

International Journal of Advanced Biochemistry Research



ISSN Print: 2617-4693
ISSN Online: 2617-4707
NAAS Rating (2026): 5.29
IJABR 2026; SP-10(5): 380-388
www.biochemjournal.com
Received: 23-02-2026
Accepted: 26-03-2026

Shilpa Mamgain
Assistant Professor,
Dev Bhoomi Uttarakhand
University, Dehradun,
Uttarakhand, India

Aditya Raj
Research Scholar, Dev Bhoomi
Uttarakhand University,
Dehradun, Uttarakhand, India

Manjeet Kumar
Research Scholar, Dev Bhoomi
Uttarakhand University,
Dehradun, Uttarakhand, India

Tannu Kumari
Research Scholar, Dev Bhoomi
Uttarakhand University,
Dehradun, Uttarakhand, India

Corresponding Author:
Shilpa Mamgain
Assistant Professor,
Dev Bhoomi Uttarakhand
University, Dehradun,
Uttarakhand, India

Organic, inorganic, and natural sunscreens: Mechanisms of photoprotection and evaluation strategies: A comprehensive review

Shilpa Mamgain, Aditya Raj, Manjeet Kumar and Tannu Kumari

DOI: <https://www.doi.org/10.33545/26174693.2026.v10.i5Se.8390>

Abstract

Sunscreens are vital dermatological and cosmetic substances that are used to protect skin against damage caused by ultraviolet radiation resulting in erythema, photoaging, and photo-carcinogenesis. This review aims to summarize the current knowledge on various types of sunscreen agents, such as organic (chemical), inorganic (physical) and naturally occurring ones with an emphasis on the mechanisms of action and contemporary assessment methods for these materials. While organic (chemical) sunscreens rely primarily on absorption mechanisms by means of conjugated chromophoric system then energy is released through non-radiative transition (internal conversion) and heat dissipation, physical sunscreens such as zinc oxide and titanium oxide are able to reflect, scatter and absorb UV light. The efficacy of these sunscreens is determined by their morphology and particle size distribution. Several plant-based phytochemicals like flavonoids, carotenoids, and phenolic acids that have been developed as natural sunscreens. They offer photoprotection by providing highly effective anti-inflammatory and antioxidant activities without absorbing UV light. The review also emphasizes various assessment methods used to determine the efficacy and safety of sunscreen formulations. These include both *in vivo* methods like minimal erythema dose (MED) and persistent pigment darkening (PPD), as well as *in vitro* approaches like UV spectrophotometry, diffuse transmittance analysis, and others. Parameters like water resistance, photostability, ratio of UVA/UVB and critical wavelength are also assessed according to established international standards. Potential advancements, which can help in improving the performance of sunscreens, are also emphasized in the review. These include hybrid formulation techniques and the use of nanotechnology in sunscreen delivery systems.

Keywords: Sunscreen, UVA, UVB, organic sunscreen, natural sunscreen, physical sunscreen

1. Introduction

1.1 SKIN

The skin covers the entire outer surface and is the largest organ of the human body. In an average adult, it has an area of about 2 m² and weighs around 4.5–5 kg, making up nearly or close to 12 to 15% of total body weight ^[1] and functions as a dynamic biological interface that separates the internal physiological environment from external stressors while actively maintaining homeostasis ^[2]. It is composed of multiple specialized cell types arranged in a highly organized structure that provides mechanical protection and metabolic functionality. In addition to acting as a physical barrier against environmental factors such as ultraviolet radiation, chemicals, and microbial agents, the skin also contributes to innate immune defense. This protective function, however, may be altered under pathological conditions. Melanocytes located in the basal layer synthesize melanin, a pigment that plays a crucial role in photoprotection by absorbing ultraviolet radiation and limiting DNA damage, thereby reducing the risk of skin carcinogenesis.

