Phytoconstituents as photoprotective novel cosmetic formulations

Saraf S., Kaur C. D.

University Institute of Pharmacy, Pt. Ravishankar Shukla University, Raipur (C.G.) - 492 010, India

Submitted: 27-01-2010 Revised: 20-02-10 Published: 10-07-10

ABSTRACT

Phytoconstituents are gaining popularity as ingredients in cosmetic formulations as they can protect the skin against exogenous and endogenous harmful agents and can help remedy many skin conditions. Exposure of skin to sunlight and other atmospheric conditions causes the production of reactive oxygen species, which can react with DNA, proteins, and fatty acids, causing oxidative damage and impairment of antioxidant system. Such injuries damage regulation pathways of skin and lead to photoaging and skin cancer development. The effects of aging include wrinkles, roughness, appearance of fine lines, lack of elasticity, and de- or hyperpigmentation marks. Herbal extracts act on these areas and produce healing, softening, rejuvenating, and sunscreen effects. We have selected a few photoprotective phytoconstituents, such as curcumin, resveratrol, tea polyphenols, silymarin, quercetin and ascorbic acid, and have discussed the considerations to be undertaken for the development of herbal cosmetic formulations that could reduce the occurrence of skin cancer and delay the process of photoaging. This article is aimed at providing specific and compiled knowledge for the successful preparation of photoprotective herbal cosmetic formulations.

Key words: Antiaging phytoconstituents, botanical antioxidants, photoprotective herbs, ultraviolet radiations

INTRODUCTION

Solar ultraviolet radiations (UV-R) comprise 3 categories depending on the wavelength—UV-A radiations (320-400 nm), UV-B radiations (280-320 nm), and UV-C radiations (200-280 nm). Ninety percent of these are UV-A radiations, which reach us and are known as aging rays as they penetrate deep into the epidermis and dermis of the skin.[1] UV-A radiations produce immediate tanning effect—darkening of the skin due to excess production of melanin in the epidermis. They cause premature photoaging, suppress immunologic functions, and cause necrosis of endothelial cells, thus, damaging the dermal blood vessels. 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 are more genotoxic than UV-A radiations. UV-C radiations are filtered by stratospheric ozone layers. To exert biological effects, UV-R must be absorbed by cellular chromophores, which transform the energy into a biochemical signal. The cellular chromophores for UV-B radiations are nucleic acids, amino acids, such as tryptophan and tyrosine, quinines, flavins, porphyrins, and urocanic acid. Only for UV-A radiations, trans-urocanic acid is reported to be a cellular chromophore.[2]

Address for correspondence:
Dr. Swarnlata Saraf
E-mail: swarnlata_saraf@rediffmail.com

DOI: 10.4103/0973-7847.65319

The topical route has various advantages over other pathways, including avoiding hepatic first pass effects, delivering drugs or phytoconstituents continuously, fewer side effects, and improving patient compliance.[3] However, stratum corneum is the main barrier of the skin; it not only prevents dehydration, but also hinders the penetration of various drugs. The intercellular lipids of the stratum corneum play a key role in establishing the permeability barrier of the skin.[4] The aim focuses on the formulation of topical preparations that could be localized in the stratum corneum, viable epidermis, and appendages but have reduced systemic absorption. It has been reported that topical tretinoin and isotretinoin reduce the coarse and fine wrinkling, hyperpigmentation, roughness, and sallowness associated with photoaging.[5]

Phytoconstituents are gaining popularity as ingredients in cosmetic formulations because they can protect the skin against exogenous and endogenous harmful agents and can help remedy many skin conditions.[6] Exposure of skin to sunlight and other atmospheric conditions causes the production of reactive oxygen species (ROS), which can react with DNA, proteins, and fatty acids, causing oxidative damage and impairment of antioxidant system. Such injuries damage the regulation pathways of skin and lead to photoaging and development of skin cancer. The effects of aging include wrinkles, roughness, appearance of fine

lines, lack of elasticity, and de- or hyperpigmentation marks. Herbal extracts act on these areas and produce healing, softening, rejuvenating, and sunscreen effects.[7]

Various synthetic agents are used as photoprotectives, but they have limited use because of their potential toxicity in humans and their ability to interfere in certain selected pathways of multistage process of carcinogenesis. Several botanical compounds have been shown to be antimutagenic, anticarcinogenic, and nontoxic and have the ability to exert striking inhibitory effects on a plethora of cellular events at various stages of carcinogenesis. Because multiple pathways are involved in photocarcinogenesis, a mixture of several botanical antioxidants working through various mechanisms, in conjunction with the use of sunscreens, could also be an effective approach for reducing UV-generated ROS-mediated photodamage, immunosuppression, and skin cancer in humans. Few examples include tea polyphenols, curcumin, silymarin, garlic compounds, apigenin, resveratrol, ginkgo biloba, beta-carotenoids, and ascorbic acid.[8] There is a need to develop herbal formulations that could combat the harmful effects of both UV-A and UV-B radiations. The present researches are aimed to develop novel strategies to reduce the occurrence of skin cancer and delay the process of photoaging.

PREFORMULATION STEPS FOR THE DEVELOPMENT OF HERBAL COSMETIC FORMULATIONS

Selection of herbal extracts

Herbal extracts produce healing, softening, rejuvenating, and sunscreen effects. Botanical extracts are multifunctional in nature because they possess various properties, such as photoprotection, antiaging, moisturizing, antioxidant, astringent, antiirritant, and antimicrobial, which are correlated with each other.[7,9] Natural compounds belong to chemical classes, such as polyphenols, monoterpenes, flavonoids, organosulfides, and indoles.[8] After a detailed literature survey of the herbs acting as photoprotectives, we could select a few herbal constituents according to their nature, availability, estimation methods, stability, and utility of the developed formulation as well as on the basis of previous research.

