IN VITRO PENETRATION AND RETENTION OF SUNSCREEN BENZOPHENONE-3 (BZ-3) ACROSS POLYSULFONE MEMBRANE FROM A RANGE OF FORMULATIONS

BY

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In Vitro Penetration and Retention of Sunscreen Benzophenone-3 (BZ-3) across Polysulfone Membrane from a Range of Formulations

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A Thesis/Practicum submitted to the Faculty of Graduate Studies of The University of Manitoba in partial fulfillment of the requirements of the degree

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Statement of Originality

The work presented in this thesis is, to the best of my knowledge and belief, original, except as acknowledged in the text, and the material has not been submitted, either in whole or in part for a degree at this or any other university.

BharatGopal

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Abstract

The penetration of sunscreen agents through a membrane was studied using polysulfone membrane (Tuffryn®, Fischer) mounted in *in vitro* Franz-type vertical diffusion cells. A range of formulations was used in the study of sunscreen agents. Infinite doses were used to examine formulation effects. Solute activity (vehicle-solute interaction) was separated from membrane specific effects (solvent-skin or solute-skin interactions) by using constant thermodynamic activity. A simple and rapid high-performance liquid chromatography (HPLC) assay was validated for the quantification of sunscreen agents. A number of studies were undertaken to define appropriate receptor conditions for sunscreen absorption through membrane and sunscreen solubility. The polysulfone membrane permeation of Benzophenone-3 (Bz-3) from a range of single solvents was examined using the same Bz-3 activity in each vehicle. The maximum fluxes of Bz-3 were observed when the solubility in the vehicle was low, and when the solubility parameter of the vehicle (δv) is different from that of the solubility parameter of the sunscreen (δi). The flux (J) and the permeability coefficient k, were lowest when the solubility of the sunscreen in the vehicle was maximum. The flux of Bz-3 from different solvents was further increased by the presence of octyl methoxycinnamate (OM) in liquid paraffin (LP) vehicle. This work has demonstrated the importance of vehicle formulation as a determinant of sunscreen agent penetration. Maximal skin retention and minimal flux can be achieved by a combination of 1) Sunscreen lipophilicity, 2) vehicle formulation in which the sunscreen agent is most soluble, 3) a vehicle formulation such that the solubility parameter of the vehicle (δv) is sufficiently similar to the solubility parameter of the sunscreen (δi), and 4) the most appropriate formulation type for topical use.

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Lists of Abbreviations

ACN	acetonitrile
BM	butyl methoxydibenzoylmethane
Bz-3	benzophenone-3
BSA	bovine serum albumin
$C_{12\text{-}15}BA$	$C_{12\text{-}15}$ benzoate alcohol
DHB	2,4-dihydroxybenzophenone
DHMB	2,2-dihydroxy-4-methoxybenzophenone
DMSO	dimethyl sulfoxide
EtOH	ethanol
FDA	food and drug administration
FTIR	fourier transform infra red
GAGs	glucosaminoglycans
GSH-Px	glutathione peroxidase
HAC	acetic acid
HDPE	high density polyethylene
HLB	hydrophilic-lipophilic balance
HPLC	high performance liquid chromatography
H_20	water
IPM	isopropyl myristate
IR	infra red

LDPE	low density polyethylene
LP	liquid paraffin
MED	minimum erythemal dose
MeOH	methanol
OA	oleic acid
OC	octocrylene
OM	octyl methoxycinnamate
OP	octyl dimethyl PABA
OS	octyl salicylate
OT	octyl triazone
O/W	oil in water
PABA	para amino benzoic acid
PBS	phosphate buffered saline
PE	polyethylene
PG	propylene glycol
ROS	reactive oxygen species
SC	stratum corneum
SOD	superoxide dismutase
SPF	sun protection factors
THB	2,3,4-trihydroxybenzophenone
TiO_2	titanium dioxide
UCA	urocanic acid

VDW	vander walls
UV	ultraviolet
VIS	visible
Volpo-N20	polyethylene glycol 20 oleyl ether
W/O	water in oil
ZnO	zinc oxide

List of Symbols

A	absorbance
a_m	activity of solute in membrane
a_v	activity of solute in vehicle
C	percentage concentration of a solute
ΔC	concentration difference of solute between vehicle and membrane
C_m	concentration of solute in membrane
C_v	concentration of solute in vehicle
Cal/ cm^3	calorie per cubic centimetre
D	membrane/vehicle diffusion coefficient
D	apparent diffusion parameter
δi	solubility parameter of solute
δm	solubility parameter of membrane
ε	molar extinction coefficient
$E^{1\%}cm$	percent extinction coefficient

h	diffusional pathlength
hr	hour
J	flux
K	partition coefficient
κ	relative partition coefficient
K_p	pathlength of light (1cm)
min	minute
Q	cumulated amount of penetrant in receptor fluid
R	gas constant
R_m	retention of solute in membrane
γ	activity coefficient
S	solubility
t	time
V_m	volume of effective exposure area of membrane

CHAPTER 1

1.1 Introduction

Sunscreen agents were first introduced as commercial products in the early 1920s (Natow, 1986; Jass, 1990). The awareness of the deleterious effects of solar radiation and evidence of the linkage between sun exposure and skin cancer have led to a sharp increase in the use of sun care products throughout the world (Fitzpatrick and Sober, 1985; Green et al., 1988a; MacLennan et al., 1992; Farmer and Naylor, 1996). In addition, the formulation of sunscreen products with very high sun protection factors (SPF up to 50) has also increased dramatically in recent years. Due to greater public health awareness, the strategy of routine use of sunscreening products has now been widely accepted in many countries. Therefore an important research topic in the area is evaluation of the percutaneous absorption of sunscreen agents from and the influence of topical formulations in which they are presented to the skin. This knowledge would facilitate optimal formulation of sunscreen products to achieve maximal skin protection from ultraviolet (UV) radiation and minimal penetration after topical application.

The literature review is focussed on the following aspects such as, structure of human skin, factors which influence skin barrier function, UV radiation and skin damage. It also summarises the fundamentals of *in vitro* absorption of sunscreens (*in vitro* model), finite and infinite dose techniques, barrier membranes (synthetic membranes), receptor fluids, and different types of sunscreen agents like physical sunscreens and chemical sunscreens. Finally, a review of the influence of formulation on skin penetration of sunscreen agents, vehicle factors that influence skin penetration, solvent effect on sunscreen efficacy,

sunscreen formulation and review of current available literature on skin penetration of sunscreen agents were done.

1.2 Skin

1.2.1. Structure of human skin

Skin can be structurally viewed as a series of layers, the three major divisions are epidermis, dermis and subcutaneous tissue (Figure 1.1). Epidermis is the outermost skin region and is made up of a number of different cell layers. The stratum corneum (SC) or horny layer is the outer surface layer of the epidermis, followed by the granular layer, malpighian layer and basal layer. The SC which is 20 –30 cell layers thick (approx.15 μm) accounts for about one-tenth of the epidermal thickness (approx.150 μm) and consists of fully keratinised, dead cells and lipids. Keratin is a tough protein, and major lipids present in the stratum corneum are ceramides, free fatty acids, cholesterol and triglycerides (Anderson and Cassidy, 1973; Bouwstra and Gooris, 1997; Marieb, 1992). The rate-limiting step for the diffusion of most chemicals through the skin occurs in the SC due to its lipophilic nature (Monash, 1957; Scheuplein, 1965; Scheuplein and Blank, 1971). The granular layer (stratum granulosum) contains a waterproofing glycolipid that is secreted into the intercellular space to slow water loss across the epidermal layer (Marieb, 1992). The malpighian layer (stratum spinosum) is several cell layers thick. Mitosis occurs there, but less frequently than in the basal layer. As the epidermis does not contain blood vessels, cells superficial to the stratum spinosum do not receive adequate nutrients, so they become less viable and begin to die as they move towards the skin surface.

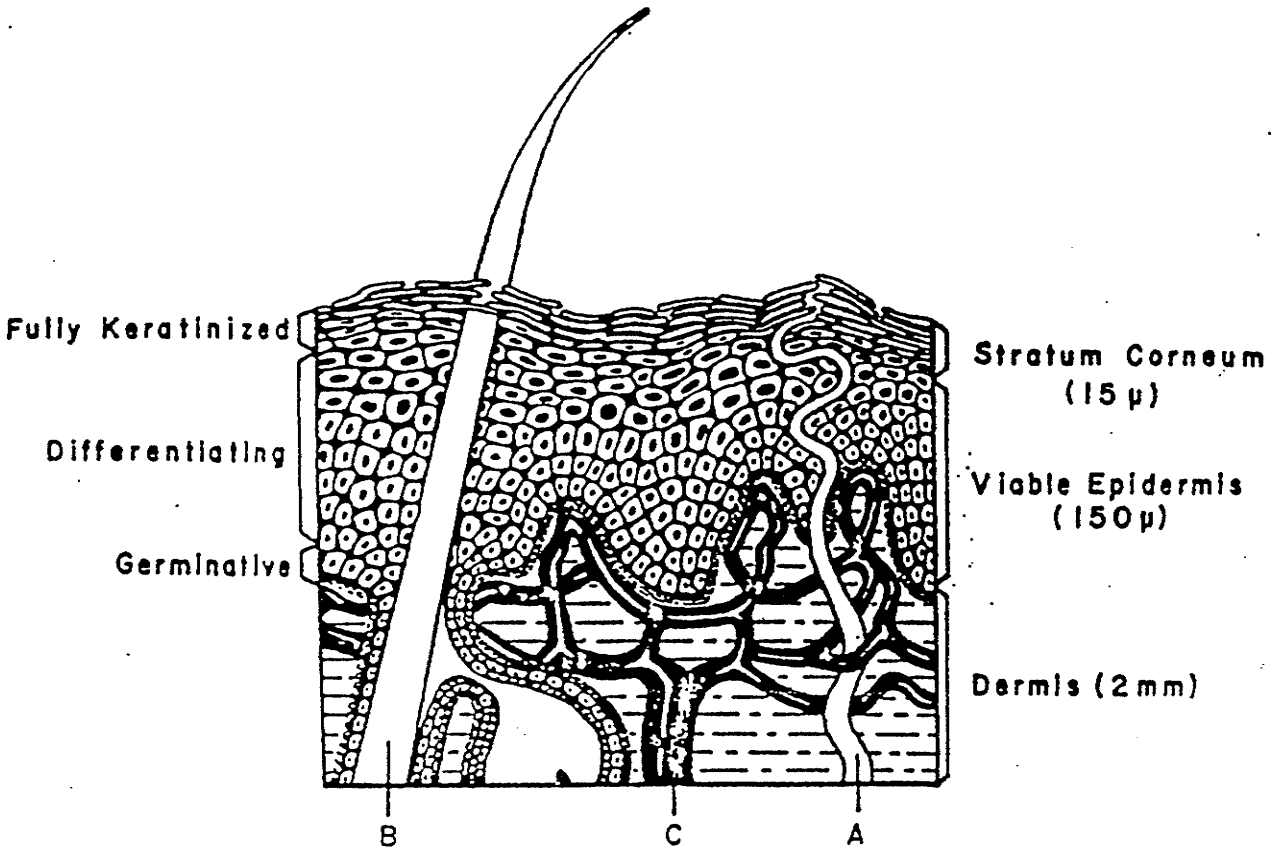


Figure 1.1 Schematic representation of skin a) sweat gland b) hair follicle and c) microvasculature (Houk and Guy, 1988).

The basal layer contains abundant melano-somes that may serve to protect the prominent nucleus from UV radiation (Wertz and Downing, 1989). The entire architecture of the epidermis constitutes a dynamic system in which each cell changes continuously during its passage from the basal layer, where it is formed, to the surface of the horny layer, where it is discarded (Wertz and Downing, 1989).

The dermis is the second major skin region and is richly supplied with nerve fibres, blood vessels and lymphatic vessels. Cutaneous receptors, glands and hair follicles reside within the dermis (Marieb, 1992). The extensive vascular supply of the dermis allows the skin to act as a blood reservoir. In most cases, chemicals that have diffused beyond the epidermis will be carried away by the dermal blood supply to the systemic circulation. It is an important area in transdermal drug delivery.

1.2.2 Barrier properties of the SC

1.2.2.1 Structure of SC bilayers

A bricks and mortar model has been used to describe the SC (Michaels et al., 1975). The bricks are the dead, flattened cells of the SC, the corneocytes that contain very little lipid. Their major structural components are aggregates of keratins that are complex mixture of proteins in which the sulphur-containing diaminoacid cysteine is predominant (Walters, 1989). The mortar consists of a structured complex containing lipid bilayers, which surround the keratin-rich interiors. The double bonds in the sphingosine moieties are located at the polar ends of the ceramides. Their straight, saturated aliphatic chains form the highly ordered, hydrophobic bilayer interior that is impermeable and resistant to oxidative damage on exposure to air at the skin surface (Wertz and Downing, 1989). The intercellular lipid sheets of the SC constitute a compact and highly organised protective covering. It acts as a barrier against the loss of physiologically essential substances and the diffusion of potentially toxic chemicals from the external environment into the body (Turner and Nonato, 1997).

1.2.2.2 Transdermal pathways and properties of penetrant

Parallel permeation paths were classically suggested to exist within the SC (Scheuplein, 1966; Flynn et al., 1974). These include-

a) the transcellular pathway- aqueous pores that are associated with the hydrated intracellular keratin or the limited aqueous phase between intercellular lipid bilayers. Transport through the skin involves a direct path in which a chemical will repeatedly partition into and out of cells together with diffusion through both inter- and transcellular phases.

b) the intercellular pathway- a hydrophobic pathway that is associated with the intercellular lipid phase only (Michaels et al., 1975; Houk and Guy, 1988)(Figure 1.2).

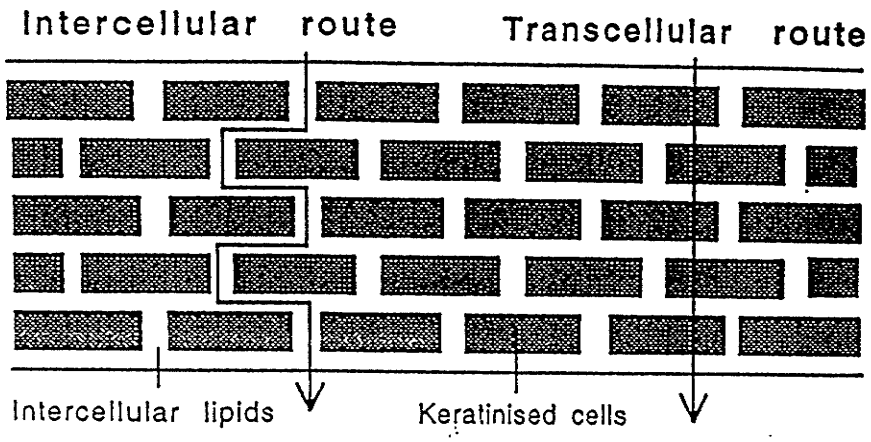


Figure1.2. Schematic diagram of penetration pathways of chemicals across the SC.

The route through which permeation occurs is largely dependent on the physicochemical characteristics of the penetrant, the most important being the relative ability to partition into each skin phase (Walters, 1989). It was thought that highly hydrophilic compounds penetrate via the so-called polar or pore route (described as the trancellular route above) as they were believed to partition more easily into the hydrophilic layers of the viable epidermis and then into the blood circulation (Houk and Guy, 1988). Whereas highly hydrophobic compounds were believed to diffuse by the lipoidal pathways of the SC (Vickers, 1963; Dupuis et al., 1986; Saunal et al., 1991; Wiechers and de Zeeuw, 1991). Recent research has demonstrated that many skin penetration enhancers promote the transport of polar compounds more than lipophilic compounds (Lopez et al., 1997; Guy, 1996). The mechanism of enhancement of these agents is by disruption of the highly ordered lipid structure between the corneocytes, thus increasing intercellular lipid diffusivity, rather than the enhancer acting on a putative polar pathway (Guy, 1996). As a result of this evidence, diffusion through the intercellular lipid phase has been suggested and is now generally accepted to be the major route of penetration through the bulk SC. Compounds penetrate the skin not only through the SC but also by way of the hair follicles or through the sweat ducts. These pathways offer only a comparatively minor route because they represent such a small fraction of the surface area (Treherne, 1956; Florence and Attwood, 1988). It has been found that only in the case of molecules that move very slowly through the SC may absorption by these routes predominate (Houk and Guy, 1988).

1.2.3 Factors which may influence skin barrier function

1.2.3.1 Effect of hydration

The state of hydration of the SC is one of the major factors, influencing percutaneous absorption of a drug. It was found that the amount of "bound water" in fully hydrated SC can be as much as five times the dry weight of the skin tissue, and was proposed to act as an endogenous plasticiser (Blank, 1952; Scheuplein and Morgan, 1967). It was reported that the penetration of certain alcohols and cortisones across the fully hydrated human skin was up to 10 and 13 times higher than across the dry skin, respectively (Scheuplein and Blank, 1973; Scheuplein and Ross, 1974). The absorption rate of salicylates across the skin was measured, and it was found that absorption of glycol salicylate and methyl salicylate greatly increased when tissue was hydrated (Wurster and Kramer, 1961). Related works have been reviewed recently by (Roberts and Walker, 1993; Wester and Maibach, 1995). One of the proposed mechanisms for the facilitation of transport is by water being absorbed into the SC where it acts as a plasticiser in its bound state (Roberts and Walker, 1993). Insertion of water molecules between polar head groups of SC lipids leads to increased fluidity (Barry, 1987) (Figure 1.3). It was also reported that an increase in water content was associated with a decrease in lipid/protein phase transition temperature and hence, induction of lipid disruption and protein denaturation (Potts, 1989). Low temperature lipid phase transitions occuring near physiological temperature were suggested to be most likely responsible for mechanical alterations of the SC, whereas transitions occuring near 70 °C were primarily responsible for barrier properties

(Potts, 1989). Water increases the fluidity of the SC lipids, and is presumed to cause a swelling of the compact structures in the horny layer, which facilitates flux (Florence and Attwood, 1988; Potts, 1989). It has been found that hydration does not always have an enhancing effect in percutaneous absorption, as the effect is dependent on the polarity of the drug (Makki et al., 1995). Poorly and moderately lipophilic molecules such as 5-methoxypsoralen showed increased absorption with increased hydration, while for molecules with a strong lipophilic character there was no effect on the absorption rate. The level of hydration is a function of the water concentration gradient between the dermis and the surface of the skin, and can be modified by many formulation vehicles. It has been found that occlusive dressings, oinments and the oils induce the greatest hydration by a mechanism of reducing water loss to the atmosphere and increasing the binding of water to the SC (Roberts and Walker, 1993). Humectants are another category of moisturiser that have long been used in topical formulation for their moisture-retaining capacity (Jellinek, 1970).

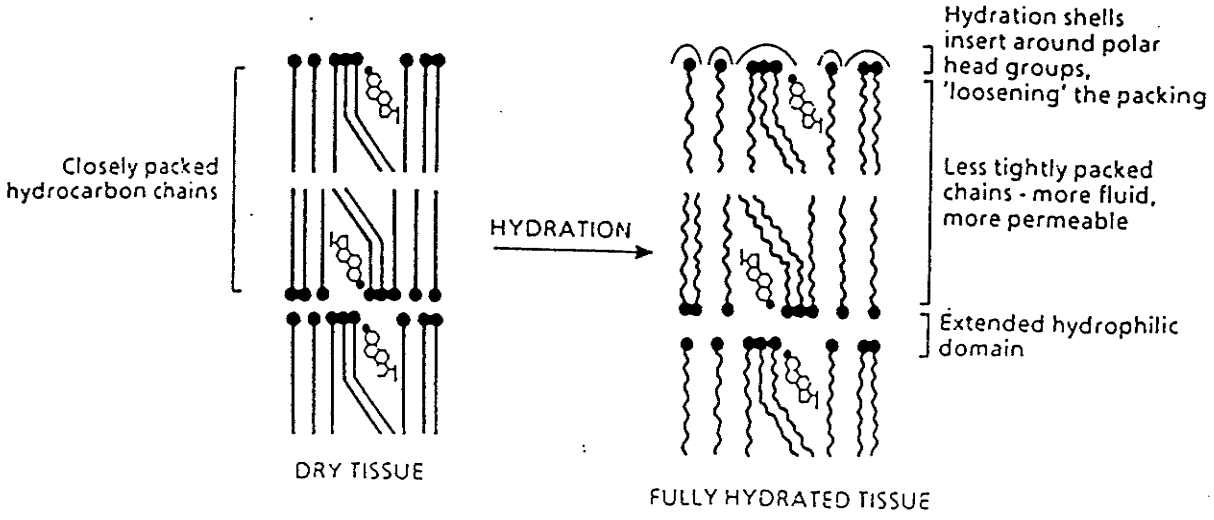


Figure 1.3 Insertion of water molecules between polar head groups of the SC lipids (Cited from Barry, 1987).

The mechanism of moisture-retention of humectants is humidity dependent (Jellinek, 1970). At a low relative humidity humectants could dehydrate the SC and decrease penetration (Roberts and Walker, 1993) e.g., glycerine.

1.2.3.2 Effect of temperature

Increase in temperature results in increasing lipid fluidity and keratin conversion (Potts, 1989). As temperature varies little in the clinical use of a topical formulation, it does not appear to affect skin barrier function under normal physiological conditions.

1.2.3.3 Effect of chemicals

1.2.3.3.1 Penetration enhancement

Skin penetration enhancers are compounds which reduce the barrier resistance of the SC, allowing a drug to penetrate more readily to the viable tissues and subsequently enter systemic circulation. Skin permeability can be modified by both altering natural conditions of the skin such as hydration and disrupting lipid and protein structure of the SC using penetration enhancers. Chemicals disturb SC barrier function via different mechanisms. Although the mechanisms are not yet fully understood, lipid perturbation and modification of the partitioning of the drug into the tissue were found to be major factors for altered barrier function by a number of investigators (Barry, 1987; Harrison et al., 1996; Brain and Walters, 1993).

Sulfoxides

The recorded history of dimethylsulfoxide (DMSO) began in 1867 following its synthesis from dimethylsulfide by a Russian chemist, Alexander Saytzeff (Franz, et al., 1995). Due to its remarkable solvent properties, it has been extensively studied and reviewed (Stoughton and Fritsch, 1964; Stoughton, 1965; Elfbaum and Laden, 1968; Barry, 1987; Chattaraj and Walker, 1995). Postulated mechanisms for the penetration enhancement properties of the chemical include elution of lipid, lipoprotein, and nucleoprotein structures, denaturation of the structural proteins and delamination of the SC (Creasey et al., 1978; Chattaraj and Walker, 1995). It was reported that DMSO increases lipid fluidity by disrupting tightly packed lipid chains, which resulted in an interaction between the polar head groups of the lipids via hydrogen bonding (Chattaraj and Walker, 1995). DMSO induced penetration enhancement was also found to be related to keratin conversion (Naik and Guy, 1997).

Alkanols

Alkanols are capable of penetrating the SC. The permeation rate of alkanols was maximal at carbon number equal to 6 and declined beyond that number (Scheuplein and Blank, 1973). When alkanols ($C \leq 3$) were applied as a pure liquid, irreversible damage to the skin tissue was demonstrated (Scheuplein and Blank, 1973). It has been shown that as the alkyl chain length of alkanols increased, the transdermal permeation rate of indomethacin increased to a maximum, and then decreased as the number of methylene groups in the alkyl chain increased to six and eight (Chein et al., 1988). It was postulated that the low molecular weight alkanols ($C \leq 6$) may act as solubilizing agents, thereby enhancing the

solubility of indomethacin in the fatty matrix of the SC hence promoting permeation (Chattaraj and Walker, 1995).

Enhancement mechanisms for phenyl alcohols were suggested via protein denaturation or plasticization of skin lipids by disrupting their highly ordered structure to increase diffusivity (Roberts et al., 1977; Chattaraj and Walker, 1995; Naik and Guy, 1997; Lopez et al., 1997). Vehicles that are permitted to remain in contact with the skin thereby are facilitating penetration (Zatz, 1991a; Naik and Guy, 1997). Solubility of lipophilic drugs such as nitroglycerin and oestradiol in the SC is linear with the concentration of ethanol in the SC (Berner and Liu, 1995). The enhancement ability of the ethanol on nitro-glycerin was found to reach a maximum at an ethanol concentration level of around 70%, where uptake of ethanol into SC or the delipidized SC is optimal (Berner and Liu, 1995). Therefore the ability of ethanol to increase penetration of lipophilic compounds was suggested to be via increased solubility of the compounds in the SC by cosolvency or delipidization of the SC.

Propylene glycol

Propylene glycol (PG) is one of the most widely used solvents in topical formulations. It is commonly used in moisturisers as a humectant. Propylene glycol binds moisture to itself and is believed to hold moisture in the skin - keeping it soft and young. Proponents quote and maintain studies showing it to be a safe, effective ingredient. It can effectively increase the skin penetration of various chemicals (Portnoy, 1965; Lorenzetti, 1979; Mollgaard and Hoelgaard, 1983; Sheth et al., 1986; Barry, 1987). The mechanism for

penetration enhancement was suggested to be via solvating α-keratin and occupying hydrogen bonding sites, thus reducing drug tissue binding and promoting permeation (Barry, 1987). Small polar accelerants such as DMSO and PG may also be accumulated in both intercellular and protein regions of the SC, thus inducing increased drug partitioning into the skin to yield increased fluxes (Barry, 1987).

Fatty acids

Attempts for transdermal drug delivery have led to extensive research of these compounds on their skin penetration enhancement effect (Cooper et al., 1985; Green et al., 1988b; Aungst, 1989; Schneider et al., 1996). Long chain fatty acids have been shown to be effective penetration enhancers *in vitro* for a number of coapplied chemicals (Barry, 1987). Oleic acid (OA) is the one mostly studied. Saturated fatty acids provide a major component of the horny layer lipids. Thus topically applied fatty acids probably readily penetrate into the tightly packed lipid of the SC. For instance, OA may penetrate the lipid region with its polar end close to the lipid polar heads. Its bent structure then disrupts the tightly packed lipid region to increase lipid fluidity and drug mobility (Barry, 1987). The ability of certain fatty acids to increase permeability of the skin appears to be related to a selective perturbation of the intercellular lipid bilayers present in the SC. It has been found that unsaturated C_{18} acids and alcohols are more effective enhancers than the corresponding saturated acid or alcohol (Aungst et al., 1986). Among stearic, oleic acid and linolenic acids, maximum enhancement was observed with linoleic acid due to the fact that more double bonds exist in its structure (Chattaraj and Walker, 1995). When SC was treated with OA, the treatment decreased the phase

transition temperatures of the lipids, thus supporting the hypothesis of increased motional freedom or fluidity of the lipids (Golden et al, 1987).