The skin comprises three main layers: the outer layer epidermis, the dermis beneath it, and the deeper subcutaneous tissue ^[2]. Each of these layers has a different structure and composition depending on its specific function ^[3]

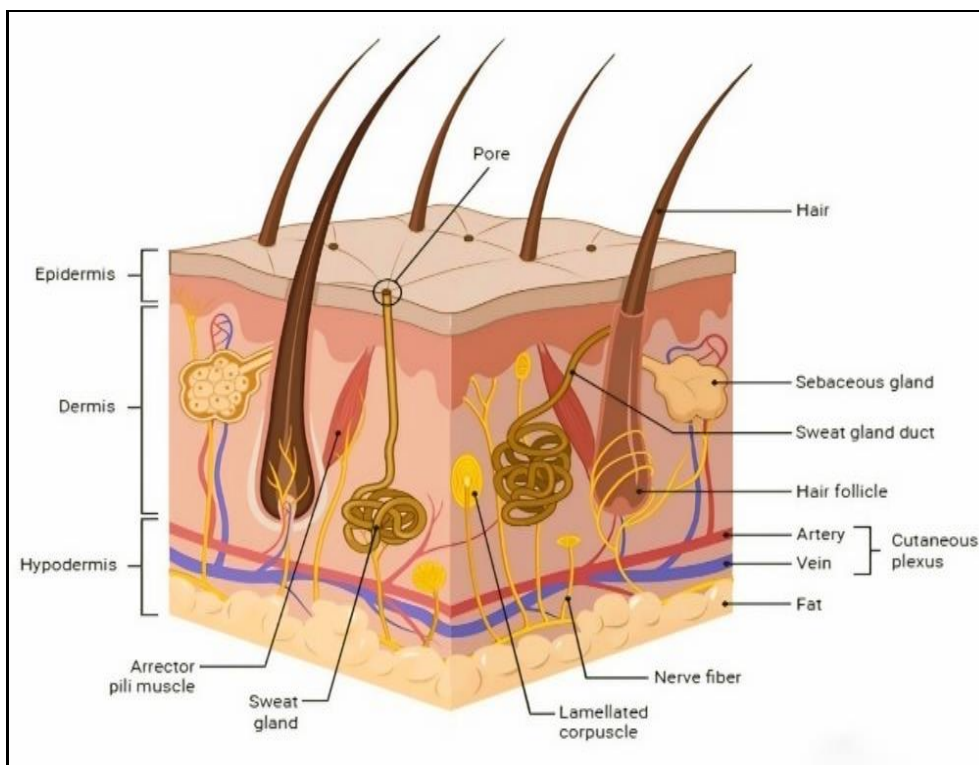


Fig 1: Structure of Skin

1.1.1 Primary function of the skin

The skin's primary functions are:

- Act as the first line of defence against foreign diseases from entering the body.
- Shield from sunlight and other dangerous chemical or physical substances.
- Stop extracellular fluid and water loss.
- Control of body temperature, sensory perception, immunological responses, absorption of certain drugs, and hormone synthesis [4].

1.2 Sunscreen

These are topical formulations developed to minimize the harmful effects of ultraviolet solar radiation by enhancing the skin's inherent defence mechanisms [5]. They function by attenuating incident radiation, thereby reducing its penetration into deeper skin layers and preventing photodamage.

Solar radiation contains ultraviolet, visible, and infrared radiation, of which ultraviolet component possesses the highest energy and most significant biological impact [6, 7]. Although it represents a relatively small fraction of total solar output, UV radiation is a major contributor to both acute and chronic dermatological damage, including erythema, premature aging, and carcinogenesis [8].

A vast range of requirements must be met by the perfect sunscreen composition [9].

- The sunburn-causing rays, which fall between 2900 and 3300 Angstroms, must be absorbed or filtered away.
- It should be stable in the presence of light, air, and moisture, or if it breaks down in these circumstances, the breakdown products should absorb light in the 2900-3300 Angstrom range similarly to the original compound.
- The substance and breakdown products that may be formed under use conditions should be nontoxic and non-irritating.

- It should have very little or no absorption for the long UV photons over 3400 Angstroms, which are thought to cause tanning without noticeable erythema.
- It should be almost neutral so that the presence of acid or base on the skin does not cause negative consequences.
- To avoid being quickly removed by perspiration, it should be low in water solubility and have good solubility in the ointment base or vehicle in which it is to be prepared.
- In order to prevent evaporation during usage, it should be comparatively non-volatile.
- The skin shouldn't absorb it quickly [9].

