

Review

Cutaneous Permeation and Penetration of Sunscreens: Formulation Strategies and In Vitro Methods

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Abstract: Sunscreens are the most common products used for skin protection against the harmful effects of ultraviolet radiation. However, as frequent application is recommended, the use of large amount of sunscreens could reflect in possible systemic absorption and since these preparations are often applied on large skin areas, even low penetration rates can cause a significant amount of sunscreen to enter the body. An ideal sunscreen should have a high substantivity and should neither penetrate the viable epidermis, the dermis and the systemic circulation, nor in hair follicle. The research of methods to assess the degree of penetration of solar filters into the skin is nowadays even more important than in the past, due to the widespread use of nanomaterials and the new discoveries in cosmetic formulation technology. In the present paper, different in vitro studies, published in the last five years, have been reviewed, in order to focus the attention on the different methodological approaches employed to effectively assess the skin permeation and retention of sunscreens.

Keywords: sunscreens; formulation; in vitro methods; cutaneous permeation; skin penetration

1. Introduction

The detrimental effects of human exposure to ultraviolet (UV) radiation have been widely investigated and can be immediate, as in the case of sunburns, or long-term, causing, in most cases, the formation of oxidizing species responsible of photo-aging, immunosuppression and chronic effects such as photo carcinogenicity [1,2]. Ultraviolet radiation of sunlight consists of UVA (315–400 nm), UVB (280–315 nm) and UVC (100–280 nm) radiations, depending on the wavelength [3]. Whereas the stratospheric ozone layer completely blocks UVC radiation and UVB wavelengths below 295 nm, 90–95% of the UV radiation that reaches the Earth’s surface is UVA, with UVB accounting for most of the remainder. At longer wavelengths, UVA penetrates deeply through the skin layers, reaching the basal layer of the epidermis and the inner dermis, interacting with endogenous and exogenous photosensitizers and generating reactive oxygen species (ROS), which are responsible for the onset of DNA mutations related to skin cancer development, of the acceleration of collagen breakdown and of the decrease of collagen synthesis, with consequent appearance of skin fragility and wrinkles [4–6].

Sunscreens are the most common products used for skin protection against the harmful effects of ultraviolet radiation [7]; they should provide broad-spectrum UV protection for the presence of active ingredients, which attenuate the transmission of UV radiation onto the skin by absorbing, reflecting or scattering the incident radiation. It is not infrequent to see different types of molecules contemporaneously present in commercially available formulations, used in combination because none of them is individually able to provide broad spectrum UVA-UVB protection [8–10]. The active molecules could be classified as either “chemical” or “physical” based on their mechanism of action: In chemical sunscreens, the active ingredient is an organic compound, with aromatic structure,

that works by absorbing UV radiation and dissipating the energy as heat or light; in physical sunscreens, the active ingredient is an inorganic compound that acts by physically reflecting or scattering the UV radiation (e.g., minerals particles such as zinc oxide and titanium dioxide) [1]. Recent advances in nanotechnology have led to the production of nano-sized particles of these metal oxides, whose ability to absorb UV radiation is increased with respect to micronized ones. Unfortunately, TiO₂ and ZnO nanoparticles, in addition to being effective sunblock and eliminating the anaesthetic formation of an opaque film on the skin (due to visible light scattering), seem to possess a photocatalytic activity associated with oxidative stress and genotoxicity [11,12]. Moreover, as frequent application and reapplication after contact to water are recommended, the use of large amount of sunscreens could reflect in possible systemic absorption. Since these preparations are often applied on large skin areas, even low penetration rates can cause significant amount of sunscreen to enter the body. As the site of action of sunscreens is restricted to the skin surface or to the uppermost part of the stratum corneum, they should not penetrate into the viable epidermis, the dermis and into the systemic circulation; furthermore, the follicular uptake should be avoided, in order to not penetrate human cells where they can cause deleterious DNA damages [13,14]. This can happen when the solar-filter has a high substantivity, intended as the capacity of adhering to and of being retained by the skin, thus resisting removal by bathing or perspiration [15].

The degree of penetration depends strongly on the physico-chemical properties of the active compound, the nature of the vehicle in which the sunscreen is formulated and several factors related to the skin. Indeed, both molecular weight and lipophilicity of the molecule play an important role in cutaneous penetration, as well as it has been demonstrated that skin permeation and retention from topical products can differ significantly among the formulations used [16,17].

Traditionally, *in vitro* percutaneous absorption studies have been carried out using animal skin (pig, hairless mouse or rat) or excised human skin from cosmetic surgery or autopsy. However, various three-dimensional cultures of human skin epithelial cells, simulating the native multilayer tissue architecture, are nowadays commercially available [18,19].

In the present paper, different *in vitro* studies, published in the last five years, have been reviewed, in order to focus the attention on the different methodological approaches employed to effectively assess the skin permeation and retention of sunscreens in the light of the entry into force of the EU Cosmetic Regulation (EU/1223/2009) with the ban of animal testing for cosmetic purposes, as well as the widespread use of nanomaterials and the new discoveries in cosmetic formulation technology.

2. Organic Filters

Organic filters are molecules with aromatic structure having a carboxyl group that undergoes isomerization under the influence of energy absorbed from radiation. Their efficacy is mainly due to the physico-chemical properties, such as absorption coefficient and absorption spectra [1].

A list of the most recently (2013–2017) investigated UV-filters, classified by the type of ultraviolet (UV) radiation they absorb, is reported in Table 1, together with their structure and the physical-chemical characteristics.

Table 1. Experimental and predicted physical-chemical parameters of the most recently investigated UV-absorbers.

