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Short communication

Correlation between *in vitro* and *in silico* determined sun protection factor of selected UV filters

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Introduction

The skin, the largest human organ, is exposed all the time to solar ultraviolet radiation (UV) which can be divided depending on wavelength into 3 types – UVA (320-400 nm), UVB – (290-320 nm), and UVC (200-290 nm). Even though UV rays can have beneficial effects on the people, such as stimulation of vitamin D synthesis, it is necessary to be aware of their harmful effects. UVB rays are responsible for the development of skin cancer and DNA damage, so substrates called UV filters that act as photo protectors were developed over the years. The UV filters are added to the formulation of sunscreens and many other cosmetics, which besides their primary cosmetic effect also offer protection from solar radiation. The effectiveness of sunscreens is expressed by the SPF factor (Sun protection factor). SPF is calculated as a ratio of minimal erythema dose of the skin protected with sunscreen (applied at a thickness of 2mg/cm^2) and minimal erythema dose on unprotected skin, therefore the SPF value represents how many times the amount of solar energy a person can be exposed to without getting sunburn after applying sunscreen compared to not applying sunscreen (Kaur and Saraf, 2009; Schalka and Reis, 2011).

There are three types of methods available for SPF determination – *in vivo* ("on living"), *in vitro* ("in glass"), and *in silico* ("in silicon"). While *in vivo* methods are the most accurate ones, they require testing on test subjects and raise some ethical concerns. However, *in vitro* methods are done in a laboratory, don't require exposure of human volunteers to UV, and can be optimized and adjusted. There are two types of *in vitro* methods – methods based on spectrophotometric measurements of dilute solutions and methods of measurement of UV transmission/absorption through sunscreen films. (Kaur and Saraf, 2009) The results of *in silico* method are obtained from computer simulation. This method is fast and easy, reproducible and cost-effective, and also doesn't require volunteers (Herzog and Osterwalder, 2011).

The aim of this study was to compare the results of *in silico* prediction and *in vitro* determination of SPF, depending on the type of UV filters used, different concentrations, and their combinations. UV filters used in the study were benzophenone-3 (BP3) and ethylhexyl methoxycinnamate (EMC) in concentrations of 5% and 10% for BP3 and 5%, 10% and 20% for EMC.

Materials and methods

Materials

In the study following chemicals were used: Ethanol (Merck), UV filters: Ethylhexyl methoxycinnamate (Avenalab), and Benzophenone-3 (Avenalab). Absorbance values were measured using UV-Vis spectrophotometer Evolution 60, Thermo-Fisher Scientific (USA).

Measurement of the sun protection factor (SPF) in silico

For *in silico* measurements, the BASF Sunscreen simulator, a freely available tool at the webpage: https://sunscreensimulator.basf.com/Sunscreen_Simulator/ was used. On the platform, the user can after choosing the UV filter and entering the concentration, obtain the *in silico* predicted SPF values.

Measurement of the sun protection factor (SPF) in vitro

In order to evaluate the efficiency of tested UV filters spectrophotometric method developed by Mansur was used. Seven ethanol solutions containing different

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concentrations of selected UV filters were prepared: Ethanol solutions with 5%, 10% and 20% EMC, solutions with 5% and 10% BP3 as well as solutions with combinations of these UV filters - 5% EMC + 5% BP3 and 10% EMC + 10% BP3 were made. Exactly 0.5 g of each solution was transferred to a 50 mL volumetric flask and diluted with ethanol. After that, 2.5 mL were transferred to a 25 mL volumetric flask and filled with ethanol, and finally, 2 mL of the obtained dilution was transferred to a 10 mL volumetric flask and diluted with ethanol. The absorbance of the obtained dilutions was recorded in the range of 290 – 320 nm at 5 nm intervals, and SPF was calculated using equation (1) developed by Mansur et al.

$$SPF_{spectrophootometric} = CF \times \sum_{290}^{320} EE\,(\lambda) \times I\,(\lambda) \times Abs\,(\lambda) \quad (1)$$

CF – correction factor (=10), EE (λ) – erythmogenic effect of radiation with wavelength λ, I (λ) – solar intensity spectrum, Abs – absorbance of the solution. Values EE x I are constants and were determined by Sayre et al.

Calculations and statistical analysis

The *in vitro* calculations of SPF were done in Microsoft Office Excel, while the statistical analysis (Spearman’s rank-order correlation test) was performed using IBM SPSS Statistics 20.

Results and discussion

UV filters used in sunscreens can be chemical (organic) that act as UV absorbers and physical (inorganic) that block UV rays through scattering and reflection. BP3 and EMC are chemical UV absorbers widely used in sunscreens (Serpone, 2007).

The maximum concentration of BP3 in cosmetics according to updated Annex VI of EU Cosmetic Regulation 1223/2009 is 6%, while the maximum allowed concentration of EMC is 10%. Before the update in 2021, the maximum concentration for BP3 used to be 10% and for EMC 20% (Cosing database) So, the aim of this study was to compare these UV filters in their maximum concentration used over the decades and to discuss whether results of *in silico* predictions and *in vitro* calculations can be related.

The SPF values obtained after *in vitro* measurements (the SPF values predicted for solutions are presented in brackets) were for BP3 5% solution 12.773 (14.3), BP3 10% solution 5.774 (7.4), EMC 10% 16.22 (12.5), EMC 5% 10.047 (7.9), combination BP3+EMC 10% 26.161 (28.9) and combination BP3+EMC 5% 13.808 (15.3). The only value that showed a large difference in relation to the *in silico* predicted values is the SPF for EMC 20% solution – 28.871 (18.9). However, all other spectrophotometrically measured values differed by an average of 2.2 compared to *in silico* calculations. Also, it was observed that *in vitro* SPF values for EMC were slightly higher than expected in *in silico* predictions, while in BP3 the opposite was the case.

Modern *in silico* models for the calculation of the SPF are based on the algorithms used during *in vitro* measurements and the approach used for *in vivo* SPF determination. Besides SPF, they also can be used for the prediction of UVA and blue light protection and are becoming important for the first stage of the process of new sunscreen formulation development (Herzog and Osterwalder, 2011). In this study, statistical analysis has shown a very strong correlation between results, since calculated Spearman's rank correlation coefficient was 0.857.

Conclusion

The results have shown a very strong correlation between the SPF values obtained *in vitro* and *in silico*, which speaks in favor of these methods being suitable as an alternative to *in vivo* methods.

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