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UV Filters and Their Distribution on the Skin through Safe, Non-Penetrating Vehicles

PANAGOULA PAVLOU, ANGELIKI SIAMIDI, MARILENA VLACHOU, and ATHANASIA VARVARESOU,
Division of Aesthetics and Cosmetic Science, Department of Biomedical Sciences, University of West Attica, Egaleo 12243, Greece (P.P., A.V.), Section of Pharmaceutical Technology, Department of Pharmacy, School of Health Sciences, National and Kapodistrian University of Athens, Athens 15784, Greece (P.P., A.S., M.V.), Laboratory of Chemistry-Biochemistry-Cosmetic Science, Department of Biomedical Sciences, University of West Attica, Egaleo 12243, Greece (P.P., A.V.)

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Synopsis

The effects of ultraviolet (UV) radiation trigger human skin reaction, which can result in erythema, photoaging, and/or skin cancer. Sunscreens play an important role against the negative effects of UV radiation on the human skin. However, they should satisfy certain criteria, with the main one being photostability, to avoid the formation of health-threatening reactive intermediates. It has to be kept in mind, however, that photo-stable UV filters have the undesirable propensity to transfer energy to molecular oxygen and generate the very reactive singlet oxygen. They should also be well tolerated, while at the same time, they should not permeate into the skin and cause toxic effects. Thus, there is an ongoing need to develop effective and safe non-penetrating sunscreen formulations. The search for innovative active substances, efficacious combinations, and the design of vehicles or carriers has led to the implementation of advanced delivery systems. This study intended to review the commonly used UV radiation thwarting agents (organic and inorganic UV filters), compile the relevant toxicity studies, evaluate their margin of safety, and assess the current situation on innovative sunscreen formulations.

INTRODUCTION

The ultraviolet (UV) radiation causes human skin reactions, which could lead to erythema, photoaging, and/or skin cancer (1). Against the negative effects of UV radiation on the human skin, sunscreens play an important role. Their safety depends on whether they cause irritation or sensitization, or on their ability to penetrate into the skin (cutaneous permeation). In this case, systemic toxicity, by allowing the product to pass into the bloodstream, could develop (2). The sunscreens should also be photostable, to avoid UV protection loss and the formation of health-threatening reactive intermediates while

Address all correspondence to Panagoula Pavlou at gipav@pharm.uoa.gr

avoiding the energy transfer to molecular oxygen and the consequent formation of singlet oxygen (3,4). What makes the evaluation of a sunscreen's photostability important is its special application type and the chemical structure of the UV filters used (5,6).

According to the European Cosmetics Regulation No. 1223/2009 (7), sunscreen products are classified as cosmetic products, whose definition is "any preparation, such as creams, oils, gels, and sprays, intended to be in contact with the human skin, to protect it from UV radiation by absorbing, scattering, or reflecting radiation." Table summarizes the UV filter substances allowed by European Union (EU) (Annex VI, last update November 24, 2020).

In the United States, sunscreens are classified as over-the-counter (OTC) drugs. This means that they must comply with all the requirements listed in the Food and Drug Administration's (FDA) OTC sunscreen monograph. Individual sunscreen active ingredients are reviewed by the FDA, and only those that are on the FDA's monograph approved list may be used in sunscreen products marketed in the United States (8). On February 21, 2019, the FDA issued a proposed rule describing the conditions under which the FDA proposes that OTC sunscreen monograph products are generally recognized as safe and effective (GRASE) and not misbranded. The FDA has proposed the following categories for the 16 sunscreen monograph ingredients (Table II).

It is worth mentioning that formulations of sunscreens are applied to a wide area on the skin when used at the beach (>1.5 m^2), where they remain for a long time (generally, after 2 h they have to be reapplied because they wash off because of the sweat, bathing, rubbing, *etc*.). The sunscreen-containing formulations are the source of a continuous and high input of the chemical substance it contains, permeating the viable skin strata and possibly the systemic circulation. Consequently, a development of formulas based on non-penetrating photo protectors is considered to be more than relevant. A non-harmful sunscreen application, which will offer the greatest possible protection from UV rays, is important. Protection against UV radiation should be combined with minimal skin penetration. This explains why the biggest challenge cosmetologists face is the development of appropriate products that could hinder skin penetration.

Microencapsulation of sunscreens is considered to have an advantage because it is safer and more effective to use. Microencapsulation exhibits no percutaneous absorption and also reduces photo degradation, while at the same time, it has a lasting effect and makes the sunscreen stable. This process leads to the creation of capsules with a diameter between one to few micrometers (9). The benefit of this technique lies on the fact that encapsulated UV filters do not come in direct contact with the skin, preventing any possible toxicological risks.

In the ongoing endeavor of overcoming problems caused by sunscreens, nanotechnology plays an important role because nanosystems are often used as vehicles to sunscreens. Cyclodextrins and nanoemulsions, liposomes, and nanoparticles (lipid, polymeric, and inorganic) are the most thoroughly examined nanosystems in photo protection (10).

Microspheres (11), micro- and nanocapsules (NCs) (12,13), lipid particles (14,15), hydrotalcite-like anionic clays (16), and inclusion complexes (17–20) have caught the attention recently as they are considered suitable vehicles for sunscreens. UV chemical blockers were integrated into microparticulate carriers, using hydrophilic (chitosan and gelatine) and hydrophobic (polymethylmetacrylate) polymers (21).

Lipid carriers minimize skin penetration and retain their satisfactory photo-protective properties. Being under investigation, as drug carrier systems for insufficiently, water-soluble

Table I
UV Filter Substances Agreed by the EU

Chemical name/INN/XAN	Name of common ingredients glossary	Maximum concentration in ready-for-use preparation (%)
N,N,N-Trimethyl-4-(2-oxoborn-3-ylidenemethyl) anilinium methyl sulfate	Camphor benzalkonium methosulfate	6
Benzoic acid, 2-hydroxy-, 3,3,5-trimethylcyclohexyl ester/Homosalate	Homosalate	10
2-Hydroxy-4-methoxybenzophenone/Oxybenzone	Benzophenone-3 (BP-3)	6
2-Phenylbenzimidazole-5-sulphonic acid and its potassium, sodium and triethanolamine salts/Ensulizole	Phenylbenzimidazole sulfonic acid	8 (as acid)
3,3'-(1,4-Phenylenedimethylene) bis(7,7-dimethyl-2-oxobicyclo[2.2.1] hept-1-ylmethanesulfonic acid) and its salts/Ecamsule	Terephthalylidene dicamphor sulfonic acid	10 (as acid)
1-(4-tert-Butylphenyl)-3-(4-methoxyphenyl) propane-1,3-dione/Avobenzone	BMDBM	5
Alpha-(2-Oxoborn-3-ylidene)toluene-4-sulphonic acid and its salts	Benzylidene camphor sulfonic acid	6 (as acid)
2-Cyano-3,3-diphenyl acrylic acid 2-ethylhexyl ester/Octocrilene	OCR	10 (as acid)
Polymer of N-{(2 and 4)-[(2-oxoborn-3-ylidene)methyl]benzyl}acrylamide	Polyacrylamidomethyl benzylidene camphor	6
2-Ethylhexyl 4-methoxycinnamate/Octinoxate	Ethylhexyl methoxycinnamate (EHMC/OMC)	10
Ethoxylated Ethyl-4-Aminobenzoate	PEG-25 PABA	10
Isopentyl-4-methoxycinnamate/Amiloxate	Isoamyl P-methoxycinnamate	10
2,4,6-Trianilino-(p-carbo-2′-ethylhexyl-1′-oxy)-1,3,5-triazine	EHT	5
Phenol, 2-(2H-Benzotriazol-2-yl)-4-methyl-6-(2-methyl-3-(1,3,3,3-tetramethyl-1-(trimethylsilyl)oxy)disiloxanyl)Propyl	Drometrizole trisiloxane	15
Benzoic acid, 4,4-{[6-[[[(1,1-dimethylethyl)amino]carbonyl]phenyl]amino]-1,3-5-triazine-2,4-diyl]diimino}bis-, bis (2-ethylhexyl)ester/Iscotrizinol	DEBT	10
3-(4′-Methylbenzylidene)-dl-camphor/Enzacamene	4-Methylbenzylidene camphor	4
2-EHS/octisalate)	EHS	5
2-Ethylhexyl 4-(dimethylamino)benzoate/padimate O (USAN:BAN)	Ethylhexyl dimethyl PABA	8
2-Hydroxy-4-methoxybenzophenone-5-sulfonic acid (benzophenone-5) and its sodium salt/sulisobenzone	Benzophenone-4 (BP-4); benzophenone-5 (BP-5)	5 (as acid)
2,2′-methylene bis(6-(2H-benzotriazol-2-yl)-4-(1,1,3,3-tetramethylbutyl) phenol)/bisoctrizole	Methylene bis-benzotriazolyl tetramethylbutylphenol (MBBT)	10
Methylene bis-benzotriazolyl tetramethylbutylphenol (nano)	Methylene bis-benzotriazolyl tetramethylbutylphenol (NANO)	10

