Behaviour of zinc oxide nanoparticles in sunscreens

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***Abstract*—**UV radiation has important biological consequences to the skin and photoprotection is therefore highly important. Topically applied sunscreens containing zinc oxide as UV filter belong to the group of potentially used photoprotective agents. In this study we have focused on the behaviour of ZnO in nanoform in cosmetic products. With regard to zinc oxide nanoparticles, there is a lack of reliable data on the percutaneous absorption of these particles. Therefore the ability of ZnO nanoparticles from commercial emulsions to penetrate skin layers was studied using dermal absorption procedure. Two methods were used for ZnO determination in commercial fat bases: spectrophotometric method and atomic absorption spectroscopy (AAS). Results showed that data obtained by AAS are very close to the actual ZnO content in commercial samples and therefore this method seems to be suitable for ZnO determination in cosmetic products. Contrary to AAS, spectrophotometric procedure is more problematic with a greater tendency to provide values higher than actual. According to the dermal absoption procedure it was found, that the majority of ZnO remains on the skin surface and do not enter the viable skin parts.

***Keywords*—**atomic absorption spectroscopy, dermal absorption, nanomaterials, spectrophotometry, zinc oxide

I. Introduction

Sunlight is composed of a continuous spectrum of electromagnetic radiation that is divided into three main regions according to its wavelength: ultraviolet, infrared and visible light. The sunlight´s composition at ground level consists of about 45% infrared light, 50% visible light and 5% ultraviolet (UV) light. Although the UV region represents only minor part of sunlight, it causes a wide range of adverse effect on human skin and health, such as erythema, inflammation, immunological or morphological changes, photoaging or skin cancer [1]-[3].

UV radiation comprises the wavelengths from 200 to 400 nm and is arbitrarily subdivided into short-wave UVC (200 - 290 nm), mid-wave UVB (290 - 320 nm) and long-wave UVA (320 - 400 nm). The energy carried by each portion of the spectrum is inversely related to wavelength. Since the depth of penetration of UV radiation into skin increases with an increasing wavelength, UVC with the highest energy is the most biologically damaging. However UVC radiation is effectively filtered out by the stratospheric ozone layer and its role in pathogenesis of human disease is minimal. UVA and UVB radiation both reach the earth´s surface in sufficient amounts to have important biological consequences to the skin [4]-[7].

UV irradiation of human skin activates a complex sequence of molecular responses that damage skin connective tissue. To exert its biological effect, chromophores in the skin must absorb UV radiation. Depending on the chromophore type, absorbed energy may cause direct chemical modification of the chromophore itself, or the energy may be transferred to another molecule which undergoes chemical modification [8]. UVA light primarily acts indirectly through generation of reactive oxygen species (ROS), which subsequently can cause lipid peroxidation, activation of transcription factors and generation of DNA-strand breaks. While UVB light may also generate ROS, its main mechanism of action is the direct interaction with DNA, i.e. cross-linking of adjacent pyrimidines. UV absorption and ROS-mediated photochemical reactions are the basis of photoaging, i.e. aging of skin attributed to continuous, long-term exposure to UVA and UVB radiation [1], [3], [5], [8].

The above mentioned deleterious effects of UV radiation make protective measures necessary. Topically applied sunscreens containing different UV filters are the most used photoprotective means. UV filters can be classified into two groups according to their nature. The inorganic UV filters (physical UV filters) principally work by reflecting and scattering UV radiation, while the organic UV filters (chemical UV filters) absorb the light. Two inorganic oxides are used for UV protection in humans: zinc oxide (ZnO) and titanium dioxide (TiO2) [9]-[11].

Although ZnO with a particle size around 500 nm provide sufficient protection against UVA and UVB radiation, such

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particle size allows also a reflection of visible light. This fact makes ZnO based sunscreen visible after application on skin ("white look"), which is cosmetically less acceptable. Acceptability of ZnO UV filters can be improved by using ZnO particles with dimensions less than 100 nm (nanoparticles) as the decrease in particle size reduces the reflection of visible light. Moreover, application of nanoparticles in cosmetics generates products with improved texture, more vibrant colour and greater skin penetration [12].

