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Research article

Application trial of a simple spectrophotometric method in determination of sun protection parameters of selected sunscreen cosmetics

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Abstract: *Knowledge of the sun protection factor SPF of a sunscreen product is essential for the safe use of solar radiation. In Poland and in the European Union, in vivo method is used to determine the SPF. This method is time-consuming, expensive, does not ensure repeatability, and raises ethical doubts. Therefore, instrumental methods that can replace the traditional in vivo method are sought. In this study an attempt to determine sun protection parameters, such as SPF, degree of protection against UVA and critical wavelength of selected sun protection cosmetics was made with the use of a simple spectrophotometric method based on measuring the absorbance of ethanol solutions of selected sunscreen cosmetics. The obtained results may be useful for future development of a new in vitro method for determination of sun protection parameters of sunscreen cosmetics.*

Keywords: *sun protection factor (SPF); sunscreen cosmetics; spectrophotometry*

Introduction

Solar radiation covers the range of infrared radiation (of wavelengths > 800 nm), the range of visible light (800-400 nm) and UV radiation. Taking into account the effects of UV radiation on living organisms, UV radiation is divided into: UVA (320-400 nm), UVB (280-320 nm) and UVC (100-280 nm). The wavelength range with the highest energy (UVC) is completely absorbed by the ozone layer in the stratosphere and does not reach the Earth's surface. UVB is only about 10% of the total amount of UV radiation that reaches the earth's surface because it is effectively absorbed by ozone molecules. The negative effects of UVB include accelerating the degeneration of connective tissue, erythema, pigment changes, sunburn and skin neoplastic changes. UVA causes the mildest biological effects (slight erythema), but it accelerates the skin aging

processes and DNA damage. It intensifies the skin's sensitivity to light in the presence of photosensitisers. UVA penetrates into the deeper layers of human skin, intensifying the effect of UVB radiation. Therefore, the development of methods to assess the degree of protection against UVA radiation is very important [1].

Solar radiation is inevitable and, in addition to ensuring well-being, it activates many important biochemical processes (for example UVB is involved in the photoactivation of vitamin D_3 synthesis). However, apart from beneficial effects of sunlight, overexposure to it is one of the most important factors leading to exogenous skin aging. Additional negative effects of excessive exposure to solar radiation include skin drying, erythema, skin burns, sunstroke, initiating the formation of free radicals, which in the long term may contribute to the appearance of neoplastic changes pigment skin changes and, in extreme cases, development of melanoma [2].

The skin has some defense mechanisms against UV radiation, which include the synthesis of melanin, a pigment capable of absorbing UV radiation, the process of keratinisation of the epidermis (thickened epidermis has a greater ability to scatter and absorb harmful radiation), synthesis of urocaine acid from histidine (which absorbs UV radiation with a wavelength of 290 nm) [2]. However, natural protective processes are not enough to neutralise all the negative effects of solar radiation on the human body. It is necessary to use sunscreen products which contain appropriate UV filters.

UV filters are the substances that have the ability to absorb, scatter or reflect UV radiation. These substances can be divided into chemical filters and physical ones. Chemical filters are the organic compounds that can absorb UV radiation. Chemical filters can be divided into UVA filters (for example dibenzoylmethane derivatives), UVB filters (for example derivatives of p-aminobenzoic acid, p-methoxycinnamic acid, salicylic acid and camphor) as well as UVA + UVB filters (for example benzophenones, phenylbenzotriazoles). Physical filters are the pigments with a particle size of 200-300 μm and micronised pigments with a particle size of less than 100 μm (zinc oxide, titanium dioxide). Physical filters scatter light. Additionally, there are also natural substances with low photoprotective ability, for example plant extracts from Moldavian beekeeper, common peach, chamomile as well as sesame oil, argan oil, shea butter and cocoa butter [3,4]. List of permitted UV filters with their maximum concentrations is included in the Annex VI of Regulation (EC) 1223/2009 [5].