Table I
Continued

Chemical name/INN/XAN	Name of common ingredients glossary	Maximum concentration in ready-for-use preparation (%)
Sodium salt of 2,2′-bis(1,4-phenylene)-1H-benzimidazole-4,6-di-sulfonic acid/bisdisulizole disodium (USAN)	Disodium phenyl dibenzimidazole tetrasulfonate (DPDT)	10 (as acid)
2,2'-(6-(4-Methoxyphenyl)-1,3,5-triazine-2,4-di yl)bis(5-((2-ethylhexyl)oxy) phenol)/Bemotrizinol	Bis-ethylhexyloxyphenol methoxyphenyl triazine (BEMT)	10
Dimethicodiethylbenzalmalonate	Polysilicone-15	10
TiO_2	TiO_2	25
TiO_2 (nano)	TiO_2 (NANO)	25
Benzoic acid, 2-[4-(diethylamino)-2-hydroxybenzoyl], hexylester	Diethylamino hydroxybenzoyl hexyl benzoate	10
1,3,5-Triazine, 2,4,6-tris(1,1′-biphenyl)-4-yl-, including as nanomaterial	Tris-biphenyl triazine/Tris-biphenyl triazine (NANO)	10
ZnO	ZnO	25
ZnO (nano)	ZnO (NANO)	25
3,3-(1,4-Phenylene)bis(5,6-diphenyl-1,2,4-triazine)	Phenylene bis-diphenyltriazine	
2-Ethoxyethyl (2Z)-2-cyano-2-[3-(3-methoxypropylamino) cyclohex-2-en-1-ylidene]acetate	Methoxypropylamino Cyclohexenylidene Ethoxyethylcyanoacetate	3

Table II
UV Filter Substances Categorized According to the FDA

GRASE[a] for use in sunscreens	Not GRASE[b] for use in sunscreens	Insufficient data for use in sunscreens[c]
ZnO and TiO_2	Aminobenzoic acid (PABA) and trolamine salicylate	Cinoxate, dioxybenzone, ensulizole, homosalate, meradimate, octinoxate, octisalate, OCR, padimate O, sulisobenzone, oxybenzone, and avobenzone

[a] GRASE = generally recognized as safe and effective.
[b] These ingredients are not currently marketed.
[c] For those ingredients in the "insufficient data" category, the FDA proposes that it needs additional data to determine that sunscreens with these ingredients would be GRASE.

compounds, lipid nanoparticles and NCs are considered colloidal carriers (22). These carriers allow shielding of chemical compounds against photodegradation phenomena, ensure bioavailability optimization, and allow controlled release, while at the same time, they can be produced in great numbers. These exact colloidal carriers have been proven to amplify the accumulation of UV filters on the upper skin layers, as they have been designed to strengthen their photo-protective properties (23). The size of lipid nanocarriers makes skincare products easy to formulate and apply. Melt-emulsified lipids are the base of solid lipid nanoparticle (SLN) formulation, are stable under room-temperature conditions, and are made of well-tolerated and biodegradable raw materials (24,25).

The harmful effects associated with the exposure to UV radiation are well documented. As a result, the development of a new generation of UV filters that can provide effective protection against the entire UV spectrum radiation has become major concern. These protective systems should be carefully designed by selecting substances of highly reliable stability, to ensure optimal safety and efficiency during the entire time of sun exposure.

The aim of this review was to present the methods (*in vitro*/*in vivo*) used for the estimation of skin penetration of sunscreens regularly used (some sunscreens listed in both FDA monograph and EU Annex are no longer used because they have an unpleasant feel, irritate skin, or are no longer produced), the studies conducted on their toxicity, the evaluation of margin of safety (MoS), and the current situation and perspectives by using new carriers.

IN VITRO AND *IN VIVO* METHODS FOR CUTANEOUS PENETRATION AND TRANSDERMAL PERMEATION OF ORGANIC UV FILTERS

The stratum corneum (SC) is the outermost layer of the epidermis. It consists of 10–25 layers of dead, elongated, fully keratinized corneocytes, which are embedded in a matrix of lipid bilayers. Ceramides form the major two-tailed component of the SC lipid matrix. Free fatty acids and cholesterol form the other two dominant components of SC lipids. On most body sites, the SC is 12–16 cell layers thick, but it can vary from as little as nine cell layers on the forehead or eyelids to as much as 25 on the dorsum of the hand and up to 50 or more on the palms or the soles of the feet. The crossing of the SC is the rate-limiting step in the sequence of percutaneous absorption. The desirable site of action of UV filters is restricted to the skin surface or within the uppermost layers of the SC. Ideally, a sunscreen should impregnate the SC and create a filter against UV radiation, but not penetrate into the underlying viable tissue.

Both *in vivo* and *in vitro* methodologies are available for the evaluation of the skin absorption and percutaneous penetration properties of sunscreen and skincare products. However, *in vitro* tests are mostly preferred over the *in vivo* because of ethical reasons and also feasibility. In cases where the crossing of the SC is considered as the foremost rate-limiting step in the process of skin absorption and percutaneous penetration, data come often from *in vitro* methods. Prediction of *in vivo* skin absorption and percutaneous permeation, for most of the compounds, is made possible, thanks to the *in vitro* data deriving from studies using skin membranes. Whereas human skin is only available from surgical sources, excised skin can be easily obtained from animals. This is exactly what makes the production of proper membranes possible, thus opening the way for conducting reproducible experiments. With passive diffusion, instead of active, being the cause of penetration, molecular transport is considered the primary route for skin permeation, whereas viability of the skin is not a requirement for penetration testing. In the rare case of dermal biotransformation, it is vital that separated tests are conducted, including fresh excised skin, which could foster a prolong viability under certain circumstances.

Test methods that are used for the estimation of the *in vitro* rates, use diffusion cells, also known as Franz cells (Figure 1). These cells consist of an upper and a lower chamber. These chambers are divided by a sample of the human or pig skin in the shape of a disk.

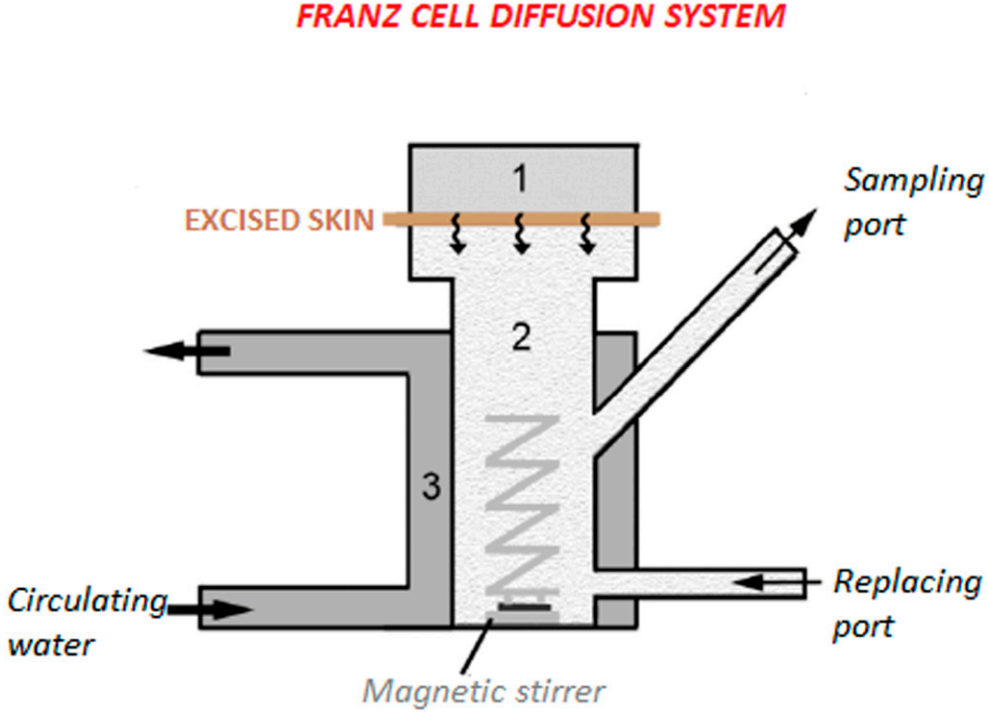

Figure 1. Schematic representation of the Franz cell diffusion system (modified from Kim et al.). The donor chamber (1) above the skin contains the applied topical agent. The chamber below the skin is the receptor chamber (2) from which samples are taken through the sampling port. The receptor chamber is surrounded by a water jacket (3) maintained at 32°C. A magnetic stirrer and stirring helix are magnetically rotated at the bottom of the receptor chamber. The topical drug, which is applied to the SC side of the skin, permeates into the dermis side and then crosses the skin (26).

A reactor fluid is present in the lower chamber, helping the simulation of the circulation in the dermis. The substance to be tested is placed on the skin, and its diffusion through the skin is evaluated by analyzing the receptor fluid, which is located in the lower chamber. After the diffusion period is terminated, several skin layers are examined for any residual materials. The UV filters are likely to exhibit different penetration rates, in contrast to pure substances, because they are examined in standardized preparations, at several concentrations. After application (the duration varies at every experiment), the samples are rinsed using a surfactant solution, to analyze the concentration of the product in every layer (SC, epidermis, dermis, and receptor fluid). The amount of the applied test substance, which is found in the SC, is estimated by the so-called SC absorption. By dermal absorption, on the other hand, we mean the amount of the applied product found in the epidermis and dermis. Last, by percutaneous absorption, we mean the amount of the applied product that is found in the receptor fluid.

