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Review Article

Sunscreens: A review

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ABSTRACT
Sunlight despite of source of life and energy creating major health challenges like sunburn, pigmentation, wrinkles, dermatitis, urticaria, ageing, immune-suppression and number of skin cancers too. Sun protective clothes and or sunglasses provide insufficient and less convenient approach to get rid of all these health hazards. So sunscreen protection is popular mean among various regions of world. Present article have summarize types and classification, regulations, terminologies, evaluation methods, labeling, dosage and controversies of sunscreens. Natural chemical classes like phenolics (tannins, flavonoids), carotenoids, vitamins, oils are also discussed.

Key words: UV rays, SPF, COLIPA, IPD, PPD, ISO, Polyphenols, Antioxidant.

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INTRODUCTION

In India, cosmetic is defined as any article intended to be rubbed, poured, sprinkled, or sprayed on, or introduced into, or otherwise applied to the human body or any part thereof for cleansing, beautifying, promoting attractiveness or altering the appearance, and includes any article intended for use as a component of cosmetic.[1] Now-a-days one cosmetic product category sunscreen have gain wide popularity due to additional health benefits apart from beautification.[2-3] Either separate sunscreens or many other sunscreen loaded cosmetic products for skin care, hair care, lips care and eye care are available in market.[4-7] This review is tried to summarize all possible issues related to sunscreens.

Ultra-Violet radiations and human skin[8-9]

Ultraviolet (UV) radiation is defined as that portion of the electromagnetic radiation lies between X-rays and visible light which is from 200 to 400 nm. This ultraviolet radiation comprises 3 categories depending on wavelength as follows:

- **UV-A Radiation**: This radiation ranges between 320 to 400 nm. UV-A is most responsible radiation for immediate tanning or darkening of the skin due to excess production of melanin in the epidermis, premature photo ageing, suppression of immunologic functions, and even necrosis of endothelial cells and damage of dermal blood vessels.
- **UV-B Radiation**: This radiation ranges between 280 to 320 nm. UV-B radiations are known as burning rays as they are 1000 times more capable of causing sunburn than UV-A. UV-B rays act mainly on the epidermal basal cell layer of the skin but more genotoxic than UV-A radiations. Ultraviolet B (UVB) rays vary with time and season are major cause of sunburn. Sunburned skin is a leading risk factor for melanoma and non-melanoma skin cancer.
- **UV-C Radiation**: This radiation ranges between 200 to 280 nm. UV-C radiations are filtered by stratospheric ozone layers so less effective and hazardous.

The human skin is the largest organ of the body of surface area of approximately 1.5–2.0 m2. Skin acts as effective barrier against the harmful effects of environmental and xenobiotic agents.[9-10] Among all factor chronic exposure of UV radiations is key factor in instigation of skin problems like cracks, burns, immune suppression, wrinkles, dermatitis, urticaria, ageing, hypopigmentation, hyperpigmentation and most complicated skin cancers.[11] Role of infrared radiations in skin damage is unclear.

Mechanism of photoreaction

Photo-oxidative mechanism depending on light-driven reactive oxygen species (ROS) generation is now accepted to cause skin photoaging and photocarcinogenesis.[12] UVA rays mediated photo-oxidative damage effectively reaches through the upper layers of skin into the human dermis and dermal capillary system. Substantial protein and lipid oxidation occurs in human skin epidermis and dermis together with a significant depletion of enzymatic and non-enzymatic antioxidants in the stratum corneum, epidermis and dermis. The immediate as well as persistent pigment darkening (IPD or PPD) responses of human skin are due to photo-oxidation of pre-existing melanins and its precursors respectively. Also up-regulation of hemeoxygenase-1 (HO-1), ferritin, glutathione peroxidase, Cu–Zn-dependent superoxide dismutase (SOD1), manganese-dependent superoxide dismutase (SOD2), and catalase occurs after solar irradiation.[13]

UV rays contact initiates photo oxidative reactions to activate protein kinase C enzyme and reactive oxygen species which further reacts with protein lipids and DNA to form cyclobutane pyridine dimmers. This leads to erythema, edema, skin sunburn and cell apoptosis. UV irradiation activates cell surface growth factor and cytokine receptors on keratinocytes and fibroblasts in human skin, critical in the regulation of cell proliferation and survival.[14] UV-driven formation of H2O2 regulates the tyrosine kinase activity of the epidermal growth factor receptor (EGF–R) and emerging evidence suggests the inhibition of protein tyrosine phosphatases as a consequence of UV-induced ROS formation. According to response to sun radiation Fitzpatrick's skin type classification[15] is most popular for decision of types of skin:

Protection:

Use of physical barriers[16] to sunlight like sun protective clothing, sunglasses, hats, umbrella, shade and possible avoidance of sunlight can be

Skin Phototype	Cutaneous reaction to UVR
I	Always burns
	Never tans
II	Always burns easily
	Tans minimally
III	Burns moderately
	Tans moderately
IV	Burns minimally
	Tans easily
V	Rarely burns
	Tans easily and substantially
VI	Almost never burns
	Tans promptly and intensely

very common options for sun protection but sunscreens are most preferred and predominant mode of sun protection due to various societal reasons like ease of application and higher efficacy of protection.[17-18] Many animals (e.g. elephant uses mud as a physical barrier to block the UV-rays and thus sunburns.