Esters

Several alkyl esters, such as ethyl acetate and isopropyl myristate, are effective skin penetration enhancers for a number of drugs. Regarding ethyl acetate, it was suggested that it might act in a similar manner to the smaller polar enhancers such as DMSO. These compounds penetrate the SC and increase lipid fluidity by forming a solvation shell around polar head groups, which leads to a disruption of lipid packing (Chattaraj and Walker, 1995). Isopropyl myristate (IPM) was suggested to have a direct action on the SC, penetrating into the lipoidal bilayers of the membrane, hence increasing fluidity of the membranes and promoting permeation of bimolecules (Sato et al., 1988). Aliphatic esters were postulated as acting mainly on SC lipids, increasing diffusivity in the SC or partition coefficient between the SC and vehicle of both the drug and solvent (Chattaraj and Walker, 1995).

Azone

Azone is a novel penetration enhancer, which can be incorporated into a variety of topical preparations at low concentration with significant accelerant effects (Stoughton and McClure, 1983; Chattaraj and Walker, 1995). It was found that azone decreased the transition temperature within the lipid bilayer, which induced the formation of a liquid phase, and a resultant increase in membrane fluidity (Beastall et al., 1988). Another interesting finding is its effect on sunscreen penetration and retention. With a 3% azone formulation, penetration of para-aminobenzoic acid (PABA) into the skin was four times

greater than PABA alone, with a maximum reservoir effect seen after 4 hr (Hadgraft and Williams, 1993). It was suggested that azone and analogues enhance penetration by a mechanism of unbalancing electrostatic potential of the head group region of the lipids (Brain and Walters, 1993; Naik and Guy, 1997). Molecules randomly get attracted to each other due to uneven distribution of electron cloud .

Effect of surfactants

Surfactants are widely used in cosmetic products as emulsifier, suspending, wetting solubilising and stabilising agents. Their effects on skin permeability have been reported since the 1960s, and their potential in transdermal drug delivery as penetration enhancers has been well studied and reviewed (Bettley, 1961; Bettley, 1965; Scheuplein and Ross, 1970; Cappel and Kreuter, 1991; Ashton et al., 1992; Walters et al., 1993; Black, 1993; French et al., 1993; Ruddy, 1995). It has been suggested that the lipid lamellae of SC are the major site of action of nonionic surfactants; the more lipophilic the surfactant, the greater its ability to penetrate into the lipid membrane (French et al., 1993). However anionic surfactants are believed to alter the skin permeability mainly by reacting with the protein and extracting lipids of the SC (Black, 1993). As a general rule, cationic surfactants elicit greater irritation and skin permeation than anionic surfactants, and less permeation was found for nonionic surfactants (Ruddy, 1995).

1.2.3.3.2 Penetration retardation

Retarders are defined as agents, which reduce the percutaneous absorption of compounds through augmentation of skin barrier function (Schaefer and Redelmeier, 1996).

Retarders should penetrate the SC or follicles to reduce either the partitioning or diffusivity of molecules through the various penetration pathways. Although augmentation of barrier function has been discussed for a number of years, there are few published studies which have demonstrated this concept. The influence of azone analogues on the physical properties of model phospholipid systems have been studied (Hadgraft, 1993). It was found that one analogue, named N0915, increased the phase transition temperature of dipalmitoyl phosphatidyl choline mixtures and lowered the area of the lipid head group in monolayer studies. Lower penetration of metronidazole from an ethanol vehicle was demonstrated when N0915 was incorporated (Hadgraft, 1993). The mechanism of action for retarder N0915 was suggested to be via attraction of both adjacent ceramide molecules due to neutralisation of electro-sites of the ceramide, thereby stabilising the lipid barrier (Brain and Walters, 1993). Similar retardation effects have been reported for two other synthetic penetration modifiers (Brain et al., 1995a). The strategy of retardation could be utilised for reducing undesirable percutaneous absorption.

1.3 UV radiation and skin damage

1.3.1 Activity of UV spectrum

The electromagnetic spectrum emitted by the sun ranges from very short cosmic rays to very long radio waves and beyond. The ozone layer in the stratosphere is an essential ecological factor reducing the atmospheric penetration of harmful radiation emitted from the sun. UV rays, which comprise the shortest of the nonionising rays, are responsible for

the most of the photocutaneous changes that occur (Epstein, 1990). UV rays have been divided into three categories:

a) UVC radiation from 200 to 290nm, is quite photoactive but is absorbed by the ozone layer in the stratosphere (assuming it is present).

b) UVB radiation ranges from 290-320nm, is energy rich and can produce intense physicopathological photodamage. It is mostly absorbed just above the dermis, inhibits DNA, RNA and protein synthesis and causes a delayed erythemal response. Chronic UVB exposure damages dermal connective tissue and is the primary carcinogenic stimulus for nonmelanoma skin cancers, and photaging (Epstein, 1990; Farmer and Naylor, 1996).

c) UVA radiation from 320 to 400nm, is of lower energy and penetrates deeply into the dermis and beyond. It causes an erythemal response which reaches a peak at 6 hr (Epstein, 1990). It has been reported that UVA radiation mediates mutagenic, genotoxic, and carcinogenic effects through cellular photosensitisers in an indirect manner and that its photobiological effects are cumulative (Lavker et al., 1995; Runger et al., 1995). Furthermore, UVA radiation is thought to act synergistically with UVB radiation in the formation of skin cancers (Anderson et al., 1997).

d) Visible (VIS) and infrared (IR) radiation ranges beyond 400nm and penetrates the skin deeply. As they are now low in energy, they are not damaging to the skin and thus are less important for sun protection. The solar spectrum and associated tissue penetration levels are summarised in Figure 1.4.

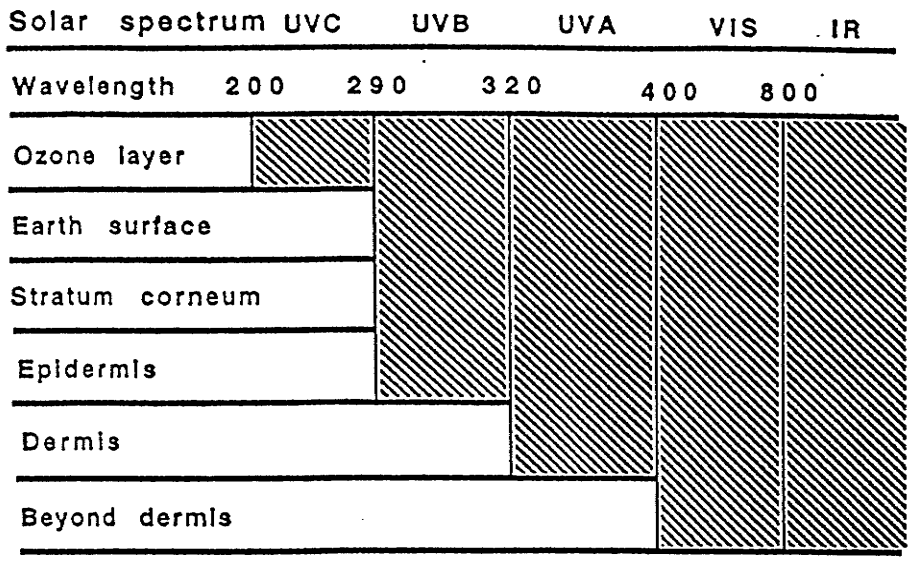


Figure 1.4. Schematic diagram of the solar spectrum and its penetration level.

1.3.2 Lipid peroxidation and skin aging

Numerous skin pathologies have been linked to UV radiation (Rundel and Nachtwey, 1978; Sober, 1987). In response to UV light, lipid peroxidation occurs resulting in elevated free radical levels and altered antioxidant defence in the skin (Pathak and Stratton, 1968; Frei et al., 1990; Nishi et al., 1991; Van Henegouwen et al., 1995). The peroxides isolated from UV-irradiated skin have also been shown to be potent carcinogens (Black and Douglas, 1973). Researchers showed an increase in lipid peroxide levels in mice that peaked 18 hr after acute UVB irradiation, and accompanied a sharp decrease in activities of superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) (Lizawa et al., 1994). Chronic irradiation caused the lipid peroxide level to fall to a minimum at 0.5-1.0 weeks and was accompanied by peaks in SOD and GSH-PX activity after 0.5 weeks. In contrast, catalase activity did not change significantly. This finding suggests that lipid peroxidation is caused by UVB-induced reactive oxygen species (ROS). The parallel decrease in enzyme activities may be due to inactivation by

finding suggests that lipid peroxidation is caused by UVB-induced reactive oxygen species (ROS). The parallel decrease in enzyme activities may be due to inactivation by ROS after acute UVB irradiation. In the chronic case, the decrease in the lipid peroxide level resulted from the scavenging effect of increasing SOD and GSH-Px activities induced by ROS. This occurrence implies that excess ROS accompanied an activation of the enzymes and lipid peroxidation, and may further alter cell function. However, less ROS activated the enzymes to defend against lipid peroxidation.

The effect of ROS induced by xanthine and the xanthine oxidase system on skin aging was investigated (Tanaka et al., 1993). It was found that ROS decreased collagen production and increased glucosaminoglycans (GAGs) synthesis effects, which were consistent with the biological alterations of connective tissue matrix components observed in photoaging skin. These results suggest that ROS may be one of the factors that cause the biological changes of skin. Catalase and alpha-tocopherol have been found to completely prevent the ROS-induced alteration; however SOD had no effect on the ROS induced changes. At a dose of UV radiation below the minimal erythemal dose, the concentration of oxygen radicals generated is sufficient to cause 54% (UVA) and (26% with UVB) deactivation of thioredoxin reductase (Sundaram et al., 1990). What role the antioxidant enzymes have on the lipid peroxidation of the skin is not totally clear. The contradictory findings for the role of SOD and catalase may be due to variability in experimental conditions.

1.3.3 Natural defences against UV radiation

Human skin has a number of natural defences against UV radiation (Pathak, 1990):

a) The process of keratinisation which leads to formation of a compact horny layer containing UV absorbing protein (e.g. amino acid of keratins). This layer not only prevents transmission of radiation to the viable cells of the epidermis but also attenuates the radiation by scattering.

b) Formation of melanin pigmentation during UV radiation. It acts as an UV absorber and scatterer as well as a ROS scavenger, hence providing a shield to protect the nuclear DNA of keratinocytes and dermal proteins.

c) The preferential accumulation of carotenoid in subcutaneous tissue to quench singlet oxygen.

d) UV radiation-absorbing urocanic acid which undergoes trans-cis isomerisation.

e) ROS scavenging – enzymes such as peroxidase-reductase enzymes.

f) Error-free DNA repair.

1.4 *In vitro* percutaneous absorption: fundamentals

1.4.1 In vitro model

In vitro assessment of percutaneous absorption is performed using diffusion cells (Figure 1.5). They include donor and receptor (either flow-through or static) compartments that are separated by a barrier membrane (Franz, 1975). The cells usually stand on a magnetic plate, which is immersed in a water bath to maintain a constant temperature. Compounds penetrating the membrane can be assessed by analyzing the amount in the receptor fluid. The membrane may be human or animal skin, or a suitable synthetic filter. This *in vitro* model represents a convenient means for evaluating skin permeation characteristics and

formulations can be screened *in vitro* in a short period of time at relatively low cost. Data obtained from *in vitro* studies are usually more complete and more precise than those from *in vivo* studies. The type of process measured in *in vitro* studies is passive diffusion of the original compound rather than assessment of metabolites in the excreta (Bronaugh and Collier, 1991; Schaefer and Redelmeier, 1996). In addition, skin distribution can be determined by assessment of the remaining penetrant in each skin layer after the penetration study. In many cases, *in vitro* data can be used to elucidate absorption mechanisms and they are often used for determining vehicle effect on the permeation of a permeant.

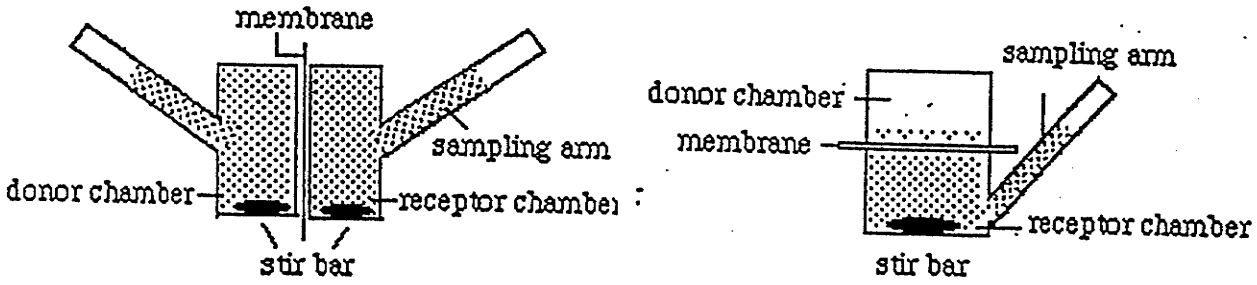


Figure 1.5. Schematic diagram of diffusion cells Left: Horizontal cells and; right: Vertical cells.

1.4.2 Finite and infinite dose techniques

A finite dose technique, e.g., application of a finite dose of penetrant to the donor, is usually employed when a clinical perspective is required. A dose of practical use in human administration is applied on excised human skin and the donor side of the

human administration is applied on excised human skin and the donor side of the diffusion device is exposed to ambient conditions during the study period. In many cases, donor concentration of the tested compound reduces as permeation proceeds, while composition of the applied preparation changes due to evaporation. This design can be used for assessing penetration of compounds under human in use conditions, but is difficult to interpret with regard to penetration parameters.

To evaluate formulation differences, an infinite dose technique is often employed. An excess dose is applied and the donor compartment is occluded (e.g., sealed using a glass cover slip). The drug concentration and the formulation composition in the donor compartment remain constant during the experiment. This design permits interpretation of the apparent permeability, partition and diffusion coefficients and thus comparison of different vehicles in terms of these parameters. Penetration mechanisms can be studied using this technique.

1.4.3 Barrier membranes

1.4.3.1 Synthetic membrane

Many studies use model membranes to evaluate the effect of changing formulation variables on the observed overall drug availability (Flynn and Roseman, 1971; Roberts and Anderson, 1975; Barry and Brace, 1977). The membrane physically separates the donor formulation from the receptor phase, and the release rate is determined by the formulation. Solute transport through a membrane takes place either by partitioning into and diffusion through a distinct membrane phase or by diffusion through a continuous

donor-membrane-receptor phase via channels. Cellulose acetate filtration and all dialysis membranes are examples of such porous barriers that provide only a physical barrier to free movement (Houk and Guy, 1988). Dimethylpolysiloxane (silicone rubber) is the most commonly used non-polar membrane. It is highly permeable and characterised as a non-porous, homogenous hydrophobic barrier (Houk and Guy, 1988). Polyethylene membrane was first introduced to investigate vehicle effects on permeation of phenol (Roberts and Anderson, 1975). *In vitro* penetration studies, using a model membrane system can provide useful information regarding vehicle effects, and can be particularly useful in the investigation of mechanism and the validation of theoretical concepts (Zatz, 1991b). Although membrane model systems have been employed for many years and will be continually used in the future, the concept of possible plasticisation of the membrane by tested chemicals must be considered as this may be misleading in the interpretation of the true mechanisms of penetration (Kowaluk et al., 1984).

1.4.4 Receptor fluids

Receptor phase receives the permeants and provides sink conditions. In skin penetration studies, phosphate buffered saline (PBS) at pH 7.4 is often employed as the receptor fluid to mimic human biological fluid. Where lipophilic compounds are being studied, a receptor fluid modifier is required to provide adequate sink conditions because of limited solubility of the compounds in the buffered saline. Modifiers such as bovine serum albumin (BSA), aqueous ethanol and nonionic surfactant are common choices as receptor fluids to enhance solubility of lipophilic compounds in diffusion cell receptor media (Bronaugh and Stewart, 1984; Scott and Ramsey, 1987; Macpherson et al., 1991; Ramsey

et al., 1994). It has been found that inclusion of 4% BSA in a physiological buffer is more similar to *in vivo* receptor fluid (blood) and is adequate for many studies (Collier and Bronaugh, 1991). Nonionic surfactants (e.g., polyethylene glycol 20 oleyl ether, Volpo-N20) are commonly used receptor fluid modifiers due to their relatively mild effect on skin with lower irritation potential than ionic agents (Bronaugh and Stewart, 1984). However, when organic solvents such as aqueous ethanol and surfactant solutions are used as receptor fluid, caution must be taken as the lipophilic properties of the solvents may increase the risk of extracting SC lipid and modifying skin barrier properties.

1.5 Sunscreen agents

The choice of sunscreen agents or combination of agents is crucial for providing the formulation with a suitable SPF value, broad UV spectrum absorption and waterproof capacity. The level of sun protection of sunscreen formulations, the SPF value has been devised by the U.S Food and Drug administration (FDA). The SPF is defined as a ratio of the UV dose required to develop erythema through the applied sunscreen, to the dose required to develop the same erythema without the sunscreen (Levine and Griego, 1993). Therefore a sunscreen product with SPF 15 would provide sun protection for fifteen times longer duration than exposure without the sunscreen product. As a general rule, sunscreens with an SPF of 15 or greater are considered high potency; those with SPF between 6 to 12 are considered to possess medium potency; and sunscreens with SPF below 6 provide little protection against UV radiation (Levine and Griego, 1993). With regard to sunscreens themselves, desired SPF value and broad UV spectrum protection

can be obtained by using a combination of sunscreen actives with different mechanisms of solar attenuation and blockage of a range of UV wavelengths.

Obtaining a high SPF value is not simply a matter of increasing the dosage of the sunscreen agents. Instead it can be optimised by a proper combination of the sunscreen compounds based on knowledge of their synergistic activity. Improper combination of sunscreen agents will influence the appearance and effectiveness of the resultant products (Klein, 1989). Ideally, an efficient sunscreen combination will minimise the concentration of sunscreen agents required. This approach is particularly important because it will reduce possible irritation and cost (Klein, 1990a). It is also important when using an agent such as Benzophenone (Bz-3), which has been shown to penetrate into the systemic circulation following topical application. Minimal concentration in the topical sunscreen formulation will minimise systemic absorption and the possible consequences.

Other factors that must be considered in the choice and formulation of sunscreen agents, are their solubility or miscibility in the formulation vehicle, and their substantivity (e.g., overall ability to resist removal by water). These factors are dependent on the nature of sunscreen and vehicle. More than 20 chemical sunscreens have been approved in the USA and European community; the most commonly used chemical suncreens are benzophenone-3 (Bz-3), octocrylene (OC), octyl methoxycinnamate (OM), butyl methoxydibenzoylmethane (BM), and octylsalycilate (OS). OctyldimethylPABA (OP) was a popular UVB absorber until recently, but its usage has declined sharply in recent

years due to questions about its safety. The following properties of sunscreen agents are discussed in the sections below, including their absorption range, solar attenuation mechanisms, structure-activity, relative advantages and disadvantages and formulation aspects.

1.5.1 Physical sunscreens

1.5.1.1 Classification and mechanisms of UV protection

Physical sunscreens have been described as agents, which scatter radiation, thereby possessing a broad UV/VIS (visible) spectrum of activity (Roelandts et al., 1983). However, it has recently been reported that many of the physical blockers provide their protective effect not only by attenuating solar radiation through scattering, but also by absorbing VIS and UV radiation (Sayre, 1990; Patel et al., 1992). Therefore the physical sunscreens can be classified into at least two different groups: one group acting purely by scattering radiation (e.g., barium sulfate, talc), and the other group of agents also absorbing selected wavelengths (eg.metallic oxides). Within the later group, some compounds such as titanium dioxide (TiO_2) and zinc oxide (ZnO) exhibit a semiconductor optical absorption gap. These compounds strongly absorb and dissipate UV radiant energy by mechanisms of excitation of electrons from the valance band to the conduction band at wavelengths shorter than the optical gap (about 400nm) and scattering radiation at wavelengths longer than this gap (Sayre, 1990; Anderson et al., 1997). Iron oxides exhibit electronic absorption bands over the UV/VIS region, thereby resulting in potential protection from a broad spectrum of solar radiation.

TiO_2 and ZnO are the most common physical sunscreens in commercial formulations. TiO_2 has gained popularity as a physical blocker since 1987, while ZnO has a long history of usage in makeup and baby products (Klein, 1992). Two grades of raw materials of TiO_2 and ZnO are available for cosmetic use, pigmentary and UV attenuating (transparent) grades. The pigmentary TiO_2 (150-300nm) and ZnO (200-400nm) scatter visible light, hence appearing white and opaque (Anderson et al., 1997). The attenuating grade TiO_2 (microfine particles, 20 -150nm) and ZnO (40-100nm) are specially designed to attenuate UV radiation by absorbing it. They are complimentary in terms of their sun protective effect. Microfine TiO_2 shows broad-spectrum protection across the UVA and UVB regions, but its primary function is for UVB protection (λ-max 300nm). High SPF sunscreen products (e.g., SPF>20) can be formed using TiO_2; ZnO provides much less protection than TiO_2 in the region of UVB radiation. However, it has a maximum absorption at 360nm and this is intended primarily for UVA protection. Combination of these two sunscreens effectively increases their protection efficacy with respect to broad UV spectrum coverage and SPF (Table 1.1) (Anderson et al., 1997).

Table 1.1 Efficacy of combined TiO_2 and ZnO

TiO_2	**ZnO**	**SPF**	**UVA/UVB ratio**
7.5	0	20	0.59-0.60
0	6	6	0.90
2.5	2.5	12	0.63
2.5	7.5	20	0.81

* Cited from Anderson et al., 1997

1.5.1.2 Physical sunscreen formulation

In general, formulation of physical sunscreens is not aesthetically acceptable because of its opaque appearance, greasy feeling and discolouring property. However, they are nonirritant, nonsensitising and effectively block solar radiation. They are therefore very good for children and for persons who are allergic to chemical sunscreens. They are also useful for application to areas that burn easily and therefore require very high protection such as the nose and lips (Pathak, 1987; Pathak, 1997). Correct formulation of physical sunscreen agents is essential to ensure the efficacy of the resultant product. In the case of sunscreen agents that attenuate UV radiation by purely scattering, the difference between the refractive index of the agents and their surrounding medium (vehicle) should be maximised to maintain their optical protection (Macleod and Frain-Bell, 1975; Sayre, 1990). As a result, the sunscreen formulation is viable on the skin surface after use, thus affecting the cosmetic elegance and acceptance of the product. To achieve effective scattering, the size of the particles can be reduced, thereby increasing the concentration of the particles on the skin surface. The optimal particle size for scattering solar radiation should be around 0.2microns (Macleod and Frain-bell, 1975). With further decrease in particle size e.g., <0.03 micron in diameter, scattering will take place, diminishing the protective potency of the sunscreen formulation by shifting the scattering range to the short UV region (Sayre, 1990). However, in the case of sunscreen agents that provide their protection by mechanisms of absorbing and scattering UV radiation, the formulation is more flexible and effective. With regard to consumer acceptability, a transparent product can be achieved by reducing particle size to minimise opacity (e.g., 20nm particle size of TiO_2 is recommended, or by matching the refractive index of the sunscreen agents with the vehicle) (Anon, 1993). Although the effects of scattering and reflection are

reduced in a transparent product, absorption of UV radiation is still sufficient to provide an effective product.

Microfine physical sunscreens with UV absorption and scattering capabilities are widely employed in suncare products, and new techniques have been developed to aid their formulation. As fine-particle materials have a tendency to form agglomerates, manufacturers have developed several technologies to minimise this effect, such as surface treatment (coating) and dispersion technologies (Anderson et al., 1997). This also aids the dispersion of TiO_2 in both organic and aqueous systems (Anderson et al., 1997). By surface treatment, the microfine physical sunscreens are made compatible and stable in formulation. Surface treated TiO_2 is easily incorporated into sunscreen formulations and is compatible with the suspending agents used to keep it from precipitating out, particularly at elevated temperatures (Klein, 1990b). Adequate dispersion usually requires suitable dispersing agents to ensure that TiO_2 is wetted by the agent and prevented from flocculating. For instance, to form an oil product, either a lipophilic coating on TiO_2 or a suitable oil to wet TiO_2 ratio is necessary in order to aid adequate dispersion. Several formulation types are commonly used for sunscreens, such as emulsions, oils, gels and sticks. Of these, emulsions are the most common type of sunscreen formulation.

There are two types of emulsions, which can be chosen for physical sunscreens: oil in water (o/w), or water in oil (w/o). A surface treated physical sunscreen such as TiO_2 can be incorporated into either the internal or external phase. It has been found that incorporation of these agents into the external phase tends to be easier to formulate and

provides higher SPF values (Anderson et al., 1997). TiO2 and ZnO are available, as raw materials in both pre-dispersion and powder forms. Predispersions are easier to use because they are liquid ingredients that can be readily mixed with the appropriate phase of the emulsion. Any aggregates should have already been broken down so that the dispersion contains active material at the optimum particle size (Anderson et al., 1997). However, since the proportion of oil phase used in emulsions tends to be relatively small (usually 10-15% for lotion and 30-40% for cream), an oil based predispersion may be problematic as it occupies too large a volume of the oil phase of the emulsion to allow flexibility to incorporate other oil ingredients. The principal advantage of powders is their flexibility for incorporating them into various formulations. However, as powder is being used, it is usually necessary to add a suitable dispersant to prevent formation of aggregates and high-energy processes may be required to disperse or prevent formulation of the aggregates during preparation (Anderson et at., 1997). All inorganic solids bear positive and negative charges at their surface. The net charge depends on the pH of the environment and surface coating. Each material has a particular pH in solvent at which there is no net charge, termed the point of zero charge. At this pH there is no electrostatic repulsion between particles so increasing the tendency for agglomeration (Anderson et al., 1997). Therefore to formulate a stable product, the pH at the point of zero charge should be avoided.

Combination of physical and chemical sunscreens can increase sun protection efficacy and decrease usage of the chemical sunscreens, thus reducing the potential for irritancy and skin penetration of the chemicals. Tabel-1.2 illustrates synergistic effects of the

sunscreen combination. One possible explanation of these synergistic effects is that scattering of light by the physical sunscreen increases the optical path length through the film and increases absorption of light by the chemical sunscreen (Anderson et al., 1997).

