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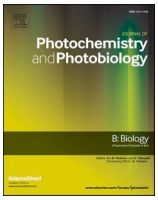

A new visible light absorbing organic filter offers superior protection against pigmentation by wavelengths at the UVR-visible boundary region

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ABSTRACT

Skin pigmentation by solar ultraviolet radiation (UVR; ~295–400 nm) is well established. More recently, visible light (VL; 400–740 nm) has been shown to induce rapid pigmentation. Such pigmentation is thought to be caused by oxidative stress, which has associations with skin cancer and photoageing. However, the UVR-VL boundary region has been less well studied. The lower back of healthy Fitzpatrick skin type II-IV individuals was irradiated with increasing doses of narrow-band 385 nm and 405 nm radiation. Pigmentation change was measured immediately, 6 h and 24 h post-irradiation using two reflectance spectroscopy devices and visual grading. Pigmentation was dose-dependently increased in all skin types and time points for both spectra. Two sunscreens, both labelled SPF 15 and UVA protective in the EU and USA (but with different Boots star rating in the UK, 2* *vs* 5*) were compared. Their formulations were the same apart from the addition of a new organic filter bis-(diethylaminohydroxybenzoyl benzoyl) piperazine (BDBP) that absorbs between 350 and 425 nm. The product that lacked BDBP provided minimal protection against pigmentation, but its addition provided almost complete protection. This demonstrates the needs to improve photoprotection at the UVR-visible border and for sunscreens to act as neutral density filters.

1. Introduction

Terrestrial solar radiation is a continuum that can be divided into defined wavebands including ultraviolet radiation (UVR), as well as visible light (VL) and infrared radiation (IR). The effects of UVR on the skin are well established, particularly within the UVB (280–315 nm) waveband. There has also been research on the effects of UVA1 (340–400 nm). However, the effects of very longwave UVA1 (*e.g.*, >380 nm) and VL (400–740 nm), that are abundant in sunlight, are much less studied. VL penetrates the skin much deeper than UVR, potentially interacting with a wider range of chromophores. One established endpoint, for both UVR and VL is pigmentation.

Skin colour (constitutive pigmentation) determines photobiological consequences, many of which seem less harmful in darker skin types [1], and is principally defined by the type, quantity and epidermal distribution of melanin that is synthesised in melanocytes. There are two major melanin types; eumelanin and pheomelanin. Fair skin has lower concentrations of melanin, contained in melanosomes clustered in keratinocytes. Darker skin has more melanin, with an increased ratio of eumelanin: pheomelanin, and is dispersed more evenly in keratinocytes as opposed to in clusters [2,3]. Evidence suggests that pheomelanin is a potential photosensitiser, resulting in an increased skin cancer risk in those with fair skin [4]. Constitutive skin pigmentation offers photoprotection, especially in darker Fitzpatrick skin types (FST) V and VI that have evolved to reside in zones of elevated insolation, and have increased skin cancer resilience [1]. UVR Sensitivity is determined by the minimal erythema dose (MED). Fair-skinned individuals may have a MED of 1–3 standard erythemal doses (SED) compared to deeply pigmented individuals who require up to 10 times this dose [5].

Facultative, or acquired pigmentation (tanning) is a photoadaptive response whereby sunlight exposure promotes skin darkening above the constitutive baseline level. Such pigmentation is thought to afford photoprotection by the UVR absorbing and scattering properties of melanin. Three discrete kinds of acquired tanning exist: immediate pigment darkening (IPD), persistent pigment darkening (PPD) and delayed tanning (DT). IPD appears immediately post-UVR exposure as a greyish hue, remaining for several hours. It is believed to occur by polymerisation and oxidation of melanin and its precursors (*e.g.*, 5,6-dihydroxyindole (DHI) and 5,6-dihydroxyindole-2-carboxylic acid (DHICA)). Action spectroscopy for IPD shows a UVA peak, most likely a

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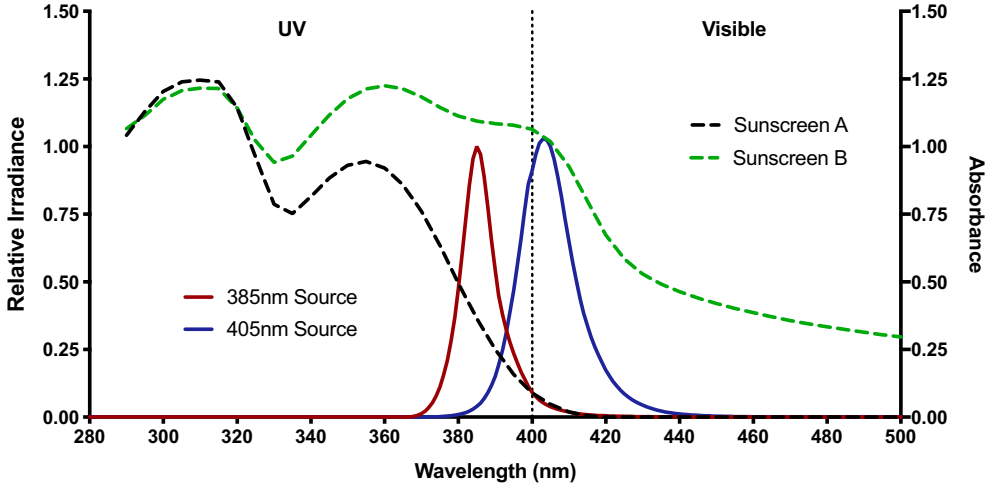