2. Ultraviolet Radiation (UV)

UV radiation can lead to a plethora of skin ailments, including but not limited to, inflammation, degenerative aging and cancer [10]. People's exposure to ultraviolet (UV) radiation has primarily occurred through occupational sunlight exposure. However, in recent years, recreational exposure to UV radiation has increased substantially due to the growing popularity of outdoor leisure activity and the international tanning of skin for cosmetic purposes [11, 12]. Electromagnetic radiation is mainly divided into infrared (IR), visible light (VIS), and UV radiation. Infrared radiation includes heat energy and cannot be seen by naked eye. UV radiation is divided into three main categories based on variations in wavelength and energy, including UVA (320-400 nano-meter (nm)), UVB (290-300 nm), and UVC (200-290 nm). These differences in spectral range result in distinct interactions and effects on biological issues [13].

2.1 Ultraviolet Radiation-A

UVA radiation, characterized by its longer wavelength, penetrates deeply into the dermal layers and remains relatively unaffected by atmospheric variations. It can also

pass through window glass, contributing to continuous exposure even in indoor settings^[14, 15].

Unlike UVB, UVA is less effective in inducing erythema but plays a dominant role in photoaging through the generation of reactive oxygen species and degradation of dermal structural proteins. Prolonged exposure has been associated with immunosuppression, ocular damage, and a heightened risk of skin cancer^[16, 17]. UVA radiation can be beneficial as it assists in vitamin D3 formation by acting on 7-dehydrocholesterol in the skin. Additionally, UVA contributes to immediate pigment darkening by oxidizing pre-existing melanin^[18].

2.2 Ultraviolet Radiation-B

UVB radiation, although less abundant than UVA at the Earth's surface, exhibits higher biological reactivity due to its shorter wavelength and greater energy. It primarily affects the epidermal layer, forming cyclobutane pyrimidine dimers which can lead to direct DNA damage, a key event in photocarcinogenesis^[13].

Clinically, UVB is responsible for erythema and sunburn and contributes to immunological alterations that may further enhance carcinogenic risk.

2.3 Ultraviolet Radiation-C

Although this radiation contains the highest level of energy and has the potential to cause significant damage to living organisms, it is almost entirely absorbed by the ozone layer in the atmosphere. Consequently, only a negligible amount reaches the Earth's surface, and it does not play an important role in human exposure to solar ultraviolet radiation. Therefore, it is generally regarded as having minimal biological significance for humans^[19].

3. Mechanism of action of sunscreens

Sunscreens are topically applied chemicals that are designed to stop UV effects so as to prevent skin damage which may result from ultraviolet (UV) radiation. They achieve protection through the presence of organic (chemical) and inorganic (physical) UV filters that interact with those radiations by absorption, reflection, scattering or a combination of these mechanisms. Efficient use of sunscreens is very important for greatly minimizing sunburns, premature aging of the skin, immune suppression and development of skin cancer in chronically UV-exposed individuals^[20, 21].

3.1 Organic (Chemical) Sunscreens

Organic UV filters function primarily through selective absorption of ultraviolet radiation. Their molecular structures typically contain conjugated systems that enable the absorption of photons within specific wavelength ranges. Upon absorption, these molecules transition to an excited state and subsequently dissipate the absorbed energy as heat or lower-energy radiation, returning to their stable ground state without inducing cellular damage^[22, 23]. This mechanism effectively reduces the effects and penetration of UV radiation into deeper skin layers, thereby protecting cellular DNA from photochemical damage and preventing the formation of mutagenic lesions associated with photocarcinogenesis^[24]. Organic UV filters commonly used in sunscreen include avobenzone, oxybenzone, octinoxate, octocrylene, hemosalate, and sulisobenzene. Avobenzone is a primary UVA filter (320-400 nm) but is photo-unstable

and requires stabilization with agents such as octocrylene or bemotrizinol^[25]. Octinoxate and hemosalate mainly absorb UVB (280-320 nm) and are effective in preventing erythema and sunburn^[26]. Oxybenzone has broad-spectrum protection capabilities against both UVA and UVB, though it has shown osteogenic, anti-osteogenic, anti-androgenic effects *in vitro* studies^[27]. Although organic filters provide efficient photoprotection, certain compounds exhibit photoinstability or may induce photoallergic responses, necessitating careful selection and formulation optimization^[28].

