CHAPTER II

LITERATURE REVIEW

RADIATION EFFECTS AND PREVENTION METHODS

1. Effects of radiation on skin

The solar spectrum can be generally divided into three region: ultraviolet (UV 290 - 400 nm), visible (400 - 760 nm), and infrared (760 - 3000 nm). The composition of the solar spectrum at the earth's surface is approximately 6% ultraviolet, 49% visible and 45% infrared (11-12).

The individual ranges are quite different with respect to their physiological effects. The invisible infrared radiation can produce heat. The heat effect is considerably lowered in the visible range, and instead the light effect comes into the foreground. Whereas ultraviolet radiation has sufficient energy content to initiate photochemical processes according to its energy which is inversely proportional to the wavelength. This means that as the wavelength decreases, the ultraviolet radiation increases in energy and the photochemical reactions brought about by ultraviolet radiation increase (13).

The portion of ultraviolet (UV) radiation can be classified by various physiological responses into three ranges, A, B, and C. The ranges from the shortwave visible light are :

The UVA range (320 - 400 nm) enhances the burning effects of UVB and is implicated in the long term effects of sun - exposure including premature skin aging and elastosis.

The UVB range (290 - 320 nm) is a narrow and energy - rich band. It causes sunburn and delayed tanning as well as being carcinogenic (14).

The UVC range (200 - 290 nm) is potentially the most dangerous. It is predominantly screened out by the ozone layer and therefore it is not normally a problem. However it reach the peaks of high mountains. With the depletion of the ozone layer by the action of chlorinated fluorocarbons; the increase of UVC are predominant (15).

Figure 1 shows the three layers of the human skin which are depicted along with the amount of UV radiation that penetrates these layers.

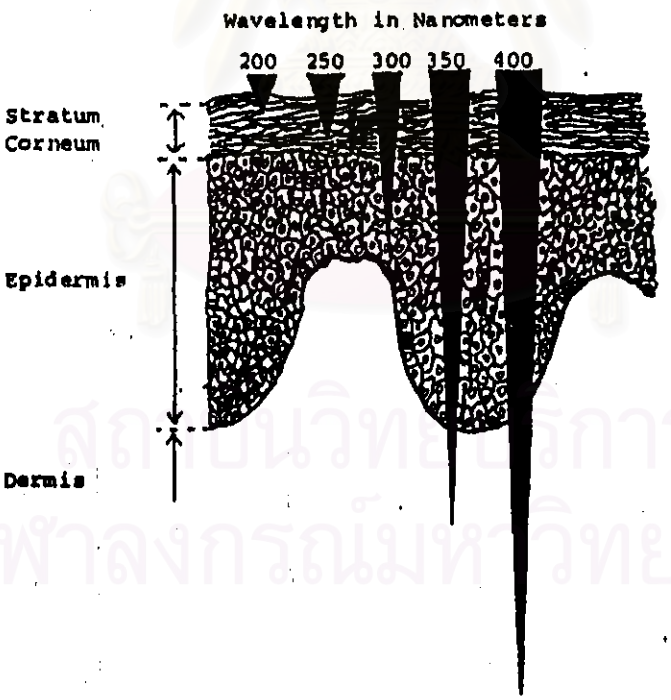


Figure 1 Schematic representation of light penetration into skin (6).

The followings are the effects of UV on the skin (1, 16).

1. Sunburn : It is the cutaneous inflammation as a result of acute exposure to solar radiation. The signals that the skin's response to such over exposure are redness, pain or tenderness, swelling, in extreme case, the blistering and peeling. The erythema reaction is within minutes or hours of exposure, reaching a maximum in transient, generally appearing in 12 to 24 hours and then subsiding over several days.

It should be known that UVA can cause delayed erythema but the dose is much higher than UVB. About one thousand times more energy is needed for UVA induced delayed erythema than that for UVB.

2. Premature aging : This happens according to the sun's damage to the skin is cumulative. By the time one has had enough exposure to produce change in the collagen and elastic fibers as well as some loss of subcutaneous fatty tissue. This results in premature aging process which macroscopic appearance is dry, scaly, sagging and wrinkled.

3. Tanning : It is the result of stimulating the formation of melanin pigment by UVA penetrating the dermis layer.

4. Skin cancer : At least eight factors influence the effectiveness of sunscreens in reducing the risk of nonmelanoma skin cancer (NMSC). Those determiners to be considered here include:

1. The relationship between dose of sunlight to which an individual is exposed and the risk of NMSC
2. The action spectrum for the development of NMSC
3. The absorption characteristics of sunscreens
4. Life-time exposure patterns to sunlight

5. Possible differences in the individual's susceptibility to the carcinogenic effects of sunlight at different periods during a life time and as a consequence of prior exposures to sunlight and other carcinogens
6. Changes in exposure habits that occur as a result of sunscreen use
7. Loss of acclimatization that may occur as a result of sunscreen use
8. The possible toxicity of sunscreens

The relative importance of each of these factors in determining an individual's risk of NMSC is likely to vary with age. Further, there are likely to be interactions between each of these factors which make it difficult to estimate precisely the utility of sunscreens for a particular individual for a given period of time.

Radiation in the range of 290 to 315 nm exerts the most profound effects on the human organism (18). It has an important role in vitamin D metabolism in human skin. UVB radiation converts the epidermal precursor, 7- dehydrocholesterol, to previtamin D_3. Previtamin D_3 is then isomerized to vitamin D_3, which is absorbed into vasculature and binds to a vitamin D binding protein which transports it to the liver and then to the kidney. It is then hydroxylated at the 1 and 25 positions by Cytochrome P - 450 dependent enzymes. The end product of this reaction is 1, 25 - dihydroxyvitamin D_3 which is the active form of the compound that regulates calcium homeostasis. Previtamin D_3 is also converted by UVB to tachysterol and lumisterol, which act as biologically inactive epidermal reservoirs of hormone as shown in Figure 2. This step provides a mechanism to regulate the release of this hormone and to prevent hypervitaminosis D.

The effect of ultraviolet radiation (UVR) on vitamin D metabolism in skin is the only known positive physiologic role of solar radiation in human beings. In contrast, however, sunlight may directly cause or aggravate many cutaneous disorders. The number of skin diseases can be classified accordingly in Table 1(18). This classification is based on the following etiologic categories of photosensitivity disease : genetic and metabolic, phototoxic and photoallergic, degenerative and neoplastic, and photoaggravated.

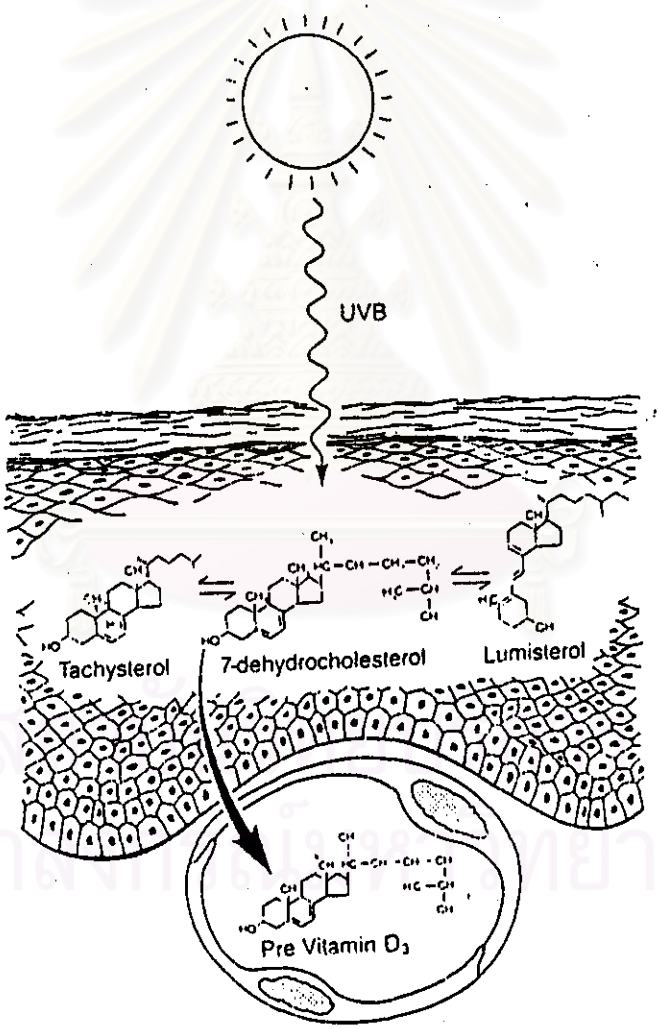


Figure 2 The inactive vitamin D precursor, 7 - dehydrocholesterol (ergosterol), forms the active hormone, vitamin D_3, following the absorption of ultraviolet energy (6).

Table 1 Classification of diseases caused by photosensitivity.

Type	Diseases
Genetic (alone)	Erythropoietic porphyria
	Erythropoietic protoporphyria
	Albinism
	Xeroderma pigmentosum
	Rothmund - Thompson disease
	Bloom's diseases
	Cockayne's diseases
	Familial porphyria cutanea tarda
	Phenylketonuria Hepatoerythropoeitic porphyria
Genetic and environmental	Sporadic porphyria cutanea tarda
	Variegate prophyria
	Hartnup's diseases
Metabolic (alone)	Kwashiorkor
	Pellagra
	Carcinoid
	Pseudoporphyria
Phototoxic (internal)	Drugs
Phototoxic (external)	Drugs, plants, food
Photoallergic (immediate)	Solar Urticaria
Photoallergic (delayed)	Drug photoallergy
	Persistent light reaction

Table 1 Classification of diseases caused by photosensitivity (continued).