An *in vivo* method, for measuring skin penetration, is the tape-stripping method. For 30 min, samples are placed on the skin surface, and then, the substance in excess is removed from the surface by swiping with a dry cloth. Seven tape strippings are used to remove the SC of the area under examination. The first tape strip is thrown away, whereas the next six are gathered, put in a beaker, containing a suitable solvent and stirred for 30 min. The levels of the sunscreen product in the solvent are then measured (27).

It is well known that there are considerable differences between animal and humans in their skin delivery systems, attributed to factors including SC thickness, hydration, and lipid composition. Using fresh frozen human skin instead of animal skin can serve as an excellent alternative to methods using animal skin while also making the result much closer to human living skin. Although the tissue has been frozen and stored at −80°C, transport and barrier mechanisms apparently remain functional. Similar distribution patterns have also been demonstrated in the porcine skin. Although the experiments are performed with the skin kept at −80°C, freeze–thaw cycles or careless storage at higher temperatures might affect the results and the permeability of the skin. Nevertheless, ethically, it is an excellent way to avoid using experimental animals in permeation studies (28).

TOXICITY STUDIES

Scientists are well aware of toxicity issues related to filters. This concern has been confirmed by a number of studies (*in vitro*/*in vivo*), according to which commonly used sunscreens were found to have an endocrine active chemical action. *In vitro* studies investigated estrogenic activity of UV filters, varying in their design and endpoints, which might explain the diverging results. Most *in vitro* studies reported that BP-3, 4-methylbenzylidene camphor (4-MBC), OMC, HMC, and OD-PABA exhibit estrogenic activity. However, not all of the UV filters exhibiting *in vitro* estrogenic activity were estrogenic in acute *in vivo* models.

In vitro, 8/9 chemicals (BP-1, BP-2, BP-3, 3-BC, 4-MBC, HMS, OD-PABA, and OMC) showed estrogenic (on MCF-7 cells) and 2/9 (BP-3 and HMS) showed antiandrogenic activity (on MDA-kb2 cells). Six/nine filters (BP-1, BP-2, BP-3, 3-BC, 4-MBC, and OMC) increased uterine weight in immature rats. 3-BC and 4-MBC displaced 16α125I-estradiol from human estrogen receptor (ER)β. Developmental toxicity of 4-MBC (0.7–47 mg/kg body weight/day) and 3-BC (0.24–7 mg/kg), administered in chow, was investigated in the Long–Evans rats. Weight gain of pregnant rats was reduced only by

3-BC, early postnatal survival rate, and thymus weight by both compounds at higher doses. 4-MBC and 3-BC delayed male puberty and dose-dependently affected reproductive organ weights of adult male and female F1 offspring, with partly different effect patterns. Thyroid weight was increased by higher 4-MBC doses. Tissue-specific changes in mRNA levels of estrogen-regulated genes in the prostate, uterus, and brain regions, determined by real-time PCR, in their response to acute estradiol challenge in adult gonadectomized offspring were observed. Lowest effective doses were 0.24 mg/kg/day for 3-BC and 7 mg/kg/d for 4-MBC. Fat tissue levels at 7 mg/kg 4-MBC (GC–MS) approached the range of UV filters in fish. A human SED of 4-MBC has been estimated as 0.23 mg/kg body weight. Such a dose would be only 1/3 of the present no observed adverse effect level (NOAEL) and 1/30 of the lowest observed adverse effect level of 4-MBC (2,29).

Other researchers also came to the conclusion that various metabolic factors, such as fat and lipid homeostasis and thyroid hormone production, mediated by non–estrogen-related methods, were affected by OMC and 4-MBC. OMC and 4-MBC in rats were shown to exert endocrine disrupting, including uterotrophic, i.e., estrogenic effects. Estrogens also have metabolic effects; therefore, the impact of oral application of the two UV absorbers at two doses (50 or 250 mg per 20 g food of OMC or 4-MBC, respectively) for 3 mo on lipids and hormones was compared with that of estradiol-17β (E2). E2, OMC, and 4-MBC reduced weight gain, the size of fat depots, and serum leptin, a lipocyte-derived hormone, when compared with the ovariectomized control animals. Serum triglycerides were also reduced by the UV screens but not by E2. On the other hand, E2 and OMC reduced serum free fatty acids and cholesterol low-density lipoproteins, and high-density lipoproteins; this effect was not shared by 4-MBC. Whereas E2 inhibited, OMC and 4-MBC stimulated serum LH levels. In the uterus, both UV filters had mild stimulatory effects. 4-MBC inhibited serum T4, resulting in increased serum thyroid-stimulating hormone (TSH) levels. On the basis of human data, the applied doses for experimental animals were relevant as they also resulted in μM concentrations (30).

Wang et al. (31) concluded that 4-MBC acted as a possible inhibitor of the pituitary–thyroid axis, as the TSH serum levels were found to be considerably high. Moreover, the weight of the thyroid glands was considerably increased. Cinnamate derivatives interfered with the TH axis in rats. The perinatal OMC exposure induced adverse effects on the reproductive and neurological development of rat offspring. The treatment with OMC for 12 weeks caused a decrease in T4 level in the blood of ovariectomized female rats and inhibited the activity of 5′-deiodinase that converts T4 to T3 in the liver [mean intake of test substances (mg/animal/day): OMC, 2.5 (low) or 12.5 g/kg (high); 4-MBC, 2.5 (low) or 12.5 g/kg (high)].

Experimental studies referring to human exposure showed that BP-3, 4-MBC, and OMC rapidly permeated intact skin and could be detected in plasma after 1–2 h following application. Interestingly, the concentrations of these compounds in the same experimental study in male urine and plasma were higher than those in female samples, indicating a gender difference in the metabolism, distribution, and possibly also in the accumulation of UV filters in adipose tissue. Was it an effect of surface? Male body has larger surface than female body. If the sunscreen was applied at the same mg/cm^2 concentration, more surface equates to more sunscreen applied, hence more sunscreen in urine and plasma? The concentrations that were used in every experiment were different. A single blinded experimental study in Denmark used 10% concentration of BP-3, 4-MBC, and OMC (whole-body application of sunscreen 2 mg/cm^2). In Sweden, an experimental study used 4% of BP-3 (whole-body application of sunscreen 2 mg/cm^2) (32).

Randomized clinical trial at a clinical pharmacology unit (West Bend, Wisconsin) was conducted in 48 healthy participants. Systemic absorption and pharmacokinetics of six active ingredients (avobenzone, oxybenzone, octocrylene (OCR), homosalate, octisalate, and octinoxate) in four sunscreen products under single- and maximal-use conditions were assessed. Participants were randomized to one of four sunscreen products, formulated as lotion ($n = 12$), aerosol spray ($n = 12$), nonaerosol spray ($n = 12$), and pump spray ($n = 12$). The sunscreen product was applied at 2 mg/cm^2 to 75% of the body surface area at 0 h on day 1 and three times on day 2 through day 4 at 2-h intervals, and 34 blood samples were collected over 21 d from each participant. In this study, all six of the tested active ingredients were systemically absorbed and had plasma concentrations that surpassed the FDA threshold for potentially waiving some of the additional safety studies for sunscreens. Geometric mean maximum plasma concentrations of all six active ingredients were greater than 0.5 ng/mL, and this threshold was surpassed on day 1 after a single application for all active ingredients (33).

Levels of UV filters measured in human seminal fluid and comparisons to levels measured in concurrently collected urine and serum samples were presented. In total, nine UV filters were analyzed by TurboFlow-LC–MS/MS (Thermo Fisher Scientific, San Jose, CA) in paired urine, serum, and seminal fluid samples from 300 young Danish men from the general population; each man collected one of each sample type within 1 h. Four of the examined UV filters could be detected in seminal fluid samples at levels above limit of detection in more than 10% of the samples. BP-1 and BP-3 were most frequently detected in, respectively, 18%, 19%, and 27% of the seminal fluid samples albeit at levels one to two orders of magnitude lower than the levels observed in urine. 4-MBP was detectable in 11% of the seminal fluid samples, whereas in <5% of the urine samples. Overall, 45% of the men had at least one of the UV filters present in their seminal fluid at detectable levels. In conclusion, chemical UV filters are present in men's seminal fluid; some of which can activate the human sperm-specific CatSper $Ca2^+$ channel, and thereby potentially interfere with the fertilization process (34).

Few human studies have investigated potential side effects of UV filters, although human exposure is high as UV filters in sunscreens are rapidly absorbed from the skin. One of the UV filters, BP-3, has been found in 96% of urine samples in the United States, and several UV filters have been found in 85% of Swiss breast milk samples. It seems pertinent to evaluate whether exposure to UV filters contributes to possible adverse effects on endocrine disruption (35).