3.2 Inorganic (Physical) Sunscreens

The inorganic sunscreens are principally made of the zinc oxide (ZnO) and titanium dioxide (TiO₂). How these materials protect the skin is by having a thin film that is more or less continuous on the skin surface which interacts with UV light. In the early days, it was thought that physical sunscreens worked mainly on reflecting and scattering UV radiation; however, recent spectroscopic and photophysical studies have shown that UV absorption is the main protection mechanism, particularly in micronized and nano-sized formulations^[23, 29, 30]. Inorganic Ultraviolet filters are extremely photostable, chemically inert, and have low skin penetration so they are suitable for sensitive skin types as well as long-term use^[31]. Particle size affects optical properties and cosmetic appearance. Nanoparticles (<100 nm) on the other hand manage to cut down significantly on the visible light scatter and at the same time they have an efficient UV absorption especially in the UVB and UVA-II regions^[29]. Studies shows that the ZnO and TiO₂ nanoparticles do not penetrate viable epidermal or dermal layers and are localized in the stratum corneum in normal conditions, which adds to their topical cosmetic safety^[32, 33].

3.3 Broad-Spectrum Sunscreen

Broad-spectrum sunscreens are designed with the ability to offer both protection against UVB (280-320 nm) and UVA (320-400 nm). UVB radiation causes the acute erythema and sunburn, and UVA radiation goes deeper to the dermis and plays a major role in photoaging, oxidative stress, immunosuppression, and the development of skin cancer^[34, 35].

To achieve broad-spectrum protection, combining various organic filters, inorganic filters, or hybrid systems is used to guarantee that the coverage of the UV spectrum is continuous. Broad-spectrum efficacy as a parameter is determined by regulatory guidelines on critical wavelength (370 nm and above) and UVA protection factor, with a balance ensuring holistic and complete photoprotection^[36, 37].

4. Sunscreen classification (Based on dosage form)

4.1 Emulsion

An emulsion is classified as a lotion or a cream based on its viscosity. Formulation with a viscosity below 50, 000 centipoises are generally classified as Lotions, and those with viscosities between 150, 000 and 500, 000 centipoises are referred as Cream^[38]. It generally consists of two immiscible liquid phases, forming either water-in-oil (W/O) or oil-in-water (O/W) system. These formulations allow easy dispensing from containers and facilitate smooth application on the skin^[39]. Emulsion-based sunscreen product is considered an elegant dosage form, as they impart a smooth, soft, and non-greasy feel to the skin. However,

maintaining their stability can be difficult, particularly under elevated temperature conditions [38].

4.2 Gel

Sunscreen gels are popular due to their clear appearance, light texture, and good aesthetic appeal. They are mainly classified into four categories: aqueous gels, hydro-alcoholic gels, microemulsion gels, and anhydrous oil-based gels [38]. Aqueous gels are mainly composed of water, and, water-soluble ingredients (like-ionic surfactants, organic agents, and phosphate esters) in appropriate amount to maintain clarity and transparency at different temperature [38, 40]. Hydro-alcoholic gels are prepared by mixing ethanol and water, which help to reduce the requirement for extra solubilizing agents [38, 41]. Microemulsion gels consist of extremely fine dispersed particles, which provide a smooth and even-toned texture, and spread uniformly on the skin, giving a pleasant feel and enhancing SPF effectiveness [38, 42]. Oil-anhydrous gels are ointment like formulation that are usually clear rather than translucent [38, 41]. They are commonly made by combining mineral oil with silica based gelling agents, but their use is limited because the manufacturing process is complex and expensive [38, 43].

4.3 Aerosols

Aerosols sunscreen is typically applied by spraying onto the skin to protect it from harmful sunlight. These formulations spread rapidly over the skin surface, distributing the active

ingredients uniformly and forming a thin protective film [44].

4.4 Sun Sticks

Sun sticks are small and light in weight, and hence portable and easy to carry around. Generally, sun sticks are made from oils and oil-soluble compounds, along with the inclusion of petrolatum and wax to give them solidity [38]. There are basically three types of sun sticks, namely matte, semi-translucent, and translucent sun sticks. The former sun sticks have only mineral sunscreen agents, while the latter have both mineral and chemical filters [45].