Table 1.2 Sun protection efficacy of combination of physical and chemical sunscreens

Physical Sunscreen	**Chemical sunscreen**	**In Vitro SPF**
4% TiO_2	-	6.8
-	3% Octyltriazone	1.5
4% TiO_2	3% Octyltriazone	14.8
-	3% Octyldimethyl PABA	4.7
4% TiO_2	3% Octyldimethyl PABA	16.2
4% TiO2	-	7.2
-	3% Benzophenone-4	5.4
4% TiO_2	3% Benzophenone-4	20.1
4% TiO_2	-	5.2
-	3% Phenylbenzimidazole sulfonicacid	8.1
4% TiO_2	3% Phenylbenzimidazole sulfonicacid	21.7

1.5.1.3 Summary

Because the efficacy of physical sunscreens, which exert their effect solely by scattering UV radiation, can be modified by formulation, sunscreen products which rely on this type of sunscreen agent must be opaque, thereby limiting their cosmetic acceptance. However, where a sunscreen agent is used that acts by scattering and absorption of UV radiation, the absorbing properties of the sunscreens are consistent regardless of the vehicle used; therefore an aesthetically acceptable product with effective UV protection can be formulated. For this reason, surface treated microfine physical sunscreens tend to be the most common physical sunscreen agents utilised. In practice, sunscreen products normally contain a combination of physical and chemical sunscreen, thereby achieving high SPF in elegant formulations.

1.5.2 Chemical sunscreens

The physicochemical properties of six common sunscreen agents namely Bz-3, BM, OC, OP, OM and OS are discussed below. Each sunscreen is named by its most common name (CTFA name), followed by its chemical and alternate names in brackets.

1.5.2.1 UVA sunscreens

UVA sunscreen agents absorb UV radiation in the range of 320-400nm. Unfortunately, only BM meets this requirement. Other UVA sunscreen agents such as benzophenones and anthranilates are effective only at shorter UVA wavelengths; their absorption reduces sharply at wavelengths over 330nm (Buescher, 1993). Bz-3 and BM are the most frequently used UVA absorbers, and are generally present in combination with UVB filters to provide broad UV spectrum protection.

1.5.2.1.1 Benzophenone-3 (oxybenzone)

Bz-3, which has been defined as a UVA absorber by the FDA since 1978 has an absorption spectrum of 270-350nm with a λmax around 290nm (O' Donoghue, 1991; Klein, 1992). In sunscreen products, Bz-3 is usually combined with a strong UVB absorber to offer a broad-spectrum coverage and a high SPF.

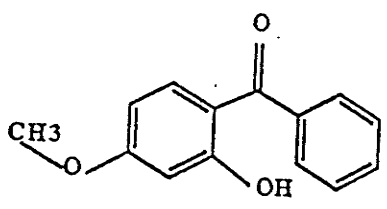


Figure 1.6 Chemical structure of Bz-3

Due to hydroxylation at the 2-position, benzophenones are photochemically stable (Levine and Griego, 1993). Bz-3 is a yellow/beige powder, which is difficult to solubilise and has a tendency to crystallise out of formulations, thereby lowering the SPF and affecting the aesthetics of resultant products (Klein, 1989; Shaath, 1997). To eliminate this undesirable effect, OS or OM are often incorporated into the formulation to solubilise Bz-3 (Klein, 1989; Patel et al., 1992). Methyl anthranilate (a weak UVA absorber) can also be added into the formulation to reduce the amount of Bz-3 required while maintaining a high SPF (Klein, 1990a). The major disadvantage of Bz-3 is its tendency to cause irritation and photoallergic reactions (Klein, 1989; Szczurko et al., 1994). The metabolism and toxicity of Bz-3 have been investigated in laboratory animals by several investigators, but there is limited data regarding metabolism and excretion in humans (Hayden et al., 1997b). After oral administration of Bz-3, three metabolites namely 2,4-dihydroxybenzophenone (DHB), 2,2'-dihydroxy-4-methoxybenzophenone (DHMB) and 2,3,4-trihydroxy-benzophenone (THB) have been identified in rats (Okereke et al., 1993). These metabolites were detectable and were eliminated primarily via urine together with the parent compound. O-dealkylation is the major pathway of Bz-3 metabolism in rats (Okereke et al., 1993).

It has been reported that no toxicity to rats was found when Bz-3 was applied dermally from a topical formulation at a dose of 100-mg/kg-body weight although an increase in GSH-reductase activity was shown after 60 minutes (Okereke et al., 1995). A study in human volunteers showed that significant amounts of Bz-3 and its metabolites (glucuronides of DHB and THB) were present in urine, following topical application

(Hayden et al., 1997a). Bz-3 is present in many topical sunscreen formulations. As they are applied frequently to large areas of the body surface, the authors suggested that further investigation of the skin penetration and safety of Bz-3 is required.

1.5.2.1.2 Butyl methoxydibenzoylmethane (Parsol 1789, avobenzone).

Prior to 1988, the range of UVA absorbers was limited to the benzophenones and anthranilates. In 1988, the FDA approved BM for safe and effective use as an UVA absorber in sunscreen formulations (Buescher, 1993). This compound is described as a true UVA absorber with an ideal broad UVA absorption band from 320-390nm (Gange et al., 1986; Johnson and Fusaro, 1987). Keto/enol tautomerism of the compound extends the UVA λmax to 358nm and contributes to the high molar extinction coefficient of >30,000 (Shaath, 1997). Therefore, it is the most effective UVA sunscreen currently available (Luftman et al., 1991).

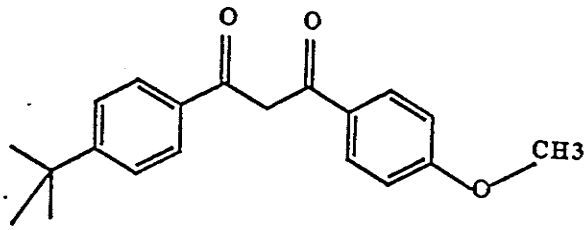


Figure 1.7 Chemical structure of BM

However, the keto/enol tautomerism of the compound also causes a loss of its activity up to 36% (Shaath, 1997). In addition, BM is subject to photoinstability. High sunscreening efficiency can only be guaranteed if a UV filter has high photostability, while the

possible formation of photoproducts, their chemical reactions and accumulation on/in human skin may have a deleterious effect. A recent report showed a large number of photoproducts that are produced when BM is exposed to UVA light in various solvents, in particular nonpolar solvents such as those used in topical vehicles (Schwack and Rudolph, 1995). The skin penetration and potential toxic consequences of these photoproducts has not been investigated. When BM is formulated with PABA and PABA esters, a bright yellow colour develops due to formation of a charge transfer complex (Klein, 1989; Givaudan-Roure, 1993). In addition, BM causes discolouration in the presence of metallic ions (Givaudan-Roure, 1993). Therefore a suitable antioxidant and sequestering agent should be used to inactivate free metal ions that can catalyze oxidation and /or/ formation of complexes with BM (Givaudan-Roure, 1993). BM has very low water solubility. In most cosmetic preparations, the incorporation of BM is accomplished by solubilising it in the heated oil phase of the preparation prior to emulsification to avoid the possible formation of crystals in the finished product (Givaudan-Roure, 1993). In practice, OM is often combined to solubilise BM, achieve a broad UV spectrum protection, and increase the SPF value of the formulation.

1.5.2.2 UVB sunscreens

UVB sunscreen agents absorb UV radiation in the range of 290-320nm and thereby give significant erythemal protection. Sunscreen agents within this group include OC, OP, OM and OS.

1.5.2.2.1 Octocrylene (2-ethylhexyl-α cyano-β-phenylcinnamate)

OC is a cyano-substituted cinnamate, which is an effective oil soluble UVB absorber offering additional absorption in the shorter –UVA range (Shaath, 1997). It has a λmax of 303nm which clearly places it in the UVB range (Klein, 1989).

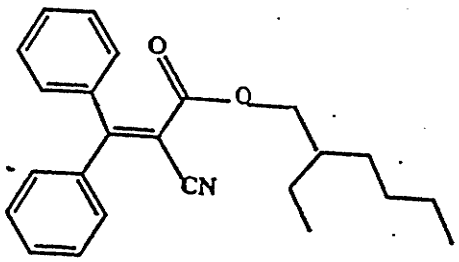


Figure 1.8 Chemical structure of OC

The eight-carbon (2-ethylhexyl) substitution diminishes its water solubility making it suitable for most waterproof sunscreen formulations (Shaath, 1997). Although OC is a cinnamate derivative, it exhibits remarkable photostability and is relatively unaffected by solvent effects. The photostability could be in part attributed to the lack of geometrical isomers in this trisubstituted double bond system (Shaath, 1997). OC is often combined with OM to achieve a high SPF value. It can also be combined with methyl anthranilate (λmax 330nm) to obtain broad-spectrum protection. When combined with OP, a yellow coloured transfer complex is formed making this combination unsuitable (Klein, 1989).

1.5.2.2.2 Octyl dimethyl PABA (Escalol 507, Padimate O, Eusolex 6007)

The parent structure of the compound is PABA which has been marketed since the 1920s and was a popular sunscreen agent in the 1950s-1960s (Natow, 1986; Shaath, 1997)

PABA has a UV λmax of 296nm and molar extinction coefficient of 13600.

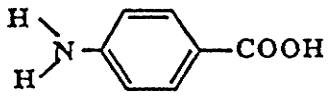


Figure 1.9 Chemical structure of PABA

However, there are a number of problems associated with PABA. Firstly, the free NH_2 oxidizes rapidly in air to produce off-colours (Shaath, 1997). Secondly, hydrogen bonding between molecules forms crystals, which readily deposit in many solvents. Improper dissolvation of PABA may yield gritty and crystalline finished formulations that are both unappealing and prevent the formation of continuous films on the skin (Shaath, 1997). Excessive hydrogen bonding of the agent with solvents to 266nm in polar solvents (Shaath, 1997). Thirdly, $-NH_2$ and –COOH are sensitive to pH changes (Shaath, 1997; Watkinson et al., 1992). These drawbacks associated with its chemical structure, in addition to its tendency towards allergic sensitivity have led to a decline in its worldwide use in recent years (Thune, 1984; English et al., 1987). New derivatives of PABA with protection of the amino and carboxylic acid groups have been developed to improve their stability, pH sensitivity, UVB screening capability, waterproofing property and cosmetic compatability. OP is a PABA derivative with increased molar extinction coefficient up to 273300 and UV λmax of 311nm.

Although the incidence of adverse effects is less frequent than with PABA itself, reports of facial stinging and sensitisation due to PABA have occurred (Klein, 1989; Bruze et al ., 1990). Another safety concern is formation of nitrosamines from the compound. Degradation of OP is the most likely source of these chemicals, although octyl monomethyl PABA, which is a possible impurity existing in OP raw material, may also contribute to the formation of nitrosamines (Klein, 1989; Pathak and Robins, 1989). No evidence has yet proved that the nitrosamines formed from OP are carcinogens (Dunkel et al., 1992). Potential carcinogenicity of nitrosamines in laboratory animals has raised concerns about their possible health risk to humans (Klein, 1989; Meyer and Powell, 1991; Buescher, 1993).

1.5.2.2.3 Octyl methoxycinnamate (Parsol MCX)

OM is a highly effective UVB absorber and currently the most popular sunscreen used for protection from UVB radiation (Patel et al., 1992; Watkinson et al., 1992). Its UV λmax is 305-310nm with a molar extinction coefficient > 23,000 (Shaath, 1997).

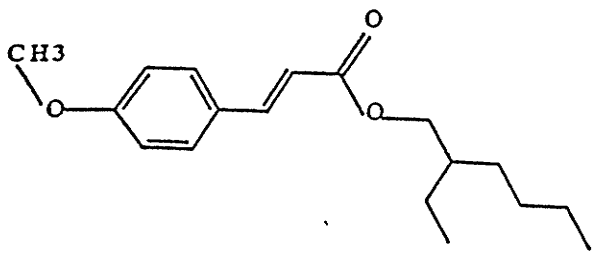


Figure 1.10 Chemical structure of OM

OM is usually present in formulation at a concentration between 2-7.5% and in combination with a UVA absorber (eg. Bz-3 or BM) to provide a broad spectrum of activity (Givaudan-Roure, 1992). OM has a number of favorable properties with respect to sunscreen formulation. It is highly compatible with cosmetic ingredients, has effective waterproofing properties, and has a good safety record (Givaudan-Roure, 1992; Haarmann and Reimer, 1993). In addition, its beneficial effect on urocanic acid (UCA) and its photoisomerization has been recognized recently. UCA is a normal constituent in the skin. Cis-UCA is formed in the epidermis by UV irradiation of trans-UCA. Cis-UCA has been shown to have immunosuppressive properties (Norval et al., 1995; Hurks et al., 1997). The trans-UCA may protect viable cells of the epidermis against actinic damage and oxidation reaction due to its UV-absorbing properties (Black, 1990; De Fine Olivarius et al., 1996). Since photoisomerisation occurs in the skin, questions have been raised regarding the immunosuppression and further photocaricinogenisis (Anderson, 1995). OM may give greater protection from UCA and its photoisomerisation than PABA esters (Levine and Griego, 1993).

Cinnamates are subject to cis-trans isomerism, resulting in loss of activity (irreversible trans formation); however, as a cinnamate derivative, OM has reasonable photostability with only a 4.5% loss in its activity (Shaath, 1997). Although the PABA derivatives have more favourable properties with regard to substantivity, questions relating to the safety record of OP have led to OM being the most widely utilised UVB sunscreen agent in products with the claim of PABA-free (Buescher, 1993). The major disadvantage of the

compound is that its λmax can be easily shifted by solvent, resulting in decreased sun protection efficacy in unsuitable formulations.

1.5.2.2.4 Octyl salicylate

Salicylates were the first UVB filters used in sunscreen preparation (Watkinson et al., 1992). Although they are relatively weak UVB absorbers, their excellent solubilising property and stability have made them continuously used in sunscreen products for more than 60years (Anderson et al., 1997). The parent structure of these compounds is shown below:

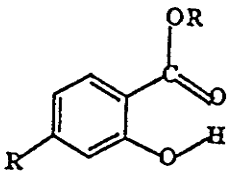


Figure 1.11 Chemical structure of salicylates

There is internal hydrogen bonding within the molecule itself, allowing for a UV absorbance around 300nm (shift to longer wavelength) and chemical stability (Shaath, 1997). Deviation from planarity of the molecule causes a lowering of the extinction coefficient, since the symmetry will dictate whether or not an electronic transition is possible (Shaath, 1997; Watkinson et al., 1992).

OS is the most suitable sunscreen in the salicylate group due to its increased lipophilicity. There is very little literature data available regarding the percutaneous penetration of the

higher salicylate esters. After mathematical modeling, it was pointed out that all the salycilates and related compounds were assumed to have a very large SC solubility, thus implying less dermal absorption (Watkinson et al., 1992). The λmax is 307nm and molar extinction coefficient around 5000. Since it is a relatively weak UV absorber, it is generally used in sunscreen products with a low SPF below 5 (Luftman et al., 1991). Nowadays OS is generally used in combination with other sunscreens, such as Bz-3 and OP, to increase SPF value but more importantly as a formulation adjunct to solubilise insoluble ingredients in the formulation vehicle (Luftman et al., 1991; Klein, 1990b; Shaath, 1997). The advantages of this compound are its emollient property, ability to solubilise for other insoluble sunscreens, and waterproofing property. As a result, OS is incorporated into numerous formulations though generally not primarily for its UV absorption capability (Shaath, 1997; Watkinson et al., 1992).

1.5.3 Summary

In summary, sunscreen agents provide sun protection by different mechanisms of solar attenuation and over varying ranges of the UV spectrum. Therefore a suitable combination of sunscreen agents with different attenuation mechanisms and UV spectrum coverage is important to ensure effective sun protection.

1.6 Skin penetration of sunscreen agents: Formulation considerations

To protect from UV radiation, great efforts have been made towards the development of safe and effective sunscreen preparations. Sunscreen agents have become one of the most popular ingredients in cosmetic formulations and are widely incorporated into skin and hair care creams as well as make ups (e.g., moisturisers, foundation and lipsticks). A

better understanding of the percutaneous absorption of sunscreens and how their formulation can influence release and skin penetration is needed.

1.6.1 Percutaneous absorption and passive Fickian diffusion

Transdermal penetration of a chemical involves partitioning between and diffusion through the SC, the viable epidermis, and the upper dermis. The total resistance to penetration is the sum of the individual resistances imposed by each layer. Experiments using excised human skin have shown that the overall resistance is characteristic of passive Fickian diffusion and that diffusion through the SC is the rate limiting barrier for a large number of chemicals (Scheuplein 1967; Scheuplein and Blank, 1973; Barry, 1983; Flynn, 1985).

Fick's First Law has been shown to be applicable to the penetration of a drug through the SC of the skin. It describes the diffusion process under conditions of steady state:

$$J = -D\, dc/dx \quad \text{.....(1.1)}$$

Where J is the flux ($\mu g cm^{-2} h^{-1}$), D is the diffusion coefficient (or diffusivity)($cm^2 h^{-1}$) of the drug in the medium, and -dc/dx is the gradient of concentration across the medium ($\mu g\ cm^{-1}$). In the case of skin penetration the medium is diffusional path length h and ΔC_v is the difference in drug concentration between the vehicle and skin. Assuming dc/dx represents a linear concentration gradient, then

$$dc/dx = \Delta C_v/h \quad \text{.....(1.2)}$$

$$\Delta C_v = C_2 - C_3 \quad \text{.....(1.3)}$$

where C_2 is the concentration of drug inside the membrane adjacent to the outside surface and C_3 is the concentration of drug inside the membrane adjacent to the inside surface.

Substituting Eq1.3 into Eq1.2, we get

$$dc/dx = (C_2-C_3)/h \quad \ldots\ldots (1.4)$$

By definition, partition coefficient K may be expressed by

$$K = C_2/C_1 \text{ or } C_3/C_4 \quad \ldots\ldots (1.5)$$

Where C_1 is the concentration of a drug on the outside of membrane and C_4 is the receiver drug concentration immediately outside the membrane adjacent to the inside surface.

Since C_2 and C_3 are not measurable, they have to be expressed in the form of measurable quantities. Hence,

$$C_2 = K \times C_1 \text{ or } C_3 = K \times C_4 \quad \ldots\ldots (1.6)$$

Substituting Eq1.4 into Eq1.1, we get

$$J = -D (C_2-C_3)/h \quad \ldots\ldots (1.7)$$

Substituting Eq1.6 into Eq1.7, we get

$$J = -D (K;C_1 - K;C_4)/h \quad \ldots\ldots (1.8)$$

Supposing the membrane permeability coefficient is Kp

$$Kp = DK/h \quad \text{.....(1.9)}$$

Permeability coefficient (Kp) (cm h^{-1}) provides a means of expressing absorption measurements. It can be affected by either altering drug diffusivity and/or partioning into the SC.

Substituting Eq1.9 into Eq1.8, we get

$$J = Kp\ (C_4 - C_1) \quad \text{.....(1.10)}$$

If we measure how much drug has been transported through the biological membrane; i.e., the rate of drug appearance instead of drug disappearance, Eq1.1 becomes:

$$J = D\ dc/dx \quad \text{.....(1.11)}$$

There is a small change in Eq1.11 in comparison to Eq1.1: the sign. The main difference between Eq1.8 and Eq1.11 is that Eq1.8 describes disappearance from the site of drug dosing whereas Eq1.11 describes appearance after the drug is absorbed (e.g., in systemic circulation). As time passes, less drug will be left at the site of dosing (hence the negative sign), whereas, more substance will be absorbed (hence the plus sign).

Since

$$J \propto \Delta C_V$$

A proportionality constant Kp may be added.

$$J = Kp\ \Delta C_v \quad \text{.....(1.12)}$$

Substituting Eq1.9 into Eq1.12, we get

$$J = DK\Delta C_v/h \quad \text{.....(1.13)}$$

Steady-state flux obtained from infinite dose experiments yield permeability parameters critical for understanding the molecular diffusional processes, which are responsible for overall barrier behavior (Houk and Guy, 1988). Studying their mechanisms of action will further aid optimisation of topical formulation.

1.6.2 Vehicle factors that influence skin permeation

1.6.2.1 Solute activity and membrane specific effects

If transport of a solute across the SC is the rate limiting step in percutaneous absorption, the diffusion of the solute transfer out of its vehicle and into the skin will depend upon thermodynamic activity of the solute in the vehicle and permeability of the SC. An increase in the activity of the solute within the vehicle will result in an increase in the permeation rate if it does not involve any interactive effect. Membrane specific effects involve a change in membrane properties that may be caused by solute and/or vehicle; such an effect is therefore, regarded as an interactive effect. Interpretation of a simple

comparison of flux values from different vehicles may be confounded by the simultaneous contribution of both effects.

It is well established that a principal driving force for diffusion across the skin is the thermodynamic activity of the solute in the donor vehicle when the vehicle has no effect on the SC barrier. This activity is reflected by the concentration of the permeant in the donor vehicle as a function of its degree of saturation within that medium. The closer to saturation concentration, the higher is the thermodynamic activity and the greater is the availability of the solute from the vehicle (Barry et al., 1985; Brain et al., 1995b; Lalor et al., 1995). To study the non-interactive vehicle effect, numerous experiments have been conducted and the *in vitro* model with synthetic membrane is often employed.

1.6.2.2 Approaches for quantifying vehicle-membrane interactions.

In order to assess vehicle-membrane interactions, it is necessary to first factor out the non-interactive effect. For instance, control the solute activity at the same level; then determine the residual interactive influence (Zatz, 1991b). A saturated solution usually serves as the reference state in assessing thermodynamic activity within the vehicle (Higuchi, 1960). Since the activity of excess solid is assigned a value of unity, the activity of a solution may be estimated by C_v/S, where C_v is the concentration of a solute within the vehicle and S is the solubility of the solute in the same vehicle. This principle leads to the assumption that saturated solutions containing the same solute should have the same noninteractive contribution to flux, despite differences in the solute concentration. In a case where the interactive contribution is minor, the fluxes will be

essentially the same (Zatz, 1991b). A number of approaches have been employed for seperating the vehicle membrane interactions.

a) Pre-treatment of the skin with solvents

For elucidating vehicle membrane interactions, skin is often pre-treated with solvents for a specified period of time, followed by application of a model solute in a standard preparation, usually a simple solution. It is assumed that the pre-treatment exerts no effect on the subsequently applied solutions; the activity of the solute is therefore constant. Differences in penetration behaviour reflect only changes in the SC structure due to the pretreatment (Zatz, 1991a). This approach is often used for assessing penetration enhancement induced by enhancers or formulation vehicle. For instance, to assess the enhancement effect of novel penetration enhancers namely 1-dodecylhexahydro-2H-azepinp-2-one, N-dodecyl-2-pyrrolidone, N-dodecanoyl-L-proline, and N-dodecyl-2-piperidione, hairless mouse skin was treated with the chemicals one hour prior to diffusion study at a concentration of 100% to avoid any vehicle effects.

A model drug in various solvents was applied following the pretreatment. Separate controls (no enhancer pretreatment) were used for each vehicle. Enhancing effects of the chemicals were demonstrated by enhancement ratio (ratio of permeability coefficient, enhancer treated: control treated) (Fincher et al., 1997). In another study, a polydimethyl siloxane membrane was pretreated with formulation vehicles (e.g., emulsion base without drug, aqueous phase of the emulsion, and oil phase of the emulsion, respectively) prior to diffusion study in order to estimate the contribution of formulation vehicles on permeability of the membrane (Lalor et al., 1994).

b) Using equal solute activity in the vehicle

A logical approach is to design experiments to compare solvents under conditions of equal activity, so that any differences in flux may be ascribed to interactions with the membrane. A reasonable reference point is the saturated solution (Nannipieri et al., 1990; Zatz, 1991b; Roy et al., 1994). Although the use of saturated solutions allows comparisons to be made at equal solute activity, the effect of high solute concentrations on solvent activity complicates the approach (Zatz, 1991a). In such a case, a low solute concentration can be used to maintain constant solvent activity, and obtained flux can be normalised by degree of saturation of the solute in donor vehicle.

c) Incorporation of a synthetic membrane.

Separation of the interactive and noninteractive vehicle effects can be achieved by cancelling the effect of differences in vehicle activity using two membrane systems to reveal the true interactive tendency towards skin. A series of parallel experiments was conducted to assess phenol permeation through both polyethylene membrane and rat skin (Roberts and Anderson, 1975). The polyethylene membrane was assumed to be inert toward the solvents. The same solutions at a given phenol concentration were applied, and the activity of phenol within a given vehicle was the same in both experiments. The non-interactive effect (solute activity in vehicle) is therefore cancelled by the ratio of the flux values or Kp values:

$$J_1/J_2 = Kp_1\Delta C_1/Kp_2\Delta C_2 = Kp_1/Kp_2 \quad .....(1.14)$$

Such a comparison provides an index of the degree of interaction with the skin.

1.6.3 Solvent effect on sunscreen efficacy

Efficacy of sunscreen agents is often influenced by the solvents in which they are dissolved. Although the solvent effect on sunscreen performance has been well documented, the resultant data are normally generated from pure or a high percentage of sunscreen in solvents; therefore the effects may not be observed in a finished formulation (Agrapidis-Paloympis et al., 1987; Meadows, 1990; Shaath, 1991). However, awareness of these effects is important to aid optimisation of the sunscreen formulation and ensure their performance.

1.6.3.1 Shift of λmax induced by solvent

The effect of sunscreens is often influenced by the solvents in which they are dissolved. (Louise et al., 1987). Polar solvents shifted the λmax of polar sunscreens to shorter wavelengths (hypsochromic) and shifted less polar sunscreens to longer wavelengths (bathochromic). λmax gets shifted to shorter wavelengths because the ground state is more polar than the excited state. Ortho-substituted sunscreen chemicals, such as salicylates and anthranilates experienced a minimum or no UV absorbance shift. Most sunscreens showed increased absorbance in both polar and non-polar solvents and decreased absorbance in semipolar solvents. In the case of three benzophenones tested (Bz-3, dioxybenzone, and sulisobenzone), a hypsochromic shift occurs within the UVA region. If the sunscreen is less polar (i.e., octyl p-methoxycinnamate, butyl methoxydibenzoylmethane and octyl dimethylPABA), the interactions with polar

solvents will shift the UV absorbance spectra to longer wavelengths. In the case of OM, formulating with nonpolar solvents should be avoided, since such solvents will shift the λmax away from 308nm.This study should aid the cosmetic chemist in selecting appropriate solvents and vehicles for sunscreen chemicals.