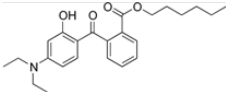
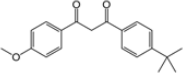
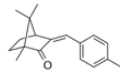
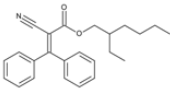
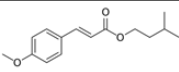
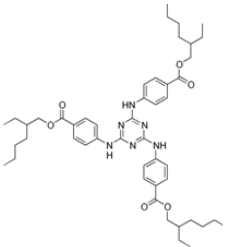
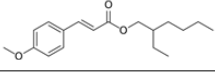
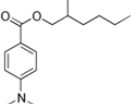
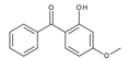
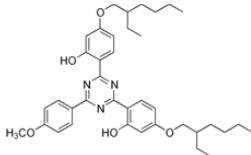
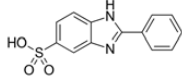
INCI Name (INN/XAN)	Chemical Structure	Brand Name	Absorption Spectrum Range	Molecular Weight (g/mol)	Log P	Water Solubility (mg/L)	Melting Point (°C)
diethylamino hydroxybenzoyl hexyl benzoate		Uvinul® A Plus	UVA1	397.515 ⁴	5.7–6.2 ¹	<0.01 (20 °C) ¹	54; 314 (dec.) ¹
Butyl methoxydibenzoylmethane (avobenzene)		Eusolex® 020, Parsol® 1789	UVA	310.393 ⁴	4.51 ⁴	2.2 (25 °C) ⁴	83.5 ⁴
4-methylbenzylidene camphor (enzacamene)		Eusolex® 6300 Parsol® 5000 Uvinul® MBC 95	UVB	258.397 ⁴	4.95	1.3 (20 °C)	66–68
Octocrylene (octocrilene)		Eusolex® OCR, Parsol® 340, Uvinul® N539T, NeoHeliopan® 303 USP	UVB	361.485 ⁴	6.78 ³	0.0038 ³	N/A
isoamyl p-methoxycinnamate (amiloxate)		Neo Heliopan® E1000	UVB	248.322 ⁴	3.6 ¹	4.9 (25 °C) ¹	N/A
Ethylhexyl triazone		Uvinul® T150	UVB	823.092 ⁴	>7 (20 °C) ⁶	<0.001 (20.0 °C) ⁶	129 ⁶
Ethylhexyl methoxycinnamate (octinoxate)		Parsol® MCX, Heliopan® New	UVB	290.403 ⁴	6.1 ⁴	0.041 (24 °C and pH 7.1) ⁴	N/A
Ethylhexyl dimethyl PABA (padimate-O)		Escalol™ 507 Arlatone 507 Eusolex 6007	UVB	277.408 ⁴	5.77 ⁴	0.54 (25 °C) ⁴	N/A

Table 1. Cont.

INCI Name (INN/XAN)	Chemical Structure	Brand Name	Absorption Spectrum Range	Molecular Weight (g/mol)	Log P	Water Solubility (mg/L)	Melting Point (°C)
benzophenone-3 (oxybenzone)		Eusolex® 4360	UVA2 + UVB	228.247 ⁴	3.7 ²	3.7 (20 °C) ²	62–65 ²
bis-ethylhexyloxyphenol methoxyphenol triazine (bemotrizinol)		Tinosorb® S	UVA1 + UVB	627.826 ⁴	12.6 ¹	<10 ⁻⁴	80.40 ¹
Phenylbenzimidazole sulfonic acid (ensulizole)		Eusolex® 232 Parsol® HS Neo Heliopan® Hydro	UVA2 + UVB	274.294 ⁵	-1.1 (pH 5) -2.1 (pH 8) ⁵	>30% (As sodium or triethanolammonium salt at 20 °C) ⁵	N/A

¹ [3]; ² [20]; ³ [21]; ⁴ Pubchem; ⁵ SCCP/1056/06 Opinion on phenylbenzimidazole sulfonic acid and its salts; ⁶ BASF safety data sheet.

2.1. Substances that Protect against UVA Radiation

Butyl methoxy dibenzoyl methane (avobenzene, AVO) is among the most common UV filters present on the market, due to the broad absorption spectrum in the UVA region. However, it suffers photo-degradation, giving rise to new compounds responsible of photoallergic and phototoxic reactions [22]. Therefore, the maximum concentration in ready to use preparation is fixed at 5% (Annex VI, Regulation (EC) No. 1223/2009).

Diethylamino hydroxybenzoyl hexyl benzoate (DHHB) is an effective UVA filter, with high compatibility with other sunscreens and it is used in solar products at a maximum concentration of 10% (Annex VI, Regulation (EC) No. 1223/2009).

2.2. Substances that Protect against UVB Radiation

Compounds from the group of camphor are characterized by high photo-stability and rarely are cause of allergic manifestations. Among them, 4-methylbenzylidene camphor (4-MBC) is authorized in Europe at the maximum concentration of 4% (Annex VI, Regulation (EC) No. 1223/2009).

A recently approved chemical compound for use in cosmetic products is 2-cyano-3,3-diphenyl acrylic acid 2-ethylhexyl ester (octocrylene, OCT), absorbing UVB radiation at 303 nm wavelength and which maximum authorized concentration is 10% as acid form (Annex VI, Regulation (EC) No. 1223/2009).

Isoamyl p-methoxycinnamate (IPMC, amiloxate), liquid at room temperature, is an efficient UVB absorber. It is a lipophilic molecule and the maximum amount that can be used in topical formulations is 10% (Annex VI, Regulation (EC) No. 1223/2009).