5. Sunscreen agents

Sunscreens help providing protection against UV radiation in two ways:

1. By preventing free radical formation (through UV filters)
2. By scavenging free radicals (via antioxidants) [46].

Sun-screening agents are broadly categorized into inorganic and organic UV filters which act in specific ways upon exposure to sunlight (Figure 2) [47]. While inorganic compounds scatter light, organic compounds absorb the UV rays. In recent times, hybrid materials that incorporate the characteristics of both organic and inorganic substances have been considered by researchers to be an efficient sunscreen compound [48, 49].

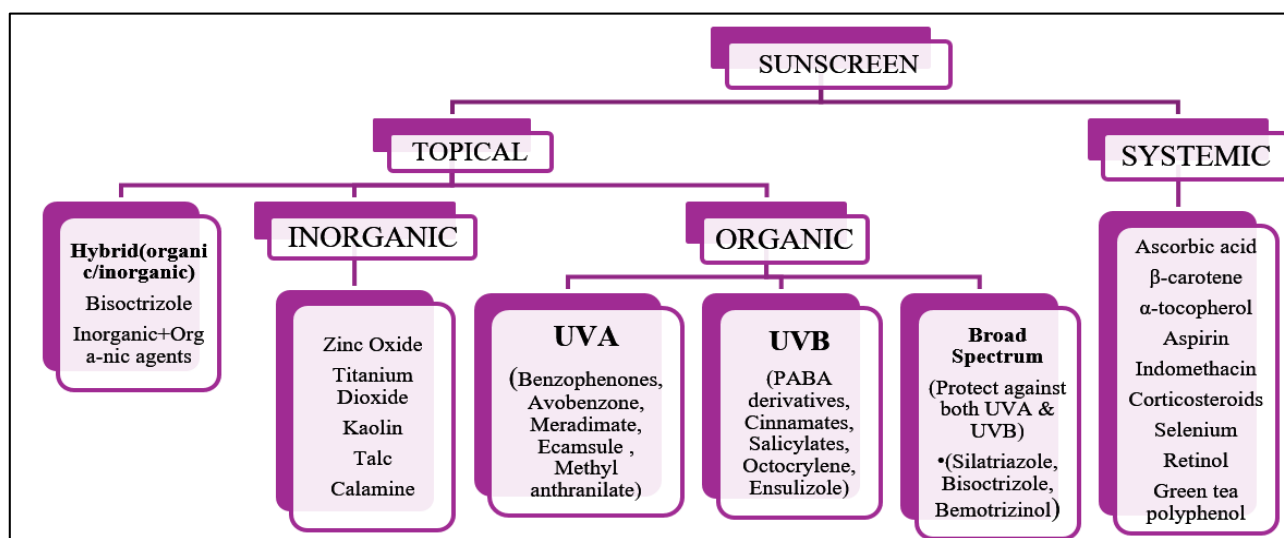


Fig 2: Classification of Sunscreen agents

5.1 Organic UV filters

Organic UV filters are further divided into UVA filters (anthranilates, dibenzoylmethanes, benzophenones) and UVB filters (salicylates, cinnamates, para-aminobenzoic acid (PABA) derivatives, and camphor derivatives), mainly operating by absorbing UV radiation. (Figure 2) [50]. These agents are widely used due to their favourable cosmetic properties, such as good stability, non-irritancy, and ease of formulation compared to inorganic filters [51]. They are often combined in formulation to achieve broad spectrum protection and enhanced SPF values, as permitted by regulatory authorities [52]. However, careful selection of filter combination is required to avoid potential incompatibilities and reduced efficacy [51]. Despite their advantages, some organic filters, like PABA derivatives and benzophenones, have been associated with adverse effects,

including skin irritation, dermatitis, and potential carcinogenic risks [50]. As a result, the use of such compounds in sunscreen formulations has been reduced or eliminated to minimize potential adverse effects on consumers. For instance, widely used organic filters like octinoxate (Ethylhexyl methoxycinnamate) and oxybenzone have been restricted in regions such as Hawaii due to their harmful impact on coral reef ecosystem [53]. Certain photo unstable filters, such as avobenzene and dibenzoylmethanes, undergo photoreactions that generate degradation products capable of absorbing across different UV regions, thereby reducing their overall photoprotective efficiency [54-56]. These photoproducts may also interact with the skin, potentially causing phototoxic, photosensitizing, and photoallergic reaction [57].