Non polar solvents shift λmax of nonpolar sunscreen agents to shorter wavelengths and shift polar sunscreen agents to longer wavelengths (Agrapidis-Paloympis et al., 1987; Shaath et al., 1990). This shift in the UV spectrum is primarily due to the relative degrees of solvation of the ground state and the excited state of the polar solvents due to hydrogen bonding, the solvation stabilising the ground state of the polar sunscreen agents and thereby inhibiting electron delocalisation. As a consequence, λmax of polar sunscreen agents is shifted to a shorter wavelength. For the non-polar sunscreen agents, the excited state is more polar than the ground state. The net effect is stabilisation of the excited state by polar solvents that lower the energy requirement for the electronic transition and hence a higher λmax would be expected (Shaath et al., 1990). OS is an ortho disubstituted compound. Its carbonyl group is conjugated to the aromatic ring, thus lowering the energy required for electron delocalisation. It decreases the ability to interact with solvent molecules, which leads to a minimum shifting effect (Shaath, 1991). Bz-3 has an orthodisubstituted group next to the UV chromophore. Therefore, as is the case with OS, different solvents produce a minimum shift in λmax (Shaath et al., 1990).

1.6.3.2 Shift of λmax induced by UV radiation

The value of λmax of sunscreen agents in solvent can be altered by UV irradiation (Shaath et al., 1990). After irradiating sunscreen agents in three different solvents for 200 seconds (200 seconds generated 5 Minimum Erythemal Dose (MED) for tested human volunteers with fair skin) λmax wavelength was examined. The λmax of OP was apparently shifted to shorter wavelength (around 280nm) after UV radiation in both IPM and LP. This decreases OP sun protection efficacy since a λmax within the range of 290-340nm is optimal for UVA and UVB protection. UV radiation in LP shifted the λmax of OM to longer wavelength, which increased sun protection efficacy. The λmax of other sunscreen agents investigated in the study was almost unchanged following exposure to UV radiation (Table 1.3).

Table 1.3. λmax (nm) shift of sunscreen agents after UV radiation in three different solvents.

Sunscreen	**Aqueous Ethanol70%**		**IPM**		**LP**	
	Before	After	Before	After	Before	After
BP	329.4	329.2	328.1	328.0	327.9	325.0
OC	329.9	305.5	299.8	300.1	299.3	299.8
OP	313.9	313.4	303.4	279.9	301.1	283.0
OM	309.7	307.4	306.3	306.3	291.0	305.9
BM	358.1	357.8	354.7	354.0	352.9	352.1
OS	305.2	304.1	307.7	307.7	308.9	308.9

Summarised from Shaath et al., 1990

1.6.3.3 Change in extinction coefficient

Solvent effects on λmax and extinction coefficient (ε) of sunscreen agents were investigated in four solvents namely Ethanol, LP, IPM and $C_{12\text{-}15}$BA. It has been found

that the polarity of the solvents not only shifted the λmax of sunscreen agents but also influenced their molar extinction coefficient to a certain degree (Agrapidis Paloympis et al., 1987).

Table 1.4. Solvent effect on λmax and excitation coefficient (ε) of sunscreen agents

Sunscreens	Ethanol		LP		IPM		$C_{12-15}BA$	
	λmax	ε	λmax	ε	λmax	ε	λmax	ε
BP	325	9400	329	7,800	328	9,000	328	8,300
OC	304	12,700	296	10,200	301	12,300	301	10,000
OP	311	27,300	300	22,400	304	27,000	305	26,700
OM	311	23,300	289	19,700	310	19,900	304	18,200
BM	359	32,500	351	27,200	357	30,500	360	29,900
OS	307	4,200	310	4,200	309	4,200	309	3,900

Summarised from Agrapidis-Paloympis et al., 1987

1.6.3.4 Stability of sunscreen agents in solvents

It has been found that photolytic and hydrolytic degradation of sunscreen agents can occur in their vehicles (Shaath, 1991; Jiang et al., 1996). Benzophenones and PABA esters showed best spectral stability followed by cinnamates. The worst one was BM. Such degradation may not influence only their SPF, but also the induced intermediates might play a role in skin damage. A thorough understanding of the behaviour of sunscreen agents in their vehicles is essential for optimal formulation. Table 1.5 summaries degradation of sunscreen agents in different media under varied experimental conditions.

Table 1.5. Percent degradation of sunscreen agents in different vehicles

5 MED UV irradiation (200 μg/ml)				Ambient light (2.5-8 μg/ml)		
Sunscreen	70% Ethanol	IPM	LP	24hr 20% Ethanol	24hr 2%BSA	120hr *lotion
BP	-	1.8	0.0	16.1	3.0	0.0
OC	0.0	1.1	2.8	-	-	-
OP	3.9	52.8	31.2	44.8	-	41.6
OM	39.1	18.7	18.7	80.4	40.9	-
BM	4.8	2.9	20.6	39.6	-	70.4
OS	1.5	9.8	0.0	62.6	0.6	-

Data from Shaath, 1991 and Jiang et al., 1996
*Copper tone lotion
- not available

1.6.3.5 Solubilisation

To provide efficient sun protection complete solubilisation of chemical sunscreen agents in formulation is essential. To achieve this requirement a solubiliser such as surfactant or ester is usually incorporated (Jellinek, 1970; Brown, 1986). Solubility parameters, the sum of all the intermolecular attractive forces, offer an effective method for assessing the activity of a surfactant (Vaughan, 1985). For two relatively immiscible solvents, a co-solubiliser may be identified by the medial location of its solubility parameter between these two solvents (Vaughan, 1985). In addition, some sunscreen agents themselves act as a good solubiliser for other sunscreens. For example, as previously discussed, OS has

excellent solubilising properties and has been widely employed in sunscreen formulations to solubilise other less soluble sunscreen agents such as Bz-3 (Patel et al., 1992).

1.6.4 Sunscreen formulation

1.6.4.1 Formulation types and their protective properties

The types of sunscreen formulation include emulsions, oils, gels sticks, aerosols and oinments. A given sunscreen combination in different formulation types will provide different SPF values. The emulsion provides high SPFs as it leaves a thick and uniform sunscreen film after spreading on the skin. As emulsions are the most common vehicles, they will be discussed in more detail in the following section. Ointments and oils are the most easily formulated sunscreen vehicles, but are relatively expensive. With single phase products stability can be achieved readily and they have excellent water resistance. Due to the fact that they are polar solvents, the λmax of non-polar solvents are shifted to shorter wavelengths, thereby decreasing their sun protection efficacy. Oils leave a very thin and transparent sunscreen film (low SPF) due to their excellent spreadability. Aqueous and hydroalcoholic gels are elegant formulation vehicles, but because they are hydrophilic in nature, they are washed off easily when exposed to water or perspiration. To maintain crystal clarity, high levels of solubiliser such as a surfactant must be incorporated to increase the solubility of nonpolar sunscreen agents, which results in increased cost and potential for irritation. Alcohol/oil based aerosol products provide low SPF due to the evaporation of the alcohol when they are applied to the skin, which leaves a thin and porous film (Klein, 1997). Sticks are mostly used on the lips. They usually

They usually consist of nonpolar sunscreen, oil or ester, and wax. They have excellent water proof properties with an oily or greasy feel suitable for application to the lips, but would be unpleasant on the skin. Sunscreen performance of the sticks is poor because they have a very thin layer of films on the skin. General advantages and disadvantages of sunscreen formulations regarding their protection properties are summarised in Table 1.6.

Table 1.6. Sunscreen formulations and their protection properties.

Formulation	**SPF for given sunscreen combination**	**Water resistance**	**Remarks**
Emulsion O/W	High, Thick and Uniform Film	Very good	Elegant, Less cost
Emulsion W/O	High, Thick, Uniform Film and It depends on oil volume	Fair	Elegant, Less cost
Oils	Low, Thin film, Shift max	Very good	Greasy feel, High cost
Gels	Low	Poor	Eye sting
Sticks	Low, Same as oils	Very good	Elegant, High cost
Mousses	High, Same as emulsions	Fair, Depends on oil volume	High cost
Aerosols	Low, Thin and Porous film	Good	Greasy feel, Expensive
Oinments	Low, Same as oils	Excellent	Greasy feel

1.6.4.2 Emulsions

Sunscreen emulsion formulations are the most common products because they allow for easy incorporation of sunscreen actives, which are typically soluble in the oil phase and can provide an elegant emulsified cream or lotion. Sunscreen emulsion formulations effectively scatter UV radiation and leave a uniform and thick sunscreen film on the skin, thus maintaining the desirable SPF. They have an elegant appearance and are cost-effective vehicles as they contain a large percentage of water, making them relatively cheaper than oil or alcohol spray formulations. The main disadvantage of emulsions is that they are relatively difficult to formulate and stabilise, particularly at elevated temperatures (Klein, 1997). Therefore they must be well designed and manufactured. Depending on their viscosity, emulsions are generally termed creams or lotions. W/O emulsions are much more water-resistant than O/W but have a more greasy feel. A combination of two emulsifiers is frequently used to provide a stronger interfacial film than could be achieved with a single emulsifier (Jellinek, 1970). Several newly developed polymers based on silicone chemistry have been introduced, which can produce very elegant W/O or O/W emulsions with outstanding waterproof properties and improved skin feel (e.g., laurylmethicone copolyol and dimethicone copolyol), while remaining very stable (eg., cyclomethicone and dimethicone copolyol). In contrast to Europe, O/W emulsions are more popular in the USA and Australia because of their elegant appearance and nongreasy feel. O/W emulsions are less substantive. To promote emulsion inversion on the skin from O/W to W/O, and to resist wash-off, a large oil internal phase and a low level of w/o emulsifier should be employed (Klein, 1989). A strategy to accomplish water resistance of a sunscreen formulation is to employ a water-insoluble substantive film forming resin. A double blind clinical study was conducted to evaluate a waterproof

sunscreen preparation (Berger et al., 1978). In this study, ammonium acrylate/acrylate ester polymer was incorporated into a sunscreen lotion containing OP. A substantive film formed after applying the formulation to the skin, which did not interfere with transepidermal water loss or normal sweat gland function and provided protection from sunburn after 60min swimming in both fresh and salt water. PVP/eicosene copolymer, an oil soluble resin, was introduced (Oteri et al., 1987). It effectively increased water repellency of a sunscreen emulsion and showed low order toxicity, excellent film forming and aesthetic properties. Acrylates/t-octylpropenamide copolymer is a multifunctional film-forming polymer. It bears a wide range of beneficial effects, such as water repellency, moisture barrier properties, rub-off resistance, fragrance retention and conditioning effects. This polymer effectively retained high SPF value of tested sunscreen formulations (>90%) after 80 minute immersion of human subjects in water (Guth et al., 1991).

To achieve a uniform and thick sunscreen film on the skin and increase stability of the emulsions, the viscosity parameter must be considered. Many techniques can be employed to increase viscosity in the external phase:

a) Adding more internal phase (both w/o and o/w) can efficiently increase emulsion viscosity at room temperature, but the resultant viscosity will be lost at an elevated temperature (Klein, 1990b).
b) Reducing particle size allows the same amount of internal phase to occupy a greater volume.

c) Adding a fatty moiety to form a liquid crystal network structure in the external phase (Suzuki et al., 1984).

d) Adding a thickener, such as carbomer to increase the external phase viscosity. This can stabilize the lotion effectively even at elevated temperatures (Secard, 1984).

1.6.4.2.1 Emulsifier

An emulsion is a dispersion of one immiscible liquid in another in the form of tiny droplets; thus it is a thermodynamically unstable system (Klein, 1989). To form and stabilise the system, an emulsifier is conventionally employed. The emulsifier forms an emulsion by significantly reducing the oil-water interfacial tension and stabilises it by minimising the kinetic energies within the system (Hemker, 1990). The most common emulsifiers in cosmetics are anionic and nonionic surfactants. Often used anionic emulsifiers are alkali metal soaps (w/o or o/w emulsifiers): triethanolamine soaps (o/w emulsifiers), and sodium or triethanolamine salt of a sulfated fatty alcohol (Jellinek, 1970). Soaps are the most effective and least expensive emulsifiers. However, care must be taken when utilising soap emulsions, since they can be irritating (high pH) and will allow the emulsion to reemulsify on the skin when water is applied (Klein, 1989). They are also sensitive to pH and salts (Jellinek, 1970). Popular nonionic emulsifiers are esters of polyalcohols (Span®, w/o emulsifiers); polyglycerol esters (o/w or w/o emulsifiers) and ethers (Tweens®, o/w emulsifiers). Their most important property is their mild effect on the skin and low toxicity. Generally, nonionic emulsifiers are insensitive to acids, alkalies and salts (Jellinek, 1970). However their hydrophilic-lipophylic balance (HLB) is temperature dependent. As temperature increases, HLB apparently decreases, since water

solubility of the emulsifiers depends on hydrogen bonding that will break down at high temperature. As a consequence, the resultant O/W emulsion may orient toward W/O when a threshold temperature is reached, termed the phase inversion temperature (Klein, 1990b). A phase inversion results in dramatic change in viscosity. A stable emulsion system requires the phase inversion temperature to be at least 20°C above the highest storage temperature (Klein, 1990b).

Conventional O/W emulsions often contain a relatively large quantity of hydrophilic emulsifiers with correspondingly high HLB values. In lotions, these are often not sufficiently counterbalanced by lipid emulsifiers. The protective films resulting from O/W emulsions almost always display an increased tendency to re-emulsify when they contact water (Dahms, 1994). Thus, these emulsions provide inadequate water resistance. However, newly developed lipid emulsifiers for O/W emulsions offer an alternative to conventional O/W emulsifiers. Even in extremely small quantities, optimally combined lipid emulsifiers can form stable O/W emulsions, and these emulsions re-emulsify in water only at the temperature above critical swelling point. It has been found that lipid emulsifiers form a lamellar structure (crystalline) when they contact water and it will not further react with water (dilute with water) at temperatures below the critical swelling point. After evaporation of unbound bulk water of thin film layer on the skin, a pure lyotropic liquid crystal phase remains which is water-repellant (Dahms, 1994). Two O/W emulsions which were formed either by a conventional hydrophilic emulsifier (a mixture of glyceryl stearate and PEG-100 stearate), or a new polymeric lipid emulsifier (polyglycerol methylglycose distearate, Tegocare® 450) were compared and a distinct

bleeding in the formulation containing the hydrophilic emulsifier was observed after immersion of thin film on a slide for approximately 2 minutes. The formulation with the lipid emulsifier did not show any redispersion (Dahms, 1994). The role of emulsifiers on permeation of sunscreen agents has not been investigated.

1.6.4.2.2 Emollients

As sunscreen solvents, the physiochemical properties of the emollients play a major role on the solubulisation and release of active agents. In addition, as a lipophilic component of the formulation, they also have a potential role in retaining the agents within the skin. Reservoir formation for lipophilic compounds in the SC has been reported previously (Vickers, 1963; Miselnicky et al., 1988; Dupuis et al., 1986; Zatz, 1992; Brown et al., 1995). The retention ability in the SC can be determined by measuring the amount of the compounds in the epidermis at the end of each diffusion experiment (Miselnicky et al., 1988). The capability of various fats and oils to penetrate the SC was tabulated to find remarkable differences amongst these compounds (Dahms, 1994). Most sunscreen emollients often used in emulsion based formulations (eg., IPM, LP and coconut oil) showed zero to slight retention in the SC; others exhibited good to excellent retention capacity (eg., OA, sesame oil, almond oil, and castor oil) (Table 1.7). It has been demonstrated that petrolatum remained restricted to the SC after topical application. (Brown et al., 1995). Likewise, labelled docosane did not penetrate through the dermis in either intact or in acetone treated hairless mouse skin regardless of the vehicle. These results suggest that topical hydrocarbons are unlikely to penetrate to deeper layers of either intact or damaged skin. This suggests that it is possible to target sunscreen agents

to the outermost layer of the skin using the penetration ability of the emollients. Reservoir formation is not only found in the skin, but also in some synthetic membranes. It was discovered that as the alkanol solubility in the membrane (silicone) increased, a significant portion of the applied dose was retained in the membrane. It prohibited attainment of the pseudo-steady-state condition necessary for calculating meaningful permeability coefficients (Houk and Guy, 1988). Taking these findings into consideration, one must question whether the limiting step for transport of these highly lipid soluble compounds is diffusion through the large aqueous stagnant layer imposed by the viable epidermis or is the slow transfer out of the SC into aqueous viable tissue (Houk and Guy, 1988).

Table 1.7 Ability of various fats and oils to penetrate the SC

Penetration capability to the stratum corneum				
Zero to slight	**Slight**	**Moderate**	**Good**	**Excellent**
Isopropyl myristate	Slight	Lard	Linoleic oil	Castor oil
Butyl myristate	Rice husk oil	Wheat germ oil	Oleic oil	Ricinoleic oil
Oleyl oleate	Olive oil	Lanolin	Sesame oil	
Lauryl alcohol	Apricot pit oil	Avacado oil	Almond oil	
Coconut oil				
Corn oil				
Peanut oil				

1.6.4.2.3 Emulsion types

The penetration behaviour of radiolabelled hydrocortisone in various vehicles was studied using human skin (Zesh and Schaefer, 1973). When a W/O emulsion and vaseline preparations were used, most of the radioactivity was found in the SC and longer penetration periods demonstrated a SC reservoir; whereas when an O/W emulsion or the polyethylene glycol oinment were employed, no distinct reservoir was present in the SC. This finding suggests a potential role of formulation type on directing solute distribution. I*n vitro* penetration assessment effect of emulsion types on delivery of ethyl p-aminobenzene was conducted (Lalor et al., 1994). Two types of emulsion (o/w and w/o) were prepared with equal volume of oil/aqueous phases. A lower release of the drug was found from the O/W emulsion as compared to the W/O emulsion, which was associated with solubility of the chemical in external phase. As the emulsifiers used in the formulations were shown to exist mainly in the external phase for both types of emulsion, solubility of the drug in the external phases was greatly increased with a value of 3.06mg/ml in the O/W emulsion and 1.87 mg/ml in the W/O emulsion. Therefore the lower release rate is suggested to be attributed to the lowering of thermodynamic activity as a result of substantial micellar solubilisation of the drug in the aqueous phase of the O/W emulsion.

1.6.4.2.4 Summary

Transport of a drug from a formulation vehicle on the skin surface to the tissue and blood stream involves both kinetic and thermodynamic determinants for

a) release of the drug from the vehicle and

b) partitioning and diffusion of the drug into and through the skin.

Clearly, the relative importance of each of these determinants is a function of the physicochemical properties of the drug, vehicle, and membrane. The overall topical delivery process is extremely complex, especially in emulsion based formulations, making it difficult to deconvolute various effects. Since an emulsion consists of two phases namely oil and aqueous, and emulsifying agents, more formulation factors may be involved in drug release and penetration behaviour. Two essential components, eg., emulsifier and emollient, seem to have a great impact on skin penetration of active compounds. Previous studies have demonstrated that the thermodynamic activity of a solute in the formulation vehicle can be altered dramatically by the choice of emollient and emulsifier, and these two components can also alter the skin barrier properties. In order to permit a better understanding of how emollients influence skin barrier function, knowledge about separation of membrane specific effects from non interactive vehicle effects and thermodynamic activity is essential. It is likely that due to the lipophilic nature of sunscreen agents, a possible SC reservoir may be formed during skin diffusion. Thus in *in vitro* experiments, sunscreen remaining in the skin must be assessed. The following questions remain to be answered regarding skin penetration of sunscreen agents:

a) Role of emollient and emulsifier on release or retention of sunscreen agents from formulations
b) Their role on skin barrier property
c) Effect of formulation type
d) Role of viscosity

1.7 Skin penetration of sunscreen agents: review of available scientific literature

There is extensive literature published mainly in cosmetic journals regarding the formulation of sunscreen products with respect to SPF, elegance, stability and substantivity (Berger et al., 1978; Secard, 1984; Klein, 1989). Acute toxic side-effects of specific sunscreen agents, which include contact irritation, allergic contact dermatitis, phototoxicity and photoallergy, have been well documented (Thune, 1984; Szczurko et al., 1994; Kimura and Katoh, 1995). Few published literatures reports are available regarding the percutaneous absorption and consequent systemic distribution of these chemicals. As sunscreen formulations tend to be applied regularly on large areas of the body, an understanding of their skin penetration and any potential hazards is essential. Studies show that the ability of a sunscreen in a topical formulation to permeate the skin and to exert its effect depends on both the physicochemical properties of the sunscreen agents and other factors related to the structure of the vehicle. On one hand the vehicle interacts with the sunscreen agent (solute): the vehicle can affect the solubility properties of a solute, and therefore its chemical potential gradient in the vehicle and in the stratum corneum, and its diffusion rate through the vehicle. On the other hand, solute could interact with the skin, mostly involving changes in the structure of the SC. Structural changes in the SC may facilitate or sustain the diffusion of the solute through the skin.

The systemic absorption from topical application of 40% zinc oxide oinment was investigated in healthy subjects (phase one) and in patients receiving total parentral nutrition (phase two) (Derry and Maclean, 1983). In the first phase, six subjects

completed a controlled, cross-over trial involving 3 hourly serum sample determinations for zinc concentration following a massive application of 40% zinc oxide oinment. There was a mean increase in serum zinc from 107.3 ± 5.32 to 116.1 ± 5.02 μg/dL 1hr after application of 40% zinc oxide oinment ($p<0.05$). Three patients receiving total parentral nutrition completed phase two of the protocol in which 40% zinc oxide oinment was applied daily to a specified area of the thigh. Analysis of these patients serum revealed that the zinc concentrations remained relatively constant over the 10-day study period. Zinc oxide is a physical sunscreen, and the above findings suggest that topical application of 40% zinc oxide oinment does not result in significant absorption. Even though this study was done due to increased interest in the potential development of zinc deficiency in patients receiving TPN (total parentral nutrition), this study helps to do further research on sunscreens.

A mathematical model was used to estimate the extent of percutaneous absorption of sunscreen agents based on their physicochemical properties (Watkinson et al., 1992). On the basis of this model it is suggested that significant amounts of certain sunscreen agents are likely to penetrate the skin and enter the systemic circulation. The above study was very useful for doing futher research on skin penetration of sunscreen agents.

An *in vivo* study using human volunteers was conducted to quantify the transdermally absorbed amount of several sunscreen agents from a propylene glycol/water mixture (Hagedorn-Leweke and Lippold, 1995). It was done by measuring the percentage of loss of the applied sunscreen agents from a donor chamber, which was attached to human skin

Their research revealed a linear relationship between the logarithm of permeability of the sunscreen agents and the corresponding octanol/vehicle partition coefficients.

More recently, a significant role of formulation vehicle on percutaneous absorption of Bz-3, OM and OS was demonstrated (Treffel and Gabard, 1996). In this study, two formulations (petroleum jelly and emulsion gel) were compared and sunscreen agents quantified in various skin layers after topical application. Greater retention of the sunscreen agents was found from the emulsion-gel in contrast to the petroleum jelly. In addition, reservoir formation was mainly found in the SC followed by epidermal tissue. Little amount of the agents was recovered in the aqueous dermis. This study demonstrated the skin penetration and retention of UV filters, as well as expected SPF, could be optimized by a suitable vehicle. The solubility study was not done in the above study, which is important because the penetration of the solute depends on the degree of saturation of the solute in the vehicle (Jiang et al., 1998a).

In another study, OM and BM release from five different emulsions {($5mg/cm^2$): conventional O/W, W/O emulsions, emulsifier free O/W emulsion (-em/o/w), water in silicone emulsion (w/s), and W/O with lamellar liquid crystals emulsion (cl o/w)} was compared using a finite dose technique (Lazar et al., 1996). Cumulated amount of the sunscreen agents in the receptor phase was quantified at 4 and 8hr and the amount that remained in each skin layer was assessed at the end of the diffusion study. A quantifiable amount was found for the OM (concentration in emulsions was four times higher than BM), and a trace amount at detection limit was found for the BM. A significant decrease

in the penetration rate was found in the lamellar liquid crystals emulsion (cl o/w) in contrast to other O/W emulsions (penetration rate: 0.124 vs 0.535 and 0.743 mg, h^{-1}, cm^{-2}); the same was found for the water in silicone emulsion (w/s) as compared to the conventional one (penetration rate: 0.12 vs 0.48 mg, h^{-1}, cm^{-2}). Skin retention of both sunscreen agents in the SC and epidermis was found to be positively correlated with their penetration rate: -emulsifier free O/W>O/W>cl O/W emulsions and W/O>W/S. Again, like the previous studies the results of this study demonstrated that penetration could be optimized by a suitable vehicle like water in silicone emulsion. The above study does not talk about the degree of saturation or vehicle solubility parameters, which is important to determine the permeation of sunscreen agents.

In vitro human skin penetration of ^{14}C labelled OS was studied using two representative sunscreen vehicles (o/w emulsion and hydroalcoholic formulation) (Walters and Brain, 1997). The average total absorption of 5%w/w salicylic acid when applied as a finite dose of o/w emulsion over a 48hr period was 0.65 ± 0.16% of the applied dose (representing a total amount permeated of 1.58 ± 0.36 μg/cm^2). When applied as an infinite dose, the average was 0.47 ± 0.22% of the applied dose (representing a total amount permeated of 27.54 ± 13.91μg/cm^2). When applied as a finite dose and infinite dose in the hydroalcoholic formulation containing 5%w/w OS, the averages were 0.23 ± 0.05% (infinite dose) (of the applied dose representing a total amount permeated of 11.28 ± 2.55 μg/cm^2). When applied as a finite dose the average was 1.14 ± 0.23% of the applied dose (representing a total amount permeated of 1.65 ± 0.39 μg/cm^2). These results suggest that *in vitro* human skin permeation of OS is relatively low. The amounts of OS and salicylic

acid permeated when applied in similar vehicles were remarkably similar over 48hr (1.58 $\mu g/cm^2$ and 1.65 $\mu g/cm^2$, respectively). This suggests that ^{14}C label appearing in the receptor fluid may in both cases represent salicylic acid. If this is the case, then it is possible that the amount of OS permeating through the skin is much less than that suggested by the data obtained here. The above study deals with only two formulations – hydroalcoholic and O/W emulsions, which underestimates the permeation of OS. A wide range of vehicles should be chosen (polar to non polar) to find the permeation of sunscreen agents.

The availability of OS, a common sunscreen agent, from liquid paraffin and the effect of OS on skin permeability was investigated (Jiang et al., 1997). A range of OS concentrations in liquid paraffin was diffused across human epidermis and synthetic membranes into 4% bovine serum albumin in phosphate-buffered saline and 50% ethanol. Absorption profiles for OS obtained from silicone and low density polyethylene (LDPE) membranes were similar to each other but higher than for the high-density polyethylene (HDPE 3 times) membrane and human epidermis (15times). The steady state fluxes and apparent permeability coefficients (Kp) obtained from the diffusion studies showed the same trends with all membranes, except for the HDPE membrane, which showed greater increase in flux and Kp at concentrations above 30%. Thus from the study it was concluded that no significant changes in permeability were found for the skin or membrane filters, except for the high density polyethylene membrane in which a plasticisation effect was found at OS concentrations above 30%. The permeability of two membrane filters remained unchanged with a range of concentrations of OS in liquid

paraffin. It indicated the suitability of the membrane filters for further diffusion studies of sunscreen agents from water immiscible vehicles. With a suitable membrane the penetration ratio of Kp-skin to Kp-membrane or fluxes can be employed to isolate vehicle effect from membrane permeability, because these ratios are independent of drug concentration. Silicone and low density polyethylene membrane filters were found to be unsuitable for further investigation, because of high absorption profiles. The above studies do not consider the drug-vehicle-skin interactions. Very few studies have been done for the development of a model system to investigate the effects of interactions between sunscreen and skin, sunscreen and vehicle, and vehicle and skin.