Fig. 1. Spectral outputs of the radiation sources and optical properties of sunscreen formulations. The absorbance spectra of sunscreen formulations tested in the photoprotection studies were simulated using the BASF sunscreen simulator [25]. Sunscreen B contains BDBP. See Table 1 for sunscreen formulations.

result of the increased potential for UVA to generate free radicals/ROS compared to UVB, as free radical formation measured *in vivo* in human skin also shows a similar peak in the UVA region [6]. PPD, which is brownish, follows IPD with UVA doses >10 J/cm^2. It lasts from several hours to days. Neither IPD nor PPD results in significant increases in melanin synthesis [2,7], unlike DT, that typically takes up to 7 days to develop as new melanin, is formed by melanocytes in the basal layer and transferred into the upper layers of the epidermis, and lasts for several weeks. The action spectrum for DT peaks about 300 nm [8], but high UVA doses will also induce melanin synthesis [9]. Pigmentation attained over the spring/summer months can decrease sunburn sensitivity by a factor of two, and UVR exposures over 4-weeks raises the MED by 1.5 [5].

IPD, PPD and DT are induced by UVR and VL [10–13], especially in darker FST (III-VI), such as Asian skin types that tan readily with pigmentation lasting many weeks [14,15]. Mahmoud et al. (2010) showed that UVA and VL exposure produced pigmentation, but VL gave 'darker and more sustained pigmentation' than UVA, lasting for 2 weeks after irradiation. However, the VL dose needed to produce a just perceptible pigmentation was around four times greater than that of UVR [16]. This validates a study by Porges *et al*, who demonstrated that VL pigmentation was present for around 10 days in FST II-IV, and Rosen *et al*, who showed IPD occurring at wavelengths up to 470 nm in FST IV-VI [17,18]. More recently, it has been demonstrated that repeated VL exposure increases pigmentation more significantly than single exposures, with increased tyrosinase activity signifying a response comparable to that of UVR-induced DT [19]. Pigmentation induced by longwave UVR/blue light (λ_{Max} = 420 nm) has also been demonstrated histologically through a substantial increase in melan-A positive cells (a melanocyte differentiation antigen) after 5 days of irradiation with a cumulative dose of 100 J/cm^2 [20]. Differences in pigmentation response between UVR (λ_{max} = 311 nm), blue (λ_{max} = 450 nm) and green (λ_{max} = 530 nm) light have also been assessed in FST I-III *ex vivo* skin cultures. The authors reported that both blue and green light induced melanogenesis in all skin types, however UVR did not induce pigmentation in FST I. Furthermore, blue and green sources did not induce DNA damage (cyclobutane pyrimidine dimers (CPD)) or apoptosis, which were observed with UVR [21]. However, other studies have demonstrated that blue light (narrowband 405 nm or broad blue 400–500 nm) decreases cell viability, increases oxidative stress and expression of number of genes linked to oxidative stress, photoageing, inflammation and opsin 3 (OPN3) gene expression [22,23]. Studies have also shown the potential for the UV–visible border region to induce delayed CPD, an emerging area of photobiological research [24].

Table 1
Protection factors and ingredients of test formulations.

Sunscreen test	Sunscreen A	Sunscreen B
SPF Measurement (ISO24444:2010)	15.1 (±2.1)	18.5 (±2.6)
SPF Calculation (Computational)	15	15.8
UVA-PF	5.2	15.8
UVA Protective (Europe)	Yes	Yes
UVA Protective (USA)	Yes	Yes
Boots Star Rating	2*	5*

Sunscreen Ingredients			
Trade name	INCI	A %	B %
Cetiol B (BASF)	Dibutyl Adipate	10.0	10.0
Lanette O (BASF)	Cetearyl alcohol	1.00	1.00
Emulgade Sucro (BASF)	Sucrose polystearate & Hydrogenated polyisobutene	3.00	3.00
Eumulgin Prisma (BASF)	Disodium Cetearyl Sulfosuccinate	0.50	0.50
Uvinul T150 (BASF)	Ethylhexyl Triazone	1.50	1.00
Uvinul A Plus (BASF)	DHHB	2.00	2.00
Water	Aqua	71.8	63.6
Glycerin (Merck)	Glycerin	3.00	3.00
EDTA BD (BASF)	Disodium EDTA	0.20	0.20
Keltrol RD (CP Kelco)	Xanthan Gum	0.50	0.50
Water	Aqua	3.00	3.00
Tris Amino Ultra Pure (Angus Chemie)	Tromethamine	1.50	0.70
Eusolex 232 (Merck)	PBSA	2.00	1.50
Protectol PE	Phenoxyethanol	1.00	1.00
BDBP Dispersion*	Bis-(Diethylaminohydroxybenzoyl Benzoyl) Piperazine	0.00	10.0

*BDBP dispersion ingredients		
Compound	INCI	%
Water	Aqua	41.65
BDBP	Bis-(Diethylaminohydroxybenzoyl benzoyl) piperazine	50
Plantacare UP2000	Decyl glucoside	4.95
Texapon K14S Special	Sodium myreth sulfate	1
Citric Acid	Citric acid	0.4
Silfoam SE 2	Polydimethylsiloxan	1
Luviskol K30	Polyvinylpyrrolidone	1

The details of the ingredients (including UVR filters) included within the formulations are defined, along with the respective SPF, UVA-PF and ratings for UVA protection in Europe, US and the Boots star rating (UK).