Sun protective clothing:[19] Sun protective clothing are generally evaluated on the basis of Clothing indices which is actually a UV protection factor (UPF) i.e the ratio of average effective UV radiation irradiance transmitted and calculated through air to the average effective UV radiation irradiance transmitted and calculated through fabric. Fabric UPF is similar to sunscreen SPF, except that during testing instead of sunscreen fabric is used to protect the skin. Such indices consider erythema as endpoint to determine how much longer a person can stay in the sun when fabric covers the skin and expresses in form of following grades:

Grade	UPF
good protection	15 to 24
very good protection	25 to 39
excellent protection	40 to 50+

Sun protective sunglasses:[20] Sun protective sunglasses are only means to protect delicate eyes from harmful effects of sun radiations. Their protection efficacy is generally evaluated on the basis of amount of light transmitted through a sunglass lens which is called as luminous transmittance. According to the Australian Standard (AS/NZS 1067:2003) sunglasses are classified as follows:

Luminous transmittance	Category	Class
80-100%	0	Fashion spectacles: providing some or no protection from UV radiation but no reduction in sunglare
60-80%	1	Fashion spectacles: providing protection from UV radiation and limited reduction of sunglare-not suitable for driving at night.
35-60%	2	Sunglasses for general use: provides medium protection from UV radiation and sunglare
10-35%	3	Sunglasses: providing good protection from UV radiation and reduces sunglare
3-10%	4	Sunglasses: providing a high level of UV radiation protection and reduced sunglare but must not be used when driving.

Australian Radiation Protection and Nuclear Safety Authority (ARPANSA) together developed eye protection factor (EPF) number ranges from 1 to 10 with respective to percent of blockage of sunrays. Sunglasses labeled EPF of 9 or 10 transmit very little UV radiation. But choice of sunglasses should depend on individual visible quality and which allows pupil to normal in light.

Sunscreens: Sunscreens are cosmetic products to protect skin from damage mediated by sunlight radiation.[21] Topical sunscreen which either absorbs or reflects radiations to protect skin from harmful effects of radiations unable to give complete sunscreen potential to organs like eyes, lips.[22] While oral sunscreen products or constituents are also available in market to be consume to avoid skin damage. Following are types or classification[23-24] of sunscreens:

Sunscreen Types on the basis of Mode of application

Topical	Organic	1. UVB filters
		PABA derivatives-Padimate O
		Cinnamates-Octinoxate, Cinoxate
		Salicylates-Octisalate, Homosalate, Trolamine salicylate
		Octocrylene, Ensulizole
		2. UVA filters
		Benzophenones (UVB and UVA2 absorbers)-Oxybenzone, Sulisobenzone, Dioxybenzone
		Avobenzone or Parsol 1789 (UVA1 absorber)
		Meradimate (UVA2 absorber)
		3. Broad spectrum (UVA+UVB) filters-Ecamsule (Mexoryl SX), Silatriazole (Mexoryl XL), Bemotrizinol (Tinosorb S), Bisoctrizole (Tinosorb M)
	Inorganic	Inorganic agents function by reflecting, scattering or absorbing UV radiation.. titanium dioxide (TiO2), kaolin, talc, zinc oxide (ZnO), calcium carbonate, and magnesium oxide
	Natural chemicals	Polyphones (tannins, flavonoids), lycopenes, fixed oils, volatile oils protects skin from UV-induced free radical generated damages by scavenging reactive oxygen species
Oral/Systemic		Phenolics, flavonoids, tannins, carotenoids, vitamins like chemicals on oral consumption exhibit antioxidant effect and thus give photo protective action.

Sunscreen Regulations

Sunscreens are evaluated generally one of following method and fulfills labeling conditions as per countries guidelines.

- ***US-FDA method***: The FDA proposal measures *in-vitro* UV transmittance through a sunscreen film using the critical wavelength method. Sunscreen products offering primarily UVB protection would have a critical wavelength less than 320 nm, whereas those providing both UVB and UVA protection would have critical wavelengths between 320 and 400 nm. FDA requires that sunscreen products have a critical wavelength of at least 370 nm (the mean

value must be equal to or greater than 370 nm) to be labeled as providing "broad spectrum" UVA and UVB protection.[25]