Zinc is generally considered a non-toxic metal as it serves as an essential nutrient present in virtually any cell. It has to be consumed in the diet as its deficiency is associated with many diseases and it does not appear to accumulate with age [1], [13]. Zinc oxide has a widespread use. Besides its authorized use as a cosmetic colorant with a Color Index CI 77947, zinc oxide is applied as a bulking agent and skin protecting UV absorber [1], [14], [15]. However, there are many problems to be discussed regarding the use of ZnO nanoparticles in cosmetics. There is a very rapid development of nanotechnologies that find applications in various fields [16], [17]Concerns about the health implications of nanomaterials have been expressed because materials in the nano size range may pose toxicological hazards due to their enhanced reactivity. The physicochemical properties of nanomaterials can modify cellular uptake or protein binding and theoretically may be also able to deposit in respiratory tract, penetrate intact skin, be absorbed through gastrointestinal tract, or pass directly through cells and ultimately translocate to various tissues and organs [18]-[20]. It should be noted, that in spite of the interest of research teams, there are still insufficient information regarding the identification of potential health risks, especially risks associated with long-term exposure to nanomaterials. With regard to zinc oxide nanoparticles, there is a lack of reliable data on the percutaneous absorption of these particles [19].

In early 2009, the Therapeutic Goods Administration (TGA) conducted an updated review of scientific literature in relation to the use of nanoparticulate zinc oxide and titanium dioxide in sunscreens. The potential for ZnO nanoparticles in sunscreens to cause adverse effects depends primarily upon the ability of nanoparticles to reach viable skin cells. The current weight of evidence suggests that ZnO nanoparticles do not enter viable skin cells [21]. On the other hand, there are literature reports claiming that these nanoparticles can cause a statistically significant DNA damage in human epidermal cells at concentrations of 5 μg/ml [22]. Therefore it is necessary to continue monitoring the emerging scientific literature to ensure appropriate action in case unacceptable risks are identified.

However, the question of nanoparticle toxicity is not as simple as examining individual particles only. Due to their high diffusivity, nanoparticles can exist as isolated particles for only a short time and they possess a tendency to agglomerate rapidly. Thus, it is also crucial to understand the fate of these agglomerates upon to ascertain whether subsequent toxicological effects are attributable to nanoparticle physical properties, or are a function of their chemical composition [23].

In our study, we tried to contribute to the effort to describe and characterize behaviour of ZnO particles in real cosmetic products applied topically on skin. In order to reach this goal, we determined ZnO content in model pig skin layers obtained by dermal absorption procedure.

II. MATERIAL AND METHODS

A. Material

Two types of commercially available nanoemulsions differing in the composition of oil base were used as a model samples for ZnO determination: SOLAVEIL CZ-100 (zinc oxide based o/w emulsion consisted of 55% of ZnO, C12 - 15 alkyl benzoate, polyhydroxystearic acid, isostearic acid) and SPECTRAVEIL FIN (zinc oxide based w/o emulsion consisted of 55% of ZnO, C12 - 15 alkyl benzoate, polyhydroxystearic acid). Both emulsions were obtained from UNIQEMA (USA).

Hydrochloric acid and toluene were purchased from Petr Lukeš (Czech Republic). Zinc oxide (99.5%), nitric acid (65%), isopropyl alcohol, xylenol orange, acetic acid and sodium acetate were acquired from Lachema (Czech Republic). Hydrogen peroxide (31%) and hexadecyl pyridinium chloride monohydrate were obtained from Sigma-Aldrich (Germany).

B. Sample preparation

Two methods were used for ZnO determination: spectrophotometric method and atomic absorption spectroscopy (AAS). Sample preparation procedure depended on the nature of samples. Three types of samples were analysed in order to evaluate the ability of both methods to determine ZnO under various conditions:

a) solutions of accurately known concentration of ZnO

b) ZnO in commercially available nanoemulsions (SOLAVEIL CZ-100, SPECTRAVEIL FIN)

c) real samples obtained after dermal absorption of commercial nanoemulsions with ZnO

Standard calibration solutions for determination of ZnO by both methods were prepared by dissolving zinc oxide in a small amount of 6M HCl with subsequent appropriate dilution.