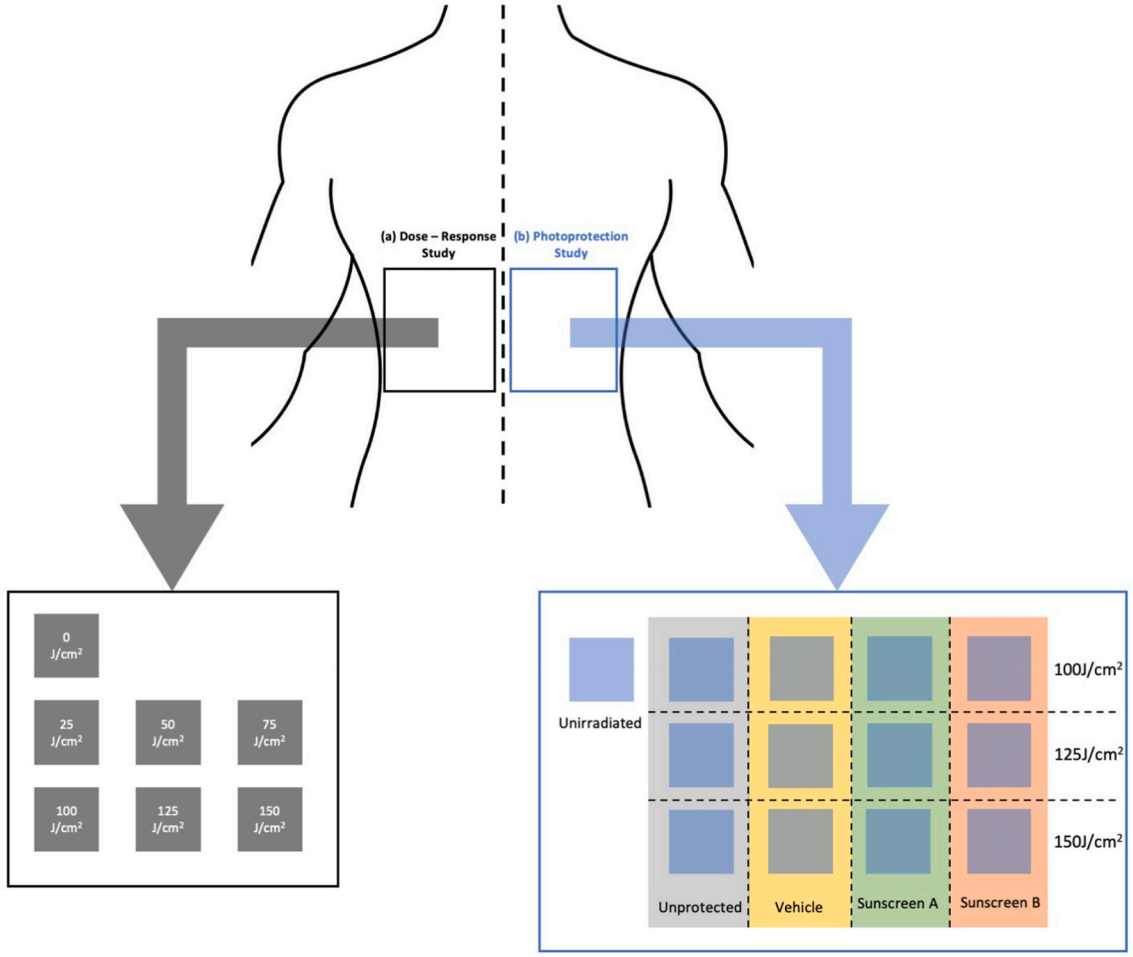

Fig. 2. Study layout for dose response and pigmentation photoprotection studies. The scheme for irradiation to measure the (a) dose response relationships and (b) pigmentation protection factors. The goal was to assess different formulations' capacity to inhibit 385 nm and 405 nm induced pigmentation.

The aims of this study were to (i) demonstrate the dose-response for pigmentation in FST II-IV by UVR-VL border wavelengths (ii) determine the ability of a new organic UVR filter to inhibit this pigmentation.

2. Materials and Methods

2.1. Ethical Approval and Recruitment

The study was conducted in accordance with the Declaration of Helsinki Principles approved by the National Research Ethics Service (NRES) London City and East (Ref 15/LO/0380) and the Guy's and St Thomas' NHS Foundation Trust Research and Development Department. Volunteers (n = 9), of FST II-IV, gave written informed consent. The average age was 26.42 ± 4.85 (SD) years, with a 50/50 gender split.

2.2. Radiation Sources

Loctite LED flood array systems (Loctite, Henkel Ltd., UK) with peak output at 385 and 405 nm (full width at half maximum (FWHM) of 6 nm and 10 nm respectively) were used for all experiments. Each array has 144 LEDs with an exposure surface of 97 mm × 96 mm. Emission spectra were determined with a DM120BC double monochromator spectroradiometer (Bentham Instruments, Reading, UK) with an integration sphere, calibrated by Public Health England (PHE) at the Centre for Radiation, Chemical and Environmental Hazards (CRCE), against a UK national standard. The spectral irradiances of both sources are shown in Fig. 1. Irradiance was regularly checked with a Loctite UVA/Vis radiometer (Loctite, Henkel Ltd., UK) calibrated against the spectroradiometer readings.

2.3. Formulations

Two sunscreens (~SPF = 15) were formulated by BASF (BASF GmbH, Grenzach-Wyhlen, Germany). Their absorbance spectra are displayed in Fig. 1 and characteristics described in Table 1. Uvinul A Plus, PBSA and Uvinul T150 are UVR filters approved by regulatory agencies used in numerous commercial sunscreen formulations. Sunscreen A was a conventional sunscreen formulation and Sunscreen B contained bis-(diethylaminohydroxybenzoyl benzoyl) piperazine (BDBP) which is a new BASF organic nanoparticle UVR filter. This filter has been regarded as safe by the European Commission (EC) Scientific Committee on Consumer Safety (SCCS). The size of the BDBP particles was 75 nm ± 28 nm (average ± SD), based on the number-weighted size distribution assessed with FOQELS (fibre-optic quasi-elastic light scattering) [26].

