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PENETRATION OF TWO SUNSCREENS IN VARIOUS FORMULATIONS THROUGH A SYNTHETIC MEMBRANE AS COMPARED TO HUMAN SKIN IN VITRO

FARKLI FORMÜLASYONLARDAKİ İKİ GÜNEŞTEN KORUYUCU MADDENİN IN VİTRO İNSAN DERİSİ İLE KIYASLANARAK SENTETİK BİR MEMBRANDAN PENETRASYONU

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The purpose of this work was to determine the skin penetration ability of ultra-violet(UV) filters from four vehicles (an O/W emulsion, a W/O emulsion, oleagenous cream, and Carbopol gel) in order to gain knowledge on the safety of sunscreen products. The UV filters tested were 2-phenylbenzimidazole 5-sulphonic acid (8%, A) and Oxybenzone (8%, B). In vitro penetration measurements were conducted by using Franz diffusion cells and Attenuated total reflectance Fourier transform infra-red (ATR-FTIR) spectroscopy. It has been shown by both the Franz diffusion cell technique and the use of ATR-FTIR spectroscopy that the investigated UV filters showed differences in penetration rate due to various vehicles. The results demonstrated that the vehicles should be optimized in order to minimize the UV filter penetration to the systemic circulation but maximise uptake into the stratum corneum (s.c.).

Bu çalışmanın amacı, güneşten koruyucu ürünlerin güvenilirlikleri konusunda bilgi edinmek üzere, çok kullanılan iki ultra-viyole (UV) filtrenin dört değişik merhem sıvağından (Y/S emülsiyonu, S/Y emülsiyonu, yağlı krem ve Carbopol jeli) deriye penetre olabilme özelliklerinin incelenmesidir. Denemeye tabi tutulan UV filtreler: 2-fenilbenzimidazol 5-sülfonik asit (8%,A) ve oksibenzon (8%,B) dur. In vitro penetrasyon ölçümleri, Franz difüzyon hücreleri ve Attenuated total reflectance Fourier transfrom infra-red (ATR-FTIR) spektroskopisi kullanılarak yapılmış ve UV filtrelerin farklı sıvağlar içinde değişik penetrasyon hızları gösterdiği bulunmuştur. Bu sonuçlar, UV filtrelerin sistemik dolaşıma penetrasyonunu en aza indirmek ve stratum korneumda kalışını arttırmak üzere sıvağların optimize edilmesi gerektiğini göstermiştir.

Keywords : *Sunscreens; UV filters; Percutaneous absorption; Human skin; Stratum corneum (s.c.); Polydimethylsiloxane (PDMS)*

Anahtar kelimeler: *Güneşten koruyucular; UV filtreler; Perkütan absorpsiyon; İnsan derisi; Stratum korneum (s.c.); Polidimetilsiloksan (PDMS)*

Introduction

Ultra-violet chemical absorbers are widely used in sunscreen preparations to protect human skin from the harmful effects of UVradiation such as actinic ageing and cutaneous cancer (1). Therefore it is important to conduct research on their various properties such as determination of sun protection factor (2,3), photostability (4) and penetration through skin. Since sunscreen preparations are often applied on large skin areas even low penetration rates can cause a considerable amount of penetrant to enter the body (2) and a study of their penetration rate is needed. Although most aspects of these compounds have been investigated, there has not been much information and published data describing their penetration into or permeation through the skin (5-7).

The purpose of our work was to determine the skin penetration profiles of UV filters in vitro in order to evaluate a possible influence of the formulations. An ideal sunscreen should exert a high extent of substantivity at the lowest possible level of transdermal penetration. It would be highly desirable if the UV filters were to remain within the outer layers of the s.c. to decrease any toxicological risk. This study was performed to compare different effects of vehicles on UV filter penetration by using a silicone membrane, s.c. and excised human skin.

Materials and Methods

Sunscreen products

An o/w emulsion, w/o emulsion, oleagenous cream and a gel containing 2% Carbopol 940 as vehicle (model formulations) were chosen according to a previous study (8). The contents of the vehicles are shown in Table I. UV filters in all formulations were: either 2-phenylbenzimidazole 5-sulphonic acid (A,8%) or Oxybenzone (B,8%). UV filters were added to the vehicles as 8% at the highest possible concentration (9). Their physicochemical properties are indicated in Table II.