Type	Diseases
Neoplastic and degenerative	photoaging
	Actinic keratoses
	Basal cell carcinoma
	Squamous cell carcinoma
	Melanoma
	Dysplastic nervous syndrome
	Bowen's disease
Idiopathic	Polymorphous light eruption
	Hydro aestivale
	Actinic reticuloid
	Solar urticaria
Photoaggravated	Lupus erythematosus
	Dermatomyositis
	Permphigus foliaceus
	Herpes simplex
	Lichen planus actinicus
	Acne vulgalis
	Atopic dermatitis
	Transient acantholytic dermatosis

2. Protective methods against solar radiation

Protection against adverse effects of solar radiation can be classified into two methods, natural protection and using topically applied sunscreen products.

The natural protection of normal skin against the effects of solar radiation are as follows (2, 3, 19) :

1. Reflection from the skin surface : Visible light and infrared are reflected from the skin surface. Some UVA is also reflected but little UVB.

2. Absorption of radiation : The stratum corneum (Horney layer) with its variable melanin content helps absorbing as well as reflecting light. The thicker the stratum corneum, the greater the protection. A number of proteins in the stratum corneum cells are good absorbers of UVB.

3. Melanin : Melanin acts as a major defense. It absorbs radiation and also scatters it, thus reducing its activity. Kollias and Bager (20) indicated that while melanin provides significant protection from UVA radiation, it provides only partial protection from UVB radiation. They also suggested that skin color does not indicate the amount of protection against UV radiation.

4. Scattering : This also occurs when radiation comes into contact with subcellular components of cells.

5. Urocanic acid : Urocanic acid is a constituent of sweat and it makes a significant contribution to the UV absorbency (21).

Another widely used method was applying sunscreen products which contained sunscreen chemicals or UV absorbing agents. Sunscreen chemicals

are designed to protect against the burning or erythemal flux from 290-320 nm (with a maximum flux around 308 nm), but permit radiation higher than 320 nm through to allow the tanning of the skin. These chemicals are known as UVB sunscreen chemicals. To achieve a total block, UVA sunscreen chemicals are required which absorb the harmful UV rays above 320 nm (1, 22).

It is accepted that the photoprotection by sunscreen products not only reduces sunburn but alco prevents premature aging of the skin and the development of cancer Kligman Lorraine and Kligman Albert (23) demonstrated that, with the use of sunscreens, further damage could be prevented and repair could occur, even in the face of continuing irradiation. Overall, the earlier application and higher efficacy of sunscreens provided the greatest amount of protection to connective tissue.

Kligman et al., (24) assessed the ability of sunscreens to protect connective tissue from actinic damage. Hairless mice were irradiated with sunlamps three times weekly for thirty weeks; each exposure consisted mainly of UVB. The unprotected irradiated animals showed a great increase in reticulin fibers and elastic fibers to the extent of elastosis, and also in the amount of neutral and acid mucopolysaccharides and melanin production. Sunscreen product with high efficacy applied to another group of animals completely prevented these changes. In 1983, the same investigators (25) reported the results of a study showing that the use of sunscreens can promote the repair of ultraviolet radiation induced dermal damage. Previously damaged dermis was repaired even during continuous irradiation. Repair occured as subepidermal reconstruction zones of new connective tissue with parallel collagen bundles and a network of fine elastic fibers.

In 1990, Young's study (26), using hairless albino mice, suggested that the routine use of sunscreens, which usually act as UVB filters, may prevent or inhibit skin photocarcinogenesis and photoaging in man. It also stated that regular use of sunscreens would prevent these long term effects but in reality the precise ability of sunscreens to inhibit these effects in human skin is not known and would be very difficult to determine.

SUNSCREENS (6)

1. Classification of sunscreen chemicals

Sunscreen chemicals may be classified according to the type of protection they offer as either physical blockers or chemical absorbers.

A. Physical Blockers

These are chemicals that reflect or scatter the ultraviolet radiation. Examples of physical blockers include zinc oxide, titanium dioxide, and red petrolatum. Physical blockers, if present in sufficient quantities, will reflect all the ultraviolet, visible, and infrared rays. They are currently being used in conjunction with chemical absorbers to achieve high sun protection factors. New forms of the metal oxides are currently being introduced that claim to enhance the sun protection without imparting the traditional opaqueness that is esthetically unappealing in cosmetic formulations. A Japanese patent discloses the use of transparent metallic oxides with an average diameter smaller than 300 angstroms. They include titanium dioxide, zinc oxide, chromium oxides, cobalt oxides, and tin oxides. Another Japanese patent reveals the use of siloxane - treated titanium dioxide with an average diameter of 40 - 70 μm. Other attempts have been made to change the physical form of the inorganic powders or to complex them with organic substances.

Of the physical blockers available, titanium oxides, iron oxides, and zinc oxides have been used in a multitude of particle sizes and suspensions and are widely used in cosmetic formulations.

B. Chemical Absorbers

These chemicals absorb the harmful ultraviolet radiation. Table 2 lists the sunscreens approved for use in the United States and Table 3 those UV filters approved in Europe (27 - 29).

Chemical absorbers are classified into either UVA or UVB blockers depending upon the type of radiation they protect :

UVA absorbers are chemicals that tend to absorb radiation in the 320 - 360 nm region of the ultraviolet spectrum, (benzophenones, the anthranilates and the dibenzoyl methanes).

UVB absorbers are chemicals which absorb radiation in the 290 - 320 nm region of the ultraviolet spectrum. (PABA derivatives, salicylates, cinnamates, and camphor derivatives).

The best classification of chemical UV absorbers is one based on the chemical properties of the sunscreens. Hence all sunscreens available on the market can be classified as follows :

1. Cinnamate derivatives are all UVB absorbers and have the following general chemical structure :

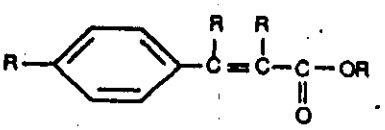


Table 2 FDA-OTC panel category I sunscreens (6).

Chemical[a]	
UVA absorbers	*% Approved*
Oxybenzone (#1)	2-6
Sulisobenzone (#2)	5-10
Dioxybenzone (#3)	3
Methyl anthranilate (#8)	3.5-5
UVB absorbers	
Amino benzoic acid (#13)	5-15
Amyl dimethyl PABA (NA)	1-5
2-Ethoxyethyl *p*-methoxy cinnamate (NA)	1-3
Diethanolamine *p*-methoxy cinnamate (#4)	8-10
Digalloyl trioleate (NA)	2-5
Ethyl 4-bis (hydroxypropyl) aminobenzoate (#5)	1-5
2-Ethylhexyl-2-cyano-3,3-diphenylacrylate (#9)	7-10
Ethylhexyl *p*-methoxy cinnamate (#11)	2-7.5
2-Ethylhexyl salicylate (#12)	3-5
Glyceryl aminobenzoate (#6)	2-3
Homomenthyl salicylate (#7)	4-15
Lawsone with dihydroxyacetone (NA)	0.25 with 33
Octyl dimethyl PABA (#10)	1.4-8
2-Phenylbenzimidazole-5-sulfonic acid (#14)	1-4
Triethanolamine salicylate (#15)	5-12
Physical	
Red petrolatum	30-100
Titanium dioxide	2-25
Zinc oxide	5-25

[a]Encyclopedia listing # is used. The numbers refer to chemicals listed in N. A. Shaath, The encyclopedia of UV absorbers for sunscreen products, Cosmet. Toilet. 3:21 (1987).

FDA-OTC panel category I sunscreens = sunscreens use in cosmetic for safety

Table 3 EEC and COLIPA numbers for UV filters used in Europe (6).

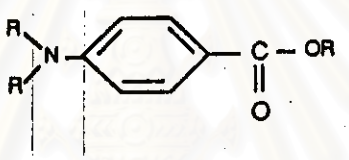
EEC #	COLIPA #	Chemical name	Encyclopedia listing #	Max % / conc
1.1	S 1	4-Amino benzoic acid	13	5
1.2	S 57	*N,N,N*-Trimethyl-4-(2-oxoborn-3-ylidene methyl) anilinium methyl sulfate	-	6
1.3	S 12	Homosalate	7	10
1.4	S 38	Oxybenzone	1	10
1.5	S 46	3-Imidazol-4-ylacrylic acid and ethyl ester	-	2
1.6	S 45	2-Phenylbenzimidazole-5-sulfonic acid and salts	14	8
2.1	S 2	Ethyl-4-bis (hydroxypropyl) aminobenzoate	5	5
2.2	S 3	Ethoxylated 4-aminobenzoic acid	-	10
2.3	S 5	Amyl 4-dimethylaminobenzoate	-	5
2.4	S 6	Glyceryl 1-(4-aminobenzoate)	6	5
2.5	S 8	2-Ethylhexyl 4-dimethylaminobenzoate	10	8
2.6	S 13	2-Ethylhexyl salicylate	12	5
2.7	S 18	3,3,5-Trimethylcyclohexyl-2-acetamido benzoate	-	2
2.8	S 19	Potassium cinnamate	-	2
2.9	S 24	4-Methoxycinnamic acid salts	4	8
2.10	S 25	Propyl 4-methoxycinnamate	-	3
2.11	S 9	Salicylic acid salts	15	2
2.12	S 27	Amyl 4-methoxycinnamate	-	10
2.13	S 28	2-Ethylhexyl 4-methoxycinnamate	11	10

Table 3 (continued).