- ***UK method of boot star rating***: The UK method, called as Boots star rating system, also measures the UV transmittance through a sunscreen film. The substrate for measurement is abraded PMMA plates. The ratio between the mean UVA and UVB absorbance measured before and after irradiation of the sunscreen products is calculated.[26]
- ***Australia***: Australian standard (AS) method uses spectrophotometer for measurements of the solar radiation transmitted by a sunscreen product to yield a percentage of UVA radiation absorbed by the product. According to this test, a product is designated as a long wave protector only if it transmits less than 10% of the incoming UV radiation between 320 and 360 nm.
- ***European countries***: COLIPA is an association within the cosmetic industry that voluntarily initiates the harmonization of labeling and product testing activities for sunscreen products. COLIPA guidelines are dedicated mainly to liquid and emulsion-type sun protection products. The test for UVA protection factors (UVAPF) evaluation should be based on the assessment of UV transmittance through a thin film (0.75 mg/cm^2) of the sunscreen sample spread on a roughened substrate, before and after exposure to a controlled dose of UV radiation from a strictly defined UV source. This method allows *in-vitro* measurements of UVAPF values, which are shown to co-relate quite well with *in-vivo* results, determined with PPD method.[27]
- ***International Organization for Standardization (ISO):*** It is an independent, non-governmental international organization in Geneva with a membership of 162 national standards bodies.[28] Following are different methods of ISO for sunscreens:
- ***ISO 24443:2012*** specifies an "*in-vitro*" procedure to characterize the UVA protection of sunscreen products. Specifications are given to enable determination of the spectral absorbance characteristics of UVA protection in a reproducible manner. In order to determine relevant UVA protection parameters, the method has been created to provide a UV spectral absorbance curve from which a number of calculations and evaluations can be undertaken. This method relies on the use of *in-vivo* SPF results for scaling the UV absorbance curve.
- ***ISO 24442:2011*** specifies an "*in-vivo*" method for assessment of the UVA protection factor (UVAPF) of topical sunscreen products. It is applicable to cosmetics, drugs and other products intended to be topically applied to human skin, including any component able to absorb, reflect or scatter UV rays. ISO 24442:2011 provides a basis for the evaluation of sunscreen products for the protection of human skin against UVA radiation from solar or other light sources.
- ***ISO 24444:2010*** specifies a method for the *in-vivo* determination of the sun protection factor (SPF) of sunscreen products. This International Standard is applicable to products that contain any component able to absorb, reflect or scatter ultraviolet (UV) rays and which are intended to be placed in contact with human skin. ISO 24444:2010 provides a basis for the evaluation of sunscreen products for the protection of human skin against "erythema" induced by solar ultraviolet rays.

In below mentioned countries Sunscreens are evaluated generally by one of above methods and fulfills labeling conditions as per countries guidelines.[29]

- ***India***: Indian being Asian population comes under Type–IV skin pattern which burns minimally and tans easily. Freckles are rare but still use of sunscreen is necessary to avoid tan. Indian regulations date from the Indian Drug and Cosmetic Act (1940) as amended from time to time considers sunscreens as cosmetics. Bureau of Indian Standards (BIS), a participating member of the ISO, sets the relevant cosmetic product standards. Key points are stability data is (similar to Australia) must and there is no maximum SPF rating for sunscreens.
- ***Japan***: Japan Cosmetic Industry Association (JCIA) provides self regulated standards. JCIA is a signatory to the COLIPA International SPF test method and JCIA has adopted ISO standards as they are published. For SPF, ISO 24444 is accepted. In Japan, for UVA, *in-vivo* testing is required and labelling is according to ratings of Protection Grade of UVA (PA) i.e PA +, PA++ and PA +++. Additionally, PA++++ was also added from 1st January 2013.
- ***China***: Sunscreens are regulated under the Hygienic Standard for Cosmetics 2007. Currently sunscreens can only be labeled up to SPF 30+. The product must be labeled in Chinese language and have a Chinese name. Water resistance norms should be followed if labelled.

Terminologies associated with Sunscreens[30-34]

- ***In-vivo* sunburn protection factor (SPF):** The Sun Protection Factor can be defined, as proposed by the FDA in 1978, as the numerical ratio between the minimal erythemal dose (MED) of sunscreen-protected skin, applied in the amount of 2 mg/cm^2 and the Minimal Erythemal dose of unprotected skin, a mathematical relation that can be represented by the equation: SPF=MED (protected skin)/MED (unprotected skin)
- ***In vitro* Sunburn Protection Factor (SPF *in vitro*):** The absolute protection performance of a suncare product against erythemal-effective UV radiation, calculated from the measured *in vitro* transmittance and weighted with the erythema action spectrum and with the "standard" output spectrum of a UV solar simulator used for SPF testing.
- ***In-vitro* UVA protection factor (UVAPF)**: The absolute UVA protection afforded by a suncare product, calculated from the measured *in-vitro* transmittance after irradiation and weighted with the PPD action spectrum and with the "standard" output spectrum of a UVA-filtered solar simulator.
- ***In-vitro* UVA protection factor before UV exposure UVAPF:** The *in-vitro* UVA protection factor measured before sample UV exposure. It is derived from the transmittance curve of the unexposed sample, weighted with the PPD action spectrum and with the "standard" output spectrum of a UVA-filtered solar simulator, after adjustment to the labeled SPF.
- **PFA (Protection Factor UVA) or UVA-PF (UVA Protection Factor):** The ratio of PPD of protected skin to PPD of unprotected skin.
- **Critical Wavelength Value (λc):** The critical wavelength λc value for the test product is defined as that wavelength where the area under the absorbance spectrum for the irradiated product (obtained using the method described above) from 290 nm to λc is 90% of the integral of the absorbance spectrum from 290 nm to 400 nm.
- **UVA-UVB Ratio**: Absorption of a 1.3 mg/square cm film is measured between 290 nm and 400 nm. The ratio of areas under the curve between 290-320 (UVB region) is compared with the area under the curve between 320 nm and 400 nm. Pre-irradiation of the sample is required. (Calculated as TPF x UVA/UVB). Various substrates can be nominated.