5.2 Inorganic UV filters

Inorganic UV filter is widely used to protect the skin by reflecting and scattering UV radiation across a broad spectrum ^[60]. Common agents include zinc oxide (ZnO), titanium dioxide (TiO₂), iron oxides (Fe₃O₄), calamine, ichthammol, talc and red veterinary petrolatum ^[61]. These filters are generally considered safe, more stable, and less toxic and safer for human than those of organic ingredients, they are visible due to white pigment residues left on the skin and stain clothes ^[61]. To improve cosmetic acceptability, micronized and nanosized particle (10-50 nm) of ZnO and TiO₂ have been developed, which reduce visible light reflection and enhanced transparency and SPF performance ^[61, 47]. Despite these advantages, nanoparticles may preferentially block shorter UV wavelengths (UVB and UVAII) and can generate reactive oxygen species (ROS), potentially leading to adverse effects such as photoallergic dermatitis and premature skin aging upon prolonged exposure ^[58]. Therefore, controlling nanoparticle characteristics-such as size, distribution, aggregation, and morphology-is crucial to ensure safety and efficacy ^[54]. Surface modification strategies, including coating or doping (e.g., with Al₂O₃ or Zr) have been employed to minimize

ROS generation and associated skin toxicity ^[59, 61].

5.3 Natural/Herbal agents

Natural product, derived as secondary metabolites from living organisms, exhibit significant antioxidant and UV-absorbing properties ^[62]. These bioactive compounds, including vitamin (A, C, and E) and plant-derived constituents such as phenolics, flavonoids, and carotenoids, are widely incorporated as inactive ingredients to protect the skin from UV-induced damage, including photoaging, wrinkles, and pigmentation ^[63]. Polyphenolic compounds particularly flavonoids, share structural similarities with organic UV filters and demonstrate both UV absorption and antioxidant activity, contributing to their photoprotective potential ^[64]. Antioxidant function by neutralizing free radicals and interrupting UV-induced oxidative chain reactions. Typically, a combination of lipid and water-soluble antioxidant is used to ensure comprehensive protection. As UV filters alone cannot completely block Ultraviolet radiation (e.g., SPF 50 + block approximately 98.3% of UV Radiation), antioxidant provide and additional protective layer against oxidative stress in sunscreen formulations ^[65].

Table 1: Comparison between Organic, Inorganic & Natural Sunscreens

Category	Type of Filter	Marketed Product (Example)	Active Ingredient(s)	Mechanism of Action	Representative Structural Features
Inorganic Sunscreens	Physical (Mineral)	The Derma Co Ultra Light Zinc Mineral Sunscreen SPF 50	Zinc Oxide (ZnO)	Reflects, scatters, and partially absorbs UV radiation (UVA + UVB)	Crystalline lattice (Zn ²⁺ -O ²⁻); hexagonal wurtzite structure
		Qurez 100% Mineral Sunscreen SPF 50	Titanium Dioxide (TiO ₂), ZnO	Scattering and reflection of UV; limited absorption	TiO ₂ in rutile/anatase crystalline forms; wide band-gap semiconductor
		UV Doux Mineral Sunscreen Gel	Titanium Dioxide	Physical UV blocking; reduced ROS via surface coating	Inorganic oxide lattice; often coated with silica/alumina
Organic Sunscreens	Chemical (UV Absorbers)	Minimalist SPF 50 Sunscreen	Avobenzene, Octocrylene	Absorbs UV → excited state → dissipates energy as heat	Conjugated aromatic system; diketone (enol-keto tautomerism)
		Deconstruct Gel Sunscreen SPF 55	Avobenzene, Octinoxate	UVA + UVB absorption via $\pi \rightarrow \pi^*$ transitions	Para-methoxycinnamate backbone (C = C conjugation)
		DOT & KEY Vitamin C Sunscreen SPF 50	Oxybenzone, Octocrylene	Broad-spectrum UV absorption and photostabilization	Benzophenone core with hydroxyl substitution
		Foxtale Glow Sunscreen SPF 50	Homosalate, Avobenzene	UV absorption and conversion to thermal energy	Salicylate ester with aromatic ring
Natural Sunscreens	Botanical/Phytochemical based	Biotique Bio Sandalwood Sunscreen SPF 50 +	Sandalwood oil, saffron extract	UV absorption + antioxidant protection reducing ROS-mediated damage	Phenolic compounds, terpenoids (aromatic rings, hydroxyl groups)
		Lotus Herbals Safe Sun SPF 40	Aloe vera, calendula, licorice	Absorbs UV and provides anti-inflammatory/antioxidant effects	Flavonoids, glycosides with conjugated π -systems
		Mamaearth Mineral Based Sunscreen SPF 50	Carrot seed oil, turmeric extract, ZnO	Combination of UV absorption (botanical) + scattering (ZnO)	Carotenoids (polyene chains), curcuminoids (diketo structure)
		Khadi Natural Herbal Sunscreen	Shea butter, cucumber extract	Mild UV absorption + skin soothing and hydration	Fatty acids, phytosterols, phenolic antioxidants
		Forest Essentials Sun Fluid SPF 50	Aloe vera, wheat germ oil	Antioxidant-mediated photoprotection; weak UV filtering	Tocopherols, polyphenols, unsaturated lipid chains