Nature of phytoconstituents

Solubility is an important criterion for the development of novel formulations. According to the nature of the phytoconstituent, that is, hydrophilic or lipophilic, best-suited formulation was selected. In Table 1, we have discussed the chemical profile and solubility of important photoprotective phytoconstituents in various solvents.

Selection criteria for suitable type of formulation

Novel technology has shown great potential for improving the effectiveness and efficiency of the delivery of nutraceuticals and bioactive compounds. Recent advances in nanotechnology show their promise as potential cosmetics for poorly soluble, poorly absorbed, and labile herbal extracts and phytochemicals. An innovative approach can improve both the esthetics and performance of a cosmetic product. The application of novel approaches can also improve its efficacy regarding continuous action of herbs on the human body.[7] The formulation and selection of the approach to be used for herbal cosmetics will depend on the purpose of preparation (ie, for topical or systemic effect; inherent properties of drug or herb extract, such as

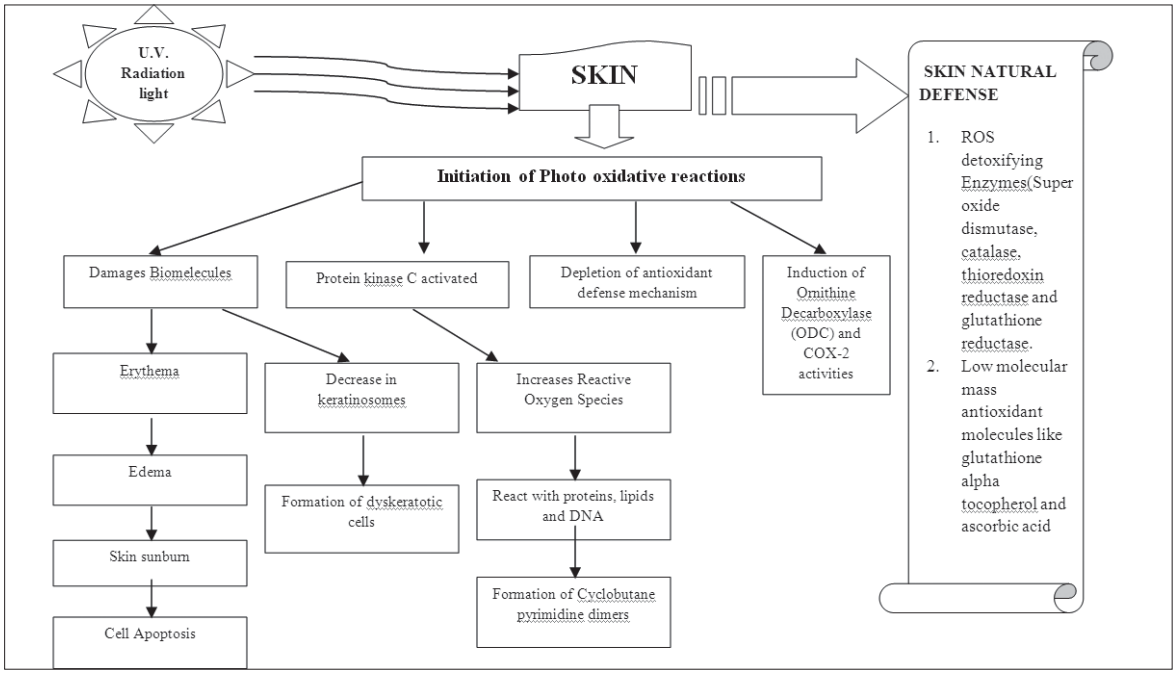
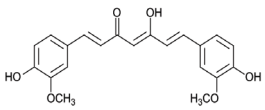
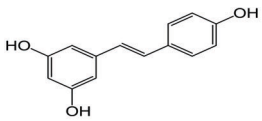
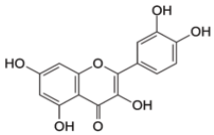
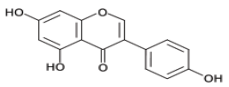
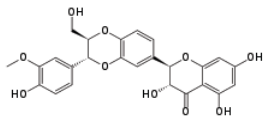
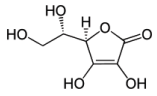