Both sunscreens were oil/water formulations with similar SPFs. SPFs were determined experimentally by Dermscan (Gdansk, Poland) according to the ISO24444:2010 methodology and regulations. *In vivo* SPFs were calculated as SPF 15.1 ± 2.1 and SPF 18.5 ± 2.6 (average ± SD, n = 5) for sunscreens A and B respectively. Simulated SPFs were calculated as SPF15 and SPF 15.8 respectively using the BASF sunscreen calculator tool [27]. Both sunscreens had different UVA/UVB ratios and UVA-PF, however both passed the European and USA requirements for labelling as UVA protective and would appear identical to the consumer.

Table 2
Linear regression analysis significance assessment of FST dose-response slopes for both sources and different timepoints.

Source	Time	FST II	FST III	FST IV	FST pooled
385 nm	0	**<0.0001**	**0.0007**	**<0.0001**	**<0.0001**
	6	**0.0206**	**0.0017**	**0.0040**	**0.0002**
	24	**0.0024**	**<0.0001**	**0.0015**	**<0.0001**
405 nm	0	**0.0003**	0.0997	**<0.0001**	**0.0044**
	6	0.2330	0.6514	**0.0001**	**0.0357**
	24	**0.0117**	0.6514	0.0928	0.0674

Significant responses are in bold.

However, sunscreen B had a higher Boots UK star rating (5*) than sunscreen A (2*). A vehicle control was also assessed alongside the test formulations. Although the SPF of the vehicle control was not tested experimentally, it is thought to be negligible based on previous experience.

2.4. Irradiation and Photoprotection Procedures

2.4.1. Dose-Response and Time-Course

Irradiation sites (1 cm^2) on the lower back were exposed to 0–150 J/cm^2 (with 25 J/cm^2 increments) of 385 nm and 405 nm, from a distance of 15 cm. Doses were selected based on pre-existing literature and *in vitro* experiments [19] and are attainable environmentally in a tropical climate on a mid-summers day (*e.g.* Rio de Janeiro) [28]. Irradiation sites were cooled with a fan to prevent skin overheating.

2.4.2. Photoprotection

Formulations were applied at 2 mg/cm^2 with a finger cot (application area 2.5 cm × 10 cm, 50 mg ±1 mg applied), after cleaning skin with ethanol wipes, and left to dry for 10 min. Areas of 1 cm^2 of skin were then irradiated with 0, 100, 125 and 150 J/cm^2 with or without sunscreens or vehicle. Ethanol wipes were used to remove sunscreens immediately after exposure. The study layouts are displayed in Fig. 2.

2.5. Pigmentation Assessment

Pigmentation changes were determined by a UV-Optimize 555 reflectance spectrometer (Matic, Naerum, Denmark) at 0, 6- and 24-h post-irradiation. This device quantifies reflected light after irradiation with 555 nm and 660 nm light to determine levels of pigmentation and redness respectively on scale from 0% to 100%. Details of the device are described elsewhere [29,30]. Three measurements of each site were made and the percentage change in pigmentation was calculated compared to baseline readings to correct for natural baseline variation in skin colour. Pigmentation was also assessed visually and with a Minolta CM-700d reflectance spectrophotometer (Konica Minolta Sensing Inc., Osaka, Japan). Similar results were observed with all three methods (data not shown). Photographs were taken for illustration purposes.

2.6. Statistical Analysis

All data are expressed as the mean ± standard error of the mean (SEM) where $n \geq 3$. Statistical analyses were performed using Graphpad Prism 9.0 (Graphpad, San Diego, CA) and were assessed using linear regression analysis and one-way and two-way ANOVA with Tukey's multiple comparisons test [31]. Slope of linear regression (response *vs.* dose) was used as a single endpoint at a given time for data presentation and comparisons. Significance was defined as: $*p \leq 0.05$, $**p \leq 0.001$, $***p \leq 0.0001$.

2.7. Protection Factor Calculations

Linear regression of outcome (increase in pigmentation) *versus* irradiation dose was used to generate a slope for each experimental condition. The ratios of slopes (untreated control/treatment) were used to calculate protection factors on a person by person basis and average values (±SD) calculated. This approach takes all the data into account.