6. Formulation consideration

6.1 Chemical Characteristics of UV Filters

A sunscreen is defined as a topical product that is applied to the skin to protect it from the harmful ultraviolet radiation ^[66]. A UV filter is a compound that protects against UV rays

by absorbing or blocking them ^[68]. A sunscreen is made up of multi-component, including one or more UV filter, often more, along with substances like surfactants, preservatives, and stabilizers ^[69, 70]. The most common way to classify UV filters is into organic and inorganic compounds types ^[66, 67].

The organic filters are often known as “chemical” filters, whereas inorganic ones are called physical filters. This classification can be confusing, as it gives the impression that some filters are not chemical in nature ^[71].

6.2 Skin permeation

The skin's fundamental properties, such as polarity, determine how well sunscreen agents and formulation ingredients penetrate the skin. Lipids, which have a preference for lipophilic (apolar) molecules, form the structure of cell membranes. These ideas are crucial because the cell will provide several routes for the sunscreen agent and formulation ingredients to enter the stratum corneum and deeper layers ^[72].

6.3 Cosmetic Elegance

Cosmetic elegance refers to the characteristics of a product that affect how it feels on the skin, as well as its pigment and fragrance ^[73]. Regarding pigment, all formulations displayed a typical milky white to light-yellow shiny appearance, with homogenous texture. Sunscreen pigment is one of the key cosmetic appeal determinants, since transparent or skin-blending formulations are generally preferred by consumers, regardless of their skin tone or Fitzpatrick skin type. A poor visual look of a product can negatively affect its value and may prevent consumers from using or purchasing it ^[74, 75]. W/O emulsions containing higher oil levels usually show reduced friction, spread more easily, and form a colourless film on the skin surface ^[76].

7. Evaluation of sunscreen

7.1 Organoleptic evaluation

The prepared formulation was evaluated by visual and sensory examination. Parameters such as appearance, color, and odor of the formulation were carefully observed ^[77].

7.2 pH Determination

The pH of the gel formulation was measured using a calibrated pH meter. The electrode of the instrument was immersed in the gel sample, and the reading was allowed to stabilize before recording the pH value displayed on the screen ^[78].

7.3 Homogeneity test

Homogeneity was assessed by spreading approximately 0.1 g of the gel on a clean transparent glass slide. The preparation was visually inspected to ensure uniform distribution. A homogeneous formulation was confirmed by the absence of visible lumps or coarse particles on the glass surface ^[79].

7.4 Spread-ability Test

For the spreadability test, 1g of the gel was placed on a glass plate measuring 20*20 cm. A weight of 125g was carefully placed over the sample. After one minute, the diameter of the spread gel was measured to determine the spreading capacity of the formulation ^[80].

7.5 Adhesion test

For determination of adhesive strength, 1g of the gel was evenly distributed on a glass slide and covered with another slide to form a uniform layer between the two surfaces. A

load of 1000 g was applied for 5 minutes to ensure proper contact. The slides were then attached to the adhesion testing apparatus, and the time required for the two-glass plate to separate was recorded ^[81].