Figure 1: Mechanism of photoreactions

Table 1: Profile of photoprotective phytoconstituents

Name of phytoconstituents	Chemical structure	Nomenclature	Chemical data	Solubility
Curcumin		1,7-Bis (4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione.	Mol. Formula: $C_{21}H_{20}O_6$ Mol. mass: 368.38 g/mol	Acetone (20 mg/mL) ethanol, DMSO, dimethyl formamide (1 mg/mL), 0.1 M NaOH (3mg/mL) soluble in alkali. Insoluble in methylene chloride, water at acidic and neutral pH
Resveratrol		(trans-3,5,4'-Trihydroxystilbene)	Mol. formula: $C_{14}H_{12}O_3$ Mol. mass: 228 g/mol	Ether, chloroform, ethanol, acetic acid, acetone. Insoluble in water
Quercetin		2-(3,4-Dihydroxyphenyl)-3,5,7-trihydroxy-4H-chromen-4-one	Mol. formula: $C_{15}H_{10}O_7$ Mol. mass: 302.24 g/mol	Order of solubility Isopropyl myristate < oleyl alcohol < propylene glycol monolaurate < oleoyl macrogol-6 glycerides < linoleoyl macrogol-6 glycerides < propylene glycol laurate (PGL) < propylene glycol monocaprylate (PGMC) < polyethylene glycol-8 glyceryl linoleate < aprylocaproyl macrogol-6 glycerides < diethylene glycol mono ethyl ether (DGME). Poorly soluble in water.
Genistein		5,7-Dihydroxy-3-(4-hydroxyphenyl) chromen-4-one. OR 4',5,7-trihydroxyisoflavone	Mol. formula: $C_{15}H_{10}O_5$ Mol. mass: 270.24 g/mol	Soluble in ethanol, DMSO, dimethyl formamide. (30 mg /mL). Poorly soluble in water
Silibinin		(2R,3R)-3,5,7-Trihydroxy-2-[(2R,3R)-3-(4-hydroxy-3-methoxyphenyl)-2-(hydroxymethyl)-2,3-dihydrobenzo[b][1,4]dioxin-6-yl]chroman-4-one	Mol. formula: $C_{25}H_{22}O_{10}$ Mol. mass 482.44 g/mol	Simulated gastric fluid (pH 1.2) (0.209 ± 0.65 mg/mL), Simulated Intestinal Fluid (pH 6.8) 0.148 ± 0.88 mg/mL, Dioxane. Poorly soluble in water.
Ascorbic acid		(5R)-[(1S)-1,2-dihydroxyethyl]-3,4-dihydroxyfuran-2(5H)-one	Molecular formula $C_6H_8O_6$ Molar mass 176.12 g /mol	Solubility in water 33 g/100 mL Solubility in ethanol 2 g/100 mL Solubility in glycerol 1 g/100 mL Solubility in propylene glycol 5 g/100 mL. Insoluble in diethyl ether, chloroform, benzene, petroleum ether, oils, fats, fat solvents.

hydrophilic or hydrophobic; surface characteristics of a system, such as permeability and charges; degree of biodegradability, biocompatibility, and toxicity; release profile and size of the product required; and antigenicity of the final product).[10] Table 2 discusses the applications of various novel formulations.

IMPORTANT PHOTOPROTECTIVE PHYTOCONSTITUENTS

The use of active photoprotectives is very beneficial in combating the deleterious effects of UV rays. Important categories of beneficial phytoconstituents include phenolic acids, flavonoids, and high molecular weight polyphenols.[21,22] Naturally occurring phenolic acids include hydroxycinnamic acid and hydroxybenzoic acid. High molecular weight polyphenols, also known as tannins, include condensed polymers of catechins or epicatechins and hydrolyzable polymers of gallic or ellagic acids.[22] Many flavonoids, such as quercetin, luteolin, and catechins, are found to be better antioxidants than ascorbic acid, alpha-tocopherol, and beta-carotene.[23] Other photoprotective phytoconstituents include curcumin, garlic compounds caffeic and ferulic acid, apigenin, genistein, resveratrol, nordihydroguaiaretic acid, carnosic acid, silymarin, tea polyphenols, *Capparis spinosa* extract, *Culcitium reflexum* H.B.K. leaf extract, *French maritime* pine bark extract, *Ginkgo biloba* extract, Grape seed extract, *K. triandra* root extract, *Prunus persica* flower extract, *S. officinalis* root extract, and *Sedum telephium* leaf extract. Mechanism of photoreactions and stages for their prevention could be understood with the help of Figure 1. We have selected few phytoconstituents and given their detailed profile. Mechanisms of action of photoprotective phytoconstituents on topical application have been depicted in Table 3.

Table 2: Types of herbal formulations with applications

Type of formulations	Applications	References
Microemulsions	They have the ability to encapsulate nonpolar molecules, such as lipids, flavorants, antimicrobials, antioxidants, and vitamins	[11]
Multiple emulsions	Prolonged release obtained Protection of the entrapped substances Incorporation of several actives in the different compartments	[12] [13] [13]
Liposomes	Amphiphilic and lipophilic substances, for example, oil soluble UV filters, can be incorporated into the lipid bilayer Charged but water soluble substances can be trapped inside the liposomes Retain moisture, restore the barrier functions of the skin Deliver active ingredients to the skin with a continuous release over a prolonged time thus maintaining skin appearance	[14] [15]
Phytosomes	Phytosomes are lipophilic in nature; they have improved topical absorption, better bioavailability and enhance delivery to the tissues	[16]
Transferosomes	Can be used for small and large hydrophobic and hydrophilic molecules Transfersomes can penetrate the stratum corneum and supply the nutrients to the skin	[17] [18]
Nanoparticles	They provide improved stability of chemically unstable active ingredients Controlled release of active ingredients, pigment effect, and improved skin hydration and protection through film formation on the skin Amount of molecular sunscreen could be decreased by 50%, while maintaining the protection level compared with a conventional emulsion	[19] [20]

Table 3: Photoprotective phytoconstituents and their mechanisms of action on topical application