The release of lipophilic agents from formulations and solutions was compared through permeable membranes (Hayden et al., 1997b). The aim of these experiments was to compare the release rate of lipophilic solutes through cellulose acetate dialysis, polysulfone and human epidermal membranes. A static vertical Franz type diffusion cell was used to mount the membranes. The receptor solution was 4% BSA in PBS at 35°C constantly agitated with a magnetic stirrer bar. The donor phase was applied to the upper surface of the membrane and samples were removed periodically in the solution and analyzed by HPLC for OM, OC, BM, OC, OP and Bz-3. The cellulose acetate membrane pre-soaked in water resulted in very low release rates (<0.005 mcg/cm^2/h) from a 5% solution of OM in light mineral oil (LP). Yet, polysulfone membrane pre-soaked in isopropyl myristate (IPM) allowed a greater release (32 mcg/cm^2/h). The skin penetration lay between that of the two synthetic membranes (0.4 mcg/cm^2/h). The synthetic

membranes can be used as model membranes for *in vitro* studies to evaluate vehicle effects.

In vitro penetration and retention of Bz-3 across epidermal and polyethylene membrane from a range of single solvent effects on permeability parameters were studied (Jiang et al., 1998a). The solubility of Bz-3 was measured in a number of solvents. Penetration of Bz-3 across human epidermis and high-density polyethylene (HDPE) membranes was studied from 50%-saturated solutions in each solvent. Maximal Bz-3 fluxes from the solvents across the two membranes varied widely. Highest fluxes were observed from Ethanol 90% for epidermis and isopropyl myristate (IPM) and $C_{12\text{-}15}$ benzoate alcohols for HDPE membrane. Both the flux and estimated permeability coefficient and skin-vehicle partitioning of Bz-3 appeared to be related to the vehicle solubility parameter (δv). The major effects of solvents on Bz-3 flux appear to be via changes in Bz-3 diffusivity through membranes. Minimal penetration of sunscreens such as Bz-3 is best achieved by choosing vehicles with a δv substantially different to the solubility parameter of the membranes δm as well as similar to that of solute δi. The above study was a breakthrough in sunscreen research, because previous studies on sunscreens concentrated only on the permeation due to formulation differences, but the above study concentrated on why the permeation is taking place and how the permeation can be stopped.

The absorption of sunscreens across human skin, an evaluation of commercial products for children and adults was studied (Jiang et al., 1998b). Topical sunscreens are routinely applied to the skin by a large percentage of the population. This study assessed

the extent of absorption of a number of common chemical sunscreen agents into and through human skin following application of commercially available products. Sunscreen products were applied to excised human epidermis in Franz diffusion cells with the amount of penetration into and across the epidermis assessed by HPLC for 8hr following application. All sunscreen agents investigated penetrated into the skin (0.25 gm^{-2} or 14% of applied dose), but only Bz-3 passed through the skin in significant amounts (0.08 gm^{-2} or 10% of the applied dose). With one exception sunscreen agents in corresponding products marketed for adults and children had similar skin penetration profiles. Limited absorption across the skin was observed for the majority of the sunscreen tested, Bz-3 demonstrated sufficiently high penetration to warrant further investigation of its continued application. Increase in penetration rate may be due to the fact that Bz-3 is relatively hydrophylic when compared to other sunscreens like OM, BM, OC and OS. Very high amounts of sunscreens were used in the above study and the increase in permeation of sunscreen agent may be due to high amounts of sunscreens applied.

The percutaneous absorption of sunscreen from liquid crystalline phases was studied (Brinon and Geiger, 1999). The purpose of the present study was to investigate the effects of two non-ionic surfactants with liquid crystalline structures on the cutaneous availability of two sunscreens. Three liquid crystalline structures were investigated: lamellar, hexagonal and cubic. The diffusion of sunscreens within the liquid crystals was determined by measuring transport kinetics into an unloaded surfactant medium from a similar system loaded with the sunscreens. The diffusion coefficients were the greatest in the cubic systems for Bz-4 (a hydrosoluble sunscreen) and in lamellar systems for OM (a

liposoluble sunscreen). So the diffusion in this surfactant system was strongly dependent on the structure of the liquid crystal and on the physicochemical properties of the solute. The transcutaneous fluxes were determined using a Franz-diffusion cell. The liquid crystalline vehicles modified the transcutaneous fluxes of Bz-4, but did not change those of OM. The solute diffusion within the vehicle was not the rate-determining step for transcutaneous permeation for either sunscreen. Bz-4 diffusion from the liquid crystalline phases is not the rate-determining step of the transcutaneous process. It is possible that liquid crystalline phases modify the vehicle-stratum corneum partition and /or the SC properties. For OM, the rate-determining step of the transcutaneous process could be its partitioning in hydrophilic parts of the skin (i.e. the dermis), which would be almost equivalent whatever the vehicle applied to the skin.

Percutaneous absorption of sunscreens through micro-Yuctan pig skin *in vitro* was studied (Gupta et al., 1999). The objectives of this study were to develop an *in vitro* model for studying sunscreen permeation in skin, and evaluate the influence of formulation differences. The sunscreens studied were two of the most widely used agents, OM and Bz-3. Preparations containing radiolabelled actives were applied to micro-Yuctan pig skin dermatomed to a thickness of 250-300μm as a finite dose in a flow – through diffusion system. At the end of each experiment the amounts removed by washing, retained inside stratum corneum and penetrated into receptor and viable skin were determined. The two sunscreens reached a peak level in SC within an hour. Bz-3 penetrated skin to a greater extent than OM. The opposite was true when comparisons of SC retention were made. The ratio of retained to penetrated amount of sunscreens from a

hydroalcoholic formulation at the end of 10hr was higher when the sunscreens were present together than alone. Despite the highly lipophilic nature sunscreens, particularly OM, SC is the rate limiting skin layer for penetration. Penetration and SC retention were formulation dependent. The ratio of SC content to the amount penetrated is a useful tool for evaluating sunscreen permeation. This study shows that the penetration of Bz-3 will increase if it is combined with another sunscreen like OM. The above study does not discuss about the degree of saturation of sunscreens in the vehicles, and previous studies show that permeation of sunscreen agents vary significantly depending on their degree of saturation in the vehicles (Jiang et al., 1998a). This study serves as a starting point for further research on a combination of sunscreen agents.

In vitro compartmental distribution and absorption of 5 UV filters was studied by using fresh human skin, after exposure times of 30min and 16hr (Potard et al., 2000). The UV filters (OM 5%, Bz-3 4.9%, OC 8% and OT 4%) were incorporated separately in simple oil-in-water emulsion at different concentrations to obtain a sun protection factor 5. They found that the quantity of Bz-3 (lipophilic compound) diffused into the deep level (receptor fluid) increased after 16hr of exposure compared to 30min. They also observed that the quantity of Bz-3 was very low in the epidermis and dermis compared to that found in the receptor fluid. The mean quantity of the other filters in the receptor fluids was close to zero or nil. OM and OC were detected in SC. This recent study has proved that Bz-3 is the only sunscreen, which penetrates deep into the skin. The above study does not consider the influence of solubility and formulation on the skin penetration of sunscreen agents.

The percutaneous absorption of sunscreens *in vitro*: interspecies comparison, skin models and reproducibility aspects was investigated (Benech-Kieffer and Wegrich, 2000). This study was designed to evaluate an *in vitro* protocol for investigating the percutaneous absorption of two sunscreens under standardized experimental conditions. OM and Bz-4 were each incorporated in a typical O/W emulsion and tested seperately. Salicylic acid was tested as a reference compound. *In vitro* percutaneous absorption was evaluated using two species, the pig and human, and two models, full thickness and split thickness skin. The reproducibility of study results was evaluated by comparing the data generated by two industrial laboratories. The correlation of quantitative data between pig skin and human skin was very good, and the split thickness skin model seemed to be more appropriate for measuring the absorption of sunscreens. Results obtained for salicylic acid demonstrated the relevance of the protocol in terms of prediction of *in vivo* percutaneous absorption. Finally, the comparison of pig skin data between the two laboratories demonstrated a good correlation and underlined the need for a standardized *in vitro* procedure. In conclusion, the study demonstrated the relevance of an *in vitro* protocol for measuring the percutaneous absorption of sunscreens. The results suggest that pigskin may be used as an alternative to human skin to predict *in vivo* human systemic exposure. Regarding the skin model, the percutaneous absorbed dose can be calculated as the amount of ingredient found in viable tissue providing that in use conditions are followed in these investigations, particularly regarding the dose applied and the length of exposure. The level of reproducibility, repeatability and correlation with *in vivo* investigations make this *in vitro* model suitable to ensure continuity and reliability

of percutaneous absorption data. Even when precautions are taken, some minor variability in the estimation of percutaneous absorption by different laboratories is to be expected; this underlines the need for the establishment of strictly standardized procedures adapted to the different classes of cosmetic ingredients.

The skin penetration of Bz-3 *in vitro* and *in vivo* was investigated to find the possible influence of formulation (Fernandez and Marti-Mestres, 2000). Six different vehicles, three solvents, and three different emulsion types were evaluated *in vitro* and *in vivo*. Each vehicle was applied to the skin model at $2mg/cm^2$. First histological studies on pig's ear skin and human skin were evaluated. *In vitro* measurements were performed with a static diffusion cell using pigskin at 1,2,4, and 8-hr. *In vivo,* Bz-3 concentration in stratum corneum was evaluated by the stripping method after 30min application on forearm of the volunteers. It was shown that pig's ear skin and human skin appeared similar and in both experiments significant difference between the vehicles was noticed. The six vehicles could be ranked in the same order of Bz-3 skin concentration. At 8hr, the highest concentration of Bz-3 in skin was obtained with propylene glycol, and o/w submicron emulsion. On the contrary, the two oily solvents, w/o emulsion and o/w coarse emulsion restrain the concentration of this UV filter in the skin. At each time, permeability *in vitro* and *in vivo* was well correlated. Low concentrations were measured in the receptor fluid, suggesting that percutaneous absorption of this UV filter across the skin would be minimal. The *in vitro* and *in vivo* skin penetration capacity of Bz-3 from six vehicles was confirmed and quantified. It has been confirmed in this work that the skin concentration and consequent systemic distribution of this sunscreen may occur at

significant levels, and the extent of this concentration will vary depending on the solvent and formulation used. The most important aspect of sunscreen vehicles is now to resist the skin damage. They may also limit the percutaneous penetration with an adapted formulation. Based on these arguments, it is believed that submicron emulsions are of little interest for sunscreen formulation. The capric-caprilic triglycerides and coconut oil appear to be interesting solvents for sunscreen formulations that limit percutaneous penetration. If cosmetic or dermatological preparations are necessary, w/o formulations which are water proofing or classical o/w emulsions appear to be the better choice to limit higher Bz-3 skin concentration. The above study gave an idea about choosing the correct vehicles for the Bz-3 permeation study across polysulfone membrane.

CHAPTER 2

AIMS AND HYPOTHESES

2.1 Aims

The specific aims of this thesis were to:

1. Validate a suitable HPLC assay for the determination of sunscreens in vehicles and *in vitro* diffusion experiment receptor fluid
2. Study the effect of a range of vehicles on the solubility of the sunscreen Bz-3
3. Study the permeation of Bz-3 from a range of vehicles across a suitable synthetic membrane
4. Determine if interactions occur between Bz-3 and other sunscreens OM and BM that influence solubility and /or permeation.

2.2 Hypotheses

1. The solubility will influence the skin penetration of sunscreen agents. Increase in solubility will decrease the permeation across membrane and decrease in solubility will increase the permeation across the membrane
2. Presence of alcohol will enhance penetration across polysulfone membrane, which is a hydrophilic membrane. Increase in permeation from an alcoholic vehicle is ascribed to increase in activity on the membrane as a result of solvent evaporation and lower solubility in the vehicle. Penetration from an alcoholic vehicle will be higher compared to water and lower when compared to oil.

3. Permeation of combined sunscreens (Bz-3 + OM) and (Bz-3 + BM) will depend on the solubility of the combined sunscreens in each vehicle. Increase in solubility will decrease permeation across the membrane and decrease in solubility increase permeation across the membrane.

CHAPTER 3

METHODOLOGY

3.1 HPLC assay validation

3.1.1 Introduction on literature assays

A simple assay method was developed for the quality control of some sunscreen products containing padimate-O and Bz-3 (Tan et al., 1984). A methanolic extract of the product containing sulfathiazole internal standard was subjected to reversed phase high-performance liquid chromatography on a 10-μm Partisil ODS-2 column with methanol-acetonitrile (90:10,v/v) mobile phase. The sunscreen - sulfathiazole peak height ratio was linear between 0.04-2.68 μg of padimate-O (r=0.9999) and 0.02-1.05μg of Bz-3 (r=0.9997) injected. All peaks were well resolved. Approximate retention times for sulfathiazole, Bz-3 and padimate-O were 3.9, 5.7 and 7.4 min, respectively. Average percent recoveries (±SD) (n=3) from simulated lotions containing 7% padimate-O and 3% Bz-3 were: padimate-O, 101.4 ± 1.5%; Bz-3, 99.9 ± 1.9%; from simulated lipsticks containing (a) 7% padimate-O and 3% Bz-3: 103.8 ± 1.2% and 100.1 ± 0.9%, respectively; and (b) 7% padimate –O and 0.5% Bz-3: 99.4 ± 0.6% and 99.3 ± 2.4% respectively. The method was successfully applied to marketed products.

A precise, accurate, selective and sensitive liquid chromatographic method was developed for the determination Bz-3 in rat biological fluids and different tissues (Abdel-Nabi et al., 1992). The minimum detection limit for Bz-3 was 2.0ng ml^{-1} and the retention

time was 6.01 min. Standard curves for Bz-3 were linear over a wide range of concentrations in methanol and different body fluids, ranging from 6.25ng ml^{-1} to 100μg ml^{-1}. To detect Bz-3 in rat whole blood after oral administration, HCL hydrolysis was required. Bz-3 produced a peak blood concentration 1hr after administration. Free Bz-3 in urine represented a very small percentage during the first 12hr after administration, while a higher concentration of the glucoronide conjugate was detected in the same time period.

In this assay, extraction procedures from plasma, RBCs (red blood cells), urine and tissues were given. No extraneous peaks from tissue or fluid extraction interfered with the quantitation of Bz-3 at 305nm. A linearity study revealed that Bz-3 was linear over a wide range of concentrations in methanol and different body fluids ranging from 6.25ng/ml to 100μg/ml. Precision, resolution, theoretical plate count, and capacity factor were calculated using US Pharmacopoeia standards. The above study was very useful in providing an idea about the extraction procedures for the quantitation of Bz-3 in plasma, RBCs, urine, and tissues.

A reversed phase high-performance liquid chromatography assay was developed for quantifying five of the most common sunscreen agents, namely OP, OM, BM, Bz-3, and OS (Jiang et al., 1996). The assay permits analysis of the sunscreen agents in formulations and in biological fluids, including human plasma. Separation was achieved using an ODS C_{18} column with a methanol-water (88:12) mobile phase. The analytes were detected by ultraviolet light

absorption at a wavelength of 315nm. The assay was linear with minimum detectable limits, calculated as greater than 3-times the baseline noise level for Bz-3 and OP, 0.05 μg/ml, for BM and OM, 0.1 μg/ml, and for OS, 1 μg/ml. Recoveries from both plasma and 2% BSA were within the range of 89-107%. The inter and intra –day coefficients of variation for the five agents were not more than 4% at the upper end of linear range and not more than 10% at the lower end. Preliminary stability studies of the sunscreen agents in a commercial product and in two diffusion cell receptor fluids were also conducted. This paper provides a reproducible and accurate assay in which 5 of the most common sunscreen agents can be resolved simultaneously. This is particularly important due to inclusion of a minimum of three sunscreen agents in their formulation. Using this assay procedure, these agents can be quantified from their formulation and biological media. One of the disadvantages is that a very low volume of sample (10 μl) was injected on to the HPLC. When all five sunscreen agents were analysed together during the stability studies, co-elution of degradation of products with other components in the receptor phase occurred. Consequently, degradation of each agent must be examined separately and a mass balance determined to account for all degradation products. The above assay was very useful in developing and validating the HPLC assay, in case of using different columns, HPLC instrument, mobile phase etc.

A rapid analytical HPLC assay was described for the quantitation of five UV filters OM, Bz-3, Bz-4, OT and OC (Potard et al., 1999). Their methods involved isocratic chromatographic mode in a RP-HPLC with UV detection. The results were validated in terms of specificity, linearity, precision, accuracy and limits of detection and

quantification. The aim of this study was to develop a simple rapid and reliable operating procedures for the quantification of UV filters and caffeine in numerous skin layer samples (stratum corneum, dermis, epidermis and receptor fluid) and in cosmetic products. The sunscreens were quantified using a Novapak C18 and a guard column and methanol: water was used as mobile phase.

Different wavelengths and mobile phases were used for different sunscreens (Table 3.1). The above assay gave an idea about choosing the correct wavelength and mobile phase for the present study.

Table 3.1 Schedule of operating conditions for chromatographic analyses

Sunscreen	OM	Bz-4	Bz-3	OT	OC
Wavelength	311nm	285nm	291nm	310nm	307nm
Mobile phase MeOH: H_20	88:12 %	40:60%	69:31%	98:2%	79:21%

Cited from Potard et al., 1999

The analytical methods were validated in terms of linearity, precision, accuracy and limits of detection and quantification. The repeatability was established by the relative standard (CV%) calculated from the ten injections of low (1mg/L) and high (50mg/L) concentrations. The intermediate precision was evaluated with the relative standard deviation of response factors obtained from the data of three calibration curves performed on three different days. The accuracy was calculated by the recovery yield between the value found with a calibration curve and the true value incorporated in the cosmetic

cream. The detection limit was calculated as the concentration that led to a signal three times the noise level, and the quantitation limit as ten times the noise level. The coefficients of linearity (r^2) were 0.999 or 0.998. The CV% of repeatability was lower or equal to 2.5%, the coefficient of intermediate precision was lower than 4% except for OT. The accuracy was between 95-102%. The limits of detection or quantitation were quite different according to components, but low enough to appreciate the quantity of product contained in each sample (Table 3.2).

Table 3.2. Data obtained by the analytical validation of all compounds.

Linearity	**OM**	**Bz-4**	**Bz-3**	**OT**	**OC**
Linearity (%) Repeatability	1	2.5	2	2.5	1.6
Intermediate(%) Repeatability	3.9	3.9	3.4	6.6	1.7
Accuracy (%)	95	97	101	101	101
Limit of detection µg/L	10	90	20	10	40
Limit of Quanititation µg/L	370	930	50	50	150
Recovery (%)	94.4	96	93.4	96.5	95.7

The above validated techniques, which respect strict requirements, are absolutely necessary before assaying all new products.

The validation of HPLC assay for the quantitation of sunscreens like Bz-3, OM, and BM was a very useful work done (Jiang et al., 1996; Potard et al., 1999). The above studies

gave an idea about chromatograms, retention times for the peaks, interday and intraday variations, wavelengths to be used, mobile phase to be used at different wavelengths, linearity, accuracy, limit of detection, recovery study, and limit of quantitation. All the above procedures helped to validate the HPLC assay for the quantitation of common sunscreen agents like Bz-3, OM, and BM.

3.2 Experimental design

a) Three sunscreen agents were used Bz-3, OM and BM for the HPLC assay validation
b) Validation was conducted by testing different mobile phases.
c) Assay precision and detectable limits were also validated.
d) Recovery study from 3.5% BSA (Bovine serum albumin) was done.
e) All samples were quantified by HPLC assay with UV detection at 315nm.

3.3 Methods

3.3.1 Experimental

The following materials and reagents were used. Bz-3 and BSA (Sigma Aldrich, USA). BM and OM (University of Queensland, Australia), HPLC grade methanol and acetonitrile (Fisher Scientific). Distilled water was filtered (0.45-μm membrane filter) (Millipore).

3.3.2 HPLC instrumentation and conditions

a) A model LC 10-AS liquid chromatograph (Shimadzu, Japan) with a 20 μl injection loop, a Novapak C18 RCM (Waters), a model SPD-10A Shimadzu UV-VIS detector

and a CR601 Chromatopac integrator (Shimadzu) were used.

b) The mobile phase was 100% methanol. Three different proportions (88:12%, 90:10%, and 95:5%) of methanol : water were tried at 315nm. The specificity was not obtained for OM and BM. However, there was no interference detected for Bz-3 samples. The specificity was obtained for OM and BM, when methanol 100% was used. The mobile phase was filtered through a 0.45-μm membrane (Nylon 66, Millipore) and degassed before use at a flow rate of 1.0ml/min. The column temperature was ambient; the injection volume was 10μl, and the detector wavelength was 315nm.

3.3.3 Validation of assay for Bz-3

Methods

a) Chromatogram for Bz-3 was plotted and the retention time was found to be 3.3 min. A stock solution of Bz-3 was made by using methanol (100 μg/ml). Five different concentrations of Bz-3 were made (0.2 to 6 μg/ml) using volumetric flasks. The samples were assayed by HPLC at 315nm.

b) Intra and interday variations for Bz-3 concentrations (0.894, 3.8 and 6 μg/ml) were calculated. The samples were assayed for 3 days for interday variation. Mean, Standard deviation and Coefficient of Variance were calculated.(n=5)

c) Recovery study in 3.5% BSA in phosphate buffer solution

Bz-3 was spiked into 3.5% BSA in phosphate buffer solution. Bz-3 stock solution was prepared. 750 µl (100 µg/ml) of Bz-3 in methanol was taken and made up to 5ml with BSA/PBS (phosphate buffer saline) in a 5ml volumetric flask. The sample solution was stirred well for 30min to ensure complete dissolution of the sunscreen agents. 100 µl of the sample was taken in a 1.5ml centrifuge tube and 200 µl of acetonitrile was added into it. The sample was centrifuged at 10,000g for 10min. Then 100 µl of supernatant was taken and mixed with 100 µl of acetonitrile and centrifuged again. The resulting supernatant was taken and injected on to the HPLC. 90-95% of Bz-3 was recovered.

3.3.4 Validation of assay for OM

Methods

a) Chromatogram for OM was plotted and the retention time was found to be 4.5 min. A stock solution of OM was made by using (100µg/ml). Five different concentrations of OM were made (0.6 to 4.8 µg/ml) using volumetric flasks. The samples were assayed by HPLC at 315nm.

b) Intra and interday variations for OM concentrations (0.6, 2.4 and 4.8 µg/ml) were calculated. The samples were assayed for 3 days for interday variation. Mean, Standard deviation and Coefficient of Variance were calculated.(n=5)

c) Recovery study was done for OM in 3.5% BSA in phosphate buffer solution. Same recovery study procedure was followed as Bz-3. 95%-99% OM was recovered.

3.3.5 Validation of assay for BM

Methods

a) Chromatogram for BM was plotted and the retention time was found to be at 4.9 min. A stock solution of BM was made by using methanol (100 μg/ml). Five different concentrations of BM were made (0.2 to 6 μg/ml) using volumetric flasks. The samples were assayed by HPLC at 315nm.

b) Intra and interday variations for BM concentrations (0.2, 2 and 6 μg/ml) were calculated. The samples were assayed for 3 days for interday variation. Mean, SD and CV% were calculated.

c) Recovery study was done for BM in 3.5% BSA in phosphate buffer solution. Same recovery study procedure was followed as Bz-3 and OM. 90 to 95% BM was recovered.

BM and OM could be separated in commercial products by using high polarity C18 columns like Hypersil ODS, Hypersil BDS C18, and Waters Spherisorb ODS1.

3.3.6 Results and Discussion

a) Chromatograms

Fig 3.1, Fig 3.2, and Fig 3.3 show chromatograms of the 3 most common sunscreen agents Bz-3, OM, and BM after sample preparation in methanol.