Ethylhexyl triazone (ETZ) possesses an excellent photostability and, thanks to its water insolubility, is often used in water resistant products. The very high extinction coefficient ($119,500 \text{ mol}^{-1} \cdot \text{cm}^{-1}$ at 314 nm) and the high molecular weight make it a very efficient solar filter [7]. The FDA does not approve its use in sunscreen products, while in Europe, it is allowed at maximum concentration of 5% (Annex VI, Regulation (EC) No. 1223/2009).

A derivative of the once-popular PABA sunscreen ingredient, ethylhexyl dimethyl PABA (Padimate O), is among the most potent UV-B absorbers. The decline in its use, along with the demand for higher sun protection factor (SPF) products, have led to the incorporation of multiple active ingredients into a single product to achieve the desired SPF, replacing single PABA esters [23]. It is suspected to release free radicals, causing indirect DNA damage, to possess estrogenic activity, and to cause allergic reactions [24]; the maximum allowed concentration is 8% (Annex VI, Regulation (EC) No. 1223/2009).

Ethylhexyl methoxycinnamate (octinoxate, OMC) is one of the most commonly used UVB filters in sunscreen products, due to its high absorption capacity in the shorter wavelength region (290–320 nm) [25]. Its safety profile has been, firstly, reviewed by the SCC (SPC/1037/93, S28) in 1993. It was concluded that the compound has a low acute toxicity, is not irritating or sensitising in animals, but can be, very rarely, responsible for allergic contact dermatitis in man (SCCS opinions 0483/01). The maximum authorized concentration in sunscreen products is 10% (Annex VI, Regulation (EC) No. 1223/2009).

2.3. Broad-Spectrum Substances (UVA + UVB)

A solid type UVA-UVB filter is represented by 2-hydroxy-4-methoxybenzophenone (oxybenzone, benzophenone-3, BP-3), a common ingredient in commercial sunscreens, thanks to the broad absorption bands in the UVA (400–315 nm), UVB (315–280 nm) and UVC (280–100 nm) regions, and therefore suitable to absorb incident solar radiation (UVA and UVB) and artificial UV sources (UVC). Although it remains photostable after being irradiated for many hours, some controversies regarding its ability to affect endocrine system and to cause dermatological problems are still ongoing [26]. In Europe, as of September 2017, the use BP-3 is allowed as a UV-filter up to 6% in cosmetic sunscreen products

and up to 0.5% in all types of cosmetic products to protect the formulation. Moreover, the consumers must be warned that the formulation contains BP-3 due to allergenic and photoallergenic potential (Annex VI, Regulation (EC) No. 1223/2009; Commission regulation (EU) 2017/238).

A recent strategy to reduce cutaneous absorption of sunscreen is the use of high molecular weight UV-filters, such as bis-ethylhexyloxyphenol methoxyphenol triazine (bemotrizinol, BMZ), one of the few chemical sunscreens with good coverage in both the UVA and UVB range. It is a new oil-soluble filter with broad-spectrum protection and high efficacy, which does not degrade under sunlight; its photostability and compatibility with many other products allow it to be used in cosmetic formulations to protect less photostable UV filters, such as AVO [7]. The maximum admitted concentration is 10% (Annex VI, Regulation (EC) No. 1223/2009).

Phenylbenzimidazole sulfonic acid (ensulizole, ESZ) is a chemical sunscreen agent that absorbs primarily UVB radiation. It provides some protection against short UVA (UVA-2) but cannot be considered a comprehensive UVA blocker. It is used as an UV-filter in cosmetic products at a maximum concentration at 8% (expressed as acid) (Annex VI, Regulation (EC) No. 1223/2009).

2.4. Natural Compounds

Nowadays, there is an increasing interest in reducing the use of synthetic UV-filters by incorporating in sunscreens natural compounds that exhibit a similar filtering activity. These so-called “natural chemicals” (i.e., polyphenols, carotenoids, vitamins and anthocyanidins) possess radical scavenger properties against ROS generated by UV-filters reaction with solar radiation, providing broad-spectrum sunscreen products with antioxidant, wound healing and anti-inflammatory properties [27]. The booster effect on the body natural reserve of antioxidants can contribute to neutralization of intrinsic and extrinsic ROS, creating a new kind of sunscreen with a two-step protection: The first operated by UV-filters as a “passive” protection by absorbing and reflecting UV radiation and the second as “active” protection by antioxidants quenching ROS generated by UV light that has by-passed UV filters [4]. Differently from synthetic UV-filters, which have to remain on the stratum corneum to be safe and effective, natural compounds should reach the viable skin layers to exploit photo-protection effect, since ultraviolet radiation penetrates deeply the skin [16].

Among the naturally occurring polyphenols, one of the most investigated products is trans-resveratrol, which possesses antioxidant, anti-inflammatory and anti-tumoral properties, as widely reported. It has also been demonstrated that it is able to inhibit UVB induced inflammation and lipid peroxidation of the skin following topical application [28].

The well known carotenoid β -carotene possesses skin protective effects against UV and IR radiation and if applied prior to irradiation is able to protect skin from UVA induced oxidative stress [29].

2.5. Inorganic Filters

Inorganic filters are inert and non-irritant substances, able to protect the skin from the incident solar radiation due to physical phenomena, such as scattering and reflection, by forming a layer over the skin that works as mechanical barrier [30]. Notwithstanding the physical filters on the market are very few with respect to the chemicals, they possess many advantages, as high photo-protection level in the longer UVA range, photostability and low photoallergic potential. The most investigated inorganic UV filters are zinc oxide (ZnO) and titanium dioxide (TiO₂).