Name of herbal constituent	Mechanisms of action on topical application	References
Curcumin	Scavange ROS, by interrupting the activation of protein kinase C Enhance glutathione content and GST activity Inhibit lipid peroxidation and arachidonic acid Inhibits Ornithine decarboxylase(ODC) activity.	[24] [25] [26] [27]
Resveratrol	Inhibits ODC and COX-2 activity Inhibits increased level of lipid peroxidation	[28]
Silymarin	Inhibits skin edema, skin sunburn, and cell apoptosis Inhibits catalase activity Inhibits induction of ODC and COX-2 activities	[29]
Genistein	Inhibits UV-B induced H2O2 production Inhibits contact hypersensitivity Reduces edema	[30]
Ascorbic acid	Inhibits solar radiations induced p53 Powerful antioxidant enhancer	[31]
Quercetin	Protects the antioxidant systems Protects activities of glutathione peroxidase, reductase, catalase, and dismutase	[32]
Green tea polyphenols	Reduced production of cyclobutane-pyrimidine dimers. Protects UV-B-induced cutaneous edema and erythema. Reduces myeloperoxidase activity, H_2O_2 and NO production, and lipid peroxidation in human skin	[33] [34] [35]

Curcumin

Curcumin (diferuloylmethane), a yellow colored polyphenol is one of the 3 curcuminoids of *Curcuma longa* (turmeric). The other 2 curcuminoids are demethoxycurcumin and bisdemethoxycurcumin. Out of which curcumin was found to be more potent showing antiinflammatory and antiproliferative properties.[36] Antioxidant and antiinflammatory properties of curcumin in mouse models are well documented.[24,37,38] Curcumin is obtained by solvent extraction from dried turmeric roots. A simple, selective, precise, and stability-indicating high-performance thin-layer chromatography (HPTLC) method of analysis of curcumin both as a bulk drug and in formulations was developed and validated. The method used TLC aluminum plates precoated with silica gel 60 F254 as the stationary phase. The solvent system consisted of chloroform:methanol (9.25:0.75 v/v). This system was found to give compact spots for curcumin (R_f value of 0.48 ± 0.02). Densitometric analysis of curcumin was carried out in the absorbance mode at 430 nm.[36]

Silymarin

Silymarin, also known as Silibinin, is a polyphenolic flavonoid derived from milk thistle. Silymarin consists of 3 phytochemicals: silybin, silidianin, and silicristin. Silybin is the most active phytochemical and is largely responsible for the claimed benefits

of silymarin. The different analytical methods that have been reported for its determination include high-performance liquid chromatography (HPLC), TLC, HPTLC, potentiometric titration, diffuse reflectance fourier transform infrared spectroscopy, and UV spectrophotometry. Few spectrophotometric methods have been reported for the assay of silymarin, based on the formation of colored complex of the drug with 2,4-dinitrophenyl hydrazine in the presence of tetramethyl amine hydroxide; reaction with diazotized sulfanilic acid in alkaline medium forms an orange-red colored chromogen. Oxidation by Fe(III) and reduced Fe(II) was estimated with 1,10-phenanthroline at 510 nm and reaction with Folin-Ciocalteau reagent to form a blue colored complex in the presence of NaOH, and subsequent determination was done at 740 nm. It has also been determined in drug formulations based on its oxidation with potassium permanganate at a neutral pH by measuring a decrease in the absorbance at 530 nm.[39]

The method is based on the oxidation of drug and 3-methyl-2-benzothiazolinone hydrazone hydrochloride with potassium persulfate in alkaline medium and subsequent coupling to form an intensely colored product. The reaction is followed spectrophotometrically by measuring the increase in absorbance with time (for 15 min) at 430 nm. The 2 calibration procedures, namely, initial rate and fixed time methods (at 12 min) are utilized for the assay of drug in the concentration range of 16.0-192.0 μg/mL.[39]

Quercetin

Quercetin belongs to the flavonoids family and consists of 3 rings and 5 hydroxyl groups. Quercetin is also a building block for other flavonoids. Quercetin occurs in food as an aglycone (attached to a sugar molecule). Only a small percentage of the ingested quercetin will get absorbed in the blood. Quercetin is found in many common foods, including apple, tea, onion, nuts, berries, cauliflower, and cabbage. Direct spectrophotometric method for the determination of quercetin in the presence of ascorbic acid was established. The influences of medium, wavelength, pH, temperature, and the ionic strengths on quercetin determination were investigated. The best conditions for calibration curve are 50% ethanol, λ = 370 nm, pH = 4.2, T = 34 °C, and $I = 7.5 \times 10^{-5}$ M. Beer's law is obeyed in the concentration range 1.0-12.0 μg/mL for quercetin. The corresponding detection limit is 0.76 μg/mL.[40] The colored complex formed on reaction of quercetin with vanadyl sulfate is used as the basis for a sensitive and reproducible spectrophotometric method for the estimation of quercetin. The 1:1 complex exhibits maximum absorption at 425 nm in aqueous ethanol (80% v/v) adjusted to pH 3.3. Beer's law is followed over the quercetin concentration range of 0.3-20 μg/mL. The molar absorptivity is 1.4×10^4 L/mole/cm. This method can be used for the determination of quercetin isolated from biological sources. In the metal complex, the 4-carbonyl and 3-hydroxy groups of the quercetin are involved.[41]

Ascorbic acid

Ascorbic acid is the main water soluble vitamin in nature. It is present in fruits and vegetables, mainly citrus plants and their juices. It possesses antioxidant activity owing to its ability to scavenge ROS. Titration with iodine has proved to be inadequate for the determination of ascorbic acid in natural products, because they contain other reducing substances and also because the color of such products interfere with the determination of the endpoint of titration. For estimation of ascorbic acid in pure solution to 5 mL of freshly prepared standard ethanolic solution of ascorbic acid (11 mg/100 mL ethanol), 1 mL of freshly crystallized perinaphthindanetrione hydrate in ethanol (2 mg/mL) is added, mixed thoroughly, and stoppered. The color gradually develops and reaches its maximum after 10 min. The stability of the color permits its measurement with ease any time from 10 min to 24 h after the reagent has been added. The percentage transmission of the sample is recorded with reference to a blank tube of ethanol set at 100% transmission. The amount of dihydroxy perinaphthindenone formed is first estimated photoelectrically and then spectrophotometrically using a 5-mm cell, and the ascorbic acid present calculated from this spectrophotometric method.[42]