BIOAVAILABILITY

Bioavailability, in the case of the sensitization process, refers to the fact that the substance is able to permeate the skin and can also activate a weak or non-sensitizing substance into a sensitizer. A first assessment is feasible by evaluating the chemical structure and the physical properties of a substance. The skin permeation potential of a substance would be allowed, e.g., in the case of low molecular weight compounds (<500 Da) (35). The physicochemical properties of the active substance and the properties of the vehicle (polarity of the solvent, particle size, and type of vehicle), exposed to the sunscreen product, affect the degree of permeation into the skin (36).

Mathematical solution of Fick's second law determines dermato-pharmacokinetic parameters of UV filters, K, and D/L^2, as follows (Crank, 1975):

$$C(x) = KC\text{veh}\left(1-\frac{x}{L}\right)-\frac{2}{\pi}\sum_{n=1}^{\infty}\frac{KC\text{veh}}{n}\sin\left(\frac{n\pi x}{L}\right)\exp\left(-\frac{D}{L^2}n^2\pi^2 t\right),$$

where C_{veh} is the permeant's concentration in the vehicle, K is the partition coefficient of the chemical between the SC surface and the vehicle, and L and D are the diffusion pathlength and the coefficient of diffusion of the chemical across the SC, respectively.

The validity of equation https://www.sciencedirect.com/science/article/pii/S0378517314009235?via%3Dihub - eq0015 also assumes that (i) all transport of chemical substance across the SC takes place by passive diffusion, (ii) the vehicle in which the chemical is presented to the SC does not modify the membrane or act as a carrier for the compound, and (iii) that no others skin layers contribute to the total barrier (37).

Presently used sun filters are lipophilic molecules with relatively low molecular weight and because of their physicochemical characteristics possess a good potential to penetrate into the deep strata of the skin and to be systemically absorbed.

It has also been proven that there are some factors that designate differences between various formulations. These factors include penetration into the skin, permeation through it, and retention of UV filters in the skin from topical products. The formulation type influences the UV filter diffusion on the epidermis. Roussel et al. (38), conducted a study according to which they managed to predict and define bioavailability of the following sunscreen agents: BP-3, 2-ethylhexyl salicylate (EHS), and OMC. The epidermis of four human volunteers was treated with petrolatum and emulsion-based formulations, which remained on the skin for 7 and 30 min. The composition of three sunscreen products applied on human volunteers included a commercial Daylong 15 formulation (i.e., BP-3, 2-EHS, and OMC-loaded liposomes in oil/water emulsion gel, Galderma-Spirig, Egerkingen, Switzerland) and 2 laboratory-produced petrolatum jellies including BP-3, OS, and OMC. All sunscreen products respected the maximal concentrations authorized by the directive adopted by the European Union (Cosmetic Directive 76/768/EEC, Annex VII. Part I). Profiles of sunscreen agents through the SC, derived from the assessment of chemical amounts in SC layers collected by successive adhesive tape stripping, were successfully fitted to Fick's second law of diffusion. Therefore, permeability coefficients of sunscreen agents were found lower with petrolatum than with emulsion-based formulations, confirming the crucial role of vehicle in topical delivery. Although no significant difference was shown for K values, likely because of the small number of volunteers, higher partition of chemicals between SC and emulsion-based vehicle was evidenced than petrolatum formulations, confirming the crucial role of physicochemical properties of vehicle for the topical delivery of lipophilic compounds. This assumption was confirmed by the comparison of permeability coefficients of UV filters showing higher values for emulsion than petrolatum formulations.

MARGIN OF SAFETY

Researchers calculated the margin of safety of UV filters by comparing the potential human SED with the NOAEL from *in vivo* toxicity studies

$$\left(\mathbf{MoS} = \frac{\mathbf{NOAEL}}{\mathbf{SED}}\right) \text{(39) see below.}$$

The accepted safety limit of an MoS value should be equal or more than 100. During *in vitro* studies, avobenzone, octinoxate, OCR, oxybenzone, and padimate O, using mineral oil as carrier, were assessed, with respect to their skin penetration properties, and were found to cause no harm to the underlying cells of the epidermis (40).

As sunscreen efficacy may depend on vehicle formulation, some researchers investigated the vehicle effects on UV filter skin penetration and permeation. *In vitro* release and skin permeation of two widely used UV filters, OMC (5% w/w) and BMBM (1% w/w), form topical formulations with different features [oil-in-water (O/W) emulsions with different viscosity, water-in-oil (W/O) emulsion, and oils with different lipophilicities]. To mimic in-use conditions, experiments were carried out repeating sunscreen application on the skin surface for three consecutive days. BMBM release from all these vehicles was very low, thus leading to poor skin permeation. The vehicle composition significantly affected OMC release and skin permeation, and slight increases of OMC permeation were observed after repeated applications. From skin permeation data, SED and MoS values of BMBM and OMC were calculated for all the investigated formulations after a single application and repeated applications.

SED was estimated, as reported by Søeborg et al. (41) and by the Scientific Committee on Consumer Safety (2016), using the following equation:

$$\mathrm{SED}\left(\frac{\mathrm{mg}}{\frac{\text{Kg body weight}}{\text{day}}}\right) = \frac{\mathrm{DA}a\left(\frac{\mu\mathrm{g}}{\mathrm{cm}^2}\right) \times 10^{-3}\,\frac{\mathrm{mg}}{\mu\mathrm{g}} \times \mathrm{SSA}\left(\mathrm{cm}^2\right) \times F\left(\mathrm{day}^{-1}\right)}{60\ \mathrm{kg}},$$

where DA_a ($\mu g/cm^2$) is the dermal absorption reported as amount/cm^2, resulting from *in vitro* skin permeation experiments; SSA is the skin surface area, expected to be treated with the formulation under investigation; *F* is the frequency of application of the investigated formulation; and 60 kg is the default human body weight.

The results showed that both vehicle composition and the pattern of application affected BMBM and OMC skin permeation. However, all formulations investigated could be considered safe under in-use conditions (42).

UV FILTER SUBSTANCE CLASSIFICATION

INORGANIC UV FILTERS

There are two inorganic filters (also known as mineral filters): titanium dioxide (TiO_2) and zinc oxide (ZnO); both are metal oxide particles. These molecules absorb, reflect, and refract UV photons but function in photoprotection primarily by absorbing UV radiation. The initial formulations of mineral filter–containing sunscreens often left a white, chalky

appearance on the skin, which is most noticeable in dark-skin individuals. New formulations were encouraged by decreasing the particle size, culminating in the usage of nanoparticles. Oxidative stress and cellular toxicity would be a serious concern about ZnO and TiO_2 in case they could penetrate the SC, enter the dermis, and ultimately the blood supply. Fortunately, both *in vivo* and *in vitro* studies have found that systemic absorption is not possible because their permeation reaches only the SCSC. This could be due to the nanoparticles' tendency to concentrate into larger structures (30–150 nm), which cannot penetrate into the skin (43–45). However, they might be dangerous when inhaled, and thus there use, as spray sunscreen products, containing nanoparticles, is restricted.

ORGANIC UV FILTERS

Organic UV filters belong to several organic categories that are classified as UVA and UVB filters because of their absorption properties. A great number of organic UV filters contain aromatic moieties conjugated with carbonyl groups. Excited delocalized electrons of the aromatic moieties make absorption into the UV range possible. Further substitution affects the absorption strength of various UV filter classes.

Dibenzoylmethane (1,3-diphenyl-1,3-propanedione) Derivatives. Cosmetic products contain the most common UVA filter, i.e., butyl methoxydibenzoylmethane (BMDBM), which replaced the former 4-isopropyl dibenzoylmethane (I-DBM), in accordance with the demands of a balanced UVA/UVB protection. The production of BMDBM was interrupted in 1993 because it was thought to be photo-allergizing. It is also implied that photodegradation products can also be reactive and may cause further contact allergies (46-48). However, photodegradation can be minimized, by using a UV filter combination or by encapsulation (16).

Benzophenone Derivatives. BP-3 and BP-4 are proven to have good photostability and broad-spectrum protection against the whole UVB and UVA ranges. That is why their use is extended to sunscreens and various skincare products. However, they do not offer complete UVA protection, unless they are combined. According to various studies, BP-3 tends to permeate the human skin, leaving traces in urine and breast milk, and is considered to have endocrine potential, as mentioned earlier (toxicity studies) (9,49,50).

Twenty-five volunteers applied a commercially available sunscreen containing 4% BP-3 morning and night for 5 d. Their urine was measured during those 5 d and further 5 d after the last application. They were divided into groups A (unirradiated) and B. Group B received UV radiation according to the skin type. BP-3 in urine was analyzed with a high-performance liquid chromatography method (HPLC). The volunteers excreted 1.2–8.7% (mean 3.7%) of the total amount of BP-3 applied. There was no significant difference between the two groups ($p < 0.99$, *t*-test).