2.6. Nanomaterials

Available literature data on skin permeation and penetration of nanomaterials should be deeply evaluated in order to perform a risk evaluation of these relatively new kinds of materials. Despite the relevant scientific data on this topic, there is still the need to provide a definite safety profile related to nanoparticles skin exposure [31]. Indeed, notwithstanding the stratum corneum represents the outermost barrier for penetration of exogenous substances, it has to be taken into account that this

layer could undergo impairment or disruption upon treatments (i.e., exposure to irritant compounds, solvents or detergents) or age related mechanisms; it is also well known that skin diseases, such as contact dermatitis or erythema, can cause an increase in skin permeability. Moreover, during the last years, the trans-appendageal route (diffusion through the hair follicle and sweat gland) has gained importance, as either potential target site or shunt for delivery of various molecules [32,33]. Therefore, the first point to clarify is the mechanism of nanoparticles penetration through the skin. Many authors proposed nanoparticles storage in the skin lipid matrix between corneocytes or in the appendages, from where they are released in a controlled and prolonged manner. On these bases, it is fundamental to further investigate how nanoparticles size, shape, surface chemistry and charge can affect skin penetration, also in relation to other physical factors concerning the environmental media (temperature, pH, vehicle etc.) [31]. A significant point to take into account is that *in vitro* alternative methods to animal testing have not yet been validated for nanomaterials, representing an obstacle to the safety assessment of these cosmetics ingredients in the European market. Anyway, given the importance of the subject and the wide literature on it, it was necessary to mention the subject, but it will not be discussed in this review.

3. In Vitro Methods

In vitro studies on sunscreens published from 2013 to 2017 have been reviewed in order to focus on the assessment of the permeation/penetration profile of the molecules not in their nano-form. A collection of these experiments is summarized in Table 2. It is a consolidated opinion that *in vitro* tests on the skin, using Franz cells and similar techniques, allow to obtain important information on penetration pathways, in order to ensure that the investigated molecules are effective and their residence time in the skin is adequate to assure UV protection. These methodologies are considered a reliable model to investigate skin diffusion, even though what happens in real condition can be underestimated. Indeed, in real condition skin permeation could be increased when superficial impairment or skin flexion happened as well as for active transportation [31].

Some controversies have been occurred regarding the skin models to be employed during permeation/penetration studies, in order to select the most appropriate to resemble human *in vivo* conditions. As reported in OECD guidelines for the testing of chemicals (OECD 427-428, 2004), skin from human or animal sources can be used, as either epidermal membranes or dermatomed skin at different thickness. During the last years, different kinds of skin have been investigated, on the general principle that rats' and rabbits' one is more permeable than that of humans, while the skin permeability of pigs is more similar [34,35]. Anyway, the selection of one species rather than another, the anatomical site and the preparative technique must be justified in performing *in vitro* tests. Full thickness skin, dermatomed at a constant depth, is the preponderant choice for *in vitro* permeation/penetration tests; some authors decided to use human abdominal epidermal membranes, removed from the dermis with a scalpel blade, dried and stored at 4 °C before experiments. The choice of stratum corneum-epidermis membrane instead of full thickness skin was justified since the dermis could act as a further barrier to permeation, distorting *in vitro* evidence [7].

Table 2. In vitro studies for the assessment of skin permeation/penetration of sunscreens.

Reference	Sun-Filter	Formulation	Substrate	Equipment	T (°C)	Receiving Phase	Length (h)	Analytical Procedure	Results
[3]	IPMC DHHB BMZ	Biomimetic O/W cream	Full thickness porcine ear skin	Franz cell	30	6% Brij PBS	12	HPLC -receiving phase -15 tape strips for SC removal -remaining tissue	None of the molecules was detected in the receiving phase after 12h and the sunscreens were largely detected in the 5 tape strips
[5]	BP-3	SLM	Porcine ear skin	Franz cell	37	Buffer 150 mM pH 7.2 + 0.5% Tween 80	12	HPLC tape stripping (SC) and E+D	SLM with natural waxes are able to inhibit permeation and reduce 3-fold penetration with respect to free BP-3
[6]	BP3	-Emulsion -Emulsion with BP3 encapsulated in mesoporous silica	Cellulose membrane	Franz cell	37	pH 7.4 buffer + 2% Tween 20	24	UVvis	Skin permeation of BP-3 was prevented due to encapsulation by MS
[7]	BMZ ETZ DHHB OMC AVO	NLC	Human skin (SC+E separated from the dermis by treatment at 60°C for 2 min)	Franz cell	32	EtOH/water 50:50	24	HPLC	Comparison NLC/nanoemulsion; NLC reduced permeation and the filter remained on the skin surface
[9]	4-MBC	-4-MBC polymeric microspheres in O/W emulsion -free 4-MBC in O/W emulsion	Episkin	Harvard apparatus	37	pH 7.4 phosphate buffer 66.7 mM + 1% Brij98	5	HPLC tape stripping (2 strips for SC) and extraction from remaining Epidermis	Encapsulation in microspheres remarkably reduced the permeation of 4-MBC and increased its retention on the skin surface
[13]	BP3 AVO ZnO	EtOH/buffer	Nude mice-8 and 24 weeks	Franz cell	37	30% EtOH/ buffer pH 7.4	24	HPLC, atomic abs. differential stripping	UVA and UVA/UVB increased follicular uptake for BP-3 and AVO, particularly for senescent skin; ZnO produced no permeation/penetration; AVO produced no permeation and penetration was higher for young skin
[14]	Padimate O	Bioadhesive nanoparticles (BNP)	Fresh pig skin	Incubation of skin with formulation in humidity chamber and subsequent washing with PBS buffer	32	/	6	HPLC tape stripping (30 strips), remaining skin chopped and extraction performed	No Padimate-O penetrated in the skin from BNPs; minimal amounts were found in the tape stripped skin, suggesting minimal epidermal penetration
[16]	BMZ OMC AVO OCT Resveratrol β-Carotene	O/W Emulsion	Porcine ear skin dermatomed at 500 μm	Franz cell	32	Phosphate buffer (pH 7.4-0.1 M) + 4% w/v BSA	12	HPLC tape stripping (16 strips) and E+D cut in little pieces	The permeated amounts were below LLOQ; over 90% was retained in the SC; the presence of both resveratrol and carotene reduced the amount of UV filters in the SC; BMZ exhibited the lowest penetration rate
[20]	BP3	SLN NLC NPLC NC	Porcine ear skin dermatomed at 600 μm	Franz cell	37	Albumina PBS solution	24	HPLC -SC (20 strips) -E and D separated with scalpel	NPLC and NC were able to significantly reduce BP-3 flux across the skin, exhibiting high in vitro SPF

Table 2. Cont.