Resveratrol

Resveratrol belongs to a class of polyphenolic compounds called stilbenes, found largely in the skins of red grapes and root of *Polygonum cuspidatum* Sieb. et Zucc (Japanese knotweed). Resveratrol is a fat soluble compound that occurs in a trans and a cis configuration. Resveratrol, a naturally occurring polyphenolic phytoalexin, is present in many plants and fruits, including red grapes, eucalyptus, spruce, blueberries, mulberries, peanuts, and giant knotweed. Also, red wine contains a lot of it. The longer the grape juice is fermented with the grape skins, the higher the resveratrol content will be. Resveratrol is an effective antioxidant with strong antiinflammatory and antiproliferative properties.[43]

Genistein

Genistein is the aglycone (without sugar component) of the glycoside genistin. Before genistein can act, it needs to be released from genistin. The main sources of genistein are soya beans, peapods, and other legumes. Other legumes, such as chickpeas, contain small amounts of genistein. Genistein is an isoflavone belonging to the group of flavonoids. Because of the similarity in the structure of genistein to that of estrogen, genistein is also a phytoestrogen. It acts as a phytoestrogen and as an antioxidant. Topical application of genistein and its gastrointestinal metabolites, such as equol, isoequol, and dehydroequol, to hairless mice skin substantially inhibited the UV-B-induced hydrogen peroxide (H_2O_2) production, contact hypersensitivity, and reduced the inflammatory edema reaction.[30] Genistein was analyzed by reverse phase HPLC with XTerra C18 column (4.6 × 250 mm, 5 μm) at 40°C and isocratic elution with the mobile phase of a mixture of acetonitrile:50 mM ammonium formate buffer (5:5, v/v) delivered at a flow rate of 1.0 mL/min. Samples (30 μL) were injected onto the HPLC system. The eluent was monitored at 260 nm with a UV-vis detector.[44]

Green tea extract

Green tea is obtained from the plant *Camellia sinensis* of the

Theaceae family. The water-extractable fraction of green tea contains several polyphenolic compounds known as catechins. Generally, a typical cup of green tea contains 100-150 mg catechins, including 50% of (-)-epigallocatechin-3-gallate (EGCG), 15% of (-)-epigallocatechin (EGC), 15% of (-)-epicatechin-3-gallate, and 8% of (-)-epicatechin (1). All these polyphenols act as potent antioxidants and can scavenge ROS, such as lipid-free radicals, superoxide radicals, hydroxyl radicals, H_2O_2, and singlet oxygen. Afaq *et al* have reported in their publications that topical application or oral feeding of a polyphenolic fraction prepared from green tea prevents photocarcinogenesis.[1] Epicatechin is also known as epicatechol. Pure epicatechin is an odorless white powder. Epicatechin is a flavonol belonging to the group of flavonoids. High quantities of it can be found in cocoa, tea, and grapes.

EVALUATION PARAMETERS OF PHOTOPROTECTIVE FORMULATIONS

Morphological studies

Penetration of the skin barrier is size dependent, and nano-sized particles are more likely to enter more deeply into the skin than larger ones;[10] hence, particle or vesicular size determination is an important aspect in designing the formulation for topical application. The $\bar{z}$-average diameters of vesicles or particles of formulations are determined by dynamic light scattering using a Malvern Zetasizer. The samples are diluted to avoid multiple scattering. As a measure of the particle size distribution, the polydispersity index is calculated, which ranges from 0.0 (monodisperse) to 1.0 (very much heterogenous). Polydispersity signifies the uniformity of droplet size within the formulation. If the polydispersity value of the formulation is very low, it indicates uniformity of the droplet size within the formulation. The scanning electron microscope produces images by probing the specimen with a focused electron beam that is scanned across a rectangular area of the specimen; can produce images that are good representations of the 3-dimensional shape of the sample. The ability to determine the positions of atoms within materials has made the transmission electron microscope (TEM), an important tool in the research and development of nanotechnologies. Formulations are visualized by TEM at an accelerating voltage of 100 kV. A drop of the sample is placed on a carbon-coated copper grid to form a thin film and negatively stained by adding a drop of 1% w/v phosphotungstic acid. The grid is allowed to air dry and the samples are viewed at ×80,000 and ×1,20,000 magnifications.[45] Differential scanning calorimetry is an important means of detecting the incompatibility between active constituents and excipients used in the formulation.[46]