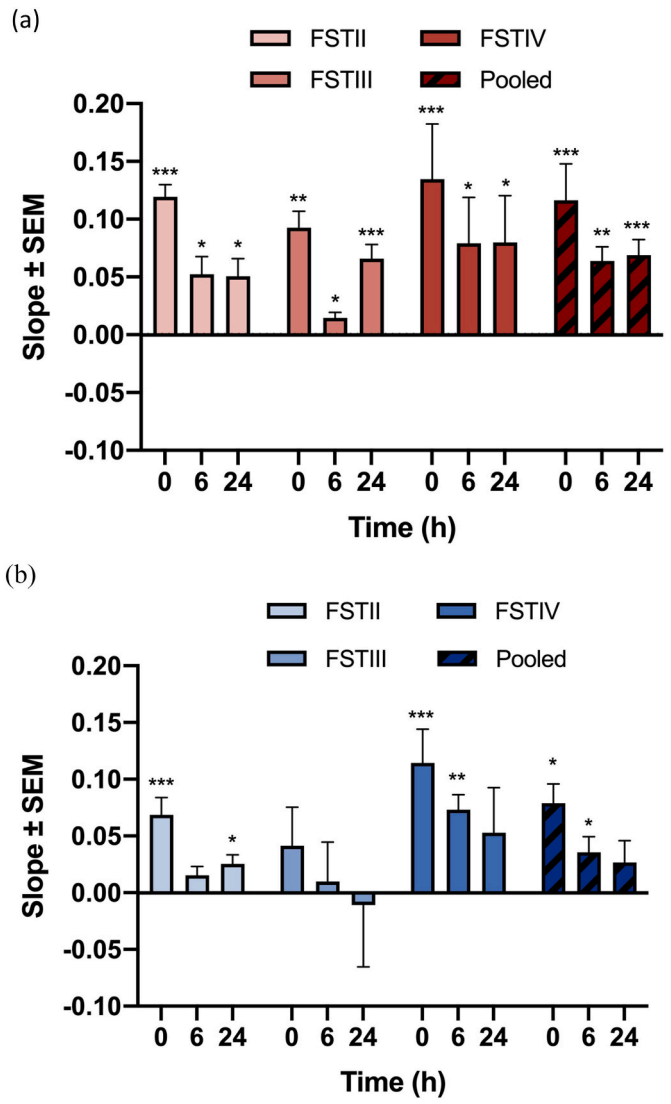

Fig. 3. Dose-responses for increased pigmentation by 385 nm and 405 nm radiation. Individuals of Fitzpatrick skin type II - IV were assessed for their pigmentation response to (a) 385 nm or (b) 405 nm radiation exposure (0–150 J/cm^2) using an UV-Optimize 555 reflectance spectrometer and slopes of response were calculated. Data points signify mean ± SEM ($n = 3$). Data were assessed by linear regression analysis. Significance values were defined as $***p < 0.001$, $**p < 0.01$, $*p < 0.05$.

3. Results

3.1. Dose Response

The 385 nm waveband significantly increased pigmentation dose-dependently for all timepoints (Table 2), although the greatest increase was observed immediately after exposure with a reduction over time (Fig. 3). Comparisons of skin type with the 385 nm source showed no significant difference ($p > 0.39$, two-way ANOVA) so data were pooled for subsequent experiments. The 405 nm source was less effective at inducing pigmentation, with a significant increase only observed immediately in FST II and IV, at 6 h in FST IV and 24 h with FST II. Lack of significance at other times and skin types was partially due to larger