For the determination of ZnO in commercial nanoemulsions, samples were hydrolysed by 6M HCl according to the method described by Salvador *et al.* [24]. Nanoemulsions were solubilized using toluene and ultrasonic bath was also used to accelerate solubilisation. Commercial nanoemulsions were applied on the surface of adhesive tape in order to simulate real samples obtained by stripping in dermal absorption procedure. Toluene as a solvent was also applied in order to extract emulsion sample from the adhesive tape in model samples as well as in samples acquired by stripping. Extraction was performed till the adhesive tape was visually completely clear with no visible residues of the sample. Toluene extracts were subsequently mixed with 6M HCl and hydrolysed.

Commercial nanoemulsions were further evaluated for their homogeneity using microwave digestion with nitric acid and

hydrogen peroxide (3:1) in microwave mineralizer Ertec Magnum (Poland).

C. Spectrophotometric determination of ZnO

The spectrophotometric determination of zinc was carried out according to the methodology described by Benamor and co-workers [25]. The method is based on the colour reaction between Zn^{2+} and xylenol orange in constant pH. Permanent environment (pH 5.5) was provided by acetate buffer and stability of the resulting colour complex was provided by addition of cationic surfactant cetylpyridinium chloride. Absorbance of a colour solution was measured in a 1 cm cell at 580 nm against a reagent blank. Readings were taken every 30 minutes using a spectrophotometer DR/2000 (Hach, USA). Such time interval is necessary to stabilize the complex formed and to enhance the accuracy of measurement.

D. Determination of ZnO by AAS

For the determination of zinc by atomic absorption spectroscopy (AAS) the AAS spectrophotometer (GBC Scientific Equipment PTY, Australia) was used with the flame type air-acetylene, Fuel lean. Samples were measured in 239 nm wavelength. Standardization was carried out using Zn-Astasol (Analytica, Czech Republic).

E. Dermal absorption

Dermal absorption procedure was performed according to Regulation SCCS/1358/10 (The Scientific Committee on Consumer Safety) and in compliance with Commission Regulation (EC) No 440/2008 laying down test methods according to the European Parliament and Council Regulation (EC) No 1907/2006 concerning the Registration, Evaluation, Authorization and restriction of Chemicals (REACH) and according to OECD methodology 428 (2004) Skin absorption: *in vitro* method.

Dermal absorption method was performed using static Franz diffusion cells. Experiments were carried out with two types of commercially available ZnO nanoemulsions SOLAVEIL CZ-100 and SPECTRAVEIL FIN, which have been specifically characterized in the preceding text. Both samples were tested on the pig skin (standard dermal substrate suitable for the procedure). Samples at the amount of 35 mg were applied on skin surface and the skin was placed horizontally on Franz diffusion cells between the upper (donor) and lower (acceptor) compartments. Sink conditions were obtained in the receptor compartment with receptor fluid composed of natrium chloride, bovine albumine and gentamycin, with a volume of receptor fluid of 15 ml. The receptor compartment was continuously homogenized using a stirring magnetic bar and the temperature was kept for 24 h at 32°C using a temperature-controlled oven. After 24 h exposure, each skin sample was divided into 13 fractions, which are described in Table 1.

Stratum corneum fractions were obtained by stripping. Strips were obtained by placing an adhesive tape to the skin surface. Pushing the tape to the skin led to an adhesion of *stratum corneum* cells to the tape. Separation of dermis and epidermis was carried out using heat. Obtained fractions were separately extracted with toluene as a solvent for *stratum corneum* strips and with isopropyl alcohol that was used for epidermis (without *stratum corneum*) and dermis.

Table 1. Fractions obtained by dermal absorption *in vitro*

Fraction number	Description
1	Rinsing of the cap
2	Swabbing of excessive product on the skin surface
3	*Stratum corneum* (adhesive tape strips) Strips 1-2
4	Strips 3-4
5	Strips 5-6
6	Strips 7-8
7	Strips 9-10
8	Strips 11-12
9	Strips 13-14
10	Strips 15-16
11	Epidermis without *stratum corneum*
12	Dermis
13	Receptor fluid

III. Results and Discussion

A. The effect of sample preparation method on the determination of zinc

ZnO solutions of accurately known concentrations were prepared by dissolving ZnO in HCl and were used for the evaluation of the correspondence between two methods, atomic absorption spectroscopy (AAS) and spectrophotometric method (SPM). Samples were diluted so that the determination was carried out within the ¼ and ½ range of the calibration curve. For each analytical method ten samples were measured for each calibration level. Differences between real ZnO content and ZnO determined by atomic absorption spectroscopy (AAS) or spectrophotometric method (SPM) are summarized in Table 2. A relatively narrow range of values were obtained, thus only average values and their percentage deviations are listed in Table 2. As it was expected, both methods AAS and SPM showed a good agreement in determination of ZnO in aqueous solutions.