Entrapment efficiency determination

Entrapment efficiency is the percentage of the initial drug or active constituent incorporated into vesicular systems. Entrapment efficiency is estimated by centrifugation method. The prepared formulations are placed in centrifugation tube and centrifuged at 14,000 rpm for 30 min. The supernatant (1 mL) is withdrawn and diluted with phosphate-buffered solution (PBS) (pH 7.4) or distilled water. The entrapped phytoconstituent (drug) is determined by UV spectrophotometer at a wavelength at which the maximum peak (λ max) is obtained for that constituent. The samples from the supernatant are diluted 100 times before taking the absorbance measurement. The free drug in the supernatant gives us the total amount of unentrapped drug. Entrapment efficiency is also determined by a Sephadex G-50 minicolumn centrifugation technique.[47] Encapsulation efficiency is expressed as the percent of drug trapped. After fusing the vesicles with Triton X-100, the amount of trapped drug was estimated by UV spectrophotometer with suitable dilution by pH 7.4 PBS. The vesicles are washed first with PBS pH 7.4 and then 3-4 times with distilled water and suspended in distilled water.[48]

$$\text{Percent entrapment} = \frac{\text{Total drug} - \text{diffused drug} \times 100}{\text{Total drug}}$$

In vitro drug release

Drug release is observed using the dialysis method at room temperature. After reconstituting the freeze-dried formulation in distilled water/PBS, an aliquot of each formulation (0.1 mL) is placed in a dialysis tube (molecular weight cutoff dialysis membrane: 12,000-14,000 Mw,), which is tightly sealed. The tube is immersed in 200 mL release medium, PBS (pH 7.4), to maintain sink condition and stirred at 300 rpm on a magnetic stirrer. Samples (0.5 mL) are taken at predetermined time intervals for 24 h, and replenished with an equal volume of fresh medium. The concentration of drug is determined by HPLC or UV after appropriate dilution with acetonitrile without further treatment.[45]

In vitro skin permeation studies

In vitro skin penetration studies are performed with human cadaver skin, using Keshary-Chien cells or Franz diffusion cells. Human cadaver skin from the abdominal region, after removing hair and subcutaneous fat tissue, is mounted on the diffusion cell. PBS serves as a receptor fluid. A small quantity (0.5 g) of the gel/cream/formulation is applied to the skin surface. At the end of 24 h, the amount of drug in the receptor compartment, the drug remaining on the skin, and the drug concentration in the skin is determined by extraction into a suitable solvent followed by spectrophotometric analysis using UV-vis spectrophotometer.[49]

In dermatologic treatment, improving the efficacy demands high drug levels in the skin. In an experiment with nanoparticle dispersion, it was found that a greater quantity of drug remained localized in the skin, with lesser amounts penetrating into the receptor compartment as compared with conventional gels. Thus, drug localizing effect in the skin seems possible with novel colloidal particulate drug carriers, such as solid lipid nanoparticle. This colloidal carrier, being submicron in size, enhances the drug penetration into the skin, and because of its lipoidal nature, the penetrated drug concentrates in the skin and remains localized for a longer period of time, thus enabling drug targeting to the skin.[49]

In vitro sun protection factor determination by UV spectrophotometer

Ratio of UV doses protected to unprotected gives the sun protection factor (SPF). The *in vitro* method measures the reduction of the irradiation by measuring the transmittance after passing through a film of product. The most common *in vitro* technique involves measuring the spectral transmittance at UV wavelengths from 280 to 400 nm.[50,51] The observed absorbance values at 5 nm intervals are calculated using the following formula:

$$\mathrm{SPF}_{\text{spectrophotometric}} = CF \times \sum_{290}^{320} \mathrm{EE}(\lambda) \times I(\lambda) \times \mathrm{Abs}(\lambda)$$

Where CF = correction factor (10), EE (λ) = erythmogenic effect of radiation with wavelength λ, Abs (λ) = spectrophotometric absorbance values at wavelength λ. The values of EE × I are constants. They were determined by Sayre *et al.*[52] The aliquots prepared were scanned between 290 and 320 nm and the obtained absorbance values were multiplied with the respective EE (λ) × I (λ) values. Then their summation was taken and multiplied with the correction factor (10)

In vitro occlusion studies

The occlusivity of formulations is determined by occlusion factor. First investigations were performed by de Vringer.[53] The *in vitro* model consists of a beaker of water covered by a filter paper; 200 mg of the formulation is spread on a filter surface of 18.8 cm^2; a reference control is maintained, which is a beaker having filter paper but no formulation. If the occlusion factor is zero, it means that there is no occlusion effect as compared to the reference. The maximum occlusion factor is 100.

An occlusion factor is calculated by the formula:

$$F = 100\ [(A - B)/A]$$

Where, A = water loss without sample (reference), B = water loss with sample.

In vivo skin hydration studies

The skin hydrating effect of the selected formulation is investigated *in vivo* and compared with the conventional gel. The topical formulations were applied to the shaved skin of female albino rats. After 24 h, the animals were humanely killed and the skin was isolated, vertically sliced using microtome, and stained with hematoxylin and eosin. The slides were observed under an optical microscope and the thickness of stratum corneum was measured. The photomicrographs were taken using an image analyzer. When there is improved hydration, the thickness of stratum corneum is increased, which is due to increased water content in stratum corneum. When the formulation is such that it covers the surface of the skin, which reduces transepidermal water loss (TEWL) and evaporation of water from the skin, thus increasing the moisture content of the skin—it also leads to increased thickness of the stratum corneum. The improved skin hydration is also responsible for the increased drug penetration into the skin.[54]

Primary skin irritation studies

Primary skin irritation studies of the selected formulation is performed using albino rabbits in accordance with the guidelines of the Consumer Product Safety Commission.8 The study has to be approved by the Institutional Ethics Committee. The scores for erythema and edema are totaled for intact and abraded skin for all rabbits at 24 and 72 h. The primary irritation index(PII) is calculated based on the sum of the scored reactions divided by 24 (2 scoring intervals multiplied by 2 test parameters multiplied by 6 rabbits). If the developed formulation showed no erythema or edema on the intact and abraded rabbit skin, the PII of the formulation is calculated to be 0.00. Thus, the formulation can be classified as a nonirritant to the rabbit skin. In addition, a subjective evaluation of product safety is conducted by a dermatologist on a predetermined 0-3 scale, that is, 0 = no sign of irritation, 1 = mild irritation, 2 = moderate irritation, and 3 = strong irritation, and a self-report diary.[55]