It has been proven that there is a positive correlation between the use of cosmetics containing BPs and their presence in human milk. Human milk samples were collected from mothers of three different cohorts in 2004, 2005, and 2006, who gave birth to a singleton child at the University Women's Hospital Basel. The age of the mothers across all three study cohorts was similar, with a mean of 32.3 years. Milk samples were taken in August/September, October, and November/December; 54.72% of the study participants used sunscreens and 60.38% used other cosmetic products containing UV filters. UV filters were detected in 46 of 54 or 85.19% of breast milk samples (BP-3: 13.21% of total) (51).

The most common photo allergens are considered to be BP-3 and BP-4. Over the 3-year period, between January 2003 and December 2005, 1,693 patients underwent patch testing to extended British Contact Dermatitis Society standard series; 553 of these patients (33%) were also patch-tested to an extended facial/cosmetic series that included a selection of chemical UV filters supplied by Trolab (Hermal, Reinbek, Germany) and Chemotechnique (Crawford Pharmaceuticals, Milton Keynes, UK). BP-4 was the most frequently positive chemical UV filter that was tested (13 patients). It also produced the third most frequently positive patch test results overall. Of the 13 patients who patch-tested positively to BP-4, five were male and eight were female. None of the other UV filters that we added, for this study, gave any appreciable numbers of positive results. BP-3, which previously had been routinely patch-tested in the British Contact Dermatitis Society facial/cosmetic series, gave positive results in three patients (0.5%) (52,53).

P-Aminobenzoate Derivatives. One of the most widely used and commercially promoted UV filters, worldwide, is the UVB filter, 4-aminobenzoic acid (PABA) (54). Nevertheless, it was proven that not only PABA but also its derivatives could cause photoallergic reactions (55,56). In the year 2008, PABA was prohibited in the EU, as a UV filter in cosmetic products. Similarly, the use of ethylhexyl dimethyl p-aminobenzoic acid (padimate O) has also been limited and almost been replaced by different UV filters.

Salicylate Derivatives. This group of analogues is typically characterized by two UVB filters, EHS and homosalate. Salicylate derivatives are not strong UVB absorbers, but they are able to enhance other UVB filters, as they are highly water resistant, because of their water insolubility. Salicylates used in cosmetics are known to cause no allergic or photoallergic reactions, while at the same time, they do not permeate into the skin (57). Photostability and solubility make EHS a particularly suitable ingredient of sunscreens. In addition, triethanolamine salicylate, a water-soluble UV absorber, acts typically as a photo-protective agent in haircare products (58).

Camphor Derivatives. The camphor derivatives, 3-benzylidene camphor and 4-MBC, were widely used, for a long time, because of their perfect photostability, as UVB filters. In 1994, almost 30% of sunscreen products contained 4-MBC. During 2004–2006, 4-MBC was still widely used in sunscreen products. However, mainly because of their endocrine potential, the camphor derivatives have been strongly criticized in the past years.

Produced and patented by L'Oréal (Paris, France) in 1982 and approved by the EU in 1991, terephthalylidene dicamphor sulphonic acid (TDSA, Mexoryl SX) has been found to be an effective UV filter. The use of Mexoryl SX in the sunscreen "Anthelios SX" was approved by the FDA in 2006. TDSA exhibits sufficient sun protection against the negative effect of UVA rays, including pigmentation, epidermal hyperplasia, and even the limitation of skin hydration and elasticity. Also, TDSA is photostable and not percutaneously absorbed (59-61).

A study was designed to investigate the systemically absorbed dose of Mexoryl SX in humans after topical application of a typical sunscreen emulsion. In addition, to assess the correlation with *in vitro* experiments, the percutaneous absorption of this UVA filter through isolated human skin was measured under identical exposure conditions. When applied *in vivo* for a period of 4 h, 89–94% of the applied radioactivity was recovered from the wash-off samples. In urine samples, the radioactivity slightly exceeded background

levels and corresponded maximally to 0.014% of the topically applied dose. No radioactivity was measured in blood or feces sampled up to 120 h after application. *In vitro*, 24 h after a 4-h application, Mexoryl SX remained primarily on the skin surface. The mean *in vitro* absorption over 24 h, adding up the amounts found in the dermis and receptor fluid, was 0.16% of the applied dose. It was concluded from the *in vivo* pharmacokinetic results that the systemically absorbed dose of Mexoryl SX was less than 0.1%. This study demonstrated that, under realistic exposure conditions, the human systemic exposure to this UVA filter is negligible and poses no risk to human health (62).

Cinnamate Derivatives. Human exposure to ethylhexyl (octyl) methoxycinnamate (EHMC/OMC) is significant, and it can pose a risk for human health. EHMC has been found to be absorbed through the skin, and it was detected in human biomatrices, such as urine, blood, and human breast milk. Several studies have proven the toxic potential of EHMC, such as endocrine-disrupting effect *in vitro* and *in vivo*. However, it is reported and established that EHMC experiences degradation by two primary pathways, photolysis and photoisomerization. One of the products of photoisomerization is also *cis*-EHMC. When EHMC is exposed to sunlight, the commonly present *trans*-EHMC may be transferred to *cis*-EHMC (*cis*/*trans* isomerization, also called geometric isomerization). The isomeric form in which EHMC appears may impact its toxicity potential.

Researchers studied the skin permeation of the parental *trans*-EHMC and its *cis*-isomer. The *trans*-EHMC geometric isomer along with its laboratory-produced *cis*-EHMC counterpart was added in a commercial sunscreen lotion, which was applied on the skin (forearm) of two volunteers (2 mg cm^{-2}). The combined product was left on the skin for 8 h, and then tape stripping was used to remove the horny layer. A total protein assay was applied, and the thickness of the given SC was measured spectrophotometrically. The HPLC-DAD method was used to estimate the concentration of the isomers present in the extracted SC. The kinetic parameters [diffusion coefficient (D), partition coefficient (K), and permeability coefficient (k)] were calculated from the measured depth-concentration profile in six replicates (six application sites on the skin) with the use of Fick's second law [D *cis*-EHMC $= 1.62 \pm 0.83 \times 10^{14}$ ($m^2\ s^{-1}$), D *trans*-EHMC $= 1.58 \pm 0.84 \times 10^{14}$ ($m^2\ s^{-1}$)]. The values of calculated diffusion coefficients and permeability coefficients of *cis*-EHMC were slightly higher than those of *trans*-EHMC. However, the Wilcoxon nonparametric test showed no statistical difference in either k or D of both isomers ($p < 0.05$). Although the Wilcoxon nonparametric test showed no statistical difference in dermatotoxicokinetic parameter of both isomers ($p < 0.05$), the studies by Necasova et al. and Sharma et al. (63,64) showed that the *cis*-EHMC can cause more significant risk than *trans*-EHMC in the scenario of female population exposure (ages 16–65) after daily application of several kinds of personal cosmetic products. Even though the permeation of both isomers seems to be similar, the emergent *cis*-EHMC causing greater DNA damage can be more harmful than *trans*-EHMC in the same depth of SC. *In vitro* genotoxic effects of *trans*- and *cis*-EHMC on adult human liver stem cells HL1–hT1 and human-derived lymphoblastoid cells TK-6 using a high-throughput comet assay were studied. TK-6 cells treated with *cis*-EHMC showed a high level of DNA damage when compared with untreated cells in concentrations 1.56–25 $\mu g mL^{-1}$. *Trans*-EHMC showed genotoxicity after exposure to the two highest concentrations, 12.5 and 25 $\mu g mL^{-1}$.

It still remains crucial to conduct different toxicological studies of isomeric forms and define their dermatotoxicokinetic parameters to decipher the risks imposed on the human skin (65).

The group of cinnamate analogues also includes the UVB filter OCR. Studies showed that 16–24 h after application of OCR (8–10%) on the surface of skin samples, most of the OCR remained on the surface of the skin as non-penetrated material (>95%), and detectable amounts of the applied dose were found in the SC, and in low amounts or below the detection limit in other skin layers (epidermis, dermis, or receptor medium). None of the authors determined a percentage of dermal absorption. Hayden's study showed that only 0.4% of OCR was found in the epidermis and approximately 0.05% in the fluid receptor (40). Therefore, it can be concluded that transdermal absorption of OCR is very low. The sensitizing potential of OCR has been extensively reviewed in the scientific literature, and contact allergy to OCR is very rare in the general population. Photocontact allergy cases to OCR have been reported but are rare in the general population (66).

It is photostable with a good photostabilizing effect, particularly toward BMDBM. BMDBM exhibits high absorptive capacity in the UVA region, but it suffers from marked decomposition under sunlight irradiation, which leads to a reduction in the protective efficacy of the sunscreen preparation during solar exposure. In addition, its photo-fragmentation results in the formation of free radicals, which may directly or indirectly initiate skin damage. The instability of BMDBM under sunlight can be reduced by the addition of UVB filters, such as OCR or methylbenzylidene camphor, with triplet energy similar to BMDBM and acting as quenchers of its triplet state. In the case of irradiation, energy transfer between OCR and BMDBM takes place in the exited state (triplet state).