Table 2. Differences between real ZnO content and ZnO determined by atomic absorption spectroscopy (AAS) or spectrophotometric method (SPM)

concentration of ZnO [µg/l]	ZnO content determined by AAS	difference [%]	ZnO content determined by SPM	difference [%]
261.3	259.7	-0.6	262.1	+0.3

In comparison with a determination in aqueous solution, a determination of ZnO content in commercial fat bases is more complicated owing to several factors. At first it is the influence of the fat component itself, further problems may arise from the presence of emulsifying agents that ensure product homogeneity. Especially for the spectrophotometric determination should be this component eliminated from the sample due to the possible formation of emulsion. To avoid

Table 3. Differences between ZnO content in commercial ZnO based nanoemulsions determined by atomic absorption spectroscopy (AAS) or spectrophotometric method (SPM)

sample	expected ZnO content [µg/l]	ZnO content determined by AAS	difference [%]	ZnO content determined by SPM	difference [%]
SOLAVEIL CZ-100	383.2	386.9	+0.9	394.1	+2.8
SPECTRAVEIL FIN	308.5	309.7	+0.4	271.4	-12

such difficulties, hydrolysis by organic solvent is highly recommended.

In this study we tried to evaluate differences between ZnO content in commercial ZnO based nanoemulsions determined by atomic absorption spectroscopy (AAS) and spectrophotometric method (SPM). Results of both methodologies are presented in Table 3. Each measurement was performed 8 times.

Results showed that data obtained by AAS are very close to the actual ZnO content in both commercial samples and therefore this method seems to be suitable for ZnO determination in cosmetic products. On the contrary, spectrophotometric method with xylenol orange is more problematic. For nanoemulsion SOLAVEIL CZ-100 the differences between expected ZnO concentration and concentration determined by SPM did not exceed 3% deviation. Regarding the other sample (SPECTRAVEIL FIN) as high deviation as 12% was detected between real content and content determined by SPM. Due to these high differences, it can be noted that spectrophotometric determination of ZnO in water-in-oil emulsions is at least problematic, if not entirely inappropriate.

Prior to performing the dermal absorption procedure and determination of zinc in samples obtained by stripping, a process of isolation and determination of ZnO from the adhesive tape was verified. Exact amount of nanoemulsions was applied on the surface of adhesive tape to simulate actual conditions of dermal absorption method. In the first instance isopropyl alcohol was used for the extraction of the sample from adhesive tape, but the extraction with this solvent was not very successful. Application of toluene as a solvent led to a better extraction process that provided clear adhesive tape with no visible residues of the sample. Results of the determination of ZnO content in commercial ZnO based nanoemulsions applied to the surface of adhesive tape are presented in Table 4. All samples were analysed by both analytic methods, atomic absorption spectroscopy (AAS) and spectrophotometric method (SPM).

Results acquired by AAS for both commercial samples may be considered to be sufficiently reliable, although the determination reached slightly lower values when compared to actually applied amount. It is likely that these differences are attributed to incomplete sample extraction from adhesive tapes. Somewhat contradictory trend (i.e. obtained values are higher than the applied amount) can be observed in the case of determination of ZnO nanoemulsions themselves (not applied to adhesive tape, see Table 3). The reason for higher values may be a probable emulsion formation caused by stabilization of colour complex by tenside. Formation of emulsions may result in higher determined ZnO content. Such explanation was supported by the results of ZnO determination in samples that were treated by mineralization in microwave mineralizer. Results of SPM analysis of microwave mineralized samples were in accordance with the results of AAS determination.

B. Dermal absorption

The ability of ZnO nanoparticles from commercial emulsions to penetrate skin layers was studied using dermal absorption procedure described in the section II.E. Only the results obtained for SPECTRAVEIL FIN sample are presented in Table 5 as the data for SOLAVEIL CZ-100 showed the same trend.

There is obviously a considerable variance of determined ZnO values. But in all cases higher values were recorded by SPM when compared to the values obtained by AAS.