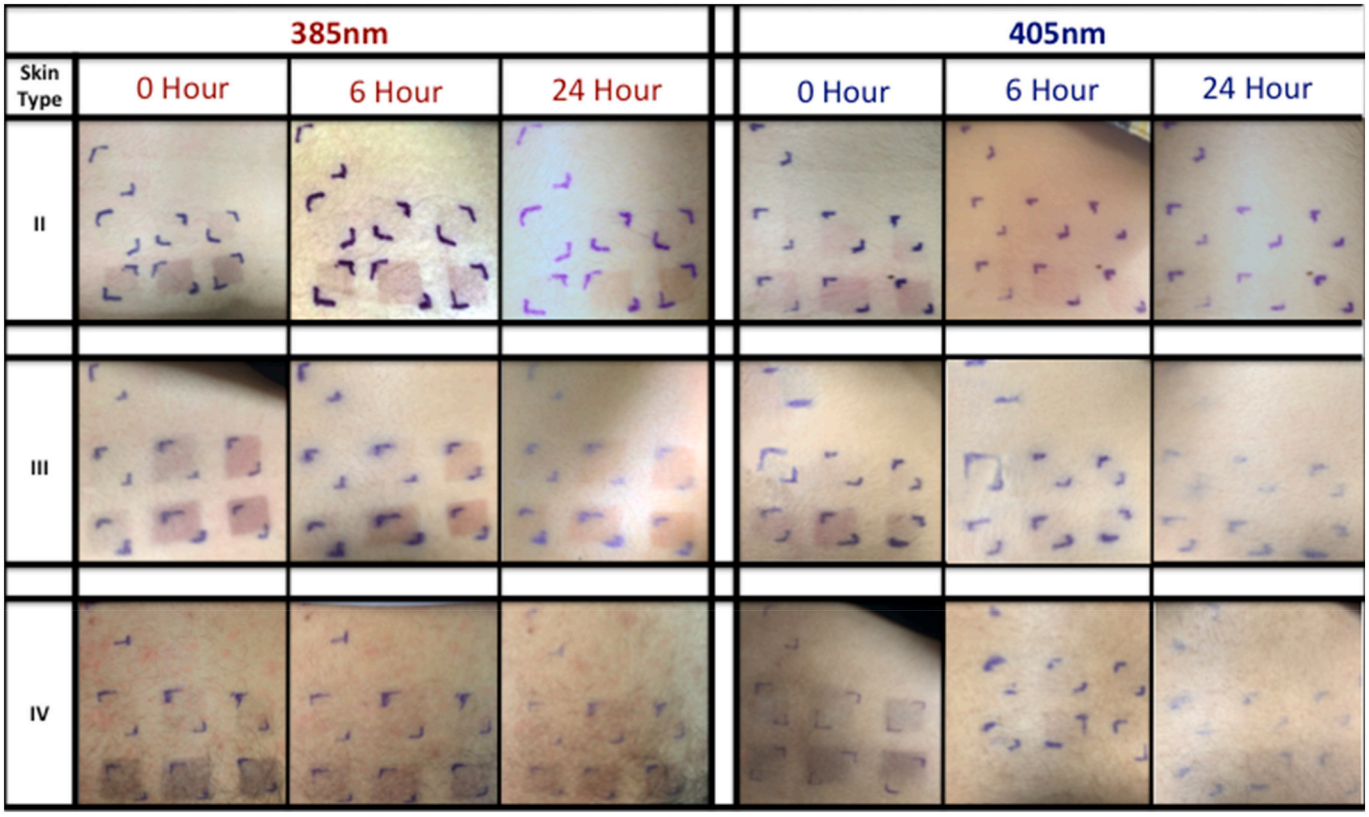

Fig. 4. Representative images of responses for each skin type and time point tested. After exposure to 385 nm and 405 nm irradiation (0–150 J/cm^2), skin responses in different skin types (II–IV) were assessed at different time points post exposure (0, 6 and 24 h). Site layout is displayed in Fig. 2a.

variation and a lesser response. As with the 385 nm source, there was no significant difference between skin types ($p > 0.07$, two-way ANOVA) so responses were also pooled. Despite a visual difference suggesting 385 nm was more efficient than 405 nm at inducing pigmentation, statistically there was no difference ($p > 0.05$, *t*-test). Example photographs are shown in Fig. 4. Raw dose response data is displayed in Supplementary Fig. S1 & S2.

3.2. *Photoprotection Against Pigmentation*

Prevention of pigmentation by three different formulations was assessed; two sunscreens with same SPF but different spectral properties, and a vehicle control that lacked any UVR filters (Fig. 5). The vehicle treatment had either no effect or slightly enhanced pigmentation compared to the irradiated control when comparing mean values, although statistically this was insignificant. Sunscreen A (conventional formulation) reduced pigmentation compared to the unprotected and vehicle control, but some pigmentation was still present. Sunscreen B (conventional formulation with the addition of BDBP) gave almost complete inhibition of pigmentation, offering significantly more protection than sunscreen A for the 385 nm source (<0.04, one-way ANOVA with Tukey's multiple comparisons test). While the same experiment with the 405 nm source appeared to provide some additional protection with sunscreen B when compared with sunscreen A, this was not statistically significant (>0.06, one-way ANOVA with Tukey's multiple comparisons test). Pigmentation protection factors were calculated comparing the slopes of the unprotected site against each treatment and displayed in Table 3. The vehicle site typically gave protection factors less than 1, suggesting enhanced damage over the unprotected site. Sunscreen A gave protection factors of 1–1.5 and sunscreen B 2.3–5.1. Representative photographs for each FST are displayed in Fig. 6.

4. Discussion

Many people sunbathe to achieve deeper pigmentation. However, others prefer to avoid pigmentation, especially in Asia where paler skin is desirable [32,33], and those prone to pigmentary disorders such as melasma [34,35]. Longwave UVA1 (385 nm) and VL (405 nm) induced dose-dependent pigmentation in FST II-IV. 385 nm was more effective than 405 nm, with a slope ratio of 1:0.65–0.81 (depending on FST), demonstrating that both wavelengths triggered responses. This effect was immediate, implicating IPD. Pigmentation was also present, to a lesser extent, at 24 h, indicating PPD. Although not determined over a longer time frame, informal follow-up with volunteers signified pigmentation lasted for >6 months, implicating DT. This confirms previous work reporting similar results with UVA1 and broad-spectrum VL [16,19] and narrowband LEDs (450 nm & 530 nm) [21,36], inducing pigmentation in darker FST. Narrowband blue light LED ($\lambda_{Max} = 415 \pm 5$ nm) has also recently been reported as a regulator of pigmentation in melanocytes through OPN3 [22,37]. Other opsins may be relevant for VL responses including blue opsin (OPN1-SW) and green opsin (OPN2), both found in skin keratinocytes and melanocytes, while UVR exposure is thought to interact with neuropsin (OPN5), also found in both cell types [38].

These results demonstrate the need for photoprotection in the UVR/VL region to prevent unwanted pigmentation. Photoprotection was assessed using three formulations: (i) a vehicle control, (ii) Sunscreen A - a conventional SPF 15 sunscreen and (iii) Sunscreen B - a sunscreen of equivalent labelled SPF that contained a new UV/visible radiation filter. The latter showed complete protection with both spectra at all time points and doses tested. The conventional sunscreen formulation provided modest protection but still allowed a significant dose-dependent pigmentation increase. Variation in protection factors was large in some cases, particularly with the BDBP formulation. This is largely