Stability studies

The formulations are sealed in vials (10 mL capacity) after flushing with nitrogen and stored at 4 and 25°C for different periods of time (10, 20, and 30 days). The stability of the formulations is assessed by monitoring the residual drug content, size, and morphology of the vesicles, with respect to time.[45]

Bioengineering methods

Viscoelasticity determination

The viscoelastic properties are measured using cutometer. The measuring principle is suction/elongation. An optical system detects the decrease of infrared light intensity depending on the distance the skin is being sucked into the probe. In this study, the strain time mode has been applied. A probe with a 2 mm opening was used and a pressure of 500 m bar was applied in order to suck the skin into the probe. Each measurement consists of 5 suction cycles (3 s of suction followed by 3 s of relaxation and was performed in triplicate on volar fore arm). The following parameters (absolute and relative) were analyzed: Ue, elastic deformation; Uv, viscoelasticity; [R0] or Uf, total deformation; Ur, retraction; [R2] or Ua/Uf, overall elasticity of the skin; [R5] or Ur/Ue, pure elasticity of the skin without viscous deformation; [R7] or Ur/Uf, biological elasticity, that is, the ratio of retraction to extension; [R6] or Uv/Ue, the ratio of viscoelasticity to elastic deformation; and R8 or (Ua), pliability, that is, the ability of the skin to return into its original state.[56,57]

Hydration measurement by corneometer

In photoaging, the epidermis becomes pale, thin, and dry.[53] So one of the cosmetic aspect is to equilibrate the moisture balance of the skin, for the hydration measurement is an important parameter. Skin hydration is measured using a corneometer, which is mounted on multiprobe adapter. Capacitance changes depending on the water content in the stratum corneum are detected and evaluated.[58]

Erythema determination by mexameter

Skin erythema is measured using mexameter, before and after a single and 1-, 6-, and 12-weeks period of daily application. Erythema can be determined by investigating the histologic, ultrastructural, biochemical, and immunologic effects of UV-R on skin and its relationship to photodamage and skin cancer.

Method: Hairless mouse model could be used for photoprotection studies. Before experimentation, 4 hairless mice are anesthetized (using ketamine) and a rectangular area approximately 2.5 × 4 cm is marked off on the dorsal area of each animal. Then sunscreen formulations are applied and one mouse is left as control, which is untreated. After 15 min drying period, UV-R are produced by a planar array of 2 UV-A 340 fluorescent lamps, which stimulate UV-R present in the sunlight from wavelength 295 to 365 nm. Irradiance could be measured using erythema UV-A and UV-B. Intensity meter and minimal erythemal dose (MED) is calculated. One MED is defined as the amount of UV-R necessary to cause a slight reddening of the skin 24 h after exposure. For hairless mouse, 1 MED is approximately 140 mJ/cm^2.[59]

Lipid concentration determination

The lipid concentration was measured using Sebumeter.® The test product is applied twice daily to the face for a period of 12 weeks. A clinical assessment and instrumental measurements are done before and after the treatment period. Casual sebum level on the forehead and both cheeks is determined with a photometric device (Sebumeter).

Biological studies

Lipid damage determination

UV-R induces the formation of ROS resulting in the damage to various components of the skin—lipid damage, which is oxidative degradation of unsaturated free fatty acids and cholesterol. It is observed that UV exposure decreases lipid melting temperature of the mouse skin and that application of sunscreens prior to UV-R would reduce this epidermal damage. The detail method as described by Felton has been already discussed in our earlier work.[53]

Quantification of UV-induced DNA damage

To estimate whether the application of the test formulation exhibits a protective effect on DNA lesions, the test formulations were applied to volunteers twice daily. To assess DNA damage by single-cell gel electrophoresis (Comet assay), epidermal keratinocytes were isolated from suction blister epidermis and embedded in low-gelling agarose gels.[53]

Histologic studies

These studies include epidermal cell turnover determination, sunburn cell count, edema determination and wrinkle determination. The decrease in corneocyte size is correlated with accelerated epidermal turnover. The determination is carried out by image analysis of D-Squame® sheets.[61] Inflammation is an acute biological response to UV-R. The vasodilatation of cutaneous blood vessels results in erythema (reddening) and edema (swelling). The treatment reduces this edema and thus, it is also an evaluation parameter for photoprotectives. Edema is calculated by the difference in skinfold thickness between the baseline and post-UV exposure data. Facial wrinkles in the crow's feet area are evaluated by means of *in vivo* topometry, using phase shift rapid *in vivo* measurement of human skin phase induction PRIMOS. The PRIMOS system represents an established and widely used method to quantify the effects on skin wrinkles.[54,60]

HERBAL PHOTOPROTECTIVE FORMULATIONS

Ashawat *et al* (2008) prepared and characterized herbal creams for the improvement of skin viscoelasticity and hydration.[61] They prepared and characterized herbal cosmetic cream comprising extracts of *Glycyrriza glabra*, *Curcuma longa* (roots), seeds of *Psorolea corlifolia*, *Cassia tora*, *Areca catechu, Punica granatum*, fruits of *Embelica officinale*, leaves of *Centella asiatica*, dried bark of *Cinnamon zeylanicum* and fresh gel of *Aloe vera* for the protection of skin against UV-induced aging. Ashawat *et al* (2006) evaluated UV absorption ability of *Boerhavia diffusa* and expressed in terms of SPF values.[51]

