]. The presence of ZnO NPs only in the sunscreen product will offer higher SPF however in the combination with organic sunscreen agents, ZnO would give remarkable SPF numbers as well as displaying broad absorption in the whole UV region. Formulation incorporated ZnO NPs into sunscreen preparations helps to avoid the decrease of SPF that can occur from the photo-instability of organic UV filter [136].