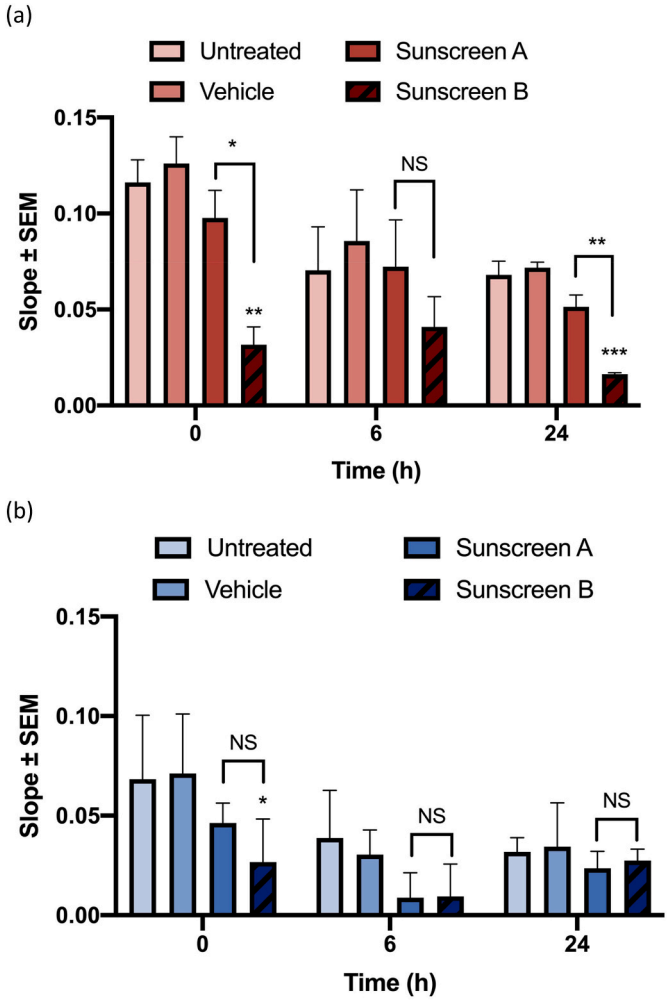

Fig. 5. Photoprotection against pigmentation in all FST pooled scored with the Optimize device. The Optimize score of pigmentation with (a) 385 nm and (b) 405 nm sources at 0, 6 and 24 h was measured for each condition: untreated, vehicle control, Sunscreen A and Sunscreen B. Dose-response data were analysed by linear regression analysis calculated slopes are plotted. Comparisons are made between all conditions against the no sunscreen control and between sunscreen A and B (mean ± SEM, $n = 3$; one-way ANOVA with Tukey's multiple comparisons test; $^{***}p < 0.001$, $^{**}p < 0.01$, $^{*}p < 0.05$).

because this formulation provided almost complete protection but, in addition, variation is expected in *in vivo* studies, especially with a small sample size. There was no obvious variation in responses between volunteers when assessed visually as constitutive pigmentation plays a role in masking some of the differences, so the change in pigmentation was used for analyses to account for this variation. The differences in prevention of pigmentation between formulations was clear which was the aim of this study.

Duteil et al. demonstrated VL-induced pigmentation and its inhibition by three different mineral based sunscreens formulated for VL protection. Measurable differences were found between the formulations, but no comparison was made with more conventional formulations [13]. Their pigmentation protection factors, obtained with broad-spectrum VL, were similar to those reported here. More recently, a study on a sunscreen with visible iron oxide pigments (yellow, red, black and white) and UVR filters showed reduced hyperpigmentation [39]. The formulation blocked the whole visible region, and it is unclear how cosmetically acceptable this would be, particularly in darker FST. Conversely, another study investigated iron oxide containing formulations and found a dual role both preventing visible light induced

Table 3
Pigmentation protection factor calculations.

385 nm			405 nm		
Condition	Slope (±SD)	Protection factor (±SD)	Condition	Slope (±SD)	Protection factor (±SD)
Immediate					
Unprotected	0.12 (0.02)	1.00 (0.00)	Unprotected	0.07 (0.06)	1.00 (0.00)
Vehicle	0.13 (0.02)	0.93 (0.07)	Vehicle	0.07 (0.05)	0.96 (1.11)
Conventional	0.10 (0.02)	1.20 (0.09)	Conventional	0.05 (0.02)	1.48 (0.70)
BDBP	0.03 (0.02)	5.13 (4.38)	BDBP	0.03 (0.04)	2.56 (5.55)

385 nm			405 nm		
Condition	Slope	Protection factor	Condition	Slope	Protection factor
6 Hours					
Unprotected	0.07 (0.04)	1.00 (0.00)	Unprotected	0.04 (0.04)	1.00 (0.00)
Vehicle	0.09 (0.05)	0.93 (0.45)	Vehicle	0.03 (0.02)	1.28 (5.58)
Conventional	0.07 (0.04)	0.99 (0.04)	Conventional	0.01 (0.02)	1.35 (4.29)
BDBP	0.04 (0.03)	2.35 (1.58)	BDBP	0.01 (0.03)	4.10 (4.80)

385 nm			405 nm		
Condition	Slope	Protection factor	Condition	Slope	Protection factor
24 Hours					
Unprotected	0.07 (0.01)	1.00 (0.00)	Unprotected	0.03 (0.01)	1.00
Vehicle	0.07 (0.01)	0.94 (0.12)	Vehicle	0.03 (0.04)	0.93 (2.78)
Conventional	0.05 (0.01)	1.33 (0.20)	Conventional	0.02 (0.01)	1.35 (1.17)
BDBP	0.02 (0.001)	4.21 (1.01)	BDBP	0.03 (0.01)	1.16 (0.62)

The slopes of each condition were calculated from linear regression analysis and the pigmentation protection factor was calculated as the ratio between the unprotected site over the treated site slopes (±SD).

pigmentation and masking existing pigmentation in individuals with FST III and above [40]. Both niacinamide (vitamin B3) and a microalgae extract have also been shown to reduce 450 nm induced pigmentation [36], along with Fernblock®, an aqueous extract of *Polypodium leucotomos*, which reduced photooxidation of melanin precursors and activation of OPN3 in melanocytes after irradiation with blue light [22].

The vehicle had no effect in some volunteers or slightly enhanced pigmentation in others, with both sources. This can be seen in the average protection factor values that demonstrated a generally consistent reduced protection factor with the vehicle control compared to the unprotected site for most conditions tested. However, the standard deviation was large, so this effect was not statistically significant. As discussed, there are several reasons for the large error values, so while further investigation is required to confirm this observation, it should not be dismissed. Vehicle enhancement of skin photocarcinogenesis in a mouse model has also previously been reported [41,42]. This suggests that some topical skin treatments, such as moisturisers, that lack UVR filters, may potentially cause more photodamage than no treatment. We propose this is likely due to a reduction in the scattering effects of the skin when products are applied topically.