EEC #	COLIPA #	Chemical name	Encyclopedia listing #	Max % / conc
2.14	S 29	Cinoxate	-	5
2.15	S 55	Digalloyl trioleate	-	4
2.16	S 39	Mexenone	-	4
2.17	S 40	Sulisobenzone	2	5
2.18	S 42	2-Ethylhexyl-2-(4-phenylbenzoyl)-benzoate	-	10
2.19	S 47	5-Methyl-2-phenylbenzoxazole	-	4
2.20	S 50	Sodium 3,4-dimethoxyphenylglyoxylate	-	5
2.21	S 52	1,3-bis (4-Methoxyphenyl) propane-1,3-dione	-	6
2.22	S 56	5-(3,3-Dimethyl-2-norbonylidene)-3-penten-2-one	-	3
2.23	S 58	α' -(2-Oxoborn-3-ylidene)-p-xylene-2-sulfonic acid	-	6
2.24	S 59	α -(2-Oxoborn-3-ylidene) toluene-4-sulfonic acid and its salts	-	6
2.25	S 60	3-(4-Methylbenzylidene) bornan-2-one	16	6
2.26	S 61	3-Benzylidenebornan-2-one	-	6
2.27	S 62	α -Cyano-4-methoxycinnamic acid and its hexyl ester	-	5
2.28	S 64	1-p-Cumenyl-3-phenylpropane-1,3-dione	21	5
2.29	S 16	4-Isopropylbenzyl salicylate	-	4
2.30	S 30	Cyclohexyl 4-methoxycinnamate	-	1
2.31	S 66	1-(4-tert-Butylphenyl-3-(4-methoxyphenyl) propane-1,3-dione	22	5

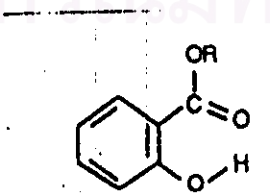
There are nine cinnamate derivatives approved for use worldwide : 2 - ethoxy ethyl-*p*-methoxy cinnamate (FDA, 2.14, S.29) , diethanolamine -*p*-methoxy cinnamate (FDA, 2.9, S.24), octyl-*p*-methoxy cinnamate (FDA, 2.13, S.28), 2-ethyl hexyl-2-cyano-3, 3-diphenyl acrylate (FDA), potassium cinnamate (2.8, S.19), propyl-4-methoxy cinnamate (2.10, S.25), amyl-4-methoxy cinnamate (2.12, S.27), α-cyano-4-methoxy cinnamic acid, hexyl ester (2.27, S.61), and cyclohexyl-4-methoxy cinnamate (2.30, S.30).

2. Para amino benzoate (PABA) derivatives are all UVB absorbers and have the following general chemical structure :



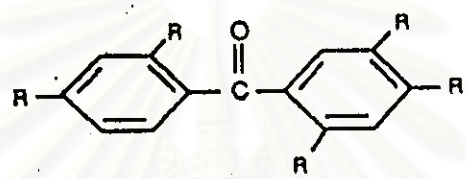
There are six PABA derivatives approved for use worldwide : glyceryl PABA (FDA, 2.4, S.6), amyldimethyl PABA (FDA, 2.3, S.5), ethyl-4-bis (hydroxy propyl) amino benzoate (FDA, 2.1, S.2), ethoxylated-4-amino benzoic acid (2.2, S.3), and octyl dimethyl PABA (FDA, 2.2, S.3).

3. Salicylate derivatives are all UVB absorbers and have the following general structure :



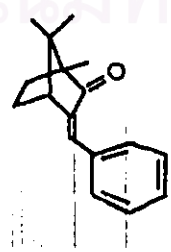
There are five salicylate derivatives approved for use worldwide : octyl salicylate (FDA, 2.6, S.13), homomentyl salicylate (FDA, 1.3, S.12), triethanolamine salicylate (FDA, 2.11, S.9), salicylic acid salts (2.11, S.9), and 4-isopropyl benzyl salicylate (2.29, S.16).

4. Benzophenone derivatives are all UVA absorbers and have the following general chemical structure :



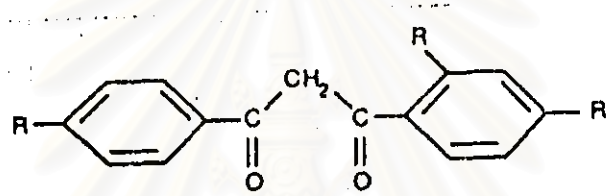
There are five benzophenone derivatives approved for use worldwide : oxybenzone (FDA, 1.4, S.38), dioxybenzone (FDA), sulisoben-zone (FDA, 2.17, S.40), mexenone (2.16, S.39), and 2-ethyl hexyl-2-(4-phenyl benzoyl) benzoate (2.18, S.32).

5. Camphor derivatives are all UVB absorbers and have the following general chemical structure :



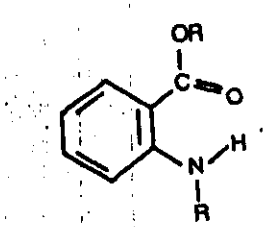
There are six camphor derivatives approved in Europe (none approved in the United States : *N,N,N*-trimethyl-4-(2-oxoborn-3-ylidene methyl) anilinium methyl sulfate (1.2, S.57), 5-(3,3-dimethyl-2-norbonylidene)-3-pentene-2-one(2.22, S.56), α'-(2-oxoborn-3-ylidene)-p-xylene-2-sulfonic acid (2.23, S.58), α' -(2-oxoborn-3-ylidene) toluene-4-sulphonic acid (2.24, S.59), 3-(4-methylbenzylidene) bornan-2-one (2.25, S.60), and 3-benzylidenebornan-2-one (2.26, S.61).

6. Dibenzoyl methane derivatives are all UVA absorbers and have the following chemical structure :



There are three dibenzoyl methane derivatives approved in Europe but not approved by the FDA for use in the United States : 1-(4-tert-butylphenyl-3-(4-methoxyphenyl) propane-1, 3-dione (2.31, S.66), 1-*p*-cumenyl-3-phenyl propane-1, 3-dione (2.28, S.64), and 1, 3-bis (4-methoxy phenyl) propane-1, 3-dione (2.21, S.52).

7. Anthranilate derivatives are UVA absorbers and have the the following chemical structure :



There are two anthranilate derivatives approved worldwide : menthyl anthranilate (FDA), and homomenthyl-*N*-acetyl anthranilate (2.7, S.18)

8. Miscellaneous compounds : The remaining six compounds are all UVB sunscreens and are approved for use in various countries : Digalloyl trioleate (FDA, 2.15, S.55), Lawsone with dihydroxy acetone (FDA), 2-phenyl benzimidazole-5-sulfonic acid (FDA, 1.6, S.45), 3-imidazol-4-ylacrylic acid (1.5, S.46), 5-methyl-2-phenyl benzoxazole (2.19, S.47), and sodium 3, 4-dimethoxy phenyl glyoxylate (2.20, S.50).

2. Mechanism of sunscreen action

Sunscreen chemicals are generally aromatic compounds conjugated with a carbonyl group . In many examples, an electron-releasing group (an amine or a methoxyl) is substituted in the ortho or para position of the aromatic ring as shown in Figure 3.

Chemicals of this configuration absorb the harmful short - wave (high energy) UV rays (250 - 340 nm) and convert the remaining energy into innocuous longer wave (lower energy) radiation (usually above 380 nm). Quantum mechanical calculations have shown that the energy of the radiation quanta present in the UVB and UVA region lies in the same order of magnitude as that of the resonance energy of electron delocalization in aromatic compounds as shown in Figure 4. Thus the energy absorbed from the UV radiaton corresponds to the energy required to cause a "photochemical excitation" in the sunscreen molecule.

Figure 3 General chemical structure of most sunscreen chemicals approved for use in the United States, where Y = OH, OCH_3, NH_2, $N(CH_3)_2$ and X = no substituent or -CH = CH- and R = C_6H_4Y, OH, OR^1 (R^1 = methyl, amyl, octyl, menthyl, homomenthyl) (6).

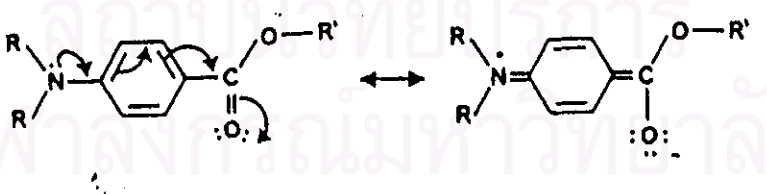


Figure 4 Resonance delocalization in a para aminobenzoate molecule (6).

In other words, the sunscreen chemical is excited to a higher energy state (π^*) from its ground state (n) by absorbing this UV radiation. As the excited molecule returns to the ground state, energy is emitted which is lower in magnitude than the energy initially absorbed to cause the excitation. Thus, the energy is emitted in the form of longer wavelengths since the energy is lower than the shorter wavelengths originally absorbed.

The longer wavelength radiation is emitted in one of several ways (Fig.5). If the loss in energy is quite large, that is, the wavelength of the emitted radiation is sufficient length that it lies in the infrared region, it may be perceived as a mild heat radiation on the skin. This miniscule heat effect is undetected since the skin receives a much larger heat effect by being directly exposed to the sun's heat.