Reference	Sun-Filter	Formulation	Substrate	Equipment	T (°C)	Receiving Phase	Length (h)	Analytical Procedure	Results
[36]	AVO BP-3 ESZ	Complex with β -cyclodextrin o/w cream	Wistar male rats abdominal skin	Franz cell	37	phosphate buffer pH7.4 and isopropyl alcohol 70:30	6	HPLC	ESZ permeated the rat skin in a higher amount; the complex BP-3-CD was found to be the safest one, both in terms of slow rate of permeation and prolonged lag-time
[37]	AVO	-AVO encapsulated in modified dextrin formulated in O/W emulsion -- free AVO in O/W emulsion	Cellulose membrane	Franz cell	37	pH 5.5 buffer + 2% Tween 20	6	HPLC	AVO encapsulated in modified dextrin and dispersed in an emulsion exhibited a transdermal flux 2.5-fold lower than free AVO.

Analysis of recent literature shows an increasing use of porcine skin with respect to the past [5,14,16,23]. Most of the studies employed the outer surface of freshly excised pig ears, after hair and underlying cartilage removal. The skin was often dermatomed to reduce its thickness and stored at $-20\text{ }^{\circ}\text{C}/-80\text{ }^{\circ}\text{C}$ for a maximum period of 30 days before use. Different receptor media were chosen, depending on the solar filter investigated and considering the very low water solubility of these molecules and their generally high lipophilicity (Table 1). The choice of an appropriate receiving phase is determinant for these studies, especially when lipophilic molecules are investigated, as it can lead to false conclusions linked to insufficient solubility of UV-filters. Moreover, it has to be taken into consideration that the receptor fluid chosen should not alter the barrier properties of the skin. In case of studies on BP-3, the receptor medium used consisted of phosphate buffer solution (pH 7.2, 150 mM) added of either albumin or 0.5% Tween 80 to ensure BP-3 solubility [5,23]. In another study, a phosphate buffer (pH 7.4, 100 mM) containing 4% *w/v* bovine serum albumin (BSA), for its ability in solubilizing lipophilic molecules, represented the receiving phase when the behaviour of different solar-filters (BMZ, OMC, AVO, OCT) after cutaneous application of an O/W emulsion [16] had been investigated.

In most cases, the permeation experiments lasted 12–24 h and, after this time, the amount of active ingredient distributed in the different skin layers was evaluated by removing SC with 15–20 subsequent tape-strips and by mechanically separating epidermis (E) and dermis (D). In all reported cases, the samples were analyzed by high performance liquid chromatography after opportune extraction treatment from the biological matrix to quantify the amount of solar-filter in the receiving fluid and the skin portions.

It was found that BP-3, with a relatively low molecular weight and a log P of 3.58, had itself a good ability to permeate and penetrate the skin. OMC, AVO, OCT and BMZ that possess the log P of 5.96, 4.51, 6.78 and 12.6, respectively, indicative of a high lipophilicity, was retained over 90% in the SC while the permeated amounts were below LLOQ. Due to these characteristics, they seem able to accumulate into the lipid phases of the stratum corneum, producing a kind of reservoir, while they appear to have difficulty in penetrating the viable epidermis, layer of predominantly hydrophilic nature.

Shokri et al. [36] investigated the fate of AVO, BP-3 and ESZ formulated in a cosmetic O/W emulsion as free filters or included in a complex with β -cyclodextrin in permeation studies through abdominal skin of Wistar rats, which were shaved with razors 24 h prior of the experiments. It is necessary to emphasize that these products are among the most common UV filters on the market and that, after cutaneous application, *in vivo* have been demonstrated to permeate the skin in significant amounts.

The receptor phase was based on phosphate isotonic buffer pH 7.4 and isopropanol 70:30 *v/v* to favour sunscreens solubility. The quantitative determination was performed by high performance liquid chromatography (HPLC). It was found that ESZ, when applied on the skin free or included in the cyclodextrin complex, was able to permeate the rat skin in a higher amount than BP-3 and AVO. In all cases, the complexation not only reduced the amount of permeated agent, but also prolonged the lag time of permeation. It is important to highlight the different behavior of the filters in relationship with their chemical–physical characteristics. In fact, both BP-3 and AVO exhibited a moderate lipophilicity and a low molecular weight, which allow the transit through the stratum corneum, but hinder a high penetration in the more hydrophilic viable epidermis and dermis. However, ESZ possesses a log P of $-1.1/-2.1$ (Table 1) and a comparable molecular weight with respect to the other filters. On this basis, it seems that the ESZ dimension are somewhat responsible of its penetration through the stratum corneum, while the hydrophilic character allow a high flux through the skin.

By comparing the results obtained from the above mentioned permeation studies through different skin models, it is clear that free BP-3 applied in O/W emulsion was able, in every case, to permeate the skin and showed the same flux through both pig and rat skin, while the time to saturate the membrane was higher in case of pig skin. Conversely, the more lipophilic AVO did not permeate the pigskin, while it was found in appreciable amounts in the receiving phase of permeation studies through rat skin.