Triazones. The UVB filters ethylhexyl triazone (EHT), diethylhexyl butamido triazone (DEBT, iscotrizinol, Uvasorb HEB), and the broad-spectrum UV filter bis-ethylhexyloxyphenol methoxyphenyl triazine (BEMT, bemotrizinol, Tinosorb S/BASF Care Creations, Germany) have a molecular weight over 500 Da because of the extension and multiplication seed in their chromophoric groups. The benefits of these UV filters include the development of high absorption coefficients, anti-inflammatory properties while being efficient and also photostable (67,68). Tinosorb S can also optimize the photostability of other UV filters in a sunscreen (69).

Triazones are an integral part of an increasing number of skincare and sunscreen products, and this has to do with their beneficial properties. A particle size less than 100 nm and a molecular weight of 823.1 Da make Tris-biphenyl triazine (Tinosorb A2B) the first UV filter to be ideally included in care products around Europe. It offers great skin protection against UV radiation between 290 and 340 nm, bridging the gap between "conventional" UVA or UVB filters. In addition, it has water-dispersible action, it is broad-spectrum, and it is micronized.

Benzotriazoles. The UV filters drometrizole trisiloxane (DTS, Mexoryl XL) and methylene bisbenzotriazolyl tetramethylbutylphenol (MBBT, Bisoctrizole, Tinosorb M) are also categorized within the 500 Da rule. They have a minor skin penetration property, while they rarely cause (photo) allergic reactions (37,70). Mexoryl XL, the first photostable UV filter, offers skin protection over the whole UVB and UVA ranges. A combination of Mexoryl SX and XL also offers a synergistic effect on their protection properties (71).

Tinosorb M is produced in the form of organic microfine particles and can be dispersed in the water phase of a sunscreen. Also, it offers all the properties of organic and inorganic UV filters, while it reflects, scatters, and absorbs UV radiation. Furthermore, it exhibits sufficient photostability and broad-spectrum ability over the whole UVB, UVA-I, and UVA-II range (72).

INNOVATIVE SUNFILTER FORMULATIONS AND CARRIERS FOR THE FUTURE.

ENCAPSULATION OF $BI_2TI_2O_7$ NANOCOMPOSITES WITH DENDRITIC SILICON DIOXIDE MICROSPHERES (DSMS), AND ORGANIC FILTERS INCLUDING SINAPOYL MALATE (SM) AND BAICALIN (BS/BTO-DSM)

TiO_2 and ZnO particles have been commonly used as inorganic filters. Although inorganic filters present less skin penetration concerns than organic filters, the harmful photocatalytic activity that can induce ROS generation and ultimately damage to cells and DNA eventually may lead to carcinogenesis. Therefore, different surface-coating or encapsulation strategies have been adopted to overcome these challenges to obtain effective and safety inorganic filters with low photocatalytic activity.

DSMs encapsulated with inorganic filter $Bi_2Ti_2O_7$ nanocomposites (BTO-DSMs) and decorated with organic filters including SM and baicalin (BS/BTO-DSM) were rationally designed to effectively enhance UV protection while effectively scavenging the generated ROS and reducing skin permeability (the UV shielding properties were investigated *in vitro* by the calcein-AM/PI double-staining assay and MTT assay on HaCaT cells). To maintain the amount of active ingredients (Ti and SM) in 0.1 mg/mL, 10 mL of product should contain 22 mg of TiO_2-DSM, 25 mg of BTO-DSM, 9 mg of SM/BTO-DSM, and 10 mg of BS/BTO-DSM with 10 mL of emulsion.

The baicalin acted as the ROS scavenger to efficiently eliminate the produced ROS generated from the organic filters. The photodegradation of MO (methyl orange) was used to assess the photocatalytic activities of samples. Before the irradiation with UV light, samples (7.5 mg) were added to MO (6 mL, 60 μM) aqueous solution and kept stirring for 0.5 h. Then, the mixture was irradiated with a UVB/UVA (254/365, 16 W) light for 3 h, and the sample was collected at a regular interval (0.5 h). The obtained samples were centrifuged, and the absorbance (465 nm) of solution was measured by a UV–Vis spectrophotometer.

The protection effects against UV irradiation on the skin of female BALB/c nude mice were further evaluated. The mice were placed under UVB/UVA (254/365 nm, 16 W) radiation for 0.5 h. Three days after UV radiation, the dorsal skin was removed and stained with hematoxylin/eosin (H&E) as well as Masson's trichome for histology. Moreover, epidermal thickness and relative keratin percentage of each group were measured.

To further evaluate the skin penetration *in vivo*, FITC (fluorescein isothiocyanate) and FITC/BTO-DSM were applied topically onto the dorsal skin of the mice. Both the FITC and FITC/BTO-DSM samples had the same amount of FITC (0.1 mg/mL). After applying the samples for 6 h at room temperature, the skin samples were wiped topically five times with PBS buffer and alcohol. After that, the dorsal skin was removed, sectioned into slices, mounted on glass slides, and imaged by a laser scanning confocal microscope.

Thus, the resulting BS/BTO-DSM presented excellent *in vitro* anti-UV performance and *in vivo* UV protection against keratinocyte apoptosis and epidermal hyperplasia without long-term toxicity. The introduction of SM into BTODSM significantly broadened the UV shield range, which also prevented the SM direct contact with the epidermis and penetration behaviors (73).

NON-PENETRATING SUNSCREENS (NPSUNS)

A form of sunscreens, the skin NPSUNs, could be used in cosmetics and pharmaceutical personal care products. The main reason that led to the design of the new photoprotectors was the immobilization of UV-absorbing moieties contained in the chemical backbone of Jojoba oil. Thus, several forms of filters were created that included conjugates of Jojoba oil with UV sunscreen molecules. Jojoba oil consists of esters of fatty acids (C18–C22) with fatty alcohols (C18–C22) and has a wax structure. Its use is rather common in cosmetics and pharmaceutical products. NPSUNs have physicochemical characteristics, which allow these derivatives to stay on the upper SC, where sunscreen molecules are activated, and, thus, no further penetration to the inner dermal strata or into the systemic circulation is feasible. It was found that OMC-NPSUN possesses a similar UV absorption spectrum as OMC and could be easily formulated in cosmetic and pharmaceutical topical products. No permeation of OMC-NPSUN across the skin was observed in 24-h *in vitro* permeation experiments after application of either neat substances or OMC-NPSUNs formulated in oil-in-water cream, in water-in-oil cream, or in Jojoba oil (74).

LIPID CARRIERS

Lipid carriers seem to be a good alternative to formulate chemical UV filters reducing their skin penetration while maintaining good photo-protective abilities. Gilbert et al. compared percutaneous absorption and cutaneous bioavailability of BP-3 (concentration 5% w/w) loaded into SLNs, nanostructured lipid carriers (NLCs), nanostructured polymeric lipid carriers (NPLCs), and NCs.

The NLCs are considered as the second generation of SLNs that allow a) more effective drug loading, b) an adjustment of the drug distribution profile, and c) an extended drug entrapment during storage. NCs and NPLCs have a characteristic hydrophobic polymer around their lipid core (Figure 2). This polymeric lipid layer of NCs allows lipophilic compounds to be released and protects the encapsulated molecules from photodegradation.

A penetration and permeation study was carried out, on porcine ear skin, according to OECD TG 428 guideline (2004). Static Franz diffusion cells were used to evaluate the percutaneous permeation of BP-3 from the developed suspensions. Porcine skin was mounted between donor and receptor compartments. Donor media were composed of 1 mL of the tested formulations containing 5% of BP-3 to ensure BP-3 infinite dose conditions. Every hour for the six first hours, and then, 22, 23, and 24 h after formulations were applied into Franz cell donor compartment, an aliquot of 500 µL of receptor medium was withdrawn and immediately replaced with an equal volume of freshly prepared one. BP-3 skin distribution study was carried out 24 h after the permeation experiment. Skin samples were removed from Franz diffusion cells and cleaned with a swab that was previously moistened into distilled water. SC was entirely removed applying 20 successive tape stripping (TS) at the skin surface. Samples were analyzed using high-performance liquid chromatography.