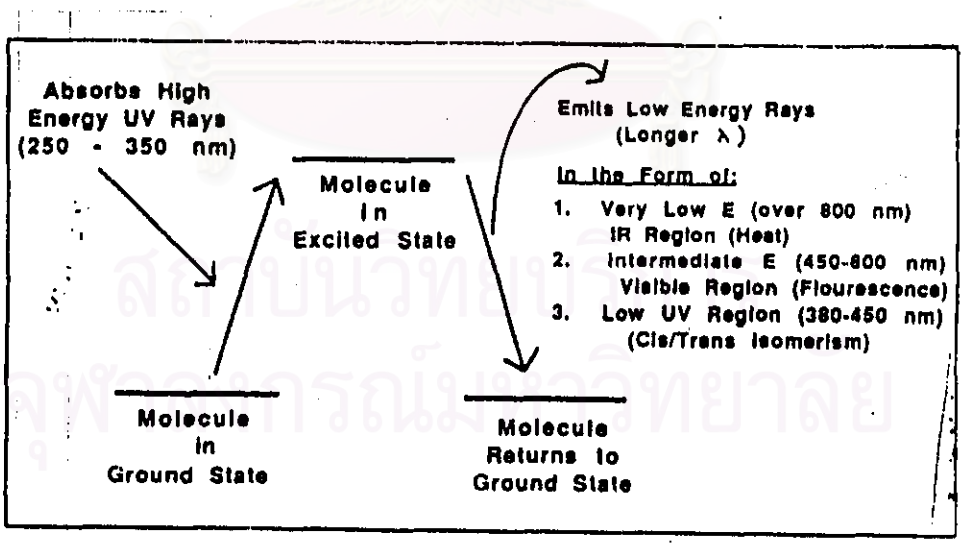


Figure 5 Schematic representation of the process in which a sunscreen chemical absorbs the harmful high energy rays and renders them relatively harmless low energy rays (6).

If the emitted energy lies in the visible region, then it may be perceived as either a fluorescent or a phosphorescent effect. This is common in the imidazoline type sunscreens where a slight bluish haze may be seen on the skin or in formulation.

In the extreme case, the emitted radiation is sufficiently energetic (lower wavelength) that it may cause a fraction of the sunscreen molecule to react photochemically. Cis-trans or keto-enol photochemical isomerization has been observed in some organic molecules causing a mild shift in the λ max of the chemical.

3. Effect of vehicle on the UV absorbance of sunscreens

A. Effect of pH

The ultraviolet absorption spectra of acidic and basic compounds may be affected by pH. In the case of acidic compounds, the use of alkaline conditions (pH over 9) will assist in the formation of anions that tend to increase delocalization of electrons . This electron delocalization would decrease the energy required for the electronic transition in the ultraviolet spectrum, and hence a bathochromic shift is observed (longer wavelength or λ max). For example, phenol in an alkaline environment will experience this anticipated bathochromic shift due to the formation of the phenolate anion as depicted in Figure 6. This phenolate anion will participate in resonance delocalization of electrons as shown in Figure 7. Acidic conditions (pH below 4) will assist in the formation of cations with aromatic amines. A hypsochromic shift toward lower wavelength occurs since the protonation of the unbounded lone pair of electrons with acid would prevent any resonance delocalization of the electrons. Thus aniline, for example, would form the anilinium cation at low pH (Figure 8) and a considerable hypsochromic shift occurs.

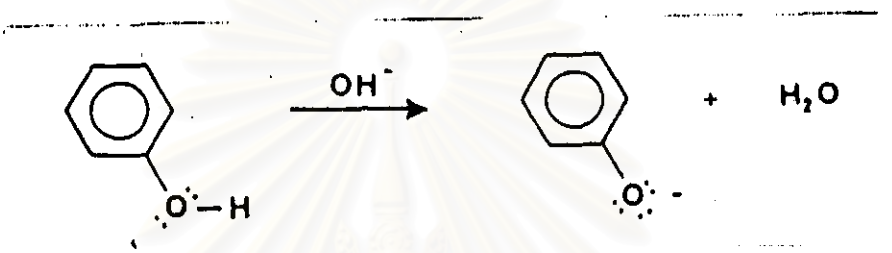


Figure 6 Phenolate anions formed by the action of alkali in phenol (6).

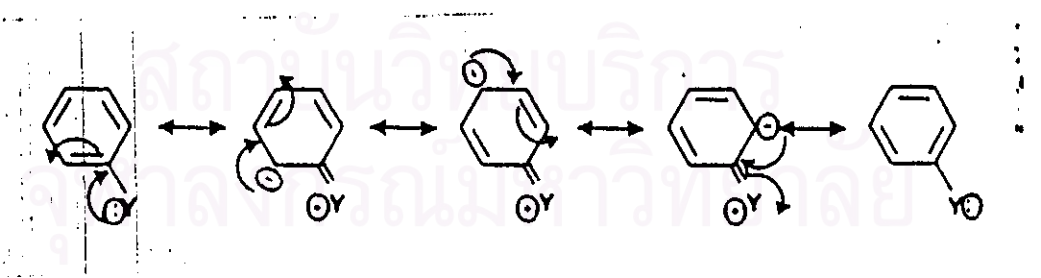


Figure 7 Resonance delocalization for monosubstituted (Y) derivatives with an unshared pair of electrons, where Y = O , $\overline{NH_2}$, NR_2, etc (6).

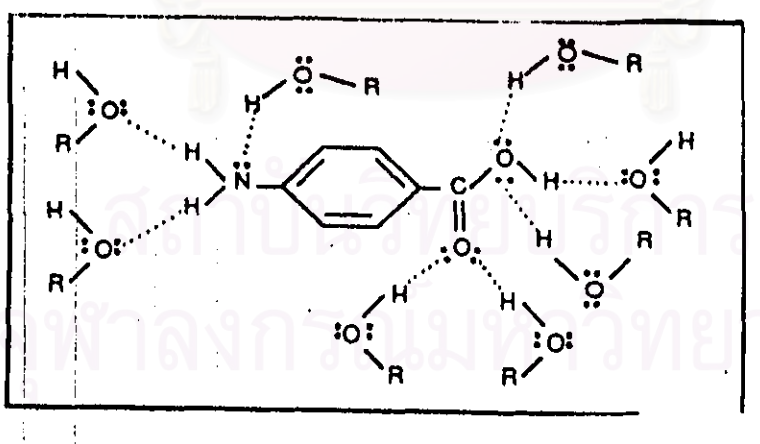
B. Effect of solvent

Solvent shifts in sunscreen chemicals have been observed and their findings have been published. The use of different solvents in cosmetic formulations may profoundly influence the effectiveness of a sunscreen chemical. The shifts in the ultraviolet spectrum are due to the relative degrees of solvation by the solvent of the ground state and the excited state of the chemical. Thus to predict the effect the solvent has on a particular chemical, the interaction (mostly hydrogen bonding) between the solvent and the sunscreen chemical must be understood.

The solvation of polar sunscreens (e.g., PABA, see Table 4), with polar solvents, such as water and ethanol, will be quite extensive. This extensive solvation stabilizes the ground state, thereby inhibiting electron delocalization, leading to the excited state illustrated in Figure 9 where $Y = NH_2$ and $R = OH$. The net result would be a hypsochromic shift to lower wavelengths. This extensive interaction (hydrogen bonding) between PABA and the solvent would hinder electron delocalization, thereby the formation of the excited state. Figure 9 pictorially represents this solvent - solute interaction.

Table 4 summarizes the results of a solvent study on sunscreen chemicals. Most commercially available sunscreen chemicals were analyzed by UV spectroscopy in several polar and nonpolar solvents. As predicted, polar compounds such as PABA, dioxybenzone, sulisobenzone, and oxybenzone all experienced a hypsochromic shift of -27 nm, -26 nm, -10 nm, and -8 nm, respectively.

Figure 8 Anilinium cation formed by the action of acid on aniline (6).



Fugure 9 Solute-solvent interaction in PABA (6).

Table 4 Summary of UV absorption data of sunscreens in combination with polar and nonpolar solvents (6).

#	Sunscreen	$\Delta\lambda$ (λ_2-λ_1)	λ_1 max nonpolar solvent[a]	λ_2 max polar solvent[a]	Extinction coefficient ($\in$)[b]
1	PABA	-27	293	266	13600
2	Dioxybenzone	-26	352	326	9400
3	Sulisobenzone	-10	334	324	8600
4	Oxybenzone	-8	329	321	9300
5	Octyl salicylate	-2	308	306	4900
6	Homomenthyl salicylate	-2	310	308	4800
7	Menthyl anthranilate	+2	334	336	5600
8	Butyl methoxy dibenzoyl methane	+9	351	360	31000
9	Octyl dimethyl PABA	+16	300	316	28400
10	Ethylhexyl *p*-methoxycinnamate	+23	289	312	24200

[a] Non-polar solvent is hexane or mineral oil.

[b] Polar solvent is a mixture of 70% ethyl alcohol and 30% water.

For less polar sunscreen compounds such as octyl dimethyl PABA, the solvent-solute interaction (hydrogen bonding) is different because the excited state is more polar than the ground state. The net result is stabilization of the excited state by polar solvents. This then lowers the energy requirments for the electronic transition, and hence a higher λ max would be expected and a bathochromic shift occurs. Molecules such as ethylhexyl *p*-methoxy cinnamate, octyl dimethyl PABA, and butyl methoxy dibenzoyl methane experienced bathochromic shifts of + 23nm, +16nm, and +9 nm, respectively (see Table 4).

Hypsochromic and bathochromic shifts in sunscreen chemicals with different solvents is pictorially represented by the energy diagram in Figure 10. Note the difference in the solvent shifts in PABA (shifts of -27 nm) as compared to the shift in its derivative, octyl dimethyl PABA (shift of + 16 nm). The reactivity of ortho-disubstituted compounds such as salicylates and anthranilates, are subject to the "ortho" effect which supersedes other resonance delocalization effects for the observed ultraviolet transitions. The six-member ring formation (Figure 11) reduces the energy requirements for the electronic transition in the molecule by loosening the electrons in the carbonyl group which is conjugated to the aromatic ring. This lower enegy transition is thus reflected in a higher than usual λ max. Most of the available electrons are involved in the six member cyclical arrangement and are not available for interaction with the solvent molecules. Thus, salicylates and anthranilates do not exhibit any significant solvent shift. The results depicted in Table 3 confirm a negligible λ max shift observed in the following chemicals; homomenthyl salicylate shifts by -2 nm, otyl salicylate by -2 nm, and menthyl anthranilate by + 2 nm.