Chemicals

UV filters were purchased from Merck, acetonitrile (HiperSolv grade) and ethanol (AnalaR grade) were

Table 1. Composition of the Vehicles (W/W)

Vehicles / Ingredients	Cetyl alcohol	White soft paraffin	Propylene glycol	Sodium lauryl sulphate	Glyceryl mono stearate	Beeswax	Cetostearyl alcohol	Liquid paraffin	Tween 20	Carbopol 940	Deionised water
o/w	25	25	12	1							37
w/o	3	50	22		3	8					14
oleagenous		40					25	33	2		
gel			40							2	58

Table 2. Physicochemical Properties of the UV Filters

UV Filters	MW [a]	MP [b] (°C)	log K_{oct} [c]	λ_{max} [d] (nm)	S [e]	log P [f]	S_{rf} (µg/ml) [g]
2-phenylbenzimidazole 5-sulphonic acid	274	70	1.81	306	10.75	0.924	99.5±0.8
oxybenzone	228	63	3.58	288	11.47	-	82.7±1.2

[a] Molecular weight
[b] Melting point
[c] Octanol/water partition coefficients calculated with MEDCHEM software (version 3.54, Daylight Chemical Information System Inc.)
[d] Maximum absorption
[e] Solubility parameters calculated according to Fedor's approach (11)
[f] Octanol/water partition coefficient determined by PCA 101 pKa and logP analyser (Sirius Analytical Instruments Ltd.)
[g] Solubility in the receptor fluid (mean sd, n=3)

also from Merck. All other reagents were of analytical grade having chemical purity.

Membrane

Polydimethylsiloxane (PDMS) (Diachema) of 330 µm thickness was used to simulate full thickness human skin (10).

Determination of Solubility in the Receptor Fluid

Excess of both UV filters were added to receptor fluid (ethanolated 25% phosphate buffer pH 7.4) and mechanically shaken at 37°C for 24 hours. After centrifugation (9000 rpm for 15 minutes) and filtration using a 0.22 µm Milipore filter, 1 ml supernatant was analysed by UV spectrophotometry.

In Vitro Skin Penetration

Full thickness female breast skin was obtained after cosmetic surgery and maintained at -20°C until ready for use. Skin was removed from subcutaneous fat layer, cut into approximately 2 cm^2 pieces and placed on Franz cells.

The penetration of UV filters through PDMS or skin was measured using static diffusion Franz cells with a 1.76 cm^2 surface area. The preparations were applied at a dose of 2.00±0.25mg/cm^2 for the vehicles. The receptor fluid was phosphate buffer pH 7.4 containing 25% ethanol maintained at 37 1°C and stirred at 600 rpm. Samples were withdrawn from the receptor phase at intervals of time (0.5h, 2h, 4h, 6h and 8h) and analysed by using HPLC.

The penetration of UV filters from s.c. was also determined by means of ATR-FTIR spectroscopy (s.c. was separated after heat treatment in a water bath at 55-60°C for one minute, removed by incubating for 24 h at 37°C on filter paper soaked in 0.0001% trypsin in phosphate buffered saline at pH 7.4 and isolated s.c. rinsed in water and air dried).

HPLC Assays

The chromatographic conditions were as follows: the analytical column used was of stainless steel (250mmx4.6mm I.D.) packed with LiChrosorb C-18 (Merck) of 5 µm. The sample injection volume was 5 µl. The detection wavelength was set at either 288 or 306 nm and the eluent was acetonitrile: water: glacial acetic acid (50:50:2).

UV filter concentrations were calculated from standard calibration curves of pure compounds. A and B in ethanolated (25%) phosphate buffer pH 7.4. Retention times were 5.2 and 19.5 min. respectively.