Results showed that BP-3 partition coefficient between the SC and SLN suspension did not significantly differ from that obtained with the BP-3 albumin aqueous solution (AAS). Compared to SLNs and AAS, BP-3 showed a better affinity for NLC, NPLC, and NC suspensions, and once BP-3 crosses the SC barrier, it showed a higher tendency to penetrate the epidermis compartment. NLCs did not permit to maintain BP-3 into the SC because

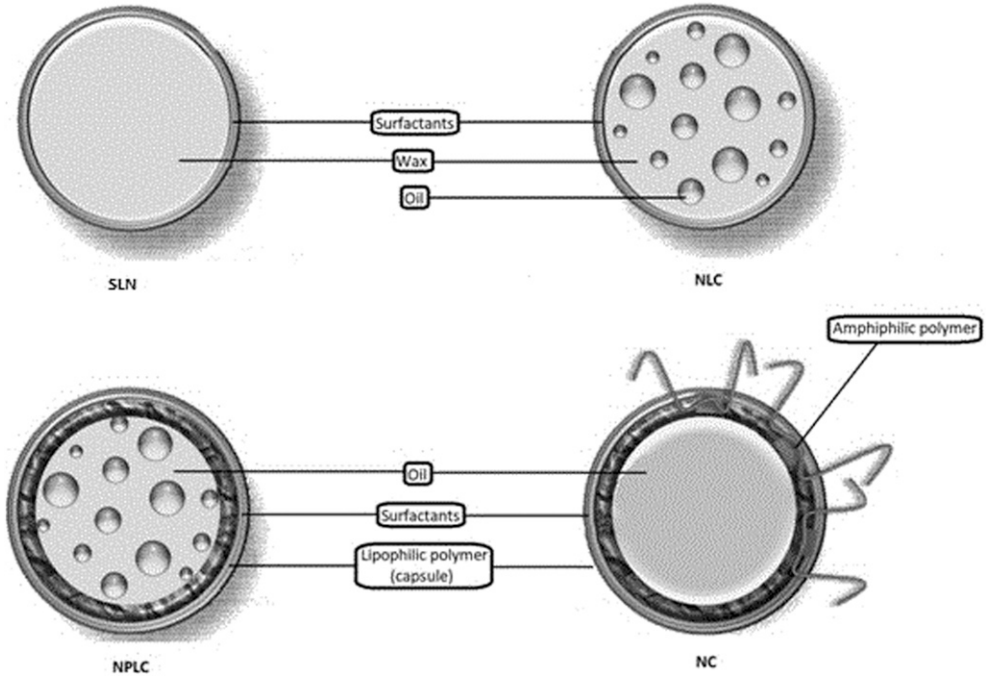

Figure 2. Schematic representation of lipid nanoparticles [SLNs and nanostructured lipid carrier (NLC)] and lipid NCs NPLC and NCs in aqueous media [modified from Kaul et al. (75)].

of the high BP-3 partition coefficient between the epidermis and the SC when BP-3 was formulated into NLC suspension (76).

The effectiveness of EHT and the ability to limit any possible toxicological effects has been made possible by the restricted percutaneous penetration of lipid microparticles (LMs). EHT encapsulation in LMs on its diffusion through the SC with glyceryl behenate and phosphatidylcholine has been examined. Creams with EHT in free or encapsulated in LM form combined with OMC and BMDBM, which are the most common UV filters and were applied on the skin of human volunteers. What was also examined was the fraction of the cream dose that was applied and had permeated in different SC layers. The cream that contained the nonencapsulated sunscreen agent presented a percentage of $21.9 \pm 4.9\%$ of the EHT dose, which was diffused in the SC. This percentage did not differ a lot from the smaller molecular weight OMC, which was found at $22.2 \pm 7.6\%$, and BMDBM, which was found at $20.5 \pm 3.7\%$. The cream that contained microencapsulated EHT gave an important reduction in 45.7% in skin permeation (77).

Nanostructured Polymer and Lipid Carriers. Polymeric (PLC) and SLNs were prepared and characterized to act as BP-3 carriers, aiming at optimizing the safety of sunscreen products. The nanoprecipitation method was used to encapsulate BP-3 (1.6% w/w) in poly nanoparticles (epsilon-caprolactone) (PCL) and hot high pressure homogenization, to encapsulate BP-3 in SLNs. In both cases, the particles remained stable for 40 d. The BP-3 encapsulated in PCL nanoparticles was released faster than BP-3 encapsulated in SLNs. A raise in the sun protection factor concurred with the encapsulation of BP-3 in both nanostructures. Also, BP-3, encapsulated in SLNs, did not seem to cause any cytotoxic

or phototoxic effects in human keratinocytes (HaCaT cells) and BABL/c 3T3 fibroblasts, whereas PCL nanoparticles with BP-3 revealed phototoxic potential in HaCaT cells.

The studies of skin permeation were made over 24 h in a Franz diffusion cell with the human skin, donated from plastic surgery. After this, the BP-3 amounts were measured in epidermis/dermis by the tapping-stripping method, as described in the receptor fluid by HPLC. During the skin permeation study, it was observed that BP-3 encapsulation in the PCL nanostructure decreased its penetration into the skin. PCL nanoparticles decreased BP-3 skin permeation by 70% in the epidermis and dermis and 80% in the receptor fluid. However, the skin permeation of SLN-BP-3 was not significantly different from that of free BP-3 (78).

Vettor et al., evaluated OMC distribution in skin compartments from OMC-loaded poly-D,L-lactic acid (PLA) nanoparticles formulated in an emulsion gel (OMC-NP emulgel) comparatively to a nonencapsulated OMC emulsion gel (OMC emulgel). Both formulations contained 5% OMC. The classical Franz cell method was first applied, and OMC amounts in each skin strata [SC, epidermis (E), and dermis (D), in the receptor fluid (RF) and at the skin surface (unpenetrated dose)] were determined after 1, 2, and 3 h exposure time.

The results showed the *in vitro* distribution of OMC in skin compartments for both formulations using either "ACET" (extraction of OMC with acetone) or "IPM" (extraction of OMC with isopropyl myristate) methods. Comparison between OMC emulgel and OMC-NP emulgel gave useful information on the cutaneous uptake of OMC in the skin depending on both formulations. When applied encapsulated in NP, the major part of OMC was retained at the skin surface over time. After 2 and 3 h, 85 and 80% of the applied OMC were, respectively, recovered on the skin surface with NP. These amounts were much higher than those obtained when OMC emulgel was applied (62 and 56%, respectively, after 2 and 3 h). This result demonstrated the nanoparticle accumulation at the skin surface. High amounts of OMC were detected in the SC (between 12 and 26% for OMC emulgel and 5–8% for OMC-NP emulgel with ACET method.

More than 80% of OMC concentrated on the top of the skin and in the SC after 3 h exposure time. The main difference between the OMC emulgel and the OMC-NP emulgel was OMC distribution between these two compartments. In the case of NP, the percentage of accumulated OMC was 10-fold higher on the top of the skin than that in the SC. This value dropped to twofold with OMC emulgel. Consequently, OMC amounts in viable skin layers (Qabs = E + D + RF) were superior for OMC emulgel than for OMC-NP emulgel (~3.5 vs. ~2% after 3 h) because the SC may play a role of reservoir. This result confirmed the higher affinity of OMC (lipophilic substance, log$p$ = 5.68) for the lipophilic skin layers, and second that the transport of NP was clearly impeded by the SC (39).

Luppi et al. synthesized lipophilic polymers composed of polyvinyl alcohol (PVA) and various fatty acids (FAs) and investigated *in vitro* the influence of the different nanoparticles prepared on percutaneous absorption of BP-3. PVA was selected as a starting material for the preparation of such polymers due to its biocompatibility and the possibility for substitution through chemical linkage to its oxy-residues able to modify its physicochemical properties. PVA was substituted, at two different substitution degrees (40 and 80%), with saturated FAs (myristic, palmitic, stearic, and behenic acid) to give to the polymer sufficient lipophilicity to allow preparation of nanomatrices for sunscreen delivery.

The diffusion of BP-3 across excised pig-ear skin was studied using a static diffusion cell based on the Franz design and analyzed by HPLC. Results indicated the nanoparticles' ability to limit sunscreen absorption. Moreover, nanoparticles with a low degree of substitution provided the highest amounts of BP-3 in the receiver compartment. Among these, nanoparticles with short chain length provided higher amounts of BP-3 than nanoparticles with high chain length. There was a correlation between the size of the nanoparticles and the fractional amount of BP-3 recovered in the skin 6 h after topical application: the amount of BP-3 decreased with increasing substitution degree and, for each degree of substitution, increasing nanoparticles size. This indicated the ability of low-substituted formulations to enhance the location of sunscreen in the epidermis, achieving high protection (79).

CYCLODEXTRIN DERIVATIVES

The interaction between 4-MBC and hydrophilic α-, β-, and γ-cyclodextrin derivatives was investigated in water by phase-solubility analysis. Among the studied cyclodextrins, random methyl-β-cyclodextrin (RM-β-CD) had the greatest solubilizing activity. The light-induced decomposition of 4-MBC, in emulsion vehicles, was markedly decreased by complexation with RM-β-CD. The influence of RM-β-CD on human skin penetration of the sunscreen was investigated *in vivo*, using the tape-stripping method. Considerable quantities (21.2–25.1% of the applied dose) of 4-MBC permeated in the SC. However, no significant differences in the amounts of UV filter in the 10 first strips of the horny layer were observed, between the formulations containing 4-MBC free or complexed with RM-β-CD. Therefore, RM-β-CD complexation did not alter the retention of 4-MBC in the superficial layers of the SC, where its action is more desirable (18). So the complexation with RM beta CD seems to be not effective.

COMBINATION OF ORGANIC UV FILTERS

Cozzi et al. carried out an investigation and made a comparison on how sunscreen formulations (whether free or encapsulated) with the common combination of organic UV filters, BMDBM and OCR, behave. This comparison was made concerning photostability, skin penetration, and retention on the surface of the skin. UV filters were enclosed in sol–gel silica glass microcapsules. Free and encapsulated UV filters were incorporated in a water-based cold lotion divided into three preparations: formulation without actives (F1), formulation containing UV filters (BMDBM 3% and OCR 9%) in free form (F2), and formulation F3 containing encapsulated UV filters. To examine the UV filter permeation in the SC and their retention on the skin surface, the Fourier transform infrared spectroscopy (FTIR) imaging spectroscopy and attenuated total reflection Fourier transform infrared spectroscopy techniques were applied.