Such results confirm what is already known from previous studies, suggesting that rat skin is generally more permeable than pigskin (and human skin) towards permeants with different physicochemical properties and in particular for the more lipophilic ones. Indeed, both the composition and packing of stratum corneum lipids, known to be key factors of skin permeability, differ between rodent and pigskin [38].

Most researchers are employing intact skin for the permeation/penetration studies, without taking into account that aging processes, diseases and sun exposure could often alter the skin structure. As some permeants could overcome a compromised barrier and penetrate through skin by inducing toxicity, it would represent an interesting tool to evaluate percutaneous absorption of solar filters through altered skin. Such an experiment was performed by Hung et al. [13], which used nude mice aged eight and 24 weeks as animal model for young and senescent skin in order to mimic chrono-aging. The use of nude mouse was justified since it has been reported an identical histology and biochemistry to human skin in photoaging studies [39]; even though it is notably more permeable, the mouse skin could be a useful model of facial skin on which the filters are applied, legitimizing the use of mouse skin in this kind of experiment [40]. Moreover, they irradiated the dorsal skin of the mouse with UVA (365 nm, 10 J/cm²) every other day for three days and with UVB (312 nm, 175 mJ/cm²) once a day for five days, at a distance of 10 cm for a period of 100 min and 1 min for UVA and UVB, respectively, in order to induce skin photo-aging. A combination of the two was also carried out to simulate UVA + UVB radiation. The evidence of aging was checked by macroscopic (pH, TEWL, lightness) and immuno-histological evaluation. The skin absorption and follicular uptake of AVO and BP-3 as chemical sunscreens and of ZnO as physical sunscreen were evaluated by performing a permeation experiment with Franz vertical diffusion cells followed by the differential stripping technique from aqueous vehicles containing the solar filters. The experimental conditions were set with a receiving phase consisting of 30% ethanol/pH 7.4 buffer, a temperature of 37 °C and an experiment length of 24 h. To completely remove the stratum corneum from the skin, at the end of the permeation experiment, 20-tape strips were performed, followed by cyanoacrylate casting to extract the hair follicle. It was found that ZnO, both in the micronized and nano-form, was not able to penetrate into the skin or the receptor, regardless of the treatment used. The behavior of BP-3 was not affected by chrono-aged skin, while irradiation, in particular UVA and UVA + UVB, increased both the permeation and the deposition of the filter in the follicle. BP-3 has been shown to penetrate skin and reach the circulation, phenomenon that appears more severe when the skin is irradiated with UV light. On the contrary, regarding the more lipophilic AVO, senescent skin showed less deposition with respect to young skin, probably due to lower sebum distribution in aged skin; the follicular uptake in senescent skin was increased by UVA or UVA + UVB radiation, thus reaching the same values of young skin. In any case, AVO was able to permeate the skin, maybe due to its high affinity for stratum corneum. The application of AVO and ZnO to photoaged skin may not increase the risk of excessive absorption; besides, when the skin is naturally aged the skin penetration of AVO is even reduced.

As stated above, many studies investigating the fate of UV filters after cutaneous application performed sequential tape stripping in order to evaluate the amount of the molecule penetrated in the stratum corneum. The evaluation of drug penetration into the stratum corneum (SC) by tape stripping requires an accurate measure of the amount of SC on each tape-strip in order to determine the stratum corneum depth. Recent studies are applying infrared densitometry (IR-D) to in vitro tape stripping using SquameScanTM 850A [3] to verify the endpoint of tape-stripping, i.e., complete SC removal. In fact, the SC depth can be extrapolated from the IR-D data of sequential tape-strips, where the protein content of each tape strip can be indirectly quantified from the tape absorbance [41]; the lower limit of quantification of IR-D indicates the complete removal of the SC (less than 5% of the total SC remaining) and can be used to know the exact numbers of tapes needed. Haque and co-workers [3] investigated the behavior of three UV-filters with different chemical-physical characteristics (BMZ, DHHB and IPMC) using full thickness pig-ear-skin and found that, at the end of the permeation study (12 h), most of the applied dose was recovered in the first five tape strips and none of the sunscreens was detected

in the receiving phase. The IR-D technique allowed the authors to affirm that the UV absorbing molecules were largely distributed in the first 1.7 μm of the SC, with smaller amounts accounting for the other 3.8 μm , confirming only superficial penetration of these materials as for their intended use.

Among all the papers reviewed, only the study performed by Monti and colleagues [19] used a reconstructed human epidermis model from normal human keratinocytes (Episkin, SkinEthic Laboratories, Lyon Cedex 7, France) as substrate for the permeation/penetration studies; this model is histologically similar to the native human epidermis. Episkin was placed between on the donor and receiving chambers of a Harvard apparatus, equipped with six thermostated cells, The receiving phase consisted of pH 7.4 phosphate buffer solution (PBS) added with 1.0% Brij 98 to increase the solubility of the sunscreen under study, 4-MBC. To assess the distribution profile of the solar filter in the skin, the tape-stripping technique was employed. As the tissue was constituted only of the epidermis without dermis, two tape strips were performed and the remaining tissue was considered as the living epidermis. In order to quantify the degree of skin penetration of 4-MBC, an extraction procedure from the tissues was performed and the samples were analyzed by HPLC. Since the Cosmetic Regulation 1223/2009 have banned the use of experimental animals for testing cosmetic products and there are, at the moment, no validated methods for the assessment of the permeation/penetration, such kind of studies could broaden the knowledge of the theme and produce important elements of evaluation.