Some argue, based on action spectroscopy, that sunscreens should be biased towards UVB protection with absorption decreasing with increasing wavelength [43]. In contrast, others believe that the model

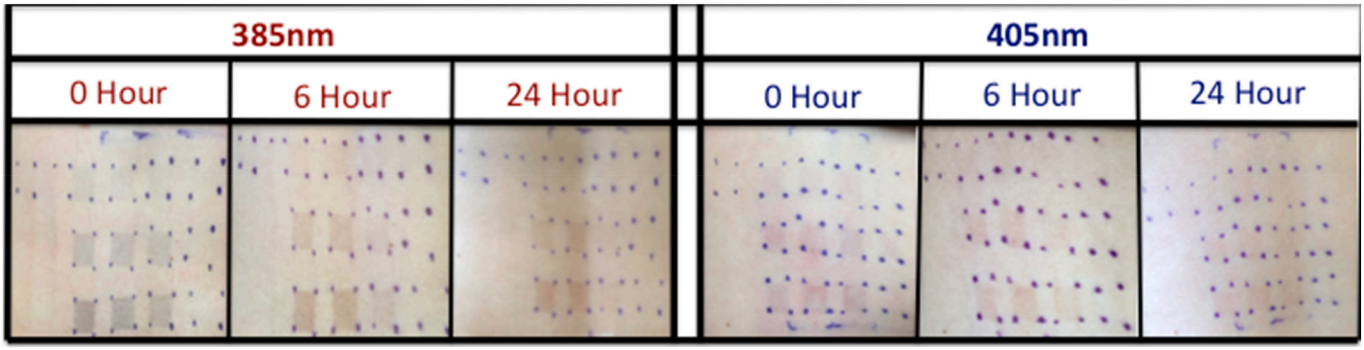

Fig. 6. Representative response over time for the pigmentation photoprotection study. The responses of one volunteer (FST II) to both 385 nm and 405 nm over time. Photographs are for illustrative purposes only – no data were obtained from the images. Differences in base colours are due to photographs being acquired in different environments. Detailed site layout is displayed in Fig. 2b. Briefly, each row represents an increasing dose of radiation (50–150 J/cm^2) and each column is representative of a different treatment, from left to right: untreated, vehicle, sunscreen A, sunscreen B.

sunscreen should be a neutral density filter [44,45]. The data reported here support the latter view, demonstrating the requirement of broad-spectrum protection. They also show that sunscreen labelling may be inadequate – two identically UVA-labelled sunscreens may provide actually provide very different levels of protection against a biological endpoint, with no indication of these differences to the consumer. This also shows the limitations of suncreen testing, where longwave UVA and visible wavelengths are not represented in the regulatory approved sunscreen testing spectrum, with mounting evidence this region contributes to the damaging effects on the skin [23,46]. This work also demonstrates potential for use in photosensitivity diseases. The disease erythropoietic protoporphyria (EPP) is associated with severe painful photosensitivity caused by wavelengths in the 400–420 nm region [47]. Similarly, many patients with solar urticaria are sensitive to longwave UVA/shortwave visible light [48]. Currently there are few treatment options available with conventional sunscreens demonstrating little protection, and the use of “Dundee cream”, while demonstrating efficacy, is unpleasant, difficult to apply and cosmetically unacceptable [49,50]. This new UV/VL filter and formulation may provide an improved treatment option for such patients.

The strengths of this study are the use of well-defined spectrally pure long-wave UVA1 and VL sources. Its weaknesses are the lack of assessment of pigmentation beyond 24 h and the small sample size of the photoprotection arm with different skin types. However, the dose-response study showed no statistically significant FST differences in slope at any time point with each source. It could be argued it would have been better to have used higher SPF sunscreens, but SPF 15 was selected for several reasons. Its application at 2 mg/cm^2 represents SPF test conditions. However, an SPF of about 15 is what might be achieved with typical use of SPF 30–50 sunscreens when products are applied at lower application densities [51,52]. Importantly, an SPF of 15 allowed the biggest difference in spectral properties with the addition of BDBP (see Fig. 1), that would optimize assessing the effect of longer wavelength protection. Finally, as this was a clinical study, a much higher SPF sunscreen would have required much higher irradiation doses requiring impractical exposure times and increased possibility of inadvertent skin photodamage.

In conclusion, this study demonstrates that 385–405 nm induces pigmentation in FST II-IV. This spectral region is typically less well protected by sunscreen formulations and is not represented in regulatory-approved sunscreen testing radiation sources. The addition of a new organic filter (BDBP) which absorbs in the UVR/VL border region, affords significantly more protection against pigmentation than a conventional formulation with the 385 nm source (with some evidence of improved protection with the 405 nm source). Both sunscreens had the same SPF, labelled as UVA protective in Europe and the USA. Better protection in the UVR/VL region is necessary to prevent unwanted pigmentation and improved *in vivo* and *in vitro* testing protocols are required to fully assess the prevention of solar radiation induced damage by sunscreen products.

Declaration of Competing Interest

BH and SA are employees of BASF GmbH who funded this research. KPL studentship was funded by BASF GmbH. KPL, SA, BH and ARY are named inventors on a patent relating to BDBP. RPES declares no conflict of interest.

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Appendix A. Supplementary Data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jphotobiol.2021.112372.

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