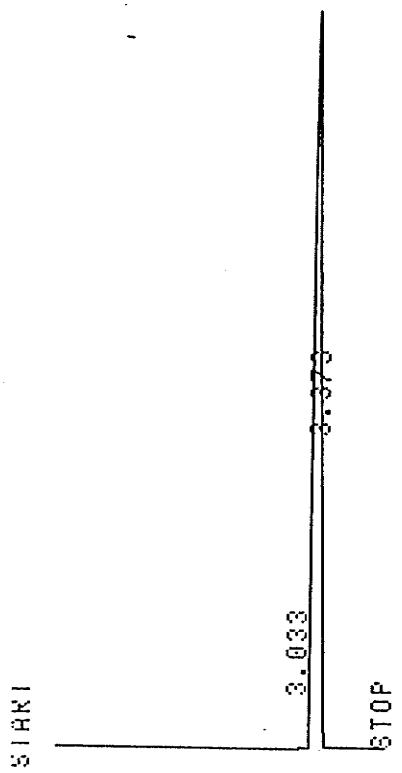


Fig 3.1. Chromatogram of Bz-3

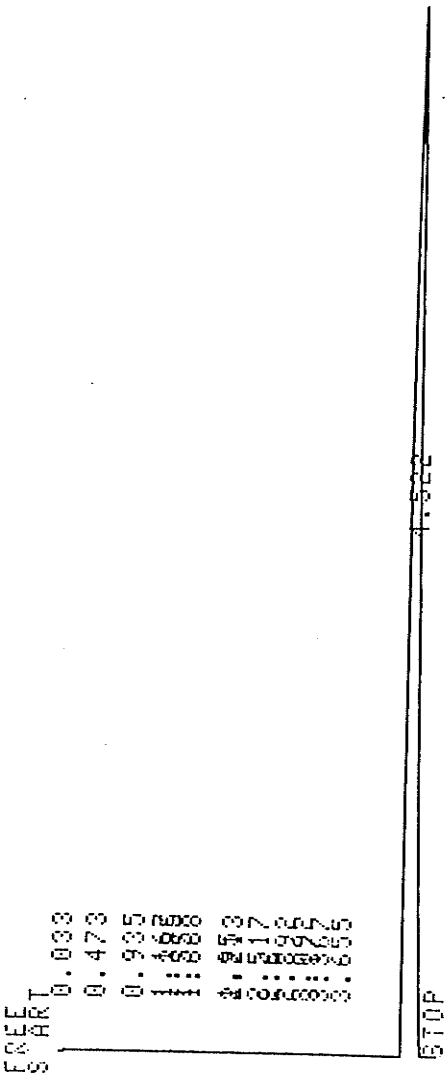


Fig 3.2. Chromatogram of OM

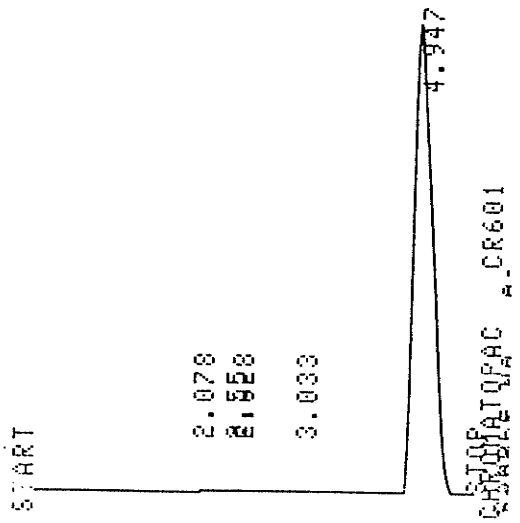


Fig 3.3. Chromatogram of BM

b) Linearity

Table-3.3 reports the results for calibration plot linearity. Excellent linearity was obtained over the range 0.2-6μg /ml for Bz-3, 0.6-4.8 μg/ml for OM, 0.2-6 μg/ml for BM.

c) Assay precision

Calibration graphs were constructed by plotting the peak area versus concentration of standards injected (Fig 3.4, 3.5, and 3.6). The best fit straight lines were determined using the method of least squares. To obtain a satisfactory UV response for all analytes, the detection wavelength was selected at 315nm, which is a compromise absorption wavelength for the three sunscreen agents. Data for calibration plots for Bz-3, OM, BM summarised in Table 3.3. The intra and inter-day precisions of the assay summarised in Table- 3.4, 3.5, and 3.6. There was no significant difference between day-day analysis. Intra and inter-day coefficients of variation (CV%) of the assay for the 3 sunscreen agents were below 10%.

d) Minimum detectable limits and low limit quantitation

The minimum detectable limits calculated as greater than three times the baseline noise level in the assay, were 0.05μg/ml for Bz-3, 0.1μg/ml for OM and BM. The lower limits of quantitation calculated as greater than 10 times, the baseline noise level in the assay were 0.1 μg/ml for Bz-3, 0.2 μg/ml for OM and BM.

e) Recovery study in 3.5% BSA in phosphate buffer.

It was necessary to evaluate the recovery of the five sunscreen agents from 3.5% BSA in PBS. A range of 90-100% recovery for the 3 sunscreen agents was observed at 2.5 μg/ml (Table-3.7, 3.8, 3.9). The coefficients of variance calculated from 3 replicates were all less than 5%.

Fig 3.4- Calibration plot for Bz-3

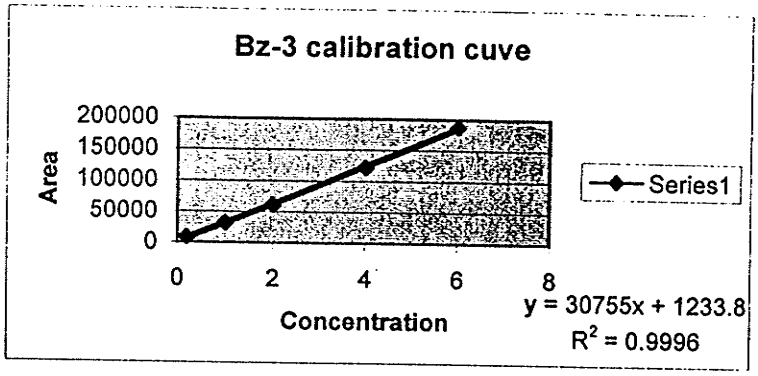


Fig 3.5- Calibration plot for OM

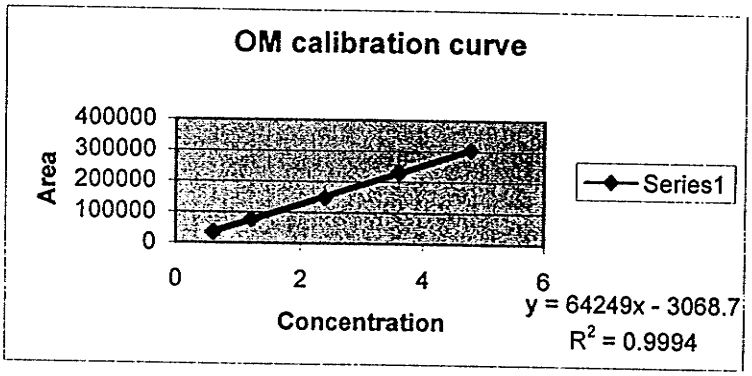


Fig 3.6- Calibration plot for BM

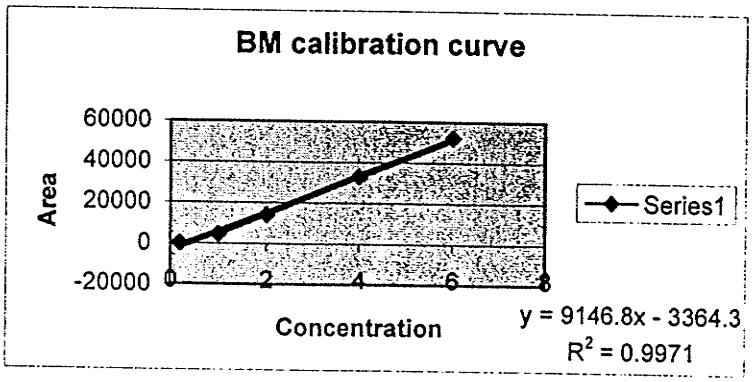


Table 3.3-Data for calibration plots.

Compound	Regression equation	Coefficient of determination	Linear range
Bz-3	Y= 30755x + 1233.8	0.9996	0.2-6
OM	Y= 64249x - 3068.7	0.9994	0.6-4.8
BM	Y= 9146.8x – 3364.3	0.9971	0.2-6

Table-3.4- Intra and inter-day variations for Bz-3 (n=6).

Bz-3	6µg	3.8µg	0.894µg
1st day			
Mean ±SD	5.78± 0.15	3.54±0.12	0.89±0.05
CV%	2.72%	3.10%	6.48%
2nd day			
Mean ±SD	5.80±0.07	3.69± 0.14	0.73± 0.06
CV%	1.23%	2.98%	8.6%
3rd day			
Mean ±SD	5.73± 0.09	3.56 ±0.11	0.74± 0.05
CV%	1.57%	2.98%	6.77%

Table 3.5- Intra and inter-day variations for OM (n=6).

OM	4.8 µg	2.4µg	0.6µg
1st day			
Mean ±SD	4.58± 0.10	2.26 ±0.11	0.40 ±0.04
CV%	2.17%	5%	8.97%
2nd day			
Mean ±SD	4.41± 0.09	2.10 ±0.09	0.48 ±0.02
CV%	2.15%	4.28%	8.43%
3rd day			
Mean ±SD	4.20 ±0.06	2.06± 0.13	0.41 ±0.03
CV%	2.14%	6.5%	10%

Table 3.6- Intra and inter-day variations for BM (n=6).

BM	6µg	2µg	0.2µg
1st day			
Mean± SD	5.79 ±0.25	1.85 ±0.22	0.86 ±0.08
CV%	4.31%	6.59%	9.3%
2nd day			
Mean± SD	5.77 ±0.19	1.81 ±0.08	0.88± 0.67
CV%	3.29%	4.475%	6.81%
3rd day			
Mean± SD	5.69 ±0.22	1.84 ±0.09	0.86 ±0.05
CV%	3.86%	4.34%	5.81%

Table 3.7 -Recovery of Bz-3 in 3.5% BSA in PBS.

Bz-3	**2.5µg/ml**
Mean ±SD	2.30 ± 0.08
CV%	3.47%
Recovery %	90-95%
Mean	94.21%
SD	1.46

Table 3.8- Recovery of OM in 3.5% BSA in PBS.

OM	**2.5 µg/ml**
Mean ±SD	2.42 ± 0.03
CV%	1.03%
Recovery %	96-98%
Mean	97.37%
SD	1.01

Table 3.9- Recovery of BM in 3.5% BSA in PBS.

BM	**2.5 µg/ml**
Mean ±SD	2.38 ± 0.06
CV%	2.52%
Recovery %	90-95%
Mean	93.43%
SD	1.40

CHAPTER 4

Solubility study

4.1 Introduction

To provide efficient sun protection complete solubilisation of chemical sunscreen agents in formulation is essential. Permeation of sunscreen agents across skin and membrane depends on the solubility of sunscreen agents in the vehicles. The efficacy of sunscreens is often influenced by the solvents in which they are dissolved, and the diffusion of sunscreens across epidermis varies significantly with formulation. It has been found that sunscreen agents were better retained in the skin after application of an emulsion gel than from petroleum jelly with the reservoir of the agents found mainly in the SC (Treffel and Gabbard, 1996). So it is necessary to find a suitable formulation. Sunscreen solubility, permeability coefficient and vehicle solubility parameter relationships were compared between the two membranes in order to estimate the degree of solvent membrane interaction.

Bz-3 solubility in different vehicles was determined (Jiang et al., 1998a). Results were explained based on solubility parameter values. The thermodynamic activity of a particular concentration of solute in a vehicle decreases when δv approaches δi (that is where the solute solubility in the vehicle is maximal) and increases when δv approaches δm.

The efficacy of sunscreens may be influenced by the solvents in which they are dissolved (Louise, 1987). Polar solvents shifted the λmax of polar sunscreens to shorter wavelengths (hypsochromic) and shifted less polar sunscreens to longer wavelengths (bathochromic)

4.2 Effect of vehicles on sunscreen Bz-3 solubility

Aim- To determine the solubility of Bz-3 in 5 vehicles liquid paraffin (LP), propylene glycol (PG), coconut oil (CO), water (H_2O), and ethanol 90% (EtOH 90%). These vehicles were chosen based on previous solubility studies, a range of polarity and pharmaceutical relevance.

Methods

A saturated amount of Bz-3 was placed in 10ml of each solvent (H_2O, Ethanol 90%, PG, LP, CO) and stirred in the dark (to maintain the stability of the sunscreen) at 24°C for 72hr. The mixtures were centrifuged at 10,000xg for 10min. This step was repeated with the resultant supernatants and the solubility of Bz-3 in each solvent was determined by HPLC assay after proper dilution of the supernatants with absolute methanol.

4.3 Results and Discussion

Solubility of Bz-3 in each vehicle studied is shown in Table 4.1.

Table –4.1 Solubility of Bz-3 in different solvents at 24 °C.

Solvents	**PG**	**Ethanol 90%**	**Coconut oil**	**H_2O**	**LP**
Solubility mg/ml	60	20	117	0.004	21
%	6	2	11.7	0.00042	2.1
Mean±SD	6.0 ± 0.6	2.0 ± 0.5	11.7 ± 0.5	0.0004 ± 0.1	2.1 ± 0.2

Bz-3 was more soluble in semipolar solvents such as coconut oil (117 mg/ml) and PG (60 mg/ml). Observed solubility decreased dramatically in polar solvents like Ethanol 90% (20mg/ml), LP (21mg/ml) and extremely polar solvents H_2O (.004mg/ml) (Table-4.1).

CHAPTER 5

Effect of vehicles on sunscreen release

5.1 Introduction

Systemic absorption of certain sunscreens has been reported. It has been found that in humans up to 2% of an applied dose of Bz-3 and its metabolites, glucuronides of 2,4-dihydroxybenzophenone and 2,3,4-trihydroxy-benzophenone, were excreted in the urine following topical application of a commercially available product over a 48hr period following a single application (Hayden et al., 1997a). The study concluded that sunscreen permeation was found to be both structure and formulation related. In *in vitro* human skin studies it was found that Bz-3 was the only sunscreen that penetrated across the skin under in-use conditions (Jiang et al., 1998b). However significant levels of all sunscreen agents studied were found to be retained in the epidermis. The amount of Bz-3 penetration across excised human skin *in vitro* at 8hr was similar to that reported for urinary excretion of Bz-3 and metabolites following a single topical application of the same products to human volunteers (0.07 gm^{-2}) (Hayden et al., 1997a). A preliminary study to assess skin tissue levels of Bz-3 using *in vivo* microdialysis further validated the *in vitro* technique and provided information regarding tissue distribution and metabolism of Bz-3 in the skin. None of the other sunscreens (OM, OC and OS) evaluated was appreciably absorbed. The above study gave an idea to do further *in vitro* studies on Bz-3. A very high Bz-3 concentration was used, and so the permeation of Bz-3 was more after 8hr.

The differences in sunscreen penetration among emulsion type formulations were demonstrated (Lazar et al., 1996). The group showed that up to 95% of sunscreens studied remained in the epidermis and that levels up to 5% diffused into the dermis. This study was done only in emulsion formulations, but previous studies showed that diffusion of sunscreen agents varied significantly with formulation (Treffel and Gabbard, 1996; Jiang et al., 1998a).

A model system was developed to investigate the effects of interactions between sunscreen and skin, sunscreen and vehicle, and vehicle and skin. The approach used was similar when penetration across an inert membrane and skin were compared in order to isolate drug-vehicle-skin interactions (Roberts and Anderson, 1975). They showed that the ratio of fluxes or permeability coefficients of phenol across polyethylene membrane and skin were independent of any effect the vehicle may have on the thermodynamic activity of the solute. In a subsequent study, using a dimethyl polysiloxane membrane, the concentration dependence of salicylic acid transport through the skin from a range of vehicles was demonstrated (Roberts and Horlock, 1978).

Before choosing a suitable model membrane for a defined drug, it is important to be aware of potential interactions that may occur between the drug and the synthetic membrane (Kowaluk et al., 1984). Such interactions may influence membrane permeability and thereby render the membrane unsuitable as a model for investigating altered skin permeability.

A previous study showed that LDPE membrane and silicone membranes absorption profiles were very high and a plasticisation effect was found with HDPE membrane, which showed greater increase in flux and Kp at concentrations above 30% (Jiang et al., 1997).

The most suitable membrane was defined as the one that least affected by either the sunscreen or the vehicle. Previous studies on polysulfone membrane show that it was least affected by the sunscreen or the vehicle. I*n vitro* release of drugs for topical dermatological drug products was evaluated (Shah et al., 1998). The purpose of this research was to evaluate different parameters that can influence *in vitro* drug release from topical dermatological drug products such as creams, gels and ointments. Experiments were designed to evaluate the influence of 1) receptor media 2) different lot numbers of synthetic membranes and 3) agitation on drug release. Among the parameters studied, the receptor medium was found to be the most important and critical variable that influenced drug release. The drug release was not influenced by agitation or by different lots of synthetic membranes. Polysulfone membrane was studied using Diprolene AF cream (DI) and Diprosone cream (DO). Both products contain the same active drug, betamethasone dipropionate, in the same concentration, 0.05%. These studies demonstrated that the polysulfone membrane yielded nearly the same release rate. These observations confirm that the characteristics of the membrane do not generally contribute to the release rate of topical dosage forms, provided that sufficient porosity of the membrane is maintained. In the present study, three lots of polysulfone membrane were used to study the *in vitro* release of betamethasone dipropionate from DI cream. Results

indicate virtually no difference in release rate from different lot numbers of the synthetic membrane. (Table-5.1)

Table- 5.1 Difference in release rate from different lots of synthetic membrane

Polysulfone Membrane Lot number	Release rate (slope) ($\mu g/cm^2/min^{0.5}$)
3080511	1.30 ±4.00
311403	1.30 ±4.79
4031705	1.32 ±3.86

Cited from Shah et al., 1998.

To establish a suitable membrane system to study drug-vehicle-skin interactions polysulfone membrane was used as a model membrane filter. The most suitable membrane was defined as the one that least affected by either the sunscreen or the vehicle. In the present study, penetration of Bz-3 through polysulfone membrane from a range of vehicles (polar to nonpolar) was investigated. Formulations chosen were LP, PG, coconut oil, Ethanol 90%, and H_2O (non polar to polar).

Saturated solutions were used through out the experiment, because it serves as the reference state in assessing thermodynamic activity within the vehicle (Higuchi, 1960). Since the activity of excess solid is assigned a value of unity, the activity of a solution estimated by Cv/S, where Cv is the concentration of a solute within the vehicle and S is the solubility of the solute in the same vehicle. The principle leads to the assumption that saturated solutions containing the same solute should have the same non interactive contribution to flux, despite differences in the solute concentration.

An increase in the activity of the solute within the vehicle will result in an increase in the penetration rate, and if it does not involve any interaction with the membrane, the solute activity in the vehicle is regarded as a non-interactive effect. Membrane specific effects involve a change in membrane properties that may be caused by both solute and vehicle. Such an effect is therefore regarded as an interactive effect. Interpretation of a simple comparison of flux values from different vehicles may be confounded by the simultaneous contribution of both effects.

It is well established that a principal driving force for diffusion across the skin is the thermodynamic activity of the solute in the donor vehicle when the vehicle has no effect on the SC barrier. This activity is reflected by the concentration of the permeant in the donor vehicle as a function of its degree of saturation within that medium. When δv approaches δi the increased solubility of a given amount of solute in a vehicle leads to reduced thermodynamic activity of the solute in the vehicle. This effect decreases the availability of the solute from the vehicle, which leads to reduction in the observed Kp. To study the non-interactive vehicle effect, numerous experiments have been conducted and the *in vitro* model with synthetic membrane is often employed.

Receptor phase 3.5% BSA in PBS was chosen based on the recovery study. 90-95% of Bz-3 was recovered from 3.5% BSA in PBS. A previous study showed that 99% of Bz-3 was recovered from 4% bovine serum albumin in PBS (Jiang et al., 1996).

5.2 Experimental design

Percutaneous absorption of Bz-3 from a range of single vehicles (polar to non polar) was investigated and the effect of sunscreen solvents on skin permeability was assessed using polysulfone membrane. A saturated concentration of Bz-3 in test solvents was employed to allow a comparison of permeation of Bz-3 under conditions of equal activity. The activity was kept constant by an infinite dose technique with occluded conditions. Permeation and retention of Bz-3 across polysulfone membrane was measured from various solvents.

5.3 Aim

To study the penetration of Bz-3 across polysulfone membrane from a range of formulations using infinite dose. Previous studies show that diffusion of sunscreen agents across epidermis vary significantly with formulation. Bz-3 release will be related to its degree of saturation in the vehicles LP, PG, CO, H_2O, and Ethanol 90%. At a given concentration, solvent which has higher solvency for the sunscreen, will lower the activity of the solute in the solvent thereby reducing the flux. Thus the solute activity will depend on the solubility of the solute in the vehicle.

5.3.1 Method

Saturated concentrations of Bz-3 were prepared in 5 vehicles (LP, PG, CO, Ethanol 90%, and H_2O). Polysulfone membrane was pre-soaked in IPM 1 hr prior to mounting between the chambers of vertical Franz-type diffusion cells. The surface diffusion area was 1.18cm^2 and the receptor chamber volume approximately 3.4ml. Saturated solutions of Bz-3 in 5 vehicles (LP, CO, Ethanol 90%, PG, and H_2O) were centrifuged and the

supernatants were placed in the donor chambers. The receptor chambers were filled with 3.5% BSA in phosphate buffered saline, pH 7.4. Diffusion cells were equilibrated at 37 ± 0.1°C in a light proof black water bath (to protect the sunscreen from photodegradation) for at least 1hr prior to vehicle application The donor chambers were sealed with silicone grease to prevent evaporation. The receptor fluids were stirred throughout with magnetic fleas and samples were taken (3.4ml) from the receptor chamber periodically and replaced (up to 2hr). Polysulfone membrane was extracted with absolute methanol (recovery >99%) by soaking the polysulfone membrane in absolute methanol for a day, and all the samples were quantified by HPLC with UV detection 315nm. All experiments were conducted in triplicate.

5.3.2 Results and Discussion

5.3.2.1 Interpretation of data

The solubility parameter of a solute, δi, is a sum of intermolecular attractive forces and has been used to predict the skin penetration of topically applied solutes (Vaughan, 1985; Waranis et al., 1987; Sloan et al., 1986; Sherertz et al., 1987). It has been suggested that solute thermodynamic activity in solution can be directly related to $(\delta i-\delta v)^2$ where δv is the solubility parameter of the vehicle (Sloan, 1992). The thermodynamic activity of a particular concentration of solute solubility in the vehicle is maximal. Membrane flux may also be a function of the relative values of δv and δm as maximum increases in flux have been found as δv approaches δm for hairless mouse skin (Sloan, 1992). Values were taken from or calculated using the one dimensional method

with δv for Ethanol 90% calculated on a mole fraction basis (Fedors, 1974; Vaughan, 1985).

Membrane flux (J, $\mu g\ cm^{-2}h^{-1}$) was assumed to be related to the permeability coefficient (Kp, $cm\ h^{-1}$) and the concentration gradient of the solute across the membrane (ΔC, $\mu g\ cm^{-3}$)

$$J_x = Kp\ \Delta C = KD\Delta C/L_x \quad (1)$$

Where x is either skin (s) or polysulfone membrane (m), and Kp is the product of the membrane/vehicle partition coefficient (K), the effective diffusion coefficient (D, diffusivity) and the membrane thickness (L). Kp values are often used to compare penetration profiles for solutes examined under different conditions and relate to a rate of diffusion of a solute within a membrane adjusted for differences in concentration. C is defined by the initial Bz-3 vehicle concentration (C_v). By definition, K_m equals C_m/C_v where C_m is the concentration of Bz-3 in the membrane. If C_m can be approximated as R_m/V_m and V_m is the volume of the membrane effectively exposed for a given application area of Bz-3, then the ratio of flux to membrane retention (J_m/R_m) defines an apparent diffusion parameter d_m of Bz-3 in the membrane.

$$d_m = J/R_m = D/V_m\ L_m \quad (2)$$

An apparent partition parameter κ_m may be defined from R_m and C_v as

$$\kappa_m = R_m/C_v \quad (3)$$

If the membrane concentration-distance profile during a steady-state penetration study is approximately linear then

$$\kappa_m = 0.5\ K_m.V_m \quad \text{..... (4)}$$

Cumulative amounts of (Bz-3)μg/ml in 5 vehicles plotted against time (hr) are presented in-Fig-5.1

5.3.2.2 Statistical Analysis

Systat (SPSS Inc, Chicago) was used to perform statistical analyses on the data. Multiple regression was done and all values were found to be significant ($P<0.05$).

5.3.2.3 Discussion

Membrane penetration and retention parameters for Bz-3 are shown in Table-5.2 with penetration - time profiles are shown in Figure-5.1. Diffusion of sunscreen agents across polysulfone membrane varied significantly with formulation. It has been found that sunscreen agents were better retained in the skin after application of an emulsion-gel than from petroleum jelly with the reservoir of the agents found mainly in the SC (Treffel and Gabard, 1996). These results show that the skin penetration of UV filters and the corresponding SPFs can be significantly modulated by product formulation. .

For the present study, the highest flux of Bz-3 across the membrane was observed with the LP vehicle followed by the Ethanol 90% and semipolar emollients coconut oil and PG. ($P<0.05$). The highest membrane retention (R_m) of Bz-3 was found following application of Bz-3 in coconut oil and Ethanol 90%. Retention was similar for LP and PG, followed by H_2O. Dm (Diffusion) depends on membrane flux and membrane retention. Dm was greater for LP, followed by Ethanol 90%, PG, coconut oil and H_2O.

Previous relationship studies between log Kp (permeability coefficient) and log k (apparent partition parameter) for Bz-3 with respect to δv (solubility parameter of the vehicle) and δi (solubility parameter of the solute) showed that log Kp decreased when δv approached δi (Bz-3 δi = 13 $(cal/cm^3)^{1/2}$) for all vehicles (LP, IPM, silicone oil, C_{12-15} BA, PG, coconut oil, and H_2O except for Ethanol 90%) (Jiang et al., 1998a). When δv approaches δi the increased solubility of a given amount of solute in a vehicle leads to a reduced thermodynamic activity of the solute in the vehicle. This effect subsequently decreases the availability of the solute from the vehicle, which leads to a reduction in the observed Kp.

In the present study the solubility of Bz-3 in LP was relatively low when compared to other vehicles like PG and coconut oil, and the flux was relatively high when compared to all the vehicles (PG, coconut oil, Ethanol 90% and H_2O). This observation may be due to the fact that δv of LP= (7.1) and the δi of Bz-3= (13) are not close to each other, which results in decrease in solubility of Bz-3, increase in thermodynamic activity and availability of Bz-3 from the vehicle (LP) and the observed Kp. In other vehicles like Ethanol 90%, Bz-3 solubility was lower than expected, and flux was high even though the δv value for Ethanol 90% = (14.9). Bz-3 was highly soluble in PG and the flux was relatively low when compared to LP. This finding may be due to the fact that δv for PG = (14), and it resulted in decrease in thermodynamic activity, increase in solubility of the solute in the vehicle and decrease in availability of solute from the vehicle and Kp value. In coconut oil, Bz-3 was more soluble than in PG, and the flux was relatively low when

compared to PG, even though δv for coconut oil was found to be 8.9. Very low solubility of Bz-3 in H_2O resulted in very low flux value. Relationship between Bz-3 flux and vehicle solubility parameter δv for polysulfone membrane is shown in Figure-5.2.