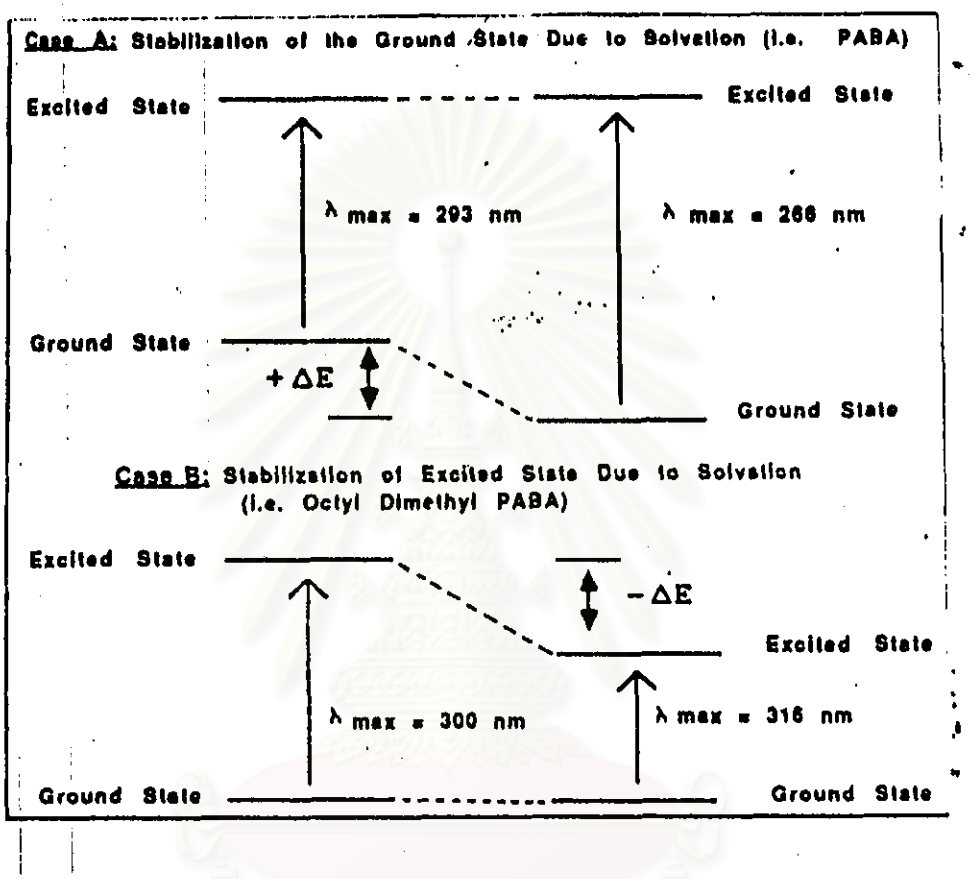


Figure 10 Energy diagram depicting the stabilization of the ground state and the excited state (6).

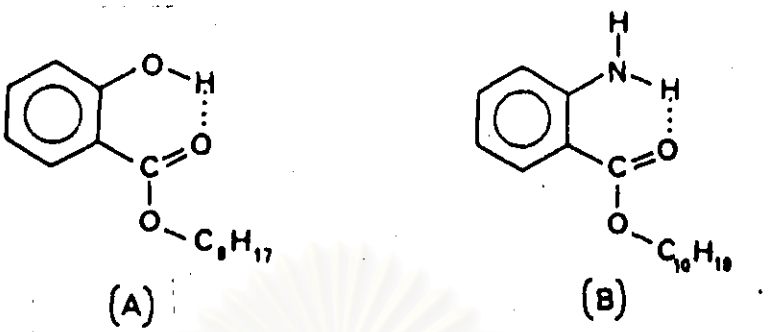


Figure 11 "Through space" hydrogen bonding interaction in octyl salicylate (A) and menthyl anthranilate (B) (6).

C. Effects on the extinction coefficient (∈)

The value of the extinction coefficient is the basis on which the effectiveness of a sunscreen chemical is assessed. Therefore, chemicals with a high extinction coefficient are more efficient is absorbing the energy of the harmful UV radiation than chemicals with a lower extinction coefficient.

All the electronic transitions for any compound may be characterized as ***symmetry allowed*** or ***symmetry forbidden***. Symmetry-allowed transitions generally have high extinction coefficients, and symmetry-forbidden transitions have lower extinction coefficients. Nevertheless, trends in extinction coefficients for sunscreen chemicals can be arrived at qualitatively by studying both the spatial requirements and the electronic transition responsible for the observed UV spectrum. The degree of resonance delocalization in a molecule can predict the relative λ max, and a similar qualitative prediction regarding its extinction coefficient is possible.

The more efficient the electron delocalization in a molecule, the higher its extinction coefficient. Compare, for example, octyl dimethyl PABA and

homomentyl salicylate. In PABA, the two substituents on the benzene ring are in a para relationship, whereas the two substituents in the case of homomentyl salicylate are in a sterically hindered ortho relationship. In ortho-disubstituted aromatic compounds, the two groups are close to one another, causing a deviation from planarity. The slightest deviation from coplanarity will significantly reduce resonance delocalization and hence a lower extinction coefficient is observed in homomenthyl salicylate as compared to octyl dimethyl PABA. For the same reason, octyl salicylate and homomenthyl salicylate (both ortho-disubstituted) have lower extinction coefficients than the para-disubstituted compouds. See Table 4 for comparative results. Increased conjugation, allowing for increased resonance delocalization, will also result in higher extinction coefficients. For example, the extinction coefficient of ethylene is 15,000, that of 1,3 butadiene is 21,000, that of 1,3,5-hexatriene is 35,000, and in the case of the highly conjugated molecule, β - carotene, it is 152,000 .

4. The future of UV filters

The ultimate sunscreen chemical should have the following characteristics:

1. The sunscreen chemical should absorb the harmful UV radiation in the region 290-360 nm. If broad-spectrum protection is not possible utilizing one sunscreen chemical, then the use of two or more ingredients which filter the 290-320 (UVB region) and the 320-360 nm (UVA region), may be necessary.

2. The sunscreen chemical should possess a large molar extinction coefficient (ε) at the wavelength (λ max) at which it absorbs maximum UV radiation. Values exceeding 20,000 would be extremely desirable. This would afford the maximum possible protection with the least amount of sunscreen added in the cosmetic formulations.

3. The λ max and molar extinction coefficient (ε) should not be affected by solvents. Excessively polar sunscreen chemicals are stabilized by polar solvents, thereby lowering the energy requirements of the ground state of the sunscreen. This, in turn, will cause a hypsochromic shift (to shorter wavelengths) in polar solvents. On the other hand, sunscreens that are not too polar in their ground state but more polar in their photochemical excited states, will experience a bathochromic shift (to longer wavelength) in polar solvents. The ideal sunscreen would be one in which the polarity of the ground state and that of the photochemical excited state are similar in nature. Hence the hypsochromic shift (due to the solvent stabilization of the ground state) will be counterbalanced by the bathochromic shift (due to the solvent stabilization of the photochemically excited state).

4. The sunscreen should have excellent photostability and be photochemically inert. If isomerization such as cis-trans or keto-enol, is possible in the molecule, then the degradation quantum yields should be low, indicating that the isomerization is reversible.

5. For waterproof formulations, the sunscreen should be totally insoluble in water. Water-soluble sunscreens will still have a role to play in sunscreen formulations, such as in hair preparations or in cases where boosting the SPF is required.

6. The sunscreen chemical should not be toxic, sensitizing, or phototoxic.

7. The sunscreen should be compatible with cosmetic vehicles and ingredients and should be easy to use and handle.

8. Since UV filters constitute a significant portion of the cosmetic formulation, occasionally exceeding 15% of the formula, then it may also be desirable to have the sunscreen impart additional characteristics. Examples of such properties include emolliency, solubilizing or emulsifying properties, moisturizing, or possibly imparting a mild pleasant aroma that can cover base notes in formulations that are fragrance free.

9. The sunscreen should not discolor skin, stain clothes, cause a stinging sensation, deposit crystals, cause drying of the skin, or produce off-odors when applied to the skin or hair.

10. The UV filter should be available isomerically pure and be chemically stable for prolonged storage and chemically inert to other cosmetic ingredients.

11. The ideal sunscreen should be inexpensive to use.

The above conditions are obiously a wish list of the theoretically ideal sunscreen candidate. Unfortunately, no sunscreen chemical on the market today can claim to possess all of the above properties. Nevertheless, the sunscreen chemicals available, whether through deliberate design or through serendipity, arm the cosmetic chemists with a reasonable arsenal of UV filters that are effective, possess a number of the "ideal" properties listed above, and have only a few undesirable effects.

The efficacy of sunscreen products

The degree of efficacy of sunscreen products is dependent on the degree to which they prevents an erythematous reaction on the human skin, which has been well represented in term of Sun Protection Factor or SPF.

The Sun Protection Factor (SPF) value is defined as the UV energy required to produce a minimal erythemal dose (MED) on protected skin divided by the UV energy required to produce a MED on unprotected skin. MED is the minimum quantity of radiant energy which produces the first detectable reddening of fair human skin following exposure. In practice, the MED is measurd as the exposure time (the time of exposure) that produces the minimally perceptible erythema at 16 to 24 hours post exposure (28). The SPF value may also be defined by the following ratio:

SPF value = MED (protected skin)/MED (unprotected skin)..... **(Eq.1)**

In order to aid consumers in selecting the type of product best suited to an individual's complexation (pigmentation) and desired response to ultraviolet light, the panel proposed the product category designation (PCD) based on the SPF value of the finished product as shown in Table 5. Table 6 describes the recommended PCD for each skin type.

Table 5 The product categories recommended by the panel.