Table 4. ZnO content in commercial ZnO based nanoemulsions applied to the surface of adhesive tape determined by atomic absorption spectroscopy (AAS) or spectrophotometric method (SPM)

sample	expected ZnO content [µg/l]	ZnO content determined by AAS	difference [%]	ZnO content determined by SPM	difference [%]
SOLAVEIL CZ-100	317.1	306.0	-3.7	348.3	+9.6
SPECTRAVEIL FIN	365.0	363.1	-0.1	359.8	-1.4

Table 5. Penetration of ZnO determined by AAS and SPM after dermal absorption method

	ZnO in individual skin fractions [%]									
	control sample		skin I		skin II		skin III		skin IV	
fraction number	AAS	SPM	AAS	SPM	AAS	SPM	AAS	SPM	AAS	SPM
1	0	0.1	-	0.5	0	1.0	0	0.5	0	1.1
2	0	0.3	35.6	59.7	67.0	82.4	70.2	128.5	81.0	113.7
3-10	0	0.1	7.4	19.4	8.2	29.4	4.2	15.3	2.1	5.9
11	0	0.1	0.2	0.1	0.2	0.1	0.1	0	-	-
12	0	0.1	0	0.1	0	0	0	0.1	0	0.1
13	0	0.1	0	0.1	-	-	0	0.1	0	0.1

Table 6. Recovery of ZnO (comparison of applied amount of ZnO and values determined by AAS and SPM)

	control sample		skin I		skin II		skin III		skin IV	
method	AAS	SPM	AAS	SPM	AAS	SPM	AAS	SPM	AAS	SPM
ZnO recovery [%]	-	-	43.2	79.9	75.4	112.9	74.5	144.5	83.1	120.9

Table 7. ZnO content in commercial ZnO based nanoemulsions determined by AAS. Samples for AAS analysis were taken from two different sampling sites for each nanoemulsion.

sample	amount of the sample [mg]	ZnO content determined by AAS	ZnO content in the sample [%]
SOLAVEIL CZ-100	360.2	204.9	56.9
	334.7	163.7	48.9
SPECTRAVEIL FIN	213.5	109.9	51.5
	278.2	104.6	37.6

Overall recovery of ZnO which represents the sum of Zn obtained from all fractions is listed in Table 6. In most cases of SPM determination, recovery higher than 100% was recorded. Regarding the recovery of ZnO determined by AAS, values obtained in this study resemble the recovery values of common tests of dermal absorption [26]. There were also significant differences in recovery values among samples tested on individual skin substrates. These irregularities are caused by inhomogeneous character of pig skin samples, which is unfortunately in biological materials quite common. Nevertheless, despite these discrepancies, it is obvious that the greatest portion of applied ZnO nanoparticles remains on the skin surface and a significant amount of zinc can be detected also in *stratum corneum.* The most important conclusion which could by drawn from dermal absorption data, is that only a negligible amount of ZnO nanoparticles penetrate to the viable skin layers.

Futhermore, it was found, that different ZnO content was determined after several months of storage even though the procedure of measuring remained unchanged. Due to this finding a more precise sample evaluation was carried out. ZnO content determination was repeated after 9 months of storage at 10°C and particle size as well as their distribution was evaluated. Results suggest that studied commercial nanoemulsions lose its homogeneity after several months of storage. Data presented in Table 6 show the differences in ZnO levels in two distinct sampling sites.

Changes in characteristics and behaviour of the samples were confirmed by the evaluation of particle size and distribution. Distribution curve of the sample SOLAVEIL CZ-100 shortly after production and distribution curves of the same sample after 9 months of storage are presented in Fig. 1 and Fig. 2.

The average particle size of the sample after production was 139 nm and low distribution curve at the range of 50 - 140 nm was acquired. After 9 months was the average particle size significantly higher (672 nm). There were particle sizes around 100 nm as well as 1000 nm particles. Such a very wide range indicates a large agglomeration of particles and instability of the sample.

IV. CONCLUSION

Determination of zinc content by spectrophotometric method and atomic absorption spectroscopy showed a good agreement solely in the case of simple ZnO solutions.