To be classified as a sunscreen, a cosmetic product meet some requirements concerning sun protection parameters, such as: the sun protection factor SPF declared on the cosmetic packaging should be at least 6, the degree of protection against UVA should be 1/3 of the sun protection factor stated on the packaging and the critical wavelength should be at least 370 nm. SPF is a measure of the assessment of the sun protection capacity of cosmetics. SPF is defined as the ratio of the minimum dose of radiation causing erythema on the skin protected by

the sunscreen product to the minimum dose of radiation causing erythema on unprotected skin. The SPF parameter shows the sun protection properties of the preparation only in relation to UVB radiation, but it does not provide any information on protection against UVA radiation, because the erythema associated with UVA becomes visible much later. Critical wavelength is the wavelength at which the absorbance of the radiation is 90% of the total area under the curve of absorbance A versus wavelength λ (A= f (λ)) in the range from 290 to 400 nm [6].

Generally, there are two kinds of methods for the SPF determination: *in vivo* and *in vitro* ones . The *in vivo* method is based on determining the minimum dose of radiation that causes erythema on the skin which is protected and unprotected by cosmetics with a UV filter. For this purpose, an appropriate amount of the sunscreen product is applied to the skin of volunteers and irradiated with a lamp imitating UV radiation. In Poland and in the European Union, the *in vivo* method described in the standard PN-EN ISO 24444:2020-06 [7] is used to determine SPF. This method is time-consuming, expensive, does not ensure repeatability, and raises ethical doubts. Therefore, *in vitro* methods are sought that could replace *in vivo* method for determining the SPF parameter.

One of the *in vitro* methods is the method described in the standard PN-EN ISO 24443:2022-06 [8]. This method is based on spectrophotometric measurement of the thin film of the sunscreener placed on a suitable carrier before and after exposure to UV radiation. The measurement is made using a spectrophotometer with an integrating sphere in the range 290-400 nm. However, in order to calculate the SPF in this method, the SPF determined by the *in vivo* method is necessary anyway.

In this study an attempt to determine sun protection parameters, such as SPF, degree of protection against UVA and critical wavelength of selected sunscreen products (oils and lotions) was made using a simple spectrophotometric method (on a laboratory scale) based on measuring absorbance of ethanol solutions of selected sun protection cosmetics. The aim of this study was to check whether this method reproduces the declared SPF values of the tested cosmetics. The obtained results indicate the need to search for new *in vitro* methods for determining the SPF of sunscreens.

Experimental

Materials

Ethanol was purchased from Chempur. The following cosmetics were used in this study:

Cosmetic 1, waterproof sun lotion, UVA+UVB, SPF 6
Cosmetic 2, waterproof sun lotion, UVA + UVB, SPF 10
Cosmetic 3, waterproof sun lotion for children, UVA + UVB, SPF 30
Cosmetic 4, waterproof oil, UVA + UVB, SPF 30
Cosmetic 5, waterproof oil, UVA + UVB, SPF 30

Cosmetics were purchased from a drugstore.

Methods

The method for determining *in vitro* SPF was adopted from Dutra et al [9]. 0.01 g/ml solutions of lotions in ethanol were prepared in 25 ml volumetric flasks and the flasks were placed in an ultrasonic cleaner until the cosmetics are dissolved. The contents of the flask were filtrated through a glass fiber filter. 0.1 ml of the filtered solution was added to a 10 ml volumetric flask and the flask was filled to volume with ethanol. 0.01% (v/v) solutions of the oils in ethanol were prepared. The absorbance spectra of the solutions of lotions and oils were recorded with the use of a Nicolet Evolution 300 double beam spectrophotometer (Thermo Electron Corporation, Cambridge, UK) equipped with a 150 W xenon lamp. All measurements were performed in quartz cuvettes at 20˚C. SPF was calculated from the Masur equation [10]:

$$\mathrm{SPF} = \mathrm{CF} \cdot \sum_{290}^{320} \mathrm{EE}\,(\lambda) \cdot \mathrm{I}\,(\lambda) \cdot \mathrm{A}\,(\lambda)$$

where: CF - correction factor determined for the standard 8% Homosolate filter (SPF 4) solution (=10,), EE(λ) – erythemal effect spectrum, I(λ) – solar intensity, A(λ) – absorbance at wavelength λ of the sunscreen product [10]. The values of EE(λ)·I(λ) are constants (Table 1) dependent on the wavelength, determined by Sayre et al. [11], so that a standard sunscreen formulation which contains 8% Homosolate has the SPF value of 4 [10].

Table 1 Normalized EE(λ)·I(λ) values [11]

λ [nm]	EE(λ)·I(λ)
290	0.0150
295	0.0817
300	0.2874
305	0.3278
310	0.1864
315	0.0839
320	0.0180

R coefficient defining the ratio of UVA radiation to UVB radiation was calculated based on the calculated areas under the absorption spectrum in the range of UVA (320-400 nm) and UVB (290-320 nm) from the following equation:

$$\mathrm{R} = \frac{\int_{320}^{400} \mathrm{A}\,(\lambda) \cdot \mathrm{d}\lambda \;/\; \int_{320}^{400} \mathrm{d}\lambda}{\int_{290}^{320} \mathrm{A}\,(\lambda) \cdot \mathrm{d}\lambda \;/\; \int_{290}^{320} \mathrm{d}\lambda}$$

The areas under the absorption spectrum in the range of UVA (320-400 nm) and UVB (290-320 nm) were calculated with the use of Origin 8 software. Based on Table 2 the star rating and the description of the protection category for the tested cosmetics was assigned [1].

Table 2 Values of R coefficient and their assigned star ratings in the Boots Star Rating System [1]

R	star rating	description of protection category
0-0.2	none	none
0.21-0.4	*	minimal
0.41-0.6	**	moderate
0.61-0.8	***	good
0.81-0.9	****	superior
>0.91	*****	ultra

Critical wavelength λ_{cr} was calculated from the absorbance spectra using Origin 8 software from the following equation:

$$\int_{290}^{\lambda_{cr}} A\,(\lambda) \cdot d\lambda = 0.9 \cdot \int_{290}^{400} A\,(\lambda) \cdot d\lambda$$

The measurements were performed in 3 replications. Standard deviations were calculated using Microsoft Excel.

Results and Discussion

Absorbance spectra of the sunscreen products are presented in Figure 1. The spectra of the sunscreen cosmetics are similar. There are two broad bands in the spectra with maxima located at about 310 and 360 nm. These bands arise from the presence of UV filters in the tested sunscreen products: Ethylhexyl Triazone, Bis-Ethylhexyloxyphenol Methoxyphenyl Triazine, Butyl Methoxydibenzoylmethane, Octocylene, Ethylhexyl Salicylate and Ethylhexyl Methoxycinnamate.

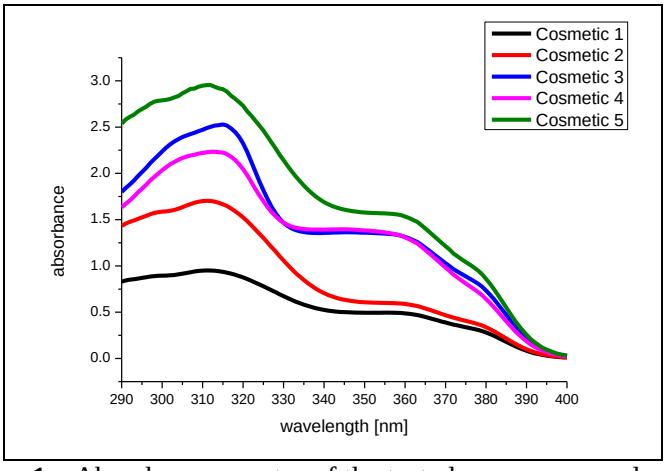

Figure 1. Absorbance spectra of the tested sunscreen products

The calculated in this work values of SPF of the tested sunscreen products are gathered in Table 3. As can be seen from Table 3 only for Cosmetic 1 (SPF 6) the