PCD of Product	SPF Rating	Description
1. Minimal Sun Protection	>2-<4	Least protection, permits suntanning
2. Moderate Sun Protection	4-<6	Moderate protection, permits some suntanning
3. Extra Sun Protection	6-<8	Extra protection, permits limited suntanning
4. Maximal Sun Protection	8-<15	Maximal protection, permits little or no suntanning
5. Ultra Sun Protection	>15	Most protection, permits no suntanning

Note : PCD Product Category Designation

Table 6 The recommended product category designation (PCD) for each skin type.

Skin Type	Sunburn and Tanning History	Recommended SPF (PCD)
I	Always burns easily; never tans (sensitive skin)	≥8 (maximal or ultra)
II	Always burns easily, tans minimally (sensitive skin)	6-7 (extra)
III	Burns moderately, tans gradually (light brown) (normal skin)	4-5 (moderate)
IV	Burns minimally; always tans well (moderate brown) (normal skin)	2-3 (minimal)
V	Rarely burns; tans profusely (dark brown) (insensitive skin)	2 (minimal)
VI	Never burn; deeply pigmented (insensitive skin)	None indicated

Determination of Sun Protection Factor

The methods for testing of efficacy of sunscreen products can be divided into two groups, namely, for *in vitro* method and *in vivo* method. The only way to guarantee an accurate SPF value is to have the actual product tested by the *in vivo* method.

A. *In vivo* testing of sunscreen products

In general, the procedure currently used for determining the SPF of a sunscreen formulation is specified by the FDA in the OTC monograph on sunscreen drug products. This test method used in the United States for evaluation of the performance of sunscreen products relative to the prevention of sunburn in humans.

The main procedures is a three-day procedure which has been outlined in the flow diagrams of Figure 12 (Day 1), Figure 13 (Day 2) and Figure14 (Day 3).

Day 1

Initiation of the evaluation of a sunscreen product begins with the enrollment of the volunteer human panel. Panelists are recruited from the normal, healthy population and screened on the basis of their skin sensitivities to sun induced damage and their medical black grounds. Skin sensitivity varies greatly from one individual to another so that all individuals can be classified into six skin types (Table 6). The skin types are based upon the individual's tendency to sunburn and subsequent ability to pigment. Each potential panelist is interviewed regarding his/her personal history of sunburn and is also asked to complete a brief medical questionaire. Individuals who have poor health or are subjected to any abnormal response to sunlight (e.g. phototoxic or photoallergic reactions) are excluded from participation. Likewise, individuals who have a skin disease such as eczema or psoriasis, or who are predisposed to skin cancer are

also eliminated from the study. Finally, any topical or systemic medications known to produce abnormal responses to sunlight must also be prohibited.

Once the skin type and medical history of each volunteer have been determined, a panel of at least twenty volunteers for FDA method is selected. As in clinical evaluation, written informed consent must be obtained from each subject. The skin of the back of each subject is also examined to determine the presence of sunburn, suntan, scars, or other conditions which might interfere with the evaluation of the SPF of sunscreens.

After qualifying for the study, each subject is irradiated to determine his/her inherent minimal erythema dose (MED). The MED is the shortest time of exposure or smallest ultraviolet dose that produces minimal perceptible erythema approximately 24 hours later. This is determined by administering a series of six dose of ultraviolet radiation to the untreated and unprotected skin of the back of each volunteer. The time or dose interval is fixed by a geometric series of 25% increments. Exposure sites are usually at least 1 cm^2 in size for artificial light sources (30) and at least 10 cm^2 in size for natural sunlight which is a specific criteria for FDA method only.

Day 2

Approximately 24 hours after exposing the skin sites to ultraviolet light, the sites of all panelists are examined for the presence of erythema. In the process of irradiating over a range of exposure times or doses of ultraviolet light, the goal is to have at least one exposure site (the shortest time) that produces no erythema and the other sites producing a progression of erythema. For each subject, the site exhibiting minimal perceptible (faint response yet having distinct edges) erythema is then selected as the MED.

Once the inherent or unprotected MED of the panelist has been determined, the test sites to be treated with sunscreen are selected. For indoor tests, the sites should be located between the beltline and the shoulder blade, lateral to the midline. Treatment areas are usually at least 50 cm^2 per one tested sample and marked for sample application either with ink or adhesive medical tapes.

In the case of outdoor testing which is recommended by FDA method only, the entire back from the top of the shoulders to the waist is generally used due to the size of the treatment/exposure sites. The test sizes are laid out using medical tapes. Size of the test sites may vary in outdoor testing depending upon the number of formulations to be tested. Usually, test sites will be at least 50 cm^2 per one tested formulation.

Sunscreen formulations (test and standard formulation) are applied to prerandomized test sites at the rate of 2 mg/cm^2 or 2 $\mu l/cm^2$ and are spread carefully to ensure complete, uniform coverage at the test site (31). Treated sites are then permitted to air dry for at least 15 minutes before proceeding to the next step.

As in the determination of the subject's inherent MED, a geometric series of six exposures (at 25% increments) is made within each treated test site. The ultraviolet exposure series of a test site is based on the subjects 's inherent MED and the estimated or claimed SPF of the sunscreen formulation applied to that test site. Consequently, the subject's inherent MED is multiplied by the expected SPF and the geometric series are established about that value. Ultraviolet exposure sites are at least 1 cm^2 in size for indoor solar simulated tests. With outdoor tests, the entire test site is irradiated rather than the 1 cm^2 subsites exposed in the indoor test. Consequently, at least four test

sites are needed per formulation, or control, to conduct the incremental ultraviolet dose exposures.

To assure the reproducibility of the test procedures being performed within a laboratory, the method in the proposed FDA guidelines specifies the use of a standard sunscreen formulation containing 8% homosalate (HMS) which has SPF 4.

Prior to releasing each subject on Day 2, the inherent MED evaluation is conducted at untreated and unprotected test sites for confirmation. This MED value is used to calculate the SPF values of the test sunscreens and the standard formulations on Day 3.

Day 3

About 16 to 24 hours after irradiation of the test sites, each panelist returns to the laboratory. The erythemic progression within each of the test sites is examined. If the progression of the erythema response at the test site is found to be acceptable (i.e., at least one exposure site with no erythema and the others exhibiting progressively stronger erythema), then the MED of each test site is selected and the SPF calculated as shown below. If, however, the erythema progression of a test site is not acceptable, then the data for that site is rejected.

Generally, good erythema progressions are obtained when sunscreen formulations are tested using solar simulators but this is not always the case with natural sunlight testing. Exposure times of up to 6 hours are frequently required, especially for the ultra protection products. Often, there is insufficient ultraviolet dosage available to obtain adequate exposures due to varying environmental conditions throughout the day. Finally, due to the lengthy exposures, some tanning is frequently observed 24 hours after exposure.

The erythema at such sites has either subsided or marked, thus yielding an irregular erythema progression. Testing in sunlight is very difficult and requires extensive effort to acquire acceptable data.

For each test site on each subject, an individual SPF is calculated using the following equation.

$$SPF = \frac{\text{MED of protected skin}}{\text{MED of unprotected skin}} \quad \text{(Eq.2)}$$

For a given test material or the standard, the SPF of the product is then determined by calculating the arithmetic mean of the panel of subjects tested. The standard error shall not exceed ± 5% of the mean for FDA method (32).

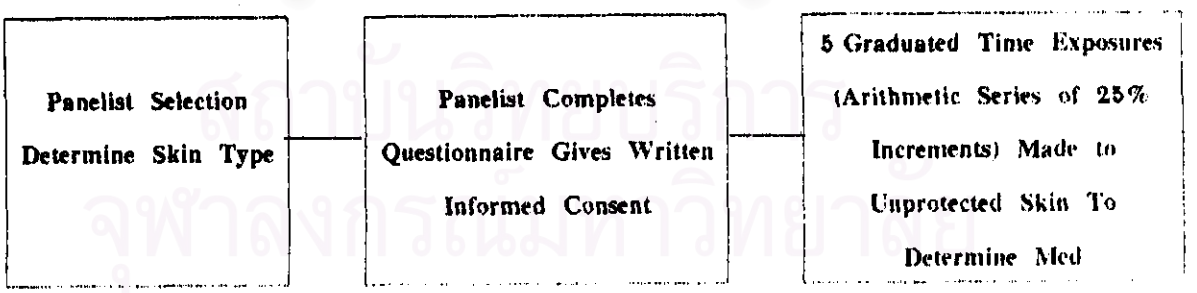


Figure 12 Sunscreen procedure, day 1.

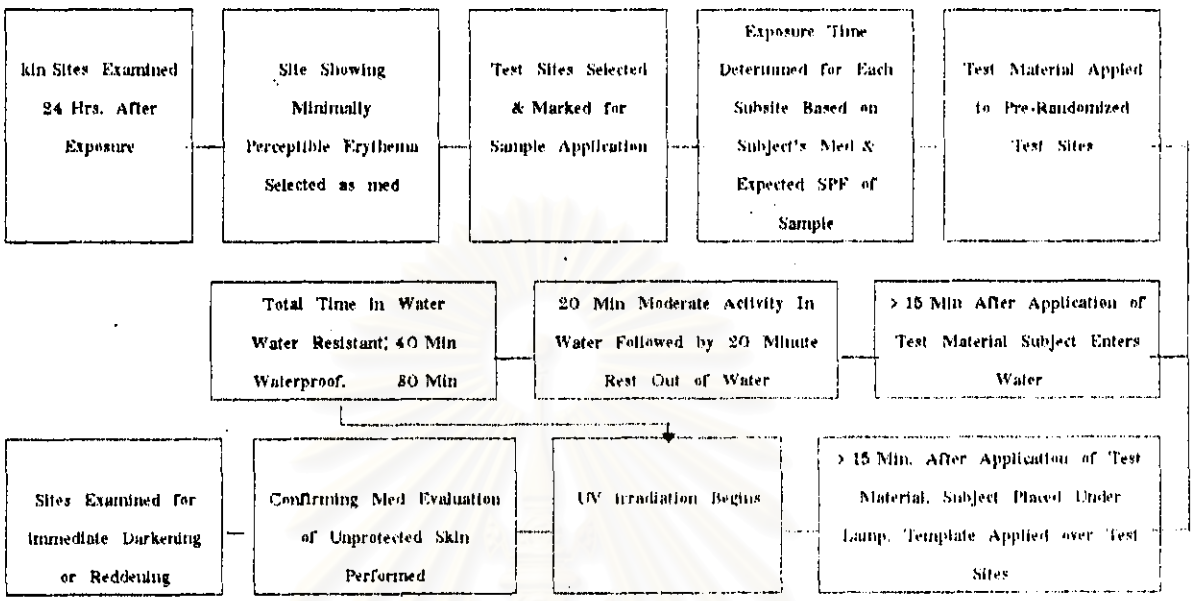


Figure 13 Sunscreen procedure, day 2.