Atomic absorption spectroscopy (AAS) seems to be a suitable method for ZnO determination in real cosmetic products and in samples obtained after dermal absorption procedure. Contrary to AAS, spectrophotometric procedure is

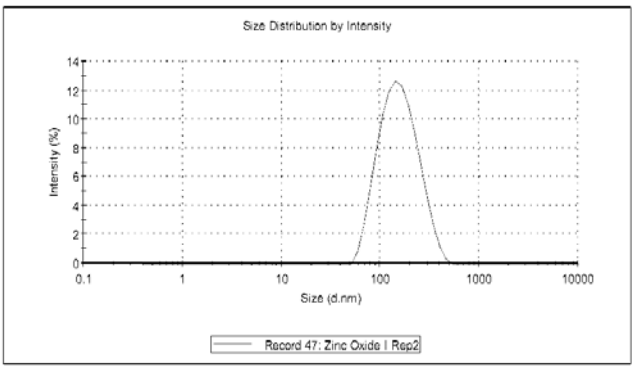

Figure 1. Distribution curve of commercial nanoemulsion SOLAVEIL CZ-100 after production

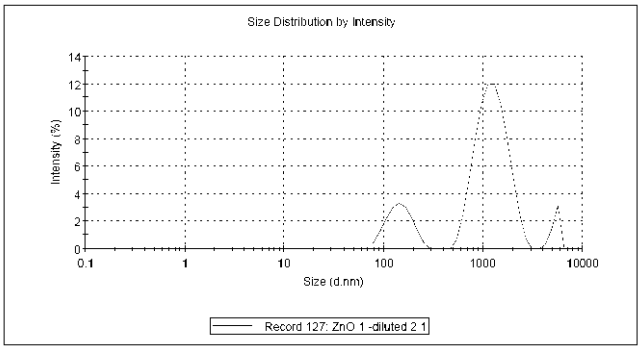

Figure 2. Distribution curve after 9 months of storage

more problematic with a greater tendency to provide values higher than actual. However, SPM may be applied provided that a faultless mineralization of the fat base is realized.

Samples obtained by dermal absorption method can be extracted by toluene, which has proven to be an appropriate solvent for this intention and subsequent ZnO determination.

Regarding the ability of ZnO nanoparticles to penetrate to lower skin layers, it is possible to conclude that the majority of ZnO remains on the skin surface and do not enter the viable skin parts.

REFERENCES

[1] A: Rougier, H. Schaefer, *Protection of the Skin against ultraviolet radiations.* John Libbey Eurotext: Montrouge, 1998, pp. 11 - 32.

[2] J. Reichrath, *Sunlight, Vitamin D and Skin Cancer*. Landes Bioscience and Springer Science+Bussiness Media, LLC: New York, 2008, ch. 1.

[3] L. Malina, *Fotodermatózy*. Maxdorf: Prague, 1999, pp. 16-25.

[4] B. Kütting, H. Drexler, "Evaluation of skin - protective means against acute and chronic effects of ultraviolet radiation from sunlight," *Curr. Probl. Dermatol*, vol. 34, pp. 87-97, 2007.

[5] P. U. Giacomoni, *Biophysical and Physiological Effects of Solar Radiation on Human Skin*. Royal Society of Chemistry: Cambridge, 2007, ch. 2.

[6] G. J. Clydesdale, G. W. Dandie, H. K. Muller, "Ultraviolet light induced injury: immunological and inflammatory effects," *Immunol. cell Biol.* vol. 79, pp. 547-568, 2001.

[7] Y. Matsumura, H. N. Ananthaswamy, "Toxic effect of ultraviolet radiation on the skin," *Toxicol. Appl. Pharmacol.,* vol. 195, pp. 298-308, 2004.

[8] S. Gonzales, m. F. Lorente, Y. G. Caldaza, "The latest on skin photoprotection," *Dermatol. Clin*, vol. 26, pp. 614-626, 2008.

[9] P. Wolf, A. Young, *Photoprotection. Dermatological Phototherapy and Photodiagnostic Methods.* Springer: New York, 2008, pp. 334-356.

[10] R. K. Chaudhuri, F. Pfluecker, I. H. Mueller, *Organic sunscreens.* Allured Bussiness Media: Carol Stream, 2002, pp. 627-629.

[11] N. A. Shaath, *The Encyclopedia of Ultraviolet Filters*, Allured Bussiness Media: Carol Stream, 2007, ch. 1-3.