- **COLIPA (European Union):** This technique involves measurement of UVAPF/SPF Ratio and Critical Wavelength. UVA-PF shown to correlate quite well with *in-vivo* results, determined with PPD method.
- **Boots star rating system:** The method used by Boots in the UK (not mandated). Absorption of a 1 mg/square cm film is measured between 290 nm and 400 nm. Pre irradiation of the sample is required. Rating scale is 3 to 5 stars. More stars mean more protection (by ratio) in the UVA are as follows:

Mean UVA/UVB ratio	Star Rating Category
0.0 to 0.59	No Rating
0.6 to 0.79	* * *
0. 8 to 0.9	* * * *
0.9 and over	* * * * *

- **Immune protection factor (IPF):** ability of sunscreen products to prevent UV-induced immune-suppression. IPF is assessed by complex methods such as the ability of a sunscreen to inhibit either the sensitization or elicitation arm of contact or delayed-type hypersensitivity reactions to allergens such as dinitrochlorobenzene (DNCB) and nickel, respectively. IPF is considered to correlate better with the UVA-protectiveness of a sunscreen than with its SPF.
- **Broad spectrum sunscreen:** Critical wavelength > 370 nm and UVA protection factor > 4
- **Water-resistant sunscreen:** Maintains the labeled SPF value after two sequential immersions in water for 20 min (40 min)
- **Very water-resistant sunscreen:** Maintains the labeled SPF value after four sequential immersions in water for 20 min (80 min)

Evaluation Methods

In 1934, Friedrich Ellinger determined the minimal erythemal dose (MED) from protected and unprotected skin by evaluating the protective efficacy of sunscreens using mercury lamp radiation on both forearms and expressed a coefficient of protection that decreased in value to the extent that protection increased. In 1956, Rudolf Schulze proposed "Schulze Factor" which been used for decades in European countries, as a reference in the evaluation of sunscreens. Schulze Factor is exposure time required for the induction of erythema on sunscreen protected and unprotected skin by incremental doses of sunlight like radiation emitted from lamps. In 1974, Greiter introduced the term Sun Protection Factor (SPF) to "Schulze factor." From then till now SPF is popular term in evaluation of sunscreens.[35] In 1978, the North-American regulatory agency (FDA) proposed the first normatization to determine the Sun Protection Factor (SPF). Following are newly accepted and followed methods of evaluation of sunscreens:

In-vitro methods

Many regulatory agencies, such as the US Food and Drug Administration (USFDA) and The European Cosmetic Toiletry and Perfumery Association (COLIPA), mandate *in-vivo* testing on human subjects, using an erythemal endpoint to determine the SPF of a topical sunscreen. The *in-vivo* tests are costly and time-consuming and may not be practical for routine product evaluation. The UV-1000S is designed to make the evaluation of SPF a simple and routine analytical procedure performed within the formulation laboratory.[31] Although *in-vivo* testing is mandatory to make a product label claim for SPF, an investment in the UV-1000S will insure that only one *in-vivo* test will have to be performed for each particular formulation. The measurement of an *in-vitro* SPF can be performed by measuring the diffuse transmittance in the ultraviolet spectrum of a carefully prepared sample. There are two objectives in a sample preparation method. The first is to simulate the application conditions used for *in-vivo* testing, both the applied quantity and substrate interaction. This would produce a reliable *in-vitro* SPF value that would positively predict the result of a subsequent *in-vivo* test. The second objective is for the method to be consistent enough to generate reproducible results sample-to-sample for the same sunscreen formulation. The spectral transmittance of a sunscreen in the ultraviolet spectral range can be used to predict an *in-vitro* SPF value based on standard erythema and solar data.[32] The Boot's Star and critical wavelength methods for categorizing the effectiveness of UVA absorbers are also performed from spectrophotometric data. Any pre-irradiation of samples to evaluate their photostability, needs to be performed with a controlled dose from a solar simulator. The flash lamp used in the UV-1000S does not expose samples to excessive light doses, keeping the spectrophotometric analysis independent of any photostability issues.[33] The recommended amount of sunscreen to apply in both FDA and COLIPA *in-vivo* methodologies is 2 mg/cm^2 or 2 µL/cm^2. Most sunscreens have a specific gravity of almost unity. The area of applicant on is measured and then the corresponding amount of sunscreen is measured using a pipette (volume) or weighed by loss. The ideal substrate for *in-vitro* SPF needs to be fairly transparent to the ultraviolet and simulate the porosity and texture of human skin, the *in-vivo* substrate. Suitable *in-vitro* substrates range from human epidermis and mice epidermis to sausage casings and natural lamb condoms. Substrates that are commonly used are Transpore, Vitro-Skin, Roughened Quartz Plate, polymethylmethacrylate (PMMA) plates, and PTFE (Teflon).[34]

1. *In-vitro* SPF determination (absorbance measurement) by UV-Spectrophotometer[35]

Weigh 1 g of all samples, transfer to a 100 mL volumetric flask, dilute to volume with ethanol, followed by ultrasonication for 5 min and then filter through cotton, rejecting the first ten mL. Transfer a 5.0 mL aliquot to 50 mL volumetric flask and dilute to volume with ethanol. Then transfer a 5.0 mL aliquot to a 25 mL volumetric flask and complete the volume with ethanol. Measure the absorptions of samples in solution in the range of 290 to 450 nm with every 5 nm increment using 1 cm quartz cell, and ethanol as a blank. Calculate average of three determinations and calculate SPF by Mansur equation. EE* I values are constant and given in Table 1.

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

Where: EE (I)–erythemal effect spectrum; I (l)–solar intensity spectrum; Abs (l)–absorbance of sunscreen product; CF-correction factor (=10).