4. Formulation Strategies

Many scientific reports confirm the interest in formulating innovative UV filters carriers to achieve high skin photo-protection, contemporaneously reducing undesirable effect linked to skin permeation. Colloidal carriers have been demonstrated to promote the accumulation of the sunscreens in the uppermost layers of the skin, where their action should occur, by enhancing their photo-protection ability. Lipid nanocarriers are almost made of well-tolerated and biodegradable raw materials, which, together with the colloidal size of the particles that facilitates the formulation in dermatological products, enable comfortable skin application. In recent years, several studies focused on the formulation of UV filters in micro and nanocarriers to protect them from photo-degradation and to prevent skin permeation [5,7,14,19,30].

Since it is noteworthy that BP-3 permeates across the skin leading to undesirable effects [23], a deeper knowledge of the influence of different formulation on its penetration properties could provide interesting implications. Martins et al. [5] showed that BP-3 incorporation in solid lipid microparticles (SLM) with natural waxes, such as carnauba wax, was able to inhibit permeation and reduce 3-fold penetration with respect to free BP-3. The importance of the stability of microparticles has been underlined, since a degradation of the carrier components could lead to a faster release or not prevent skin penetration.

Moreover, it has been demonstrated [19] that the cutaneous penetration of 4-MBC decreased when it was incorporated in polymeric cationic microspheres with respect to that obtained from free sunscreen, without change in SPF. The microspheres formulated in a W/O emulsion appeared to bind to keratin for a long period of time, thereby increasing the uptake of 4-MBC on the skin surface, especially stratum corneum, where it can explicit its action.

Nanostructured lipid carriers (NLC) are characterized by a solid lipid matrix, in which a liquid lipid is added; on the other hand, a wall of hydrophobic polymer surrounding their lipid core typically characterizes both nanostructured polymeric lipid carriers (NPLC) and nano-capsules (NC). It has been reported [7] that NLC dramatically reduced the skin permeation and favored sunscreens localization in the superficial layers of the skin when compared to a nanoemulsion formulation. Among the sun filters tested, AVO and DHHB exhibited the higher flux at the steady state when formulated in nanoemulsion (log P 4.5 and 5.7, respectively) and showed a reduced flux when encapsulated in NLC. However, ETZ and BMZ, in any case permeated through the epidermis after 24 h, maybe due to their high substantivity for the stratum corneum as highlighted also by the value of log P that was >7 and 12.6 for ETZ and BMZ, respectively. It is interesting to underline that the degree of flux reduction

after encapsulation, more considerable for DHHB, seems to be independent of both molecular weight and lipophilicity of the original molecule. It could be interesting for further works to deepen the physical-chemical characteristic of the complexes themselves, as well as their mechanisms of interaction with the skin.

Moreover, since, in commercially available formulations, two or more sun filters are often combined to broaden the solar spectrum coverage in both the UVA and UVB regions, the effect of NC and nanoemulsion was evaluated on OMC and AVO simultaneously present in the same formulation. It was found that, when the sun filters were incorporated in NLC, they exhibit a lower flux than the nanoemulsion containing the same amounts of molecules. Furthermore, the application of NLC, both containing some filters, did not produce an appreciable increase in the amount of substance permeated through the full epidermal layer; on the contrary, the use of nanoemulsion led to a significantly higher amount of AVO and OMC with respect to the same emulsion containing only one of the two. Besides, Gilbert et al. [23] demonstrated that, when BP-3 was formulated into NPLC and NC suspensions, the polymeric envelope retained the molecule in the lipid matrix and the presence of poloxamer 188 in the aqueous phase could solubilize free BP-3, thus reducing BP-3 flux through the skin with respect to the albumin aqueous solution of the filter. Moreover, it was observed a better efficiency of polymeric nanoparticles to reduce BP-3 penetration in the skin layers and to show the highest *in vitro* SPF.

Another interesting formulation tool is represented by bioadhesive nanoparticles (BNPs), described by Deng et al., [14]. Starting from polylactic acid-hyperbranched polyglycerol (PLA-HPG) nanoparticles, the HPG was converted into an aldehyde-rich corona with bioadhesive properties, and padimate-O was incorporated in these new highly skin adherent and not penetrant BNPs. The BNPs, thus prepared, remained on the stratum corneum after topical application, from which they could easily be removed with active towel drying because of water-resistance, and the deposition into hair follicle was prevented.

Among the properties that influence partition/dissolution of sunscreens agents into the surface of the stratum corneum and their diffusion through the lamellar lipid layers, it can be mentioned both the molecular weight and the lipophilic characteristics. In order to increase the dimension of the solar filter and its concentration in the upper stratum corneum, the formation of inclusion complexes with cyclodextrins has been investigated, also to increase the sunscreen photostability. Shokri et al. [36] prepared an inclusion complex of β -cyclodextrin with three different UV filters (AVO, BP-3 and ESZ) by different methods, i.e., co-evaporation, grinding and kneading. They found that the complexation reduced the rate of permeation of sunscreens with respect to when the free filter was taken in account, also increasing the lag time, while the physical mixture affected permeation only for a little extent.