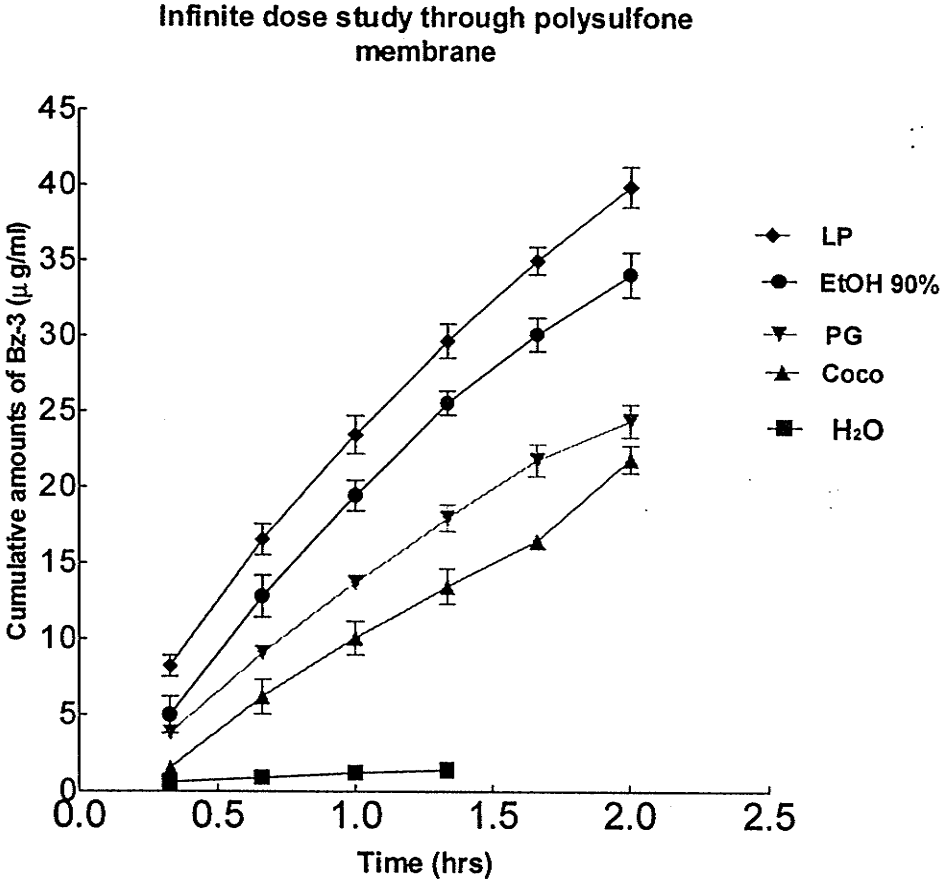
The previous study suggests that the permeability coefficient of Bz-3 from the series of vehicles studied is dominated by vehicle solubility (Jiang et al., 1998a). The amount of solute sorbed into the membrane as a consequence of vehicle uptake will be related to the solute concentration in the vehicle. A similar effect was observed when examining the effect of vehicles on the movement of phenol through epidermal and polyethylene membranes (Roberts and Anderson, 1975). Although dimethylsulfoxide (DMSO) markedly increased skin diffusivity, the overall permeability coefficient appeared to be dominated by the high solubility of phenol in the vehicle as shown by the low flux of phenol from DMSO through the inert polyethylene membrane relative to other vehicles (Roberts and Horlock, 1978).

The highest membrane retention R_m was found with application of Bz-3 vehicles like coconut oil, and R_m was similar for vehicles PG, Ethanol 90%, and LP. The trend toward higher membrane retention seems to be dependent on Bz-3 solubility (Fig –5.3). No relationship was apparent between dm and R_m (Fig 5.4). The increase in diffusivity of Bz-3 within the polysulfone membrane appeared to be related to the interaction between the membrane and the vehicles as defined by δv values. A previous study shows that d for skin was relatively constant for different δv values with the exception of Ethanol 90%, as it alters skin permeability. The optimal diffusion for the polysulfone membrane

was at δv of approximately 7.1-8.9. There was no evidence of increase in d with either Bz-3 retention in the polysulfone membrane.

Thus we can conclude that minimal penetration of sunscreens such as Bz-3 is most likely achieved by choosing vehicles with a (δv) value similar to that of the sunscreen (δi) and vehicles in which Bz-3 is more soluble.

Fig-5.1-Cumulative amounts of (Bz-3)µg/ml in 5 vehicles plotted against time (hr)



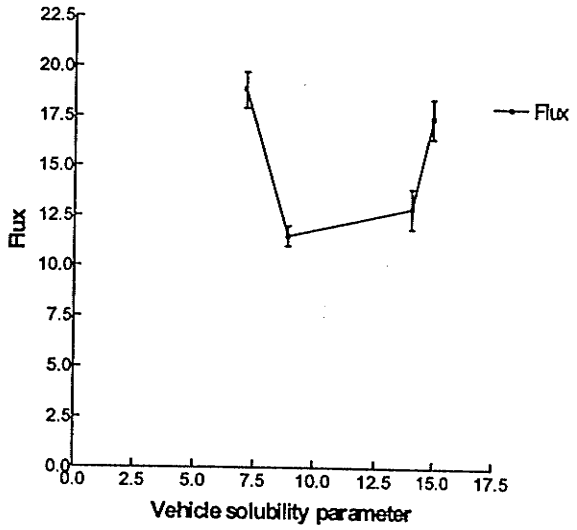
Penetration profiles of Bz-3 across polysulfone membrane. Data represents mean ± sd of 3 replicates for the polysulfone membrane.

Table-5.2-Penetration parameters of Bz-3 across polysulfone membrane from 5 solvents studied (mean ± sd of 3 replicates).

	PG	**Ethanol 90%**	**Coconut oil**	**H_20**	**LP**
Jm	12.5 ±1.0	17.4 ±1.0	11.5 ± 0.5	0.68	18.8 ± 0.9
R_m	3.5 ± 0.2	4 ± 1.5	11.2 ± 0.6	1.5 ± 0.3	3.0 ± 0.5
dm	3.5	4.3	1.0	0.45	6.2

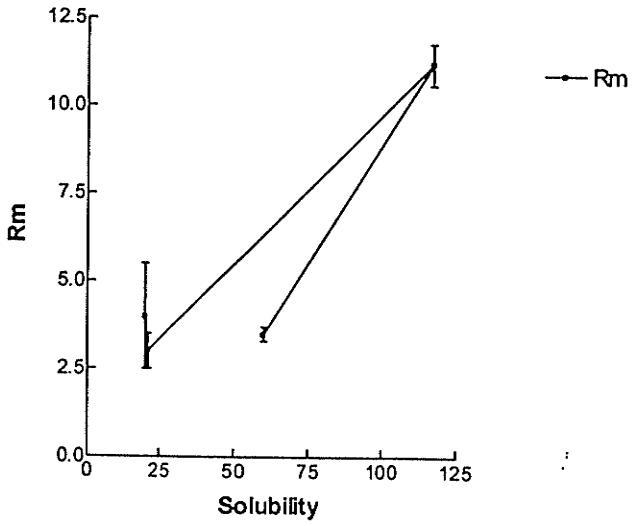
Jm = Flux ($\mu g cm^{-2} h^{-1}$), R_m = Membrane Retention, (μg), dm = Apparent Diffusion Parameter ($cm^2 h^{-1}$).

Fig-5.2. Relationship between Bz-3 flux and vehicle solubility parameter across polysulfone membrane.



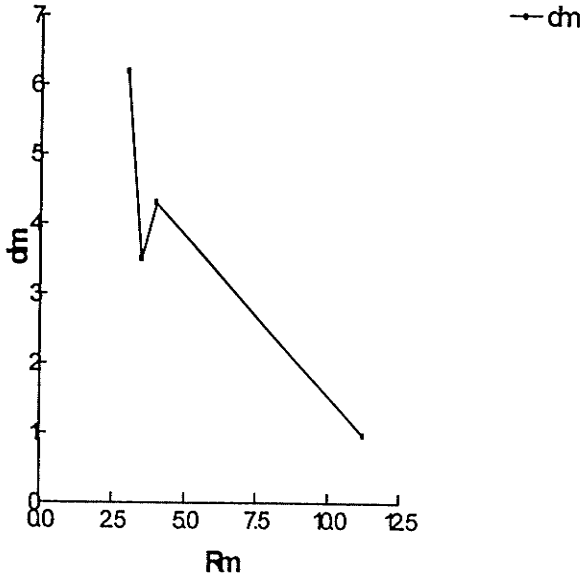
Data represents mean ± sd of 3 replicates for the polysulfone membrane.

Fig-5.3. Relationship between experimental solubility and membrane retention of Bz-3



Data represents mean ± sd of 3 replicates for the polysulfone membrane.

Fig-5.4. Relationship between diffusivity and membrane retention of Bz-3



Data represents mean ± sd of 3 replicates for the polysulfone membrane.

CHAPTER 6

Interaction between two sunscreens and its influence on solubility and release

6.1 Introduction

In practice, Bz-3 is often combined with OM and/or other sunscreens to achieve a desired SPF value and broad UV spectrum protection. It has been reported that certain sunscreens increased the rate of penetration of compounds through animal skin from various species *in vitro* (Reed and Finnin, 1997). The role of OM on permeation of Bz-3 at given concentrations (2% Bz-3 and 7.5% OM) was investigated in several emollients and both HDPE membrane and human epidermis were used to assess solvent-membrane interactions (Jiang et al., 1998a). Fluxes from the combined solvents (OM+emollient) were compared with the fluxes from each corresponding single emollient in order to estimate the extent of the solvent-membrane interactions. OM was regarded as a co-solvent in this study due to its excellent solvation ability for Bz-3. A solubility study was conducted in both combined solvents (coconut oil/OM, IPM/OM, LP/OM, and C_{12-15}BA/OM) and single solvents (coconut oil, LP, IPM, and C_{12-15} BA) for Bz-3. Solubility of Bz-3 slightly increased in LP and C_{12-15} BA, and was almost the same in cocoanut oil and IPM in the combined solvents as compared with the corresponding single solvents.

A permeation study across a HDPE membrane and skin from the sunscreen combination (2%Bz-3 and 7.5%OM) in three emollients namely LP, C_{12-15}BA and IPM was conducted with an infinite dose technique for (HDPE) and finite dose technique for

(human epidermis) (Jiang et al., 1998a). The control experiment was carried out with the emollients containing 0% OM. Bz-3 permeation from a range of concentrations of OM in LP (0%, 2%, 3.75% and 7.5%) was examined and observed fluxes were compared with the control (0% OM). The HDPE study was carried out in triplicate and the human epidermis study was carried out in six replicates. Across HDPE the permeation was in the order of LP>IPM>C_{12_15} BA, which suggests that activity of Bz-3 in these emollients primarily drove its further permeation. At a given concentration, solvent which has higher solvency for the sunscreen, will lower the activity of the solute in the solvent, thereby reducing the flux. The enhancement effect was consistently found throughout the entire range of OM concentrations studied, and the greatest was towards the lowest concentration. Bz-3 fluxes from 2% and 3.75% OM were 41 and 36 ($\mu g\ cm^{-2}$) respectively, while from 7.5% OM was 32 ($\mu g\ cm^{-2}$). For epidermis Bz-3 was the only sunscreen that penetrated across the epidermis over 8hr in the finite dose study. The release order of Bz-3 from the control solvents was found to be positively related to its degree of saturation, which again indicates that thermodynamic activity of Bz-3 in these emollients dominated its further permeation. The flux of Bz-3 was increased modestly from all solvents by the presence of OM relative to solvent alone. The extent of the increase in Bz-3 flux varies among the solvents. The reason for this increase is not clear, but may be related to the OM acting as a penetration enhancer in the epidermis.

Epidermal retention of OM was significantly higher than the retention of Bz-3 for all solvents, coconut oil> $C_{12\text{-}15}$ BA>IPM and LP. It suggests that sunscreen itself has a primary role in skin retention, which is structure related. Although Bz-3 has the lowest

thermodynamic activity in coconut oil and C_{12-15} BA (highest solubility in the emollients), which resulted in the lower fluxes at given concentration, highest skin retention observed in these vehicles suggests a potential role of formulation vehicle on skin retention of sunscreen agents. It confirms that a high membrane retention seems to be related to the solvents in which Bz-3 was most soluble. Due to the fact that OM can be blended with LP, IPM, C_{12-15} BA and coconut oil in any ratio, the varied epidermal retention of OM in different vehicles seems to be not directly related to its solubility in each solvent. Such retention might result from membrane–solvent (eg, LP, IPM, C_{12-15} BA, and coconut oil) interactions.

Table 6.1. Penetration parameters of Bz-3 across epidermis from single and combined solvents.

Penetration parameters	**Coconut oil**	**IPM**	**C_{12-15}BA**	**LP**
Flux (2%Bz-3 +7.5%OM)	0.29 ± 0.07	0.64 ± 0.25	0.32 ± 0.06	0.41 ± 0.16
Control	0.08 ± 0.01	0.25 ± 0.01	0.12 ± 0.01	0.32 ± 0.12
R_m	7.9 ± 2.6	4.0 ± 0.2	7.3 ± 2.1	3.2 ± 0.9
Control	4.2 ± 1.9	3.6 ± 1.2	4.1 ± 0.9	6.5 ± 1.4
d	0.037	0.16	0.044	0.12
Control	0.02	0.07	0.03	0.05

Unit: J = μg, cm^{-2}; R_m = μg; d = cm^{-2}

(Cited from Jiang et al., 1998a)

Penetration parameters of Bz-3 from single and combined solvents have been shown in Table 6.1. Thermodynamic activity was the primary factor that controls Bz-3 release from vehicle for the HDPE membrane. However, skin permeation of Bz-3 was significantly altered by skin-solvent interaction by showing a different rank of permeation as compared to HDPE membrane. It was found that cocoanut oil and C_{12-15}BA are desirable sunscreen emollients due to their greater solvency property, which reduced Bz-3 activity, thereby lowering Bz-3 release. While they decreased diffusivity of Bz-3 within the skin, the desired high skin retention was achieved.

Permeation of Bz-3 and OM from hydroalcoholic and diisopropyl adipate model formulations, when present individually and in combination at 10hr was studied (Gupta et al., 1999). In all cases, for comparable formulations, penetration of Bz-3 exceeded that of OM. The opposite was true when comparisons of SC retention were made; OM quantities in SC exceeded those of Bz-3. Bz-3 retention by SC was significantly greater when this sunscreen was present in combination with OM than with no other sunscreen present. This trend was not observed for OM. The penetration of OM was greater when it was alone than when it was in combination, only in the hydroalcoholic vehicle. The ratios in hydroalcoholic formulations containing the sunscreens were present alone in the formulation. There were no statistically significant differences between the ratios when the sunscreens were present alone and in combination in the diisopropyl adipate formulations.

The greater SC retention of OM coupled with its decreased penetration may be explained on the basis of the relative partition-coefficient values of two sunscreens. OM is the more lipophilic sunscreen, with a log octanol-water partition coefficient of 5.65 (compared to a value of 2.63 for Bz-3). The higher amounts of Bz-3 in receptor fluids agree with the *in vivo* percutaneous absorption studies in rats (Okereke et al., 1993). The greater skin permeation of both sunscreens from hydroalcoholic vehicle is ascribed to an increase in their activity on the skin surface as a result of solvent evaporation and lower solubility in the vehicle. This finding should be compared to the persistence of diisopropyl adipate on the skin surface, and the affinity of this vehicle seems unlikely due to the short contact time prior to evaporation.

The increase in retention to penetration ratio noted, when sunscreens were combined rather than utilized separately, particularly in hydroalcoholic formulations, is of great practical importance. The ratio of SC retention to the amount penetrated was chosen as the index of skin distribution of sunscreen. The higher this ratio, the better the skin distribution in terms of the function of sunscreen agents. To intercept UV radiation, sunscreen molecules must be present either on the skin surface or in the SC. Certainly, penetration to the viable tissues and beyond represents a loss from the desired deposition site and is counterproductive. Material on top of the skin may be subjected to removal by rubbing or contact with water. Ideally, the sunscreens would be bound to the SC, particularly its outer section, so as to immobilize them near the skin surface. The reason for this phenomenon is not known, but may be related to affinity of the two sunscreens. (Carpenter et al., 1996). With all these considerations, the above ratio was chosen. The

difference in the ratio between combinations and single sunscreens was greater for Bz-3 than OM. Therefore permeation and SC retention were formulation dependent. The ratio of SC content to the amount penetrated is a useful tool for evaluating sunscreen permeation. The ratios were higher when sunscreens were presented to the skin as a mixture rather than as formulations containing a single sunscreen. The above study was very useful in designing the combination studies. The reason for the increase in permeation was not known when sunscreens were combined. A wide range of vehicles was also not chosen.

6.2 Aim

To study the effect of addition of sunscreen on solubility Bz-3/OM in 4 vehicles at 24°C.

6.2.1 Methods

Saturated concentrations of Bz-3 were combined with 5% OM and 5% BM and placed in solvents like LP, Ethanol 90%, coconut oil, and PG. The mixtures were stirred in the dark at 24°C over 72hr. After centrifugation for 10min, (2000 rpm) the resultant supernatants were diluted with methanol and solubility of Bz-3 in the combined solvents was quantified by HPLC assay.

6.2.2 Results

Solubility of Bz-3 increased significantly in LP in the presence of OM ($P<0.05$). There was no increase in solubility of Bz-3 in Ethanol 90%, PG, and coconut oil in the presence of OM and BM (Table 6.2).

6.2.3 Statistical Analysis

E views, was used to perform statistical analysis on the data. ANOVA (analysis of variance) on data showed significant differences among observations. Bz-3 solubility in the combined solvents LP/OM was found to be significantly increased, ($P<0.05$) when compared to the single solvent LP. There was no significant increase in solubility in other combined solvents (Ethanol 90% + OM, PG + BM, LP + BM) (Table 6.2).

6.2.4 Discussion

Bz-3 solubility with OM increased significantly in LP and with BM the increase was not significant in LP and PG. Solubility of Bz-3 increased in LP, in the presence of OM. Previous studies show that Bz-3, when combined with OM, permeates more (Gupta et al., 1999). The release of Bz-3 is positively related to its degree of saturation (Jiang et al., 1998a). Even though the solubility increased in the presence of OM, the solubility of Bz-3 in LP is relatively less when compared to vehicles like coconut oil and PG ($P<0.05$).

Minimum penetration is more likely achieved by choosing vehicles in which Bz-3 is more soluble, and by choosing vehicles, that have δv values close to that of the δi value of sunscreen. The vehicle solubility parameters δi of LP = 7.1, Ethanol 90% = 14.9, PG = 14.0, and Bz-3 =13. The increase in solubility of a sunscreen in the presence of another sunscreen may depend on the type of formulation, concentration of sunscreens added, and also on the type of sunscreen added (lipophilic or hydrophilic). Therefore, 3 vehicles (LP, PG and Ethanol 90%) were chosen for the penetration study of Bz-3 from combined solvents (Bz-3+OM and Bz-3+BM).

Table 6.2- Solubility of Bz-3/OM and Bz-3/BM in 4 vehicles at 24°C (mean ± sd of 3 replicates).

Sunscreen	Coconut oil	LP	Ethanol 90%	PG
Bz-3+OM (Combined)	11.2 ±1.7	3.3 ± 0.5	2.7 ± 0.2	6.2 ± 0.1
Bz-3 (Single)	11.7 ± 0.5	2.1 ± 0.2	2.0 ± 0.5	6.0 ± 0.6
Bz-3+BM (Combined)	11.4 ±1.2	2.4 ± 0.1	1.9 ± 0.9	6.3 ± 1.3
Bz-3 (Single)	11.7 ± 0.5	2.1 ± 0.2	2.0 ± 0.5	6.0 ± 0.6

6.3 Penetration study-Combined solvents

6.3.1 Aim

To study the penetration of Bz-3 in combined solvents in the vehicles LP, Ethanol 90% and PG.

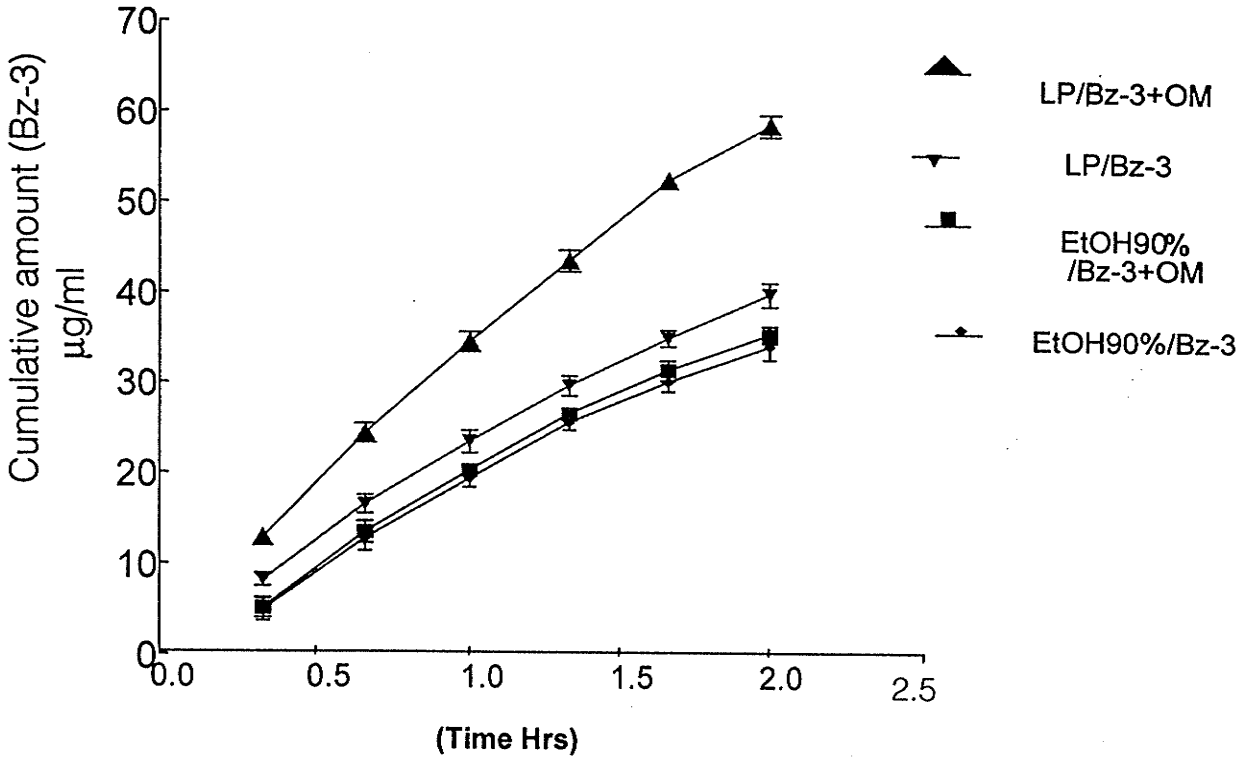
6.3.2 Methods

Saturated concentrations of Bz-3 were combined with 5%OM and 5%BM separately and placed in vehicles like LP, PG and Ethanol 90%. Polysulfone membrane was pre-soaked in IPM 1 hr prior to mounting between the chambers of vertical Franz-type diffusion cells. The surface diffusion area was 1.18cm^2 and the receptor chamber volume approximately 3.4ml. The solutions were centrifuged and the supernatants were placed in the donor chambers. The receptor chambers were filled with 3.5% BSA in phosphate buffered saline, pH 7.4. Diffusion cells were equilibrated at 37 ± 0.1°C in a light proof black water bath (to protect the sunscreen from photodegradation) for at least 1hr prior to vehicle application. The donor chambers were sealed with silicone grease to prevent evaporation. The receptor fluids were stirred throughout with magnetic fleas, and samples (3.4ml) were taken from the receptor chamber and replaced periodically (up to 4hr). Polysulfone membrane was extracted twice with methanol (recovery >99%) and all the samples were quantified by HPLC with UV detection. All experiments were conducted in triplicate.

6.3.3 Results

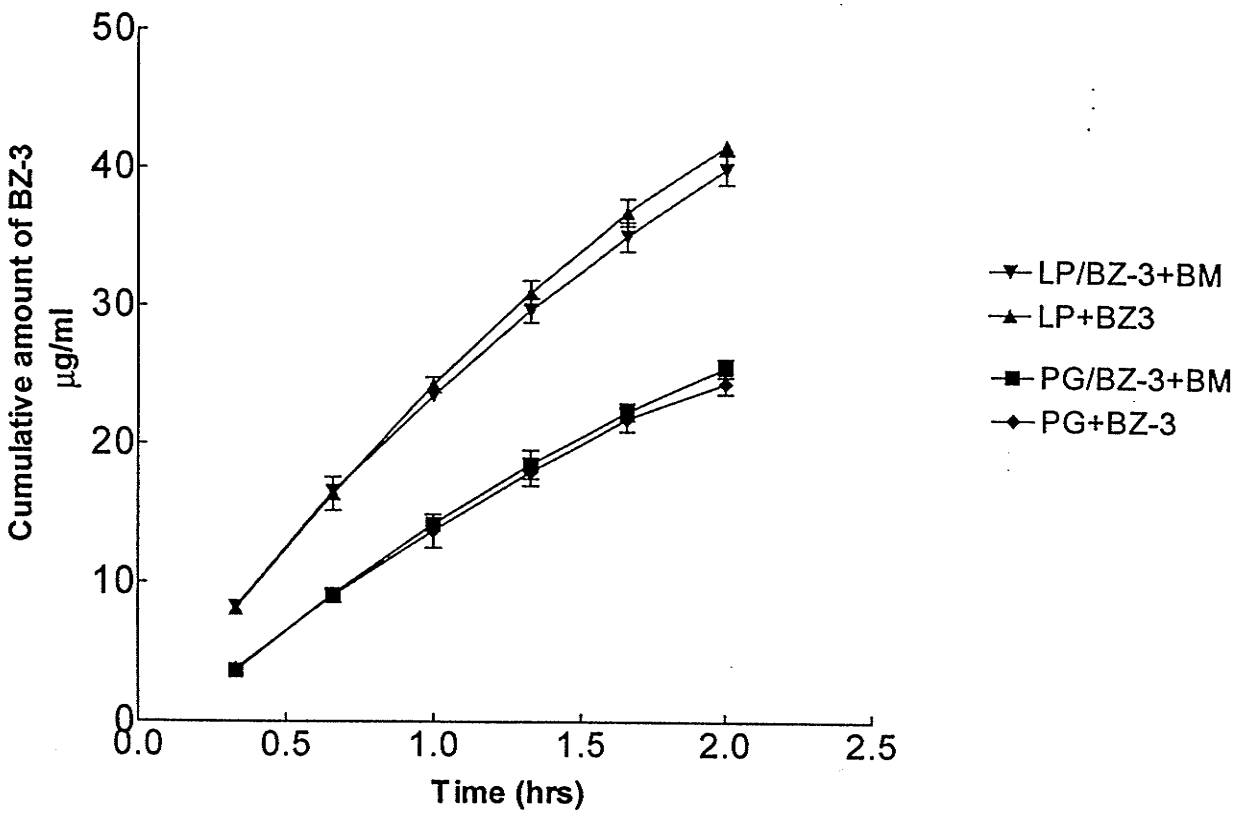
Cumulative amounts of Bz-3 (μg/ml) plotted against time (hr) shown in Figs 6.1 and 6.2. Penetration parameters of Bz-3 in the presence of OM and BM summarized in Tables 6.3 and 6.4.

Fig-6.1. Cumulative amounts of (Bz-3)μg/ml in 2 vehicles plotted against time (hr).



Penetration profiles of Bz-3 across polysulfone membrane. Data represents mean ± sd of 3 replicates for the polysulfone membrane.

Fig-6.2. Cumulative amounts of (Bz-3)µg/ml in 2 vehicles plotted against time (hr).



Penetration profiles of Bz-3 across polysulfone membrane. Data represents mean ± sd of 3 replicates for the polysulfone membrane.