[12] P. Kullavanijaya, H. W. Lim, "Photoprotection," *J. Am. Acad. Dermatol.*, vol. 52, pp. 937-958, 2005.

[13] S. R. Rolfes, K. Pinna, E. Whitney, *Understanding normal and clinical nutrition.* Wadsworth, Cengage Learning: Belmont, pp. 452-456, 2009.

[14] S. J. Castillo, M. C. Acosta-Enríquez, M. E. Zayas, H. Arizpe, T. Mendívil-Reynoso, A. Garcia-Juárez, m. E. Alvarez-Ramos, E. Larios-Rodríguez, "Formation of ZnO in or on glasses by using the sol-gel and chemical bath deposition techniques," *WSEAS Transactions on Circuits and Systems*, vol. 9, pp. 143-152, 2010.

[15] J. Díaz-Reyes, J. Martínez-Juárez, R. Galeazzi, G. Juárez-Díaz, M. Galván-Arellano, P. Rodríguez-Fragoso, E. Lopez-Cruz, "Structural and optical characterization of ZnO layers grown by chemical bath deposition activated by means microwaves." *Advances in Sensors, Signals and Materials - 3rd WSEAS International Conference on Sensors and Signals, SENSIG'10, 3rd WSEAS International Conference on Materials Science, MATERIALS'10* , pp. 105-109, 2010.

[16] J. M. Köhler, P. M. Günther, A. Funfak, J. Cao, A. Knauer, S. Li, S. Schneider, G. A. Gross, "From droplets and particles to hierarchical spatial organization: Nanotechnology challenges for microfluids." in *10th WSEAS International Conference on EHAC´11 and ISPRA´11, 3rd WSEAS Int. Conf. on Nanotechnology´11, 6th WSEAS Int. Conf. on ICOAA'11, 2nd WSEAS Int.Conf. on IPLAFUN'11*, 2011, pp. 237-245.

[17] T. Shao-Pu, „An experimental comparing analysis research on the nano-vectors for drug delivery and for gene therapy," *WSEAS Transactions on Biology and Biomedicine*, vol. 7, pp. 11-20, 2010.

[18] M. D. Newman, M. Stotland, J. I. Ellis, "The safety of nanosized particles in titaniumdioxide and zinc oxide - based sunscreens," *J. Am. Acad. Dermatol.*, vol. 61, pp. 685-92, 2009.

[19] The Scientific Committee on Consumer Products, Statemen on Zinc oxide used in sunscreens. [Online]. Available: http://ec.europa.eu/health/ph_risk/committees/04_sccp/docs/sccp_o_00m.pdf.

[20] M. Hull, D. Bowman, *Nanotechnology Enviromental Health and Safety: Risks, Regulation and Management.* Elsevier: Oxford, ch. 1, 2010..

[21] The Australian Government TGA, "A review of the scientific literature on the safety of nanoparticulate titanium dioxide or zinc oxide in sunscreens," [Online]. Available: http://www.tga.gov.au/pdf/review-sunscreens-060220.pdf.

[22] V. Sharma, R. K. Shukla, N. Saxena, D. Parmar, M. Das, A. Dhawan, "DNA damaging potential of zinc oxide nanoparticles in human epidermal cells," *Toxicology Letters*, vol. 185, pp. 211–218, 2009.

[23] B. A Wong, D. G. Nash, O. R. Moss, "Generation of Nanoparticle Agglomerates and their Dispersion in Lung Serum Simulant or Water," *Journal ofPhysics: ConferenceSeries*, vol. 151, pp. 1- 7, 2009.

[24] A. Salvador, M. C. Pascual-Martí, J. R. Adell, A. Requeni, J. G. March, "Analytical methodologies for atomic spectrometric determination of metallic oxides in UV sunscreen creams," *J. Pharm. Biomed. Anal.*, vol. 22, pp. 301-306, 2000.

[25] M. Benamor, K. Belhamel, M. T. Draa, "Use of xylenol orange and cetylpyridinium chloride in rapid spectrometric determination of zinc in pharmaceutical products," *J. Pharm. Biomed. Anal*., vol. 23, pp. 1033 – 1038, 2000.

[26] L. Kotogová, L. Borská, Z. Fiala, "Testování trandermální absorpce chemických látek *in* vitro. " *Chem. Listy*, vol. 103, pp. 533-539, 2009.