determined value of SPF is consistent with that marked on the package. When analysing the measurement results, it can be noticed that the selected measurement method overestimates the SPF values for cosmetic 2, and in the case of cosmetics with 30 SPF value, this factor is underestimated. Similar tendency was obtained in the study of Dutra et al [9]. The determined in this study critical wavelengths and the values of R (which is associated with the ratio UVA/UVB) together with star rating and description of the UVA protection category are gathered in Table 4. All the tested cosmetics are characterised by the critical wavelength value close to 370 nm and moderate protection against UVA.

Table 3. The measured absorbance A and the SPF values determined in this study

λ [nm]	290	295	300	305	310	315	320
Cosmetic 1	0.831	0.871	0.895	0.916	0.949	0.936	0.876
	0.753	0.753	0.769	0.776	0.790	0.769	0.713
	0.685	0.685	0.703	0.714	0.734	0.717	0.666
SPF_{calc}				7.985±1.032			
designation*				6			
Cosmetic 2	1.432	1.525	1.586	1.635	1.701	1.666	1.525
	1.785	1.892	1.956	2.002	2.073	2.008	1.817
	1.389	1.443	1.469	1.499	1.550	1.504	1.354
SPF_{calc}				17.004±2.569			
designation*				15			
Cosmetic 3	1.800	2.014	2.237	2.392	2.471	2.528	2.323
	1.962	2.195	2.438	2.607	2.693	2.756	2.532
	1.500	1.678	1.870	1.995	2.057	2.108	1.900
SPF_{calc}				22.738±3.030			
designation*				20			
Cosmetic 4	1.633	1.834	2.034	2.166	2.221	2.225	2.046
	1.564	1.749	1.942	2.060	2.112	2.120	1.934
	1.952	2.181	2.406	2.546	2.590	2.594	2.265
SPF_{calc}				21.963±2.478			
designation*				20			
Cosmetic 5	2.538	2.695	2.789	2.865	2.947	2.909	2.730
	2.700	2.850	2.945	3.018	3.121	3.066	2.857
	1.900	2.001	2.069	2.116	2.175	2.132	1.985
SPF_{calc}				26.471±4.801			
designation*				25			

* designation of the protection factor based on [6].

Table 4. Determined in this study critical wavelength, R value and star rating for the tested cosmetics

	λ_{cr} [nm]	R	star rating	description of protection category
Cosmetic 1 SPF 6	369	0.454±0.019	**	moderate
Cosmetic 2 SPF 10	369	0.454±0.005	**	moderate
Cosmetic 3 SPF 30	369	0.464±0.008	**	moderate
Cosmetic 4 SPF 30	369	0.497±0.009	**	moderate
Cosmetic 5 SPF 30	369	0.468±0.005	**	moderate

Only for cosmetic 1 the determined value of SPF was consistent with that declared on the package. The reason for this discrepancy may be the value of correction factor CP. This correction factor was determined for solution of Homosolate UV filter, which has SPF of 4 [10] and is not present in the tested cosmetics.

The adopted in this study *in vitro* method does not work for sunscreen products containing physical filters, since ethanol-insoluble solids do not enter the filtrate, which is then subjected to spectrophotometric measurement. This method is also not suitable for the determination of SPF of cosmetics containing new generation chemical filters, which, apart from pure chemical filters, also contain other substances, often solids, as well as micronized and nanoparticle filters, in the form of dispersed suspensions insoluble in ethanol. The obtained in this study results may be useful for future development of a new spectrophotometric *in vitro* method for determination of sun protection parameters of mixtures of pure ethanol-soluble UV filters while selecting their percentages in the recipe.

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