Table 1: Normalized product function used in the calculation of SPF

Wavelength (λ) in nm	EE x I (normalized)
290	0.0150
295	0.0817
300	0.2874
305	0.3278
310	0.1864
315	0.0839
320	0.0180
	Total 1

2. *In-vitro* Determination of SPF by UV 2000S Ultraviolet Transmittance Analyzer (Labsphere)

The principle based on the sample transmittance measurement, where transmittance is defined as the ratio of the illumination passed through a sample to the illumination impaging on the sample. *Procedure*: Weigh 100 mg of the investigational sample and spread on the 56 cm^2 area to obtain a sample even film thickness of 2 µl/ cm^2 on Transpore Tape as suggested in the operation manual of the UV-2000S Ultraviolet Transmittance Analyzer for the sample preparation and application technique.[33-34] Expose the prepared sample to Xenon flash lamp for determining the Sun Protection Factor as follows:

$$SPF = \frac{\int_{290}^{400} E\lambda\, S\lambda\, d\lambda}{\int_{290}^{400} E\lambda\, S\lambda\, d\lambda}$$

Where, E (λ) is the erythema action spectrum, S(λ) is the solar spectral irradiance, T(λ) is the spectral transmittance of the sample with the integral is calculate across the 290-400 nm wavelength limits.

Critical wavelength method/Broad spectrum rating method United States (FDA):[33-34] Critical wavelength is the wavelength, at which 90% of the area under the extinction curve between 290 and 400 nm are obtained or just a measure of the 'breadth' of UVA protection using a test method called 'critical wavelength'. The higher the extinction in the UVA, the higher will become λc. This is proposed alternative to the Boots Star System. This evaluates the uniformity of a sunscreen product's absorbance spectrum. The result is based on a number called the critical wavelength which is determined spectrophotometrically from the absorbance spectrum. The technique is not as sensitive to sample preparation as the *in-vitro* SPF or Boots Star measurements, since it only depends on the relative values of spectral absorbance and not the absolute values. In this test proposal, the absorbance of the thin film of the sunscreen is integrated (summed) from 290 nm across the UV wavelengths until the sum reaches 90% of the total absorbance of the sunscreen in the ultraviolet region (290-400 nm). The wavelength at which the summed absorbance reaches 90% of total absorbance is defined as the 'critical wavelength' and is considered to be a measure of the breadth of sunscreen protection. Filters are then classified as 'broad spectrum', having a significant part of their absorbance in the UVA, when the critical wavelength is longer than 370 nm. The critical wavelength is defined across the 290-400 nm spectrums by the following equation:

$$\lambda C = \text{Min}(\lambda') \quad 0.9 \le \frac{\sum_{\lambda}^{\lambda'} = 290\, A_{\lambda}}{\sum_{\lambda=290}^{400} A_{\lambda}}$$

Where, A (λ) is the absorbance at wavelength λ and results of broad spectrum rating method of United States should be predicted as follows:

λc	Level of Protection
340 nm ≤ λχ<370 nm	Some (UVA/UVB)
λc>370 nm	More (broad-spectrum)

UVA/UVB ratio: A recent concern with the SPF rating system for sunscreens is that it is based on erythema as an endpoint. Therefore, active ingredients that serve primarily as UVB blockers substantially improve a product's SPF. There is a need to add a product label system that describes the UVA protection offered in addition to the SPF. The spectral transmittance values, Tλ, are converted to spectral absorbance values Aλ=-log (Tλ). A term called the UVA ratio is calculated as the ratio of the total absorption in the UVA to that in the UVB.[33-34] The star rating, and its associated claim for UVA protection, is determined as follows:

UVA Ratio	Star Category Descriptor
0.0 to <0.2	Too low for UVA claim
0.2 to <0.4	Moderate
0.4 to <0.6	** Good
0.6 to <0.8	*** Superior
≥0.8	**** Maximum

Another stipulation to using this method is to first evaluate the photostability of a sunscreen formula containing UVA absorbers. The samples must be pre-irradiated, using a solar simulator light source before the spectral transmittance is measured. The pre-irradiation exposure dosage is measured in units of MED (minimal erythemal dose) and is equal to one third of the SPF value for the particular formulation under test. The photostability concerns for certain sunscreen formulas support the use of a flashlamp in the Labsphere UV-1000S. Pre-irradiating samples must be done with a continuous source whose spectrum and exposure are closely monitored. The light source of the analyzing spectrophotometer should not be used for sample irradiation. Broad Spectrum Rating method relies only on the shape of the UV absorption spectrum and not on its amplitude. The problem of this test is quite small correlation with *in-vivo* results.[32-33]

In-vivo methods

Following are commonly used in-*vivo* methods for SPF determination. All three methods have somewhat similar procedure except their endpoints and expression of results. Procedure and endpoints are as follows:

Procedure: Human volunteers are irradiated with a UVA light source (320÷400 nm) and skin changes, yielding in a immediate or persistent pigment darkening or eryhema or tanning are observed after desired time following irradiation has been stopped.