The study was done to develop quercetin-loaded nanoparticles (QUEN) by a nanoprecipitation technique with Eudragit E (EE) and polyvinyl alcohol as carriers, and to evaluate the antioxidant effects of quercetin and its nanoparticles. The release of the drug from the QUEN was 74-fold higher compared with the pure drug. In addition, the antioxidant activity of the QUEN was more effective than pure quercetin on DPPH (2,2-Diphenyl-1-Picrylhydrazyl) scavenging, antisuperoxide formation, superoxide anion scavenging, and antilipid peroxidation.[62]

Plianbanchang *et al,* studied the efficacy and safety of curcuminoids-loaded solid lipid nanoparticles facial cream as an antiaging agent.[54] Curcuminoids are easily degraded by acid and alkali hydrolysis, oxidation, and photodegradation, whereas solid lipid nanoparticles (SLN) promote its stability, prolong the release,[63] enhance the penetration of active substances through the stratum corneum by increasing its hydration and forming an intact film while drying.[64] Wissing and Muller (2003) confirmed the higher effectiveness of creams containing SLN on skin hydration and viscoelasticity as compared with conventional creams.[65]

The quercetin self-emulsified formulation was optimized based on the quercetin solubility in different oils, and the self-microemulsified efficiency of various combinations of emulsifiers and coemulsifiers was evaluated using the pseudoternary phase diagram.[66] The solubility of quercetin is significantly increased in the self-emulsified system and the formulation is stable and easy to prepare. Casagrande *et al* took quercetin-loaded formulations of non-ionic emulsion with high lipid content and anionic emulsion with low lipid content and found that quercetin remains functionally stable in formulations, and measuring the antioxidant activity is an efficient approach to evaluate quercetin

skin retention with minimal interference of the tissue products. Furthermore, these data suggest that formulations containing quercetin may be used as topical active products to control UV-B-mediated oxidative damage of the skin.[67] Martelli *et al* in their invention used curcumin at concentrations between 0.0005 and 10% of the total composition weight for cosmetic or pharmaceutic-dermatologic use and found it suitable for maintaining skin cells and enabling them to effect regeneration of the skin.[68]

Resveratrol, the main active polyphenol in red wine, was incorporated into various combinations of emulsions and liposomes to examine its physicochemical characteristics and cardiovascular protection. They concluded that encapsulation by the emulsion-liposome blends is a potent way to enhance the preventative and therapeutic benefits of resveratrol.[69] Sreekumar *et al* in their patent took a cosmetic skin care composition comprising resveratrol in an amount from 0.00001 to 10 wt%, a retinoid selected from the group consisting of retinoic acid, retinol, retinyl acetate, and retinyl linoleate, and a cosmetically acceptable vehicle. It was a cosmetic method of improving the appearance of wrinkled, lined, dry, flaky, aged, or photodamaged skin and improving skin thickness, elasticity, flexibility, and plumpness—the method comprising applying to the skin.[70]

The possibility of improving the efficacy of resveratrol, a polyphenol with strong antioxidant and free-radical scavenging properties, on cell proliferation and photoprotection by liposomal incorporation was investigated. Interestingly, liposomes prevented the cytotoxicity of resveratrol at high concentrations, even at 100 μM, avoiding its immediate and massive intracellular distribution, and increased the ability of resveratrol to stimulate the proliferation of the cells and their ability to survive under stress conditions caused by UV-B light.[71]

Zhang *et al* reported that green tea extract and EGCG exhibit antiangiogenic activities in various experimental tumor models.[72] Huang *et al* reported that EGCG is a potent agent against UV-B-induced damage in HaCaT keratinocytes.[73] Camouse *et al* have experimentally determined that topical application of green tea and white tea extracts prevent simulated solar radiation-induced oxidative damages to DNA and Langerhans cells that may lead to immune suppression and carcinogenesis.[74]

CONCLUSION

There are sufficient number of photoprotective phytoconstituents, which could be an important part of photoprotective formulations, but very less work has been done thus far and there is need for more research taking these phytoconstituents, establishing more effective formulations as several botanical compounds have been shown to be antimutagenic, anticarcinogenic, and nontoxic and have the ability to exert striking inhibitory effects on a plethora of cellular events at various stages of carcinogenesis. Because multiple pathways are involved in photocarcinogenesis, a mixture of several botanical antioxidants working through various mechanisms, in conjunction with the use of sunscreens could also be an effective approach for reducing UV-generated ROS-mediated photodamage, immunosuppression, and skin cancer in humans. Further development of novel delivery systems along with the use of botanical extracts will be a good approach for fighting against photocarcinogenesis. This article would be helpful for the development of stable herbal photoprotective formulations with least side effects, more efficiency, and longer duration of action.

ACKNOWLEDGEMENTS

Authors are thankful to UGC [major project, F.No 32-133/2006(SR)], New Delhi for financial support and Head, Cosmetic laboratory, University Institute of Pharmacy, Raipur,CG for providing instrumental and other facilities. We also pay gratitude to National Medical Library, New Delhi for library facility and NISCAIR, New Delhi for online journals facility.

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Source of Support: UGC, New Delhi.
Conflict of Interest: None declared