Table-6.3. Penetration parameters of Bz-3/OM and across polysulfone membrane from two solvents studied (mean ± sd of 3 replicates).

Solvent	Flux Bz-3	Flux OM	R_m Bz-3	R_m OM	dm Bz-3	dm OM
LP(Bz-3+ OM)	27.4 ± 1.3	19.6 ± 0.2	4.1 ± 0.1	14.5 ±1.6	6.7	1.4
LP(Bz-3)	18.8 ± 0.9	-	3.0 ± 0.5	-	6.2	
Ethanol 90%(Bz-3 +OM)	19.0 ± 0.1	12.3 ± 0.1	4.2 ± 1.8	12.5 ± 0.5	4.5	0.9
Ethanol 90% (Bz-3)	17.4 ±1.0	-	4.0 ±1.5	-	4.3	-

Table-6.4. Penetration parameters of Bz-3/BM across polysulfone membrane from two solvents studied (mean ± sd of 3 replicates).

Solvent	Flux Bz-3	Flux BM	R_m Bz-3	R_m BM	dm Bz-3	dm BM
LP(Bz-3 +BM)	20.1 ± 1.5	4.8 ± 0.2	3.1 ± 0.9	6.0 ± 0.7	6.5	0.8
LP(Bz-3)	18.8 ± 0.9	-	3.0 ± 0.5	-	6.2	-
PG(Bz-3 +BM)	14.0 ±1.3	9.1 ± 0.1	2.9 ± 0.8	2.2 ±1.2	4.8	4.1
PG(Bz-3)	12.5 ±1.0	-	3.5 ± 0.2	-	3.6	-

6.3.4 Statistical analysis

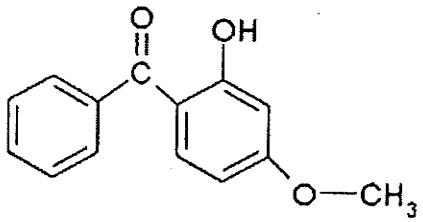
Systat (SPSS Inc, Chicago) was used to perform statistical analyses on the data. Multiple regression on data showed significant increase in flux for (Bz-3+5%OM) in LP (liquid paraffin) vehicle ($P<0.05$) when compared to the single solvent (LP). There was no significant increase in flux for other combined solvents (Ethanol 90% + 5%OM), (LP + 5%BM), and (PG + 5%BM).

6.4 Discussion

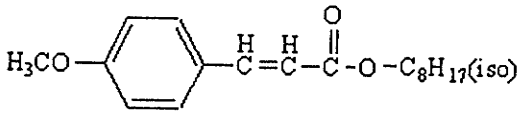
The release of Bz-3 was positively related to its degree of saturation, with LP>Ethanol 90%>PG>coconut oil. It suggests that activity of Bz-3 in these emollients primarily drove its permeation. At a given concentration, the solvent, which has higher solvency for the sunscreen, will lower the activity of the solute in the solvent, thereby reducing the flux (Table 6.3).

In the case of the solubility study for the combination of sunscreens Bz-3+OM, the solubility study was done first in 4 vehicles (LP, PG, Ethanol 90%, and coconut oil) in the presence of 5% OM. Bz-3 solubility increased significantly in LP ($P<0.05$) and no increase was found in Ethanol 90%. It was most likely due to hydrogen bond formation between OM and Bz-3.

Bz-3



OM



Hydrogen bond formation between the hydroxyl group of BZ-3 and the carboxyl group of OM molecules of OM and Bz-3, could result in an increase in solubility of Bz-3 in LP vehicle. The increase was observed only in LP, which was the least polar vehicle investigated.

The solubility of Bz-3 in Ethanol 90% is supposed to be high, because of solubility parameter values. However the actual solubility of Bz-3 in the aqueous Ethanol 90% was lower than expected. There was no significant increase in the solubility of Bz-3 in Ethanol 90% even though OM was added to it. Previous studies show that Ethanol 90% is a deviant relationship of log KP and log k (apparent partition parameter) to δv for the various vehicles used in the infinite dose study (Jiang et al., 1998a).

LP, PG and Ethanol 90% were chosen for the diffusion study across polysulfone membrane. Previous studies as well as the present study (infinite dose) show that Bz-3 permeation depends on its solubility in the vehicles. Bz-3 permeation across polysulfone membrane was significantly increased by OM in the LP vehicle ($P<0.05$). This was due to the significant increase in solubility of Bz-3 in the presence of OM thereby increasing the thermodynamic activity of Bz-3 available for diffusion across the membrane. No significant enhancing effect was found on release from ethanol 90%. The greatest fluxes were also found to be associated with highest diffusivity of the sunscreen in the membrane. The flux of Bz-3 in Ethanol 90%/OM was less than the flux in LP/OM ($P<0.05$), even though the solubility of Bz-3 in Ethanol 90%/OM was less than the

solubility in LP/OM ($P<0.05$). Ethanol 90% ($\delta v=14.9$) has a theoretical solubility parameter around that of the solute used in the present study, Bz-3, ($\delta i=13$).

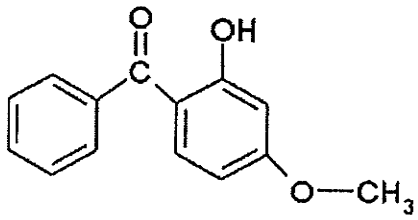
Membrane retention of OM was significantly higher than the retention of Bz-3 for both the solvents studied (LP and Ethanol 90%) ($P<0.05$). It suggests that sunscreen itself has a primary role on skin retention, which is structure related. Bz-3 retention was higher for Ethanol 90% than LP (refer to data and p value), and the result was consistent with the infinite dose study. A previous infinite dose study for Bz-3 showed that although Bz-3 has the lowest thermodynamic activity in the single solvents coconut oil and PG (high solubility), which resulted in the lower fluxes at given concentration (Jiang et al., 1998a). The highest skin retention observed in these vehicles suggests a potential role of formulation vehicle on skin retention of these agents, except for Ethanol 90%, but the present study confirms that higher membrane retention seems not to be related directly to its solubility in (Ethanol 90%, LP), and it might have resulted from membrane-solvent interaction.

Before the diffusion study of Bz-3 in the combined solvents (Bz-3+ 5%BM) was done, solubility of Bz-3 was determined in 4 vehicles (LP, PG, Ethanol 90%, and coconut oil) in the presence of BM. Solubility of Bz-3 was not increased in 2 vehicles LP and PG. These two vehicles were chosen for the diffusion study, even though the increase was not significant. Again the release of Bz-3 from the combined solvents (LP/BM and PG/BM) related to its degree of saturation, LP>PG (Table 6.4). There was no increase in flux in

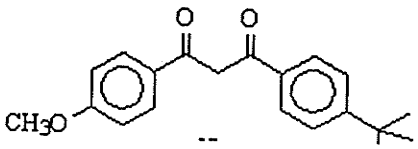
both the solvents in the presence of BM when compared to the single solvents (absence of BM).

The solubility of Bz-3 was not significantly influenced in the presence of BM. Hydrogen bond formation between Bz-3 and BM is unlikely.

Bz-3



BM



BM is a highly lipophilic sunscreen. In anhydrous conditions, in the presence of a strong base, the CH group of BM can produce its own proton, which can increase the basic nature of BM and the interaction effect between Bz-3 and BM. Since Bz-3 is a weak acid, the CH group of BM cannot produce its own proton and increase its basic nature. Therefore hydrogen bond formation between the molecules of Bz-3 and BM is unlikely, and the addition of BM had no effect on Bz-3 solubility in all the vehicles.

No interaction effect was observed between Bz-3 and BM, and so no significant increase in Bz-3 flux was observed in all the vehicles.

Effect of solvents on OM and BM flux.

In the presence of Bz-3, high flux values were observed for OM in the vehicles LP, PG and Ethanol 90% and the values were relatively different. High flux values may be due to the formation of hydrogen bonds between the molecules of OM and Bz-3 and low

solubility of BM in the vehicles. A diffusion study for OM and BM (single sunscreen) should be done in a range of solvents to compare the flux values with the combined sunscreens.

The ratio of sunscreen SC retention to the amount penetrated was used as an index of skin distribution of sunscreen agents (Gupta et al., 1999). The higher the ratio, the better the skin distribution in terms of the function of sunscreen agents. The reason for this is unknown, but may be due to mutual affinity of the two sunscreens (Carpenter et al., 1996). The interaction between the two sunscreens could be different for different sunscreens. The difference in the ratio between combinations and single sunscreens were greater for Bz-3 than OM (Gupta et al., 1999). This finding is very important because sunscreens are active within the SC (Treffel and Gabard, 1996). Therefore, combining different sunscreens in a single preparation not only widens the UV absorption spectrum but may also enhance efficiency. The greater SC retention of OM coupled with its decreased penetration may also be explained on the basis of the relative partition-coefficient values of two sunscreens. OM is the more lipophilic sunscreen, with a log octanol-water partition coefficient of 5.65 (compared to a value of 2.63 for Bz-3) (Gupta et al., 1999).

The increase in penetration of a sunscreen in the presence of another sunscreen may depend on the structure of sunscreens, type of sunscreen added (lipophilic or hydrophilic), vehicle formulation in which the sunscreen agent is least soluble, a vehicle formulation such that the solubility parameter of the vehicle (δv) is sufficiently different

to the solubility parameter of the sunscreen (δi), and log octanol-water partition coefficients of the sunscreens.

Conclusion

Thermodynamic activity was the primary factor that controlled Bz-3 release from the vehicles across polysulfone membrane. Coconut oil and PG are desirable sunscreen vehicles due to their great solvency property. These two vehicles lowered the Bz-3 release by reducing the Bz-3 activity. δi values for coconut oil and PG are 8.9 and 14, and they are close to δi of Bz-3 = (13). Minimum penetration is more likely achieved by choosing vehicles in which Bz-3 is more soluble, and by choosing vehicles, that have δv values close to that of the δi value of sunscreen.

CHAPTER 7

7.1 Conclusions

A rapid, simple HPLC assay was validated and described in chapter 3. The sunscreen agents could be simultaneously quantified by the assay with adequate resolution and precision. This assay was successfully employed throughout the research for quantifying sunscreen agents in BSA. Excellent linearity was obtained over the range of 0.2 –6μg /ml for Bz-3, OM and BM. The intra and inter-day precisions of the assay summarized in (Table-3.4, 3.5, 3.6). There was no significant difference between day-day analysis. Intra and inter-day coefficients of variation (CV%) of the assay for the 3 sunscreen agents were below 10%. The minimum detectable limits calculated as greater than three times the baseline noise and the lower limits of quantitation calculated as greater than 10 times, the baseline noise. A range of 90-100% recovery for the 3 sunscreen agents was observed at 2.5 μg/ml. The coefficients of variance calculated from 3 replicates were all less than 5%.

The membrane diffusion methodology is described in chapter 5. As the sunscreen agents were found to be unstable in ambient light, all studies were carried out in a "blacked out" water bath. Polysulfone membrane was employed as the diffusion membrane. 3.5% BSA in PBS was used in the receptor phase. Solute-vehicle-membrane interactions were examined using polysulfone membrane. The relationship between penetration (experimental) and solubility (theoretical) parameters was assessed in an attempt to predict the most appropriate sunscreen agents and vehicle formulations for sunscreen protection in practice.

The highest flux of Bz-3 across the membrane was observed with the LP vehicle followed by the Ethanol 90% and semipolar emollients coconut oil and PG ($P<0.05$). The highest membrane retention (R_m) of Bz-3 was found following application of Bz-3 in cocoanut oil and Ethanol 90%. Retention was similar for LP and PG, followed by H_2O. Dm (diffusion) depends on membrane flux and membrane retention. Dm was greater for LP, followed by Ethanol 90%, PG, coconut oil and H_2O.

The results show that membrane penetration of sunscreen agents can be significantly modulated by product formulation. *In vitro*, liquid paraffin formulation led to a greater penetration over time across polysulfone membrane than other vehicles (PG, coconut oil, Ethanol 90%, H_2O). LP vehicle appeared to favour diffusion through the skin. Penetration enhancement may be explained by changes occurring in the formulation after application, such as evaporation, acting directly on the thermodynamic activity of the UV filters. The minimal penetration of sunscreen such as Bz-3 is most likely achieved by choosing vehicles in which Bz-3 is more soluble and vehicles with a δv value similar to that of the sunscreen δi.

The permeation of Bz-3 across polysulfone membrane from single solvents and combined solvents at a fixed concentration was compared in chapter-7. The release order of Bz-3 from each single solvent was found positively related to its degree of saturation, suggesting that the thermodynamic activity of the solute in the solvent defines the permeation of Bz-3.

When Bz-3 was combined with 5% OM, a significant increase in penetration of Bz-3 was found from LP vehicle ($P<0.05$). No increase was demonstrated in other vehicles (Ethanol 90%, and PG) Thus the penetration of Bz-3 depends on the degree of saturation in the vehicles and vehicle solubility parameters. OM acted as a penetration enhancer when it was combined with Bz-3 in LP. Membrane retention of OM was found significantly higher than the retention of Bz-3 for both the solvents studied. It suggests that sunscreen itself has a primary role of skin retention which is structure related. Bz-3 retention was higher for Ethanol 90% than LP, and the result was consistent with the infinite dose study. Thus the release of Bz-3 from the vehicles depends on product formulation and the vehicle solubility parameters of the vehicles as well as the sunscreen.

7.2 Summary

1. Significant amounts of Bz-3 penetrated across polysulfone membrane.
2. Bz-3 permeation across polysulfone membrane was found to be associated with the solubility of Bz-3 in the vehicles.
3. Highest flux was seen for LP followed by Ethanol 90% for all the studies.
4. Bz-3 permeation across polysulfone membrane was significantly enhanced by OM in the LP vehicle.
5. Minimal penetration of Bz-3 is most likely achieved by choosing a vehicle with a δv value similar to that if the sunscreen δi and by choosing vehicles in which Bz-3 is more soluble.

Bibliography

Abdel Nabi IM, Kadry AM, Davis RA et al. Development and validation of a high performance liquid chromatography method for the determination of benzophenone-3 in rats. J Appl Toxicol 1992; 12: 255-359.

Agarpidis-Paloympis LE, Nash RA, Shaath NA. The effect of solvents on the ultraviolet absorbance of sunscreens. J Soc Cosmet Chem 1987; 38: 209-221.

Anderson FA. Final report on the safety assessment of urocanic acid (review). J Am Coll Toxicol 1995; Oct.14 (5): 386-423.

Anderson MW, Hewitt JP, Spruce SR. Broad spectrum physical sunscreen: titaniumdioxide and zinc oxide. In Sunscreens: development, evaluation, and regulatory aspects, Second Edition, eds Lowe NJ, Shaath NA and Pathak MA, New York: Mercel Dekker, Inc., 1997: 353-397.

Anderson RL, Cassidy JM. Variations in physical dimensions and chemical composition of human stratum corneum. J Invest Dermatol 1973; 61: 30-32.

Anon. Formulators fine tune TiO_2-based screens. Manuf Chem 1993; July: 26-29.

Ashton P, Walters KA, Brain KR et al. Surfactant effects in percutaneous absorption Part I. Effects on the transdermal flux of methyl nicotinate. Int J Pharm 1992; 87: 261-264.

Aungst BJ. Structure/effect studies of fatty acids isomers as skin penetration enhancers and skin irritants. Pharm Res 1989; 6: 244-247.

Aungst BJ. Fatty acids as skin permeation enhancers. In percutaneous penetration enhancers, eds Smith EW and Maibach HI, Boca Raton: CRC Press Inc., 1995: 277-287.

Aungst BJ, Rogers NJ, Shefter E. Enhancement of naloxone penetration through human skin *in vitro* using fatty acids, fatty alcohols, surfactants, sulfoxides and amides. Int J Pharm 1986; 33: 225-234.

Barry BW. Basic principles of diffusion through membranes. In Dermatological Formulations, New York: Mercel Dekker Inc., 1983: 49-94.

Barry BW. Mode of action of penetration enhancers in human skin. J Controlled Release 1987; 6: 85-97.

Barry BW, Brace AR. Permeation of oestrone, oestrodiol, oestriol and dexamethasone across cellulose acetate membrane. J Pharm Pharmacol 1977; 29: 394-400.

Barry BW, Harrison SM, Dugard PH. Correlation of thermodynamic activity and vapour diffusion through human skin for the model compound, benzyl alcohol. J. Pharm Pharmacol 1985; 37(2): 84-90.

Beastall JC, Hadgraft J, Washington C. Mechanism of action of azone as a percutaneous penetration enhancer: lipid bilayer fluidity and transition temperature effects. Int J Pharm 1988; 43 (3): 204-207.

Benech-Kieffer F, Wegrich V. Percutaneous Absorption of Sunscreens *in vitro*; Interspecies Comparison, Skin models and Reproducibility Aspects. Skin Pharmacol Appl Skin Physiol 2000; 13: 324-335.

Berger RS, Mezick JA, Papa CM. Design and evaluation of a water-resistant sunscreen preparation. J Soc Cosmet Chem 1978; 29: 641-649.

Berner B, Liu P. Alcohols. In Percutaneous Penetration Enhancers, eds Smith EW, Maibach HI, Boca Raton, FL: CRC Press, Inc., 1995: 45-60.

Bettley FR. The influence of soap on the permeability of epidermis permeability. Br J Dermatol 1961; 73: 448-454.

Bettley FR. The influence of detergents and surfactants on epidermal permeability. Br J Dermatol 1965; 77: 98-100.

Black HS. Antioxidants and carotenoids as potential photoprotectants. In Sunscreens: development, evaluation, and regulatory aspects, eds Lowe NJ, Shaath NA, New York: Mercel Dekker, Inc., 1990: 267-278.

Black HS, Douglas DR. Formation of a carcinogen of natural origin in the etiology of ultraviolet light -induced carcinogenesis. Cancer Res 1973; 33 (9): 2094-2096.

Blank IH. Factors which influence the water content of the stratum corneum. J Invest Dermatol 1952; 18: 145-173.

Black JG. Interaction between anionic surfactants and skin. In Pharmaceutical skin penetration enhancement, eds Walters KA, Hadgraft J, New York: Marcel Dekker, Inc., 1993, Vol. 59: 145-173.

Bouwstra JA, Gooris GS. X-ray analysis of the stratum corneum and its lipids. In mechanisms of transdermal drug delivery, eds Potts RO, Guy RH, Redwood City: Cygnus, Inc., 1997, Vol.83: 41-85.

Brain KR, Green DM, James VJ et al. Preliminary evaluation of novel penetration retarders. In prediction of percutaneous penetration, eds Brain KR, James VJ, Walters KA, Cardiff: STS, 1995a, Vol. 4a: 38.

Brain KR, Walters KA. Molecular modeling of skin permeation enhancement, eds Walters KA, Hadgraft J, New York: Mercel Dekker, Inc., 1993: 389-416.

Brain KR, Walters KA, James VJ et al. Percutaneous penetration of dimethylnitrosamine through human skin *in vitro*: application from cosmetic vehicles. Fd Chem Toxic 1995b; 33 (4): 315-322.

Brinon L, Geiger S. Percutaneous absorption of sunscreens from liquid crystalline phases. Journal of Controlled Release 1999; 60: 67-76.

Bronaugh RL, Collier SW. Preparation of human and animal skin. In *in vitro* Percutaneous absorption: principles, fundamentals, and applications, eds Bronaugh RL, Maibach HI, Boca Raton: CRC Press, Inc., 1991: 1-6.

Bronaugh RL, Stewart RF. Methods for *in vitro* percutaneous absorption studies 3: hydrophobic compounds. J Pharm Sci 1984; 73 (9): 1255-1258.

Brown H. Sunscreens need vehicles. Cosmet & Toiletries 1986; 101: 51-54.

Brown BE, Diemdeck W, Hoppe U et al. Fate of topical hydrocarbons in the skin. J Soc Cosmet Chem 1995; 46: 1-9.

BruzeM, Gruvberger B, Thulin I. PABA, benzocaine, and other PABA esters in sunscreens and after sun products. Photodermatol Photoimmunol Photomed 1990; 7(3): 106-108.

Buescher LS. Sunscreens and Photoprotection. Otolaryngol Clin North Am 1993; 26 (1) 13-22.

Cappel MJ, Kreuter J. Effect of nonionic surfactants on transdermal drug delivery. Part 2. Poloxamer and poloxamine surfactants. Int J Pharm 1991; 69: 155-167.

Carpenter T, Howe V, Orfanelli J. Protection from sun protectors. Drug Cosmet Ind 1996; 158: 56-103.

Chattaraj SC, Walker RB. Penetration enhancer classification. In Percutaneuous Penetration Enhancers, eds smith EW, Maibach HI, Boca Raton: CRC Press, Inc., 1995: 5-20.

Chein YW, XU H, Chiang CC et al. Transdermal controlled administration of indomethacin. I. Enhancement of skin permeability. Pharm Res 1988; 5: 103-106.

Collier S, Bronaugh R. Receptor fluids. In *in vitro* percutaneuous absorption principles, fundamentals, and applications, eds Bronaugh RL, Maibach HI, Boca Raton CRC press, INC., 1991: 31-49.

Cooper ER, Marritt EW, Smith RL. Effect of fatty acids and alcohols on the permeation of acyclovir across human skin *in vitro*. J Pharm Sci 1985; 74: 688-689.

Creasey NH, Battensby J, Fletcher JA. Factors affecting the permeability of skin. The relation between *in vivo* and *in vitro* observations. Curr Probl Dermatol 1978; 7: 95-106.

Dahms GH. Choosing emollients and emulsifiers for sunscreen products. Cosmet & Toiletries 1994; 109: 45-52.

De Fine Olivarius F, Wulf HC, Crosby J et al. The sunscreening effect of urocanic acid. Photodermatol Photoimmuno delivery system. Pharm Acta Helv 1996; 68: 215-219.

Derry J.E, Maclean W.M. A study of percutaneous absorption from topically applied zinc oxide oinment. J Parentr Enteral Nutr 1983; Vol 7 (2): 131-135.

Dunkel VC, San RH, Harbell JW et al. Evaluation of the mutagenicity of an N-nitroso contaminant of the sunscreen Padimate O: N-nitroso-N-methyl-p-aminobenzoic acid, 2-ethylhexyl ester (NPABAO). Environ Mol Mutagen 1992; 20(3): 188-198.

Dupuis D, Rougier A, Roguest R et al. The measurement of the stratum corneum reservoir: a simple method to predict the influence of vehicles on *in vitro* percutaneous absorption. Br J Dermatol 1986; 115: 233-238.

Elfbaum SG, Laden K. The effect of dimethyl sulfoxide on percutaneous absorption; a mechanisitc study, Part-1. J Soc Cosmet 1968; 19: 841-847.

English JSC, White IR, Cronin E. Sensitivity to sunscreens. Contact Dermatitis 1987; 17: 159-162.

Epstein JH. Biological effect of sunlight. In Sunscreens: development, evaluation, and regulatory aspects, eds Lowe NJ, Shaath NA, New York: Mercel Dekker, Inc., 1990: 43-54.

Farmer KC, Naylor MF. Sun exposure, sunscreens and skin cancer prevention: a year round concern. Ann Pharmcother 1996; 30(6): 662-673.

Fedors RF. A method for estimating both the solubility parameters and molar volumes of liquids. Polym Eng Sci 1974; 14: 147-154.

Fernandez C, Marti-Mestres G. LC analysis of benzophenone-3: 11 application to determination of *"in vitro"* and *"in vivo"* skin penetration from solvents, coarse and submicron emulsions. J Pharm Biomed Ana 2000; 24(2000): 155-165.

Fincher TK, Yoo SD, Michniak BB. Percutaneous penetration of dideoxycytidine through hairless mouse skin using novel penetration enhancers. In perspectives in Percutaneous

Penetration, eds Brain KR, James VJ, Walters KA, Cardiff: STS Publishing, 1997, Vol. 5a: 81.

Fitzpatrick TB, Sober AJ. Sunlight and skin cancer. New Eng J Med 1985; 25: 818-819.

Florence AT, Attwood D. Physicochemical principles of Pharmacy, Second Edition, London: Macmillan Academic and Professional Ltd, 1988: 425, 205.

Flynn GL. Mechanism of percutaneous absorption from physico chemical evidence. In Percutaneuous absorption. eds Bronaugh RL, Maibach HI, New York; Mercel Dekker, Inc., 1985: 17-42.

Flynn GL, Roseman TJ. Membrane diffusion 2: Influence of physical absorption on molecular flux through heterogeneous dimethylpolysiloxane barriers. J Pharm Sci 1971; 60: 1788-1796.

Flynn GL, Yalkowsky SH, Roseman TJ. Mass transport phenomenon and models: theoretical concepts. J Pharm Sci 1974; 63: 479-509.

Franz TJ. Percutaneous Absorption. On the evidence of *in vitro* data. J Invest Dermatol 1975; 64: 190-195.

Franz TJ, Lehman PA, Kagy MK. Diethylsulfoxide. In Percutaneuous Penetration Enhancers, eds Smith EW, Maibach HI, Boca Raton: CRC press, Inc., 1995: 115-127.

Frei B, Kim MC, Ames BN. Ubiquinol-10 is an effective lipid-soluble antioxidant at physiological concentrations. Proc Natl Acad Sci USA 1990; 87: 4879-4883.

French EJ, Pouton CW, Walters KA. Mechanisms and prediction of nonionic surfactant effects on skin permeability. In Pharmaceutical skin penetration enhancement, eds Walters KA, Hadgraft J, New York: Mercel Dekker, Inc., 1993, Vol.59: 113-143.

Gange RW, Soparkar A, Matzinger E et al. Efficacy of a sunscreen containing butyl methoxydibenzoylmethane against ultraviolet. A radiation in photosensitized subjects. J Am Acad Dermatol 1986; 15: 494-499.

Givaudan-Roure. Parsol MCX: the effective non-PABA UV-B filter. Givaudan-Roure Corp, 1992.

Givaudan-Roure. Parsol 1789: the safe and effective UV-A filter. Givaudan-Roure Corp, 1993.

Golden GM, Mckie JE, Potts RO. Role of stratum corneum lipid fluidity in transdermal drug flux. J Pharm Sci 1987; 76(1): 25-28.

Green A, Beardmore G, Hart V et al. Skin cancer in a Queensland population. J Am Acad Dermatol 1988a; 19(6): 1045-1052.

Green PG, Guy RH, Hadgraft J. *In vitro* and *in vivo* enhancement of skin permeation with oleic and lauric acids. Int J pharm 1988b; 48:103-112.

Gupta VK, Zatz JL, Rerek M et al. Percutaneous Absorption of Sunscreens through Micro-Yucatan PigSkin *In Vitro*; Pharm Res