Observations: Within 60 sec after each exposure (IPD test), and again approximately 2 h after exposures (PPD test) and 16-24 h after exposures (PFA), the irradiated sites were evaluated under bright "warm while" illumination (approximately 1000 Lux at 6000 K) for pigmentation response and erythema, using the following scales:

Pigmentation	Erythema
0=No response	0=No response
0.5=Equivocal response	0.5=Equivocal response
1.0=Unambiguous dark grey or brown pigmentation	1.0=Unambiguous erythema
1.5=Well define dark gray or brown pigmentation with sharp borders	1.5=Well defined erythema with sharp borders
2.0=Deep pigmentation	2.0=Bright erythema

- *IPD (Immediate Pigment Darkening) by Kaidbey and Barnes:36* where UVA protection factor from the ratio of the sunscreen protected minimal immediate pigment darkening dose to the un-protected minimal immediate pigment darkening dose within 60 sec after each exposure is determined. Endpoint for this method is pigmentation producing a grade ≥1 within 1 min after each UVA exposure.
- *PPD (Persistent Pigment Darkening) by Chardon et al.:37* where UVA protection factor from the ratio of the sunscreen protected minimal immediate pigment darkening dose to the un-protected minimal persistent pigment darkening dose, evaluated approximately 2 h after UVA exposure is determined. Endpoint for this method is pigmentation producing a grade ≥ 1, 2 h following UVA exposure. The advantage of the PPD method, when comparing with

IPD, is that the residual colour that has developed after exposure to the radiation is stabilized and allows more precise readings.

- *PFA (Protection factor in UVA) by Cole et al:38* where UVA protection factor from the ratio of the sunscreen protected minimal response dose (eryhema or tanning) to the unprotected minimal response dose, approximately 24 h after UVA exposures is determined.
- *PPF (Phototoxic Protection Factor) by Lowe et al:33* where ratio of sunscreen protected minimal phototoxic dose, measured 72 h after UVA exposure. This method uses 8-methoxypsoralen to increase sensitivity of UV-light. Endpoint for this method is erythema or tanning producing a grade ≥ 1 within 16-24 hr after UVA exposure.

Photo stability evaluation of sunscreens[39]

From study it is observed that after exposure to sunlight many sunscreen chemicals undergo degradation and losses their photo protective properties and thus efficacy of product. Hence it is must to determine photo stability of sunscreens. Photo stability evaluation is done by measuring area under the curve index [AUCI] of sunscreens after either natural UV exposure (UVnat) or artificial UV exposure (UVart). Weigh 0.5 mg/cm^2 of sunscreen and place between two plates of polished fused quartz silica with diameter 25 mm and thickness 5 mm. Expose samples for 120 min in outdoors especially in sunny weather as UVnat or use any artificial sunlight radiation like lamp source as UVart. Measure absorption as follows: before exposure, after 30 min, 90 min and 120 min exposure of natural or artificial UV sunlight. To eliminate the degradation possibility of the photoactive compounds by a temperature increase, try to heat plate for 20 min at constant temperature of sample upto 50°C ± 2°C on hot plate which is about 15°C higher than the temperature of the skin. The spectra of prior to and after heating should be same if the photoactive chemicals do not undergo degradation. Calculate the AUC for UVB (290-320 nm), UVA1 (340-400 nm), UVA2 (320-340 nm) for each spectrum before [AUC before] and after [AUC after] before and after UVnat. The AUC Index (AUCI), defined as AUCI=AUCafter/AUCbefore. If AUCI greater/equal to 0.80 then sunscreen is considered as photostable.

Dosage and Application[40-42]

It is found that sunscreen efficacy fails due to under application of defined dose or less practice of reapplication after simple wipe, sweating, swimming and or vigorous activity. The dose used in FDA sunscreen testing is 2 mg/cm^2 of exposed skin. If one assumes an "average" adult build of height 5 ft 4 in (163 cm) and weight 150 lb (68 kg) with a 32-inch (82-cm) waist, that adult wearing a bathing suit covering the groin area should apply approximately 30 g (or 30 ml, approximately 1 oz) evenly to the uncovered body area. Larger or smaller individuals should scale these quantities accordingly. Considering only the face, this translates to about 1/4 to 1/3 of a teaspoon for the average adult face. Sunscreen should be applied properly in a concentration of 2 mg/cm^2 to all sun exposed areas and allowed to dry completely before sun exposure. It should be reapplied every 2 h, and after sweating, swimming, vigorous activity or exercise and or after each wipe.

Labeling[43-44]

The critical wavelength of a sunscreen needs to be equal to or higher than 370 nm in order to claim "broad spectrum," and only "broad spectrum" sunscreens with an SPF equal to or higher than 15 can claim benefits against skin cancer and skin aging as directed in the monograph. Sunscreens previously qualified as water resistant now labeled as "water resistant (40 min)." Those previously qualified as very water resistant now labeled as "water resistant (80 min)." Terms such as "waterproof," "sweatproof," and "sunblock" not allowed and prohibition ARE enforced.

Controversies[45-46]

Non-uniformity in SPF and related ratings of sunscreen products confuses consumers. Sunscreens, particularly those with high SPF, may lead to a significant decrease in vitamin D production. Few of sunscreen chemicals like cinnamates, PABA derivatives, benzophenones, and octocrylene observe to cause acute or chronic allergic symptoms. Vary small size inorganic filters found to have percutaneous absorption and endocrine disrupting activity. Blockage of skin pores even causes acne and rosacea like adverse effects. Opaque nature and skin whitening effects are another inherent disadvantage of inorganic filters. Sunscreens cannot be applied on cracked or wounded skin. Pediatric use of sunscreens is under study or with high precautions. Cost of sunscreen products always inclines to higher side and hence year round regular use of sunscreens being expensive cannot be afforded by every population.