Another strategy to improve the effectiveness of sunscreens is the combination of organic and inorganic filters, as performed by Li et al. [6,42]. The authors encapsulated BP-3 into the inorganic UV-filter mesoporous silica (MS) by an *in-situ sol-gel* process using tetraethyl orthosilicate as a precursor and an ionic liquid as solvent and pore-forming agent. Moreover, they tuned up a cheaper and timesaving procedure, consisting in adsorbing BP-3 onto MS, used as drug delivery systems with a high surface area. A synergistic effect on the UV-absorption ability was observed and was ascribed to the lowered crystallinity of the BP-3 molecules and the additional light scattering induced by the mesoporous structure, which led to a greater optical density. Furthermore, it was found that an O/W emulsion containing the BP-3 adsorbed or included in MS exhibited *in vitro* SPF and UVA-PF higher than the free BP-3 containing emulsion and, at the same time, the *in vitro* release profile BP-3 through a cellulose membrane was significantly reduced. In particular, the encapsulation of BP-3 in MS produced the lowest flux through the membrane, suggesting a tightly entrapment of the filter in the MS matrix. The same authors in another paper used a modified dextrin as drug carrier for AVO [37]. The dextrin was modified via reactions with alkyl oxiranes to create a biodegradable molecule and more stable against protein denaturation, with a decreased skin affinity [43]. As previously reported for other products, encapsulation eliminates crystallinity of AVO, suggesting the entry of the molecule into the

cavities of the modified dextrin; moreover, the release of AVO through a cellulose membrane from a cold-process prepared O/W emulsion containing the encapsulated filter was even slower than from the same emulsion containing the free-AVO, suggesting a low degree of skin penetration.

5. Discussion

Recent studies confirm that exposure to solar radiation is associated with adverse effects on the skin, such as aging and cancer. As a result, effective sun protection and improved body defence system have become important research topics. Currently, most of the solar protection products on the market contain organic or inorganic UV filters that are primarily directed against radiation induced sunburn and DNA damage. However, some of these UV filters can penetrate the skin and at high concentrations can accumulate in the tissues, causing allergies and/or contact dermatitis [43].

In addition, filters can undergo photo-degradation following sunlight or artificial light exposure, leading to a decrease in their UV protection capability and to the generation of harmful photolytic products, such as free radicals and ROS. Therefore, recent studies aimed at searching for encapsulation or incorporation methods for organic UV filters in order to reduce skin penetration and to design an effective carrier based on new technologies in controlled delivery.

In the past, the stratum corneum was considered the only permeation barrier for chemicals to enter in the skin, but, in recent years, also viable epidermis and dermis has gained importance for their role in skin absorption of small molecules [32,44]. Alterations of the biochemistry and the structure of the skin layers may have a role in molecular delivery through the skin. Recent studies highlight the role of UVA and UVA + UVB radiation in the expression of E-cadherin in the stratum granulosum, which contributes to epidermal barrier by governing tight junctions and whose levels are reduced by irradiation [13] provoking epidermal thickening and wrinkled appearance. Moreover, radiation can induce some proliferative activity in the epidermal layers and an up-regulation of epidermal COX-2 expression in chrono-aged and photo-aged skin, suggesting inflammatory processes. Anyway, it has been demonstrated that not necessarily intrinsic and extrinsic aging increase skin permeation, especially in case of lipophilic permeants. An important role is represented by UVA radiation, the main responsible of percutaneous absorption during solar exposure.

The physicochemical properties of the solar filters are determinant in the process of penetration into and permeation through the skin layers. In particular, the log P value is a crucial element to assess if a molecule is able to permeate across the skin or not. Generally, a log P value above 2 indicates a high lipophilicity of the compound and it is likely that such molecules are capable of accumulating and forming reservoirs within the lipid phases of the stratum corneum. Additionally, these agents would have difficulty in penetrating the viable epidermis and dermis because of the hydrophilic nature of these layers. However, highly hydrophilic molecule would remain above the stratum corneum, while molecule that exhibited both aqueous and lipophilic properties are candidate to permeate the skin. Several studies pointed out on the tuning of micro- and nano-carriers to formulate chemical solar filters, in order to reduce their skin permeation and penetration, favoring the retention in the outer stratum corneum, where they are desired to act, and to protect them from photo-degradation.

The vehicle chosen to deliver filters to the skin is fundamental in influencing dermal absorption. The studies mentioned above suggest that the choice of high molecular weight filters and the use of O/W emulsions can contribute to obtain low skin permeation rates and high UV filter retention in the stratum corneum. Moreover, combination of UV filters and antioxidants could influence the skin retention of the filters, by reducing the amount of filters penetrated in epidermis and dermis. While filters must remain on the skin surface and have high substantivity for stratum corneum, antioxidants, present in the sunscreens formulation, should penetrate the skin to act as radical scavengers in the deeper skin layers, without reaching the systemic circulation. As well as penetration of UV filters in the skin layers, in addition to compromise the protective effect on the skin, can cause photosensitivity and an increased risk of allergic reactions, the combination of UV filters and

antioxidants in a sunscreen can improve the efficacy of the product, with a synergistic effect in UV skin protection and antioxidant activity.

In the majority of the cases analyzed in this review, the *in vitro* evaluation of the filters behavior towards the skin is performed with vertical diffusion cells, in order to establish the entity of permeation and penetration of the molecule through and into the skin. In the last years, the follicular route has gained much importance and the differential stripping technique has been proposed, in order to differentiate transepidermal and transfollicular penetration, allowing the quantification of substances in all the skin compartments.

The entry into force in the European Union of the Cosmetic Regulation 1223/2009, with the ban for animal testing for cosmetics and the absence of validated alternative test for the skin permeation/penetration studies has led researches to increment the use of human tissues from abdominal or aesthetical surgery. Anyway, due to the lack of suppliers and the difficulties in availability, many researches in Europe are using pig ear skin, allowed because considered as waste material from slaughter, or *in vitro* reconstructed human epidermis from normal human keratinocytes.

There is still a long way to go, because, although formulation strategies are improving and the road to reduce the penetration of sunscreens seems to have been found, the methods to assess their skin permeation/penetration with a margin of accuracy and reproducibility and with reduced time and costs, in the respect of ethical principles, are still so far.

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