Skin samples were treated with 2 mg/cm^2 of sunscreen formulations applied topically with 1 min of massage to cover the entire skin surface uniformly and mounted in diffusion Franz cell system. The skin samples were maintained in this condition for 2 h for the penetration measurement and during 4 h for the retention measurement on the skin surface. At the end of the 2-h treatment, the skin samples were removed from the diffusion cells, and the sunscreen remaining on the skin surface was gently removed before analysis. The tape-stripping technique was used.

The technique of FTIR imaging, in combination with a tape-stripping procedure, gives the opportunity to picture and compare the UV filter permeation of a specific sunscreen product in the SC. A different penetration behavior for the BMDBM between the traditional sunscreen formulation and the sunscreen formulation based on encapsulation technology was detected. With the regular formulation, the UV filters presented not only a high concentration on the skin surface as expected but also a significant concentration deep inside the SC, indicating the BMDBM under "free" formulation did not remain on the skin surface but penetrated deep inside the skin. UV filters were detected up to the layer six under free formulation after just one single topical application. On the other hand, the same UV filters combined with encapsulation technology were observed on the skin surface, and almost no penetration was detected inside the SC. Encapsulated BMDBM (avobenzone) was not detected after the layer one, clearly indicating that the encapsulation technology allowed to keep the UV filters at the surface of the skin where they will exert their purpose most efficiently (69) (see Figure 3).

Microparticles loaded with BMDBM or with combined BMDBM and OCR were produced by the hot emulsion technique, using glyceryl behenate as the lipid material and poloxamer 188 as the surfactant. The LMs were characterized by release studies, scanning electron microscopy, and powder X-ray diffractometry. The BMDBM and OCR loading was 15.2 and 10.6%, respectively. To reproduce the conditions prevalent in commercial sunscreen products, the photo-protective efficacy of the LMs was evaluated after their

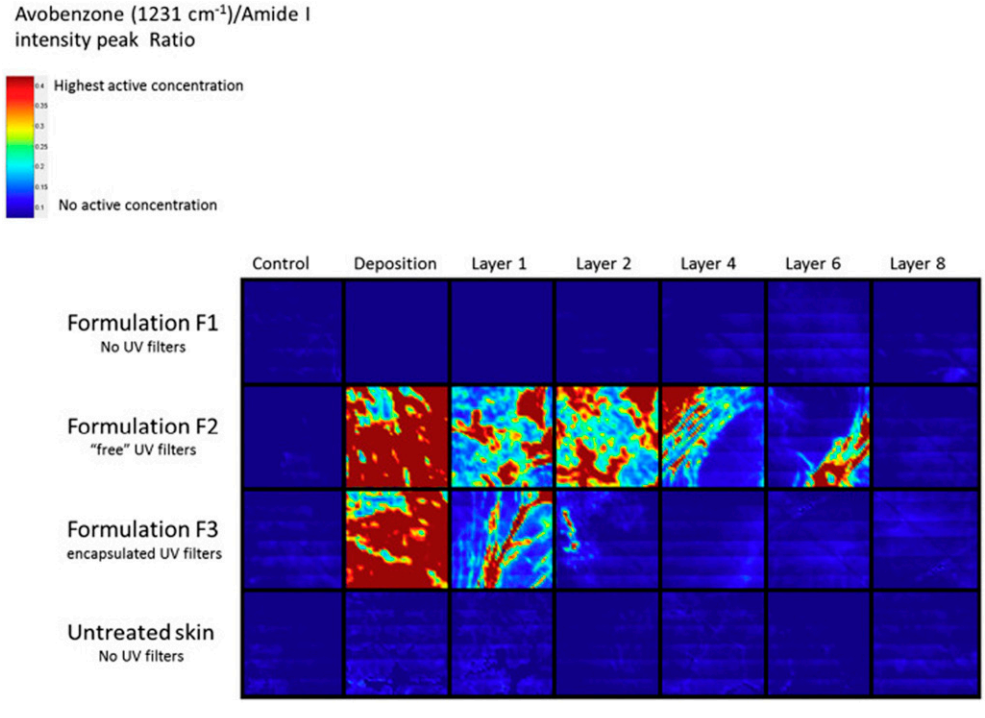

Figure 3. FTIR images allow to visualize and compare the BMDBM penetration inside the SC for different skin samples: skin samples treated with formulations F1, F2, and F3 compared with the untreated skin. For each sample, the FTIR images were scanned before (control), after topical application on the sunscreen formulation, and after eight sequential tape strips [Cozzi et al. (69)].

introduction in a model cream (oil-in-water emulsion), containing a mixture of UVA and UVB filters. A small but statistically significant decrease in BMDBM photodegradation was obtained when the UVA filter was encapsulated alone into the LMs (the extent of degradation was 28.6% ± 2.4 for nonencapsulated BMDBM and 26.0% ± 2.5 for BM-DBM-loaded microparticles). On the other hand, the co-loading of OCR in the LMs produced a more marked reduction in the light-induced decomposition of microencapsulated BMDBM (the UVA filter loss was 21.5% ± 2.2). Therefore, incorporation in LMs of BMDBM together with the sunscreen OCR is more effective in enhancing the UVA filter photostability than LMs loaded with BMDBM alone (16,80).

INCORPORATION IN MESOPOROUS SILICA

The effect of incorporating avobenzone (AVO/BMDBM), oxybenzone (OXY) and OMC in mesoporous silica (SBA-15) was investigated by Daneluti et al. Stick formulations containing "free" and "incorporated" UV filters (SF1 and SF2, respectively) were prepared. Different physicochemical analytical techniques including N_2 adsorption isotherm, small-angle X-ray scattering, and thermogravimetry/derivative thermogravimetry (TG/DTG) were used to confirm that OMC had been successfully entrapped in SBA-15.

Cutaneous delivery experiments using the porcine skin with quantification by UHPLC-MS/MS demonstrated skin deposition of avobenzone and oxybenzone after different application times (6, 12, and 24 h). The amounts of OMC and AVO permeated across the porcine skin were below the limit of quantification of the UHPLC-MS/MS method (i.e., concentrations < 10 ng mL^{-1}) for application times of 6 and 12 h. However, after application for 24 h, both UV filters were detected in the receiver compartment, and permeation from SF1 and SF2 was not significantly different at 24 h. Regarding the OXY results, this was detected in the receiver compartment after application for 6, 12, and 24 h for both SF1 and SF2 although transdermal permeation from both formulations was significantly lower at each time point with SF2: SF2 having a 30-, 12-, and 1.5-fold lower OXY permeation than SF1 after 6, 12, and 24 h, respectively. OXY showed the highest capacity to permeate the skin at all exposure times. After 24 h, the OXY amount detected in the receptor compartment after application of SF1 was 18.7-fold and 21.5-fold greater than that of AVO and OMC, respectively, whereas for SF2, it was 33-fold and 16.5-fold greater than that of AVO and OMC, respectively. OXY has a slightly lower molecular weight (228.25 g mol^{-1}) than OMC and AVO, but more importantly, it is less lipophilic (log $K_{o/w}$ 3.79 vs. 5.96 and 4.51, for OMC and AVO, respectively), and this may facilitate partitioning into the viable epidermis and hence transdermal permeation (81).

DISCUSSION

Extensive research on the tests used for estimating the permeation behavior of various groups of UV filters has been carried out. This has led to the discovery of safe vehicle systems that prevent skin absorption of efficacious UV filters and the development of advanced ones with high photostability and low toxicity.

Lipid carriers seem to be a good alternative to formulate chemical UV filters reducing their skin penetration while maintaining good photo-protective abilities.

Nanoparticles seem to be interesting carriers of sunscreen, as demonstrated by the good stability, lower toxicity, lack of phototoxic effect in cells, and no allergic reaction in mice.

In addition these particles, because of their crystallinity, can scatter/reflect incoming UV radiation, increasing the sun protection factor.

A beneficial characteristic of NPSUNs is their high skin substantivity which could minimize the need for repetitive applications. Such a characteristic, along with the factor of non-penetrability, makes NPSUNs highly applicable.

Moreover, incorporation in LMs of combination of sunscreen substances is significantly effective in enhancing the UVA filter photostability.

SBA-15, an innovative mesoporous material, increased photoprotection by UV filters while reducing their cutaneous penetration and transdermal permeation. Mesoporous silica materials of type SBA-15 are nontoxic and biocompatible, and the presence of an ordered pore network with homogeneous pore size enables a good and reproducible control of drug loading and a beneficial release profile. Furthermore, the high pore volume and large surface area facilitate drug loading and drug adsorption.

Consequently, a development of formulas based on non-penetrating photo protectors is considered to be more than relevant. This explains why the biggest challenge cosmetologists face is the development of appropriate products that could hinder skin penetration.

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DECLARATION OF INTEREST

The authors have no declaration of interest. The authors alone are responsible for the content and writing of the manuscript.

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