Natural chemicals as sunscreens

Natural chemicals like polyphenols (flavonoids, tannins), carotenoids, anthocyanidins, few vitamins, fixed oils, volatile oils from vegetables, fruits, medicinal plant parts (leaves, flowers, fruits, berries), algae and lichens are more effective over synthetic chemicals which is due to their long term beneficial effects especially against free radical generated skin damages along with UV-rays blocking.[47-49] All of these possess strong antioxidant activity. Most of them have moisturizing and cooling (aloe vera juice, fixed oils), antimicrobial (volatile oils), wound healing and anti-inflammatory (polyphenols like curcumin), anticancer (tannins and resveratrol), anti ageing or cell rejuvenating (anthocyanidins, carotenoids, vitamins) type of activities too.[50-52] These all effects make them choice ingredients in cosmetics. Photo-radiation mediated skin damages require multiple protection means to produce long term benefits and avoidance of chronic conditions like cancers. Hence following natural chemicals[47-55] use can be ideal in sunscreen products.

Consumer expectations

Consumers are in demand of all-in-one sunscreen product which should be non-toxic, non-allergic, water or sweat proof, moisturizing, cooling, antioxidant and UV-A as well as UV-B protective with high SPF values.[56] Skin radiating, anti-acne and anti-ageing sunscreens are also in demand. In many sports, players spend their maximum time under sun and hence better served with above mentioned improved products.

Recent technology

Sunscreens are more popular in the form of lotions, creams, gels, sprays, sticks and oils. Recently microsponges, microsphere, dendrimer, liposome, nanoparticle incorporated more photo-stable and effective sunscreens products are available in market. Sunscreens not remain a special cosmetic but many other photo-protective chemicals added cosmetics in hair care (e.g. shampoo), skin care (e.g. moisturisers, foundations and concealers), lip care (e.g. lipsticks, lip balms) and even in eye care (e.g. eye creams) with more than 30 SPF are available in market.[57-58]

CONCLUSION

Thus it can be concluded that there is great market potential for sunscreen chemicals either synthetic or natural or in combination due to awareness of protection from hazardous UVA as well as UVB rays. Photo-stable, uniform UVA/UVB protective sunscreen product with high SPF can be minimum ideal requirement but natural chemicals like polyphenols (flavonoids, tannins), carotenoids, anthocyanidins, few vitamins, fixed oils and volatile oils from vegetables, fruits, medicinal plant parts (leaves, flowers, fruits, berries), algae and lichens are more effective due to their long term beneficial effects especially against free radical generated skin damages along with UV-rays blocking. These natural chemicals incor-

Class	Sources	Mode of action of photo protection
Flavonoids		
Apigenin (5,7,4'-trihydroxyflavone) is a widely distributed plant flavone	Cereal grains and aromatic herbs (parsley, rosemary, thyme), fruits (apples, cherries, grapes), vegetables (beans, broccoli, celery, leeks, onions, barley, tomatoes) and beverages (tea, wine)	Inhibits UV mediated induction of ornithine decarboxylase activity, down-regulates COX-2 expression in macrophages
Chrysin (5,7-dihydroxyflavone), an analog of apigenin, is a natural flavone	Propolis and honey	Inhibits UV mediated induction of ROS
Quercetin (3,5,7,3',4'-pentahydroxyflavone, is one of the most potent antioxidant compounds	Fruits and vegetables (apples, grapes, lemons, tomatoes, onions, lettuce, broccoli, kale, cottonseed etc.), beverages (tea, red wine), herbs (Gingko biloba, Apocynum venetum, Poacynum hendersonii, Opuntia ficusindica) , olive oil, and propolis from bee hives.	Protects skin's antioxidant systems (glutathione peroxidase, glutathione reductase, catalase and superoxide dismutase activities), prevention of UVC radiation-induced liposome peroxidation SPF of quercetin matches to homosalate, a synthetic sunscreen agent
Silymarin is a standardized extract of flavonolignan diastereoisomers silibinin A and silibinin B in a roughly 1:1 ratio, the diastereoisomers isosilibinin A and isosilibinin B, silicristin, and silidianin	Seeds of the milk thistle	Inhibition of UVB-induced oxidative stress, inflammation and suppression of immune system
Genistein (4',5,7-trihydroxyisoflavone,	Soybean isoflavone	Through enhancement of antioxidant enzyme activities and scavenging of oxygen free radicals, specific inhibitor of protein tyrosine kinase, and phytoestrogen
Isoflavones like daidzein, genistein, and glycitein	Byproduct of soybean (*Glycine max* L) oil processing and also present in Red clover (*Trifolium pratense* L.)	Able to inhibit UVB induced keratinocyte death, release of hydrogen peroxide (H2O2), and UVB induced MAPK phosphorylation
Tannins		
Catechins including (−) epicatechin (EC), (−) epicatechin-3-gallate (ECG), (−) epigallocatechin (EGC), (−) epigallocatechin-3-gallate (EGCG), (+) catechin, and (+) gallocatechin (GC)	Green tea, pomegranate, Amla,	Reduces DNA damage and erythema formation due to protection of DNA repair enzymes from inactivation by ROS and due to UVB absorption ability of green tea polyphenolic
Anthocyanidins		
Anthocyanidins mixtures	Colored (range from yellow to purple (except green) fruits, flowers and berries, vegetables, cereal grains, (e.g. Pomegranate (*Punica granatum*)	Inhibits the adverse effects of UVB exposure including translocation of transcription factors NF-kB and AP-1, over expression of the pro-inflammatory cytokine IL-8, cleavage of procaspase-3 (a key step in apoptotic pathway), and DNA fragmentation
Cyanidin 3-glycosides	Citrus species	Protects skin via transcriptional mechanisms of NF-κB and MAPK signaling
Pelargonidin	Strawberries and other berries	Blocks collagen destruction and inflammatory responses via transcriptional mechanisms of NF-κB and MAPK signaling
Carotenoids		
β-carotene, lycopenes	Tomatoes (*Solanum Lycopersicum*), Carrots (*Daucus carota*) and in many red-orange colored fruits and vegetables	As a chain breaking antioxidant in a lipid peroxidation.
Fucoxanthin, astaxanthin	Brown algae	As a chain breaking antioxidant in a lipid peroxidation.
Other poly-phenoilc compounds		
Resveratrol (Trans-3'4'5'-trihydroxystilbine)	Grape (*Vitis vinifera*) Nuts, fruits	Inhibits ODC and COX-2 activity. Inhibit increased level of lipid peroxidation
Curcumin	Roots of *Curcuma longa Zingiberaceace*	Scavenge ROS, by interrupting the activation of protein kinase-C. Enhance glutathione content and GST activity. Inhibit lipid peroxidation and arachidonic acid. Inhibit Ornithin decarboxylase (ODC) activity