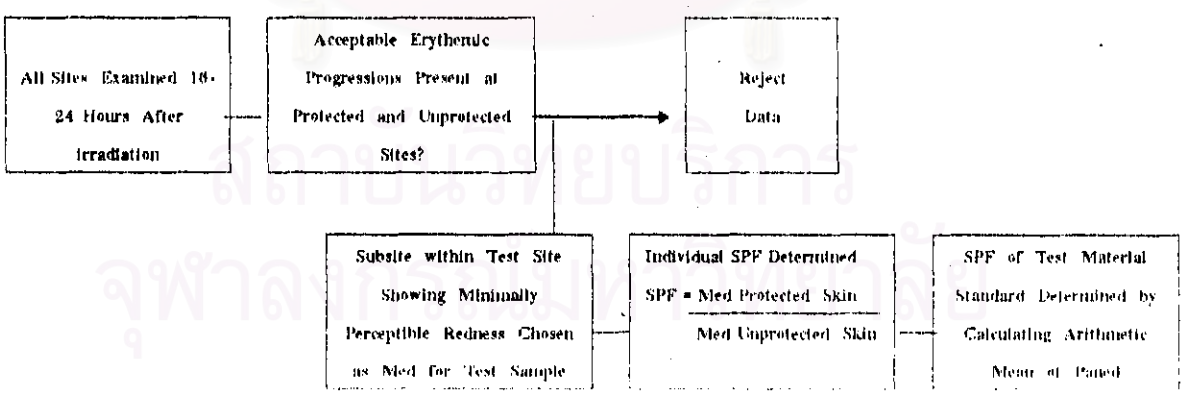


Figure 14 Sunscreen procedure, day 3.

The comparison between the proposed FDA and other methods is summarized in Table 7 and 8.

Table 7 Comparison of American and German sunscreen testing standard.

	US (FDA)*	German (DIN)**
Light Source	Filtered Xenon Arc (Continuous Emission Spectrum in UVB Region)	Osram Vitalux (300w) (Mercury Vapor Line Spectrum) Sunlight Testing Not Addressed
Application Rate	2.0 mg/cm^2	1.50 ± 0.15 mg/cm^2
UV Dose increments	25% increments Arithmetic mean	40% increments Geometric mean
Calculation of SPF Standard Reference	8% Homosalate SPF 4.1 ± 0.8	2.7% P-methoxy-2-ethylhexyl Cinnamate SPF 3.7 ± 0.3

* Proposed OTC Standard Method 8/25/78

** Approved Standard Method DIN 67501 (1985)

Table 8 Comparison of American and Australian sunscreen testing standards.

	US (FDA)*	Australian (SAA)**
Light Source	Filtered Xenon Arc (Continuous Emission Spectrum in UVB Region) Natural Sunlight	Filtered Xenon Arc (Continuous Emission Spectrum in UVB and UVA Regions) Natural Sunlight too Variable and Unpredictable
UV Dose increments	25% increments	26% increments
Test Panel	At Least 20 Subjects	At Least 10 Subjects
Data Evaluation	Standard Error of Mean Not to Exceed ± 5%	Standard Error of Mean Not to Exceed ± 10%

* Proposed OTC Standard Method 8/25/78

** Approved Australian Standard AS2604-1986

Lawson et al. (33) constructed the instrument for measuring erythema based on the optical properties of the skin structure which absorbed and scattered light. It used to measure erythema induced in the skin by exposure to ultraviolet radiation. Their assessment made using this instrument were more reproducible and sensitive than judgments made by eyes.

Edward (34) has introduced the Mexameter MX 16 ™ in order to measure the content of melanin and hemoglobin (erythema) in the skin. It is a dual instrument incorporating a melanin index meter and an erythema index meter. Both of these are based on the diffuse remittance spectrometry principle, whereby a measurement is made of the absorbance of a volume of tissue at specific wavelengths, from which the concentration of absorbing pigment can be estimated and used to construct a pigment index.

The erythema index is measured from diffused remittance of two different wavelengths that were directed onto the skin. One (green light) is strongly absorbed and the other one (red light) strongly reflected by blood pigments. Thus if a ratio of the remitted light intensities is taken, then the optical effects of the overlying epidermis is accounted for and the ratio is mainly due to blood pigment absorbance. After some simplifying assumptions this absorbance measurement is related to the logarithm of the pigment concentration. More precisely, the erythema index (Ei) is formed by substracting the log of the inverse reflectance of the red light from that of green light, or:

Ei = log (Red signal/Green signal)..... (Eq.3)

The melanin index is constructed in the same way as the erythema index, but utilizing red and near infrared wavelengths to acheive this value. The red and the near infrared wavelength lie on minima of blood pigment absorbance. Thus they give excellent isolation of the melanin index from the vascular state of the skin.

The Mexameter MX 16 ™ is constructed using LED light sources and a silicone diode detector. It is computer controlled, with ambient light rejection, automatic and simultaneous calculation of each index from the same skin area. Its output is displayed on two LCD screens. Thus it is easy to use.

B. *In vitro* testing of sunscreen products

The sun protection factor determination is in fact a biological measurement of the transmission of erythemically effective light which can be expressed as SPF = $1/T_{\text{effective}}$. (35, 36). This relationship is particularly useful in comparing test results generated by different testing systems. By using this formula, any measurement of light transmission should be directly convertible into SPF units (37).

A number of *in vitro* testing procedures has been suggested by Sayre et al., (38) for estimating the human efficacy of sunscreening products. The most widely used *in vitro* test is the determination of the absorption characteristics of sunscreening agents based on spectrophotometric analysis. There are five main methods which have been employed as described in the following series.

1. The dilute-solution method

This method was used to predict sunscreen efficacy based on the solution absorbance data alone. In this procedure a known weight of the sunscreen preparation is dissolved in sufficient solvent in order that its spectrum can be readily determined. Sayre et al.,(38) suggested that this method is unable to demonstrate the contribution to product efficacy providing by the presence of physical sunscreens such as talc, titanium dioxide and zinc oxide, because such ingredients are not soluble in solvents suitable for spectral analysis.

Sayre et al. (38) suggested that this method estimated the effectiveness of all products were higher than those obtained under in-use conditions. Apparent SPF values obtained from this method can be calculated by using the relationship SPF = $1/T_{effective}$ and assumed that no influenced other factors on predicting SPF values. In fact, the skin surface phenomena may be a major cause of error. On the skin, the ingredients may shield one another, may be absorbed into the skin, or may exhibit local concentrations making them different from their presence in solution alone. In order word, the behavior of the active ingredients and the vehicle on the skin is quite different from that of the product in solution. From these results, this method is not suitable for accurately prediction SPF values and extrapolating the SPF meaning to product efficacy based on human use.

2. The thin-film spectrophotometric method

This method was developed by Robertson and Groves who used it to evaluate several commercial sunscreen preparations in Australia in 1972. Unlike the dilute-solution method, this procedure determines the transmission spectrum

of the sunscreen preparations in the exact form in which it will be applied to the skin. The advantage claimed for this method is that the spectrum obtained is theoretically more comparable to that of the sunscreen preparation on the skin. The main reason is because the solvent environment of the ultraviolet absorber has not been changed as it has in the dilute-solution procedure. The thin-film method does have an advantage in that it can be used for pigmented products which cannot be evaluated by solution technique. To determine the transmission spectrum, a small quantity of the preparation is squeezed between a quartz plate and the flat surface of a quartz prism (8).

This method used the Lambert-Beer Law to relate the data obtained on thick films to thinner films as would actually be used on the skin. Theoretically, both of these methods should be valid, assuming that absorption of UV light by the sunscreen product while in actual use does follow the Lambert-Beer relationship and that the interaction of the product with human skin is negligible. These assumptions, however, are not entirely correct and this method still has been unable to accurately predict the efficacy of sunscreen ingredients in products designed to prevent sunburn.

3. The epidermal hairless mouse method

This method uses the epidermis from hairless mouse to determine the ability of a film of sunscreen preparation to prevent the transmission of ultraviolet radiation. This modification of the basic thin-film technique to include skin was found to give values indicative of the protective ability of several sunscreen preparations closely comparable to those obtained by in vivo methods. The SPF values obtained by using the hairless mouse epidermis procedure agree with the SPF values obtained by using human subject and the xenon arc solar simulator in *in vivo* method. These results would indicate that the

hairless mouse epidermis procedure is a reliable and useful *in vitro* method for assessing the protective capabilities of sunscreen preparations.

4. The photoacoustic spectroscopy method

This method is similar to the epidermal hairless mouse method. It allows the measurement to be made directly on the sunscreen formulation applied to the excised full-thickness, newborn rat skin. Thus the parameters which govern the spectral properties of the skin-sunscreen agent complex are maintained close to those of the "in use" situation.