7.6 Viscosity

Viscosity is typically measured using a Brookfield viscometer. The evaluation process generally involves:

- **Procedure:** A specific amount of the formulation (e.g., 50 g) is placed in a beaker, and a spindle is dipped into it.
- **Rotation:** The spindle is rotated at various speeds, such as 5, 10, 20, 50, or 100 rpm.
- **Units:** The resistance to flow is recorded in centipoise (cP) ^[79].

7.7 Centrifugation test

The centrifugation test is a critical procedure used during the stability testing of sunscreen formulations to evaluate their physical integrity and predict shelf-life. The standard protocol involves:

- **Speed:** Spinning the sunscreen cream at 3000 rpm.
- **Duration:** The process typically lasts for 15-20 minutes.
- **Observation:** Following the spin, the formulation is inspected for any signs of phase separation, which occurs when the oil and water components of the cream begin to detach from one another ^[79].

7.8 In-vitro Methods for SPF Determination

Regulatory authorities such as the United States Food and Drug Administration (USFDA) and the European Cosmetic, Toiletry and Perfumery Association (COLIPA) recommended *in-vivo* testing on human volunteers using the erythral end point to determine the Sun Protection Factor (SPF) of topical sunscreen products. However, *in-vivo* testing procedures are expensive, time consuming, and not always suitable for routine product screening during formulation development ^[82]. Therefore, *in vitro* spectrophotometric methods are commonly used for preliminary evaluation of Sunscreen formulations. These methods provide a simple, rapid, and reproducible approach for estimating SPF in laboratory conditions. The UV-1000S spectrophotometer is frequently used to perform these measurements during the formulation stage. Although regulatory approval of SPF claims generally requires *in-vivo* testing, *In-vitro* evaluation helps reduce the number of *in-vivo* studies required for each formulation. *In-vitro* SPF determination can be performed by measuring the absorbance of sunscreen samples using a UV-visible spectrophotometer ^[83].

7.9 In-vivo Methods for SPF Determination

In-vivo testing methods are widely used to evaluate the sun protection efficacy of sunscreen formulations under controlled conditions using human volunteers. These methods are based on measuring biological skin responses after exposure to ultraviolet radiation. Although the general procedure is similar across different techniques, they differ in their evaluation endpoints and the expression of results ^[83].

Table 2: Comparison between *In-vitro* and *In-vivo* SPF Determination

S. No.	Section	Parameter	<i>In-vitro</i> SPF Determination	<i>In-vivo</i> SPF Determination
1	Methodology	Principle	Based on UV absorbance measurement of sunscreen solutions using UV-Vis spectrophotometry.	Based on biological skin response (erythema/pigmentation) following controlled UV exposure.
		Technique	Spectrophotometric analysis across UVB/UVA range (290-400 nm).	Application of sunscreen on human skin followed by irradiation with a solar simulator.
2	Procedure	Sample Handling	Weighing, dilution (commonly in ethanol), ultrasonication, and filtration prior to analysis.	Standardized application (2 mg/cm ²) on human volunteers with defined skin types.
		Exposure Conditions	No biological exposure; laboratory-based optical measurement.	Controlled UV exposure (typically UVB for SPF; UVA for PA testing) under clinical conditions.
3	Evaluation	Calculation/Assessment	SPF calculated using the Mansur equation incorporating erythral weighting factors and absorbance values.	SPF determined by ratio of Minimal Erythema Dose (MED) on protected vs. unprotected skin.
		Output	Estimated SPF value (predictive).	Actual SPF value (biologically validated).
4	Advantages	Efficiency	Rapid, cost-effective, reproducible, suitable for high-throughput screening.	Provides realistic efficacy data reflecting human skin physiology.
		Application	Ideal for formulation optimization and preliminary screening.	Required for regulatory claims and product labeling.
5	Limitations	Regulatory Acceptance	Not accepted as a standalone method for labeling claims.	Mandatory for regulatory approval in most jurisdictions.
		Practical Constraints	Does not account for skin interaction, photostability, or formulation spreadability.	Expensive, time-consuming, ethical considerations, inter-individual variability.

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