Continued.....

Class	Sources	Mode of action of photo protection
Caffeic, coumaric, cinnamic and ferulic acids	Vegetables, Olive oil (*Olea europoea)* Oleaceae, caper (*Capparis spinosa*),	Inhibits formation of hydroxyl radicals, scavenges both hydroxyl and superoxide radicals.
Saffron	Stigmas of crocus flowers (*Crocus sativus*) crocin-(responsible for the color), picrocrocin-(responsible for the bitter taste), and safranal-(responsible for odor and aroma)	Ameliorates antioxidant enzymes and suppresses lipid peroxidation and nitric oxide formation
Usnic acid, 1-chloropannarine, epiphorelic acid I and II, calicin, depsides, and depsidones	Lichens (symbiotic organisms of fungi and algae)	As a chain breaking antioxidant in a lipid peroxidation.
Phlorotannins and dieckol, Plastoquinones, sargaquinoic acid, and sargachromenol	Green red alga	As a chain breaking antioxidant in a lipid peroxidation.
Vitamins		
Vitamin C (Ascorbic acid)	Most fruits and vegetables (amla, papaya, orange, lemon, grapes, tomatoes, mango)	Inhibits solar radiation induced p53 powerful antioxidant enhancer.
Vitamin E (Tocopherol)	Green leafy vegetables and fortified cereals	Interrupts free radical chain reactions by capturing the free radical
Fatty oils	Shea butter, Jojoba oil, Olive oil, Coconut oil, Castor oil, Almond oil, Mustard oil, Chaulmoogra oil, Sesame oil	
Volatile oils	Peppermint oil, Tulsi oil, Lemon grass oil, Lavender oil, Orange oil, Lemon oil, Eucalyptus oil, Tea-tree oil, Rose oil	

porated sunscreens might provide cost effective, truly broad spectrum sunscreen products with antioxidant, wound healing, anti-inflammatory and many more skin protective effects.

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None.

CONFLICT OF INTEREST

Authors do not have conflict of interest.

ABBREVIATION USED

UVA: Ultraviolet-A radiation; **UVB:** Ultraviolet-B radiation; **Ultraviolet-C radiation:** UVC; **IPD:** Immediate pigment darkening; **PPD:** Persistent pigment darkening; **ROS:** Reactive oxygen species; **UPF:** Ultraviolet protection factor; **SPF:** Sunburn protection factor, **PFA:** Protection Factor UVA, **IPF:** Immune protection factor, **MED:** Minimal erythemal dose, **COLIPA:** European Cosmetic Toiletry and Perfumery Association, **ISO:** International Organization for Standardization.

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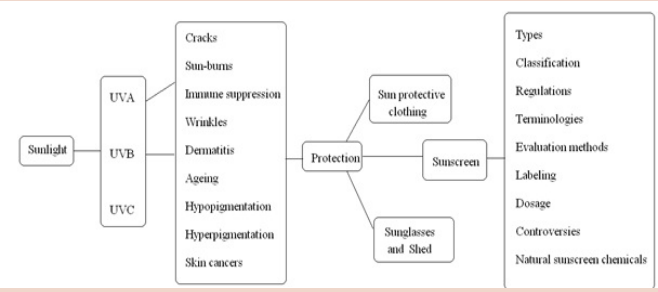
PICTORIAL ABSTRACT



SUMMARY

- Over exposure to sunlight causes number of skin problems.
- There is rise in sunscreen demand all over the world.
- Sunburn protection factor (SPF) is measurable output to calculate efficacy of sunscreens.
- *In vitro* determination of SPF is capable to predict in-vivo efficacy of sunscreens.
- Labeling conditions are different for different regions of world.
- Natural photoprotective chemicals along with antioxidants can provide broad spectrum protection.
- Natural antioxidant chemicals possesses additional antiageing, anti-inflammatory and many more protective effects.
- Consumer demand is of all-in-one sunscreen product.

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