In photoacoustic spectroscopy, the sample to be studied is placed inside a sealed chamber, a photoacoustic cell. The cell contains a very sensitive microphone and is filled with a gas, such as air, at ambient temperature and pressure. The sample is irradiated with monochromatic light which is chopped at some acoustic frequency (50 to 5000 Hz). If the sample absorbs any of the incident radiation, some energy level in the sample is excited and this energy level must subsequently de-excite, usually by means of a non radiative or heating mode of de-excitation. The periodic input of light thus results in a periodic heating of the sample and subsequent periodic heat flow from the sample to the surrounding gas. The gas at the sample-gas interface responds to this periodic heat flow with an oscillatory motion that produces a periodic pressure change in the sealed photoacoustic cell. The microphone in turn detects this pressure change as an acoustic signal which is then processed electronically and recorded (39).

By using this method, the photoacoustic signal bears a close resemblance to the true absorbance of sunscreen product in actual "in use" situation.

5. The skin cast method

This method uses resin casts taken from replicas of human skin. Resin (Luviset Cap-X) casts are formed from silicone rubber impressions of human skin. They have the same topography as the surface of the skin which is a important factor for light that impinged on the surface of scatter. The surface texture also facilitated product application which resulted in similar distribution of product as in actual "in use" situation. The sun protection factor is calculated as the ratio of transmission of ultraviolet light before and 10 minutes after application of sunscreen products. The SPF values obtained from this procedure have some correlation with the SPF values obtained from *in vivo* procedure (40).

However, this technique is time-consuming and demands special care to make resin cast a good substrate onto the test products will be applied. Thus a new method was established by Diffey and Robson who used a transpore tape, a readily available, inexpensive substrate. Spectroradiometric measurement of the transmission of ultraviolet radiation through this substrate with and without the sunscreen applied allowed for rapid determination of SPF of tested sunscreen product. This *in vitro* method was found to have a good correlation with *in vivo* SPF results of tested products having SPF values less than 20 (41). Diffey and Robson suggested that the transpore tape was a useful medium for rapid screening of sunscreen photoprotection. Unlike either mouse or human epidermis, it required no preparation and was readily available. However, this transpore tape was inappropriate for testing sunscreens which are in either oil or alcohol vehicles due to their absorption into the tape (42).

C. An instrument for *in vitro* determinations of SPF

Sellers and Carpenter (43) developed an instrumental sun protection factor (SPF) analyzer. This SPF analyzer is a microcomputer-controlled UV-VIS scanning spectrophotometer. The systemic analyzer automatically scans the wavelengths from 290 nm to 400 nm and accumulates and stores data every 5 nm interval. A monochromatic protection factor (MPF) is calculated and plotted after each run using the data collected at 23 specific wavelengths. To compensate for variations in sample application and for wavelength-dependent variables of the substrate, as many as 12 separate areas of a sample can be analyzed. The calculations are based on the work of Diffey and Robson. Transpore surgical tape is used as the medium to which the sample is applied. Advantages gained by using this analyzer include:

- SPF values that correlate well with published data.
- Fast results, typically within five minutes from sample application to calculated results.
- Low consumable costs.

1. Optical system

The analyzer's optical system is comprised of a continuous UV-VIS source, color compensating filters, diffusion plates, a grating monochromator and a photomultiplier detector as shown in Figure 15.

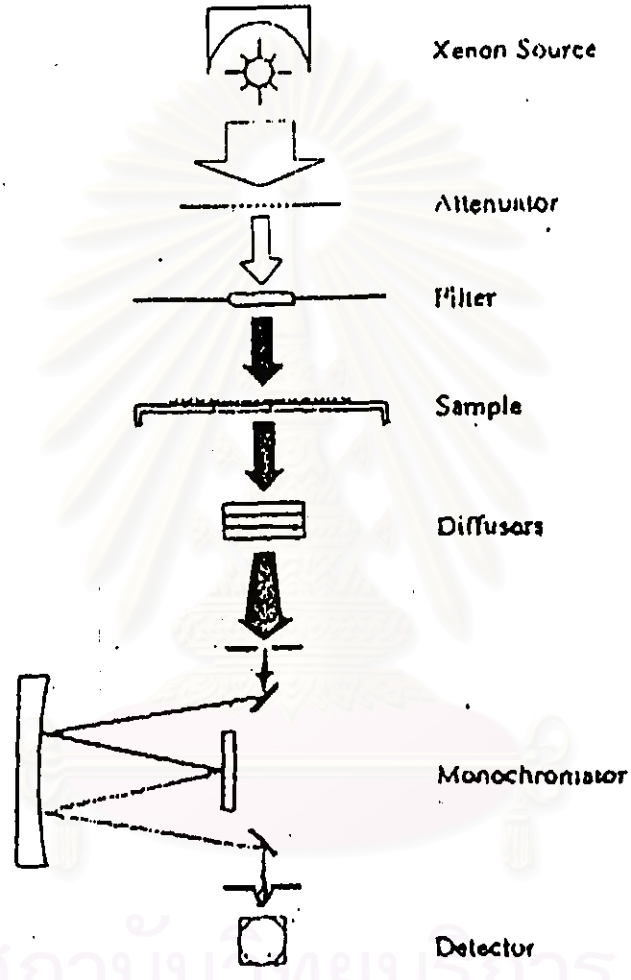


Figure 15 optical path diagram of sun protection factor (SPF) analyzer (9).

A compact 125 W xenon arc lamp provides UVB and UVA radiations. Partially collimated radiation from the source passes through a special attenuator, filter and aperture before striking the sample. The filter alters its spectral distribution so that it approximates the solar spectrum. The beam of violet radiation (16 mm in diameter) incident on the sample is transmitted, absorbed, and reflected by the sample and substrate. Transmitted radiation passes through a series of quartz diffuser plates (five ground surfaces) where the beam is homogenized and attenuated. The beam then enters a grating monochromator producing the monochromatic radiation which impinges on the photosensitive surface of the detector; generating signal is proportional to the intensity of the radiation striking its surface.

2. Computer and operation

Prior to running an analysis, the system must be calibrated. After calibration is completed, the system is optimized to ensure the best signal-to-noise ratio over the UVB and UVA wavelength range by adjusting the gain of the detector.

To initiating a run, the user is asked to input the information about operator, sample identification, and sample volume. The first step in analysis is to make a reference run. A piece of transpore surgical tape is placed over the open frame of sample holder and then the sample holder is placed in the incident beam. After the user presses "b" on the keyboard to "begin" a run, the software drives the monochromator to 290 nm the lowest wavelength at which SPF data will be obtained. Any 290 nm monochromatic radiation that is not absorbed or reflected by the substrate strikes the detector. This generates a current which is converted to a voltage, and then amplified, digitized

and stored as the data point at 290 nm. The monochromator is then driven from 5 nm to 295 nm and another data acquired. All 23 wavelengths covering 290 to 400 nm at 5 nm interval are monitored. The reference data is used to compensate for wavelength-dependent variables in the source substrate, monochromator, and detector.

A sample is applied to the substrate. A sample (100 μl) is applied in rows of small "dabs" or "spots" to transpore tape supported on the open metal frame of sample holder. A 1 cc syringe or pipette (Manual or automatically) can be used for dispensing the sample. An area approximately 2.75 x 2.75 inches (7.0 x 7.0 cm) should be covered with 100 or more dabs of sample, which is then spread evenly over a 50 cm^2 area of the substrate. This technique will distribute a layer of sample 2 $\mu l/cm^2$ over the specified area. A sample thickness equivalent to that used in the standard *in vivo* SPF tests.

The sample is placed in the incident beam and the first run initiates. Data is accumulated at each wavelength in the same manner as the reference run. The MPF, as defined by Diffey, is the ratio of the signal at a specific wavelength of the substrate with sample (1%T). The MPF for each wavelength of the 290 nm to 400 nm scan is calculated and displayed in the plotting window. If multiple runs were requested, the sample holder is repositioned exposing a different area of the sample to the incident radiation and a second run started. After each run, the new MPF are calculated. The process is repeated until all runs are completed.

After all runs are completed, the mean (average) MPF and their standard deviations are calculated for each wavelength. Then SPF is calculated from the MPF as described by Diffey and Robson and is shown below:

$$SPF = \frac{\sum_{290}^{400} E_\lambda B}{\sum_{290}^{400} \frac{E_\lambda B_\lambda}{MPF_\lambda}} \qquad \text{.....(Eq.4)}$$

where E_λ is the spectral irradiance of terresterial sunlight under defined conditions (midday, midsummer sunlight at 40^0 north, solar zenith angle 20^0; B_λ is erythemal effectiveness (CIE) and the MPF_λ is the mean monochromatic protection factor as previously described.

The mean absorbance ratio (UVA/UVB ratio) is also calculated and displayed on the summary data plot. The ratio is calculated by taking the mean (average) absorbance of the integrated area covering the UVA (320 to 400 nm) and dividing by the total absorbance of the integrated area covering to UVB (290 to 320 nm). Absorbance values at each of the 23 measuring wavelengths are calculated from MPF_λ. The area per unit wavelength interval is calculated by using Simpson's Rule for irregular areas. Mean absorbance ratios range from 0 to 1, with 0 indicating no UVA absorbance and 1 indicating equal absorbance of UVA and UVB. The summary data currently include valued for the average UVA protection factor, erythemal UVA protection factor, and a unity UVA protection factor.

The SPF analyzer is designed to ease manufacturers of active sunscreen products in rapidly sunscreening for the SPF value of new formulations and for quality control. Sellers and Carpenter (43, 44) claimed that SPF values obtained from this instrument correlated well with those from in vivo testing.