ATR-FTIR Studies

ATR-FTIR studies for some cosmetic and sunscreen products have been performed previously (12,13) and this technique is also used in our research for UV filters A and B. S.c. membranes were used after heat separation and subsequent trypsinisation as previously described (14). The membranes were placed in direct contact with the surface of a ZnSe attenuated total reflectance crystal (Spectra-Tech Inc.) mounted on a Nicolet 710 FTIR spectrometer. S.c. samples were placed onto the surface of the ATR crystal with a PVC trough on top. The trough and membrane were sealed with petroleum jelly and the join monitored for leakage of UV filters during the experiment.

The s.c. membrane was treated with the sunscreen products having an equivalent dose per unit area to that used in diffusion cell experiments. The Omnic software program was used for automated collection and evaluation of spectra. Spectra were collected every 30 seconds and 50 sweeps for each spectrum. Three replicates were run for each treatment. After each run, peak areas associated with A stretch at 1640 cm^{-1} and B stretch at 1625 cm^{-1} were calculated to give measurements of UV filter permeation.

Experimental values of penetrant peak areas against time were evaluated using Ultrafit program. Values of D/h^2 and A_o were allowed to vary until the best fit was achieved as measured by minimisation of X^2 (D=permeant diffusion coefficient; h=film thickness; A_o = area of penetrant peak)

ATR-FTIR experiments were conducted to assess the effects of different vehicles on UV filter penetration from s.c.

Results and Discussion

Skin Penetration Using Franz Cells and ATR-FTIR

Determined physicochemical properties of UV filters are shown in Table 1. As indicated both A and B were freely soluble in the receptor fluid. Penetration data from Franz cell experiments and ATR-FTIR studies are listed in Table III. Penetration rate constants (log k_p) were calculated by using HS in Franz cell and s.c. in ATR-FTIR experiments. PDMS was used to measure the diffusion coefficients in Franz cell studies. It was stated that PDMS could be used instead of skin in penetration studies (10). One of the aims of this study was to compare the results obtained by using skin and PDMS in penetration studies. It was observed that similar diffusion coefficients resulted when s.c. and PDMS used.

Significant differences regarding penetration profiles of the UV filters due to various vehicles were found. Data obtained show that the skin penetration of UV filters can be significantly changed by product formulation. In the literature, there has been very little information about the penetration of UV filter A(15).

Table 3. Penetration values of UV filters detected from Franz cell experiments and ATR-FTIR studies

Sunscreen (8 %)	Vehicle	Membrane	Diffusion coefficient in Franz cell (cm^2/h)	Diffusion coefficient in ATR - FTIR (cm^2/h)	log k_p
B	w/o	PDMS-HS*	9.06×10^{-3}	-	-2.69
B	oleagenous	PDMS-HS	5.72×10^{-4}	-	-2.78
B	o/w	s.c.	-	1.82×10^{-4}	-3.06
B	oleagenous	s.c.	-	1.46×10^{-4}	-3.08
B	gel	PDMS-HS	1.57×10^{-3}	-	-3.28
B	gel	s.c.	-	$7.22\text{-}x\ 10^{-3}$	-3.4
B	o/w	PDMS-HS	5.43×10^{-5}	-	-3.6
B	w/o	s.c.	-	8.76×10^{-2}	-3.66
A	o/w	s.c.	-	9.54×10^{-5}	-4.02
A	o/w	PDMS-HS	2.01×10^{-4}	-	-4.24
A	w/o	s.c.	-	1.97×10^{-4}	-4.25
A	gel	PDMS-HS	1.07×10^{-4}	-	-4.28
A	w/o	PDMS-HS	6.24×10^{-5}	-	-4.48
A	oleagenous	PDMS-HS	1.55×10^{-4}	-	-4.61

The term "HS" implies the full thickness human skin (Franz cell experiments) in the table but stratum corneum (s.c.) was used as a membrane for ATR-FTIR experiment. In Franz cell work, both PDMS were used to calculate diffusion coefficient and HS for determination of $\log k_p$ and fluxes.

Therefore it seemed necessary to conduct research to determine the penetration ability of this compound from skin.

In this study, it was shown that the penetration rate and absorbed amounts of oxybenzone was higher than 2-phenylbenzimidazole 5-sulphonic acid in all formulations. Diffusion coefficients were found to be similar in Franz cell experiments and ATR-FTIR studies. logK_p values in Table III demonstrate the significant variability that may exist between the degree of percutaneous penetration of UV filters in different formulations.

In this research, as can be seen from Table III, diffusion coefficients and log k_p values demonstrated that for UVfilter B, the penetration rate and absorbed quantity was higher in lipophilic vehicles. It was contrary for UV filter A where it was high in hydrophilic vehicles. This situation could mean that the UV filter A with low k_{oct} (partition coefficient between water and octanol) value has more penetration ability in a hydrophilic vehicle whereas UV filter B with high log k_{oct} value has more tendency to penetrate in a lipophilic vehicle.

The results of the skin penetration study carried out with the UV filters in different vehicles are illustrated in Figures 1 and 2, each data point represents the the mean of three experiments. Regression analysis of the penetration data within the experiment allowed calculation for each compound of the flux in $\mu g\ cm^{-2}h^{-1}$.

Discussion

These results show that the skin penetration of UV filters can be significantly changed by product formulation. For UV filter A, o/w emulsion produced the highest penetration rate. For oxybenzone, the opposite trend was found. Higher concentrations were measured after oleagenous and w/o emulsion applications. According to the results, it could be concluded that for B, o/w and gel formulations led to less penetration tendency whereas for UV filter A, oleagenous and w/o vehicles are better for keeping the sunscreen from penetration.

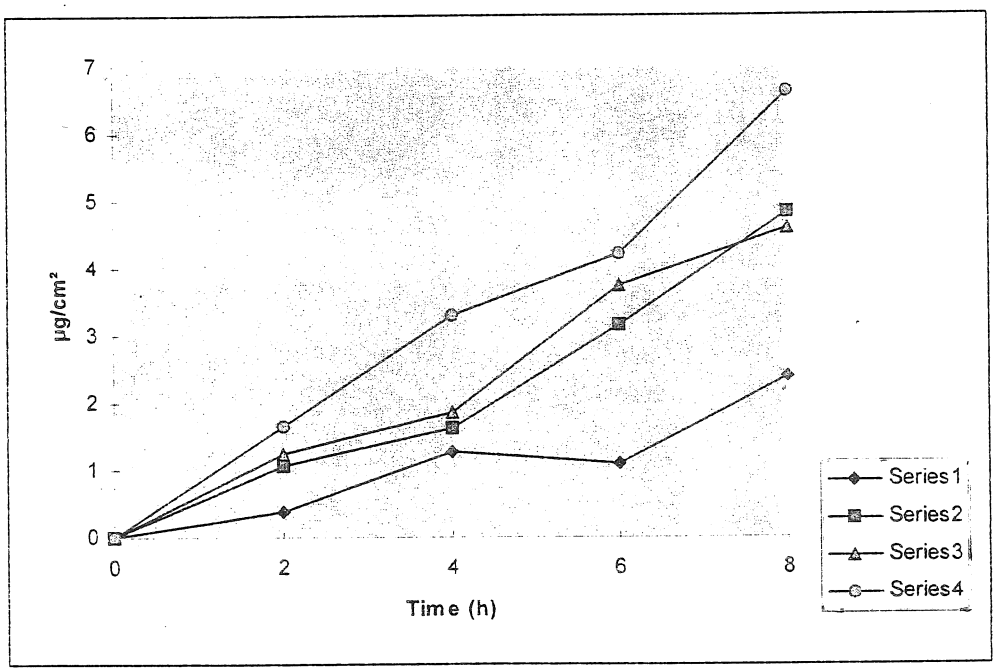

Fig.1. Penetration profiles of UV filter A in various formulations through HS
Series 1-oleagenous
Series 2-w/o
Series 3-gel
Series 4-o/w

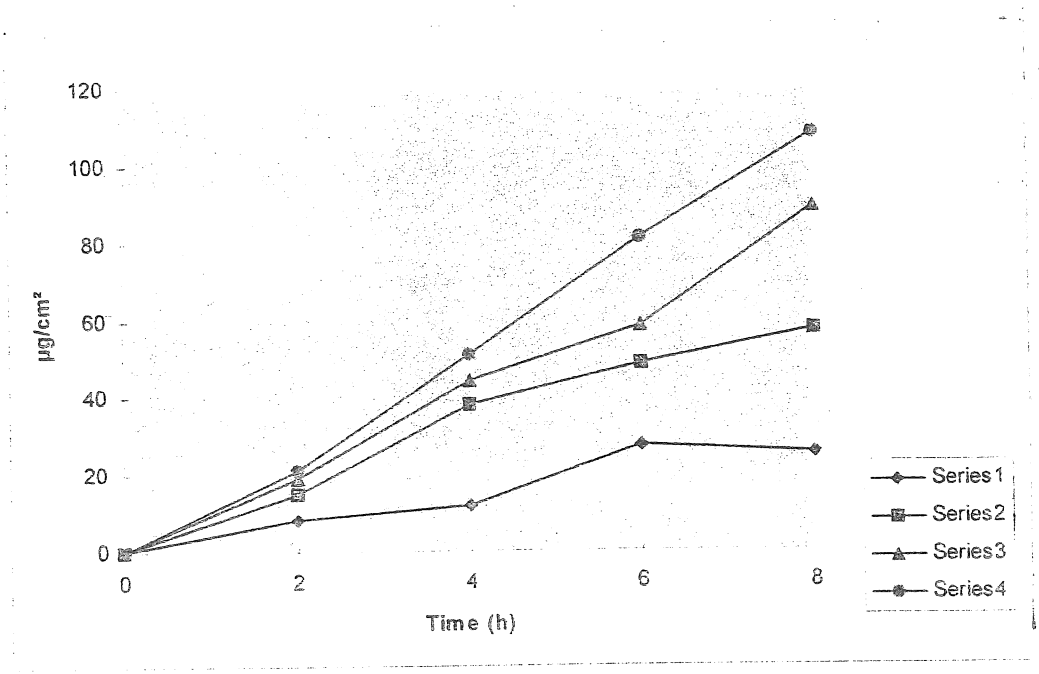

Fig.2. Penetration profiles of UV filter B in various formulations through HS
Series 1-o/w
Series 2-gel
Series 3-oleagenous
Series 4-w/o

In this study, ATR-FTIR spectroscopic instrumentation was used to compare the results obtained from it with Franz cell work. It was found out that this system has advantages as increased sensitivity, rapid analysis, ease of operation and computerized data storage.

In Franz cell experiments, PDMS and HS whereas in ATR-FTIR studies, s.c. were used as membranes. As shown in Table III, diffusion coefficient values were slightly different in Franz cell and ATR-FTIR studies whether PDMS or skin were used. The data obtained from both techniques have shown that they could be used as alternatives.

Table 4. Permeation fluxes and absorbed amounts of the compounds in various vehicles

Compund in Vehicle	J (µg cm^{-2} h^{-1})	Quantities absorbed over the whole skin surface* (mg/h)
A-oleagenous	0.274±0.08	3.836 ± 0.52
A-w/o	0.524±0.076	7.336 ± 0.84
A-gel	0.622±0.093	8.708 ± 1.09
A-o/w	0.827±0.065	11.578 ±2.13
B-o/w	4.18±1.19	58.520 ± 9.08
B-gel	7.54±1.58	105.560 ± 21.24
B-oleagenous	11.047±2.11	154.658 ±43.4
B-w/o	13.46±4.47	188.44 ± 50.36

* Whole skin surface area was estimated as 1.4 m^2
J=flux calculated from linear regression at steady-state and expressed as geometric mean±SD (n=3)

Since sunscreen preparations containing the investigated UV filters are nearly applied to whole body surface, maximum amounts of the permeants which are absorbed per hour assuming treatm of a total skin surface as 1.4 m^2 are also listed in Table 4. Extrapolation results are between 3.836 (oleagenous)- 11.578 mg (o/w) and 58.520 (o/w)-188.44 mg (w/o) for UV filters A and B respectively. However this extrapolation is bold, it still gives an impression of the maximum strain on toxicologic aspect.

In conclusion, we could demonstrate that the penetration of UV filters into the skin can be optimized by choosing a suitable vehicle due to the filter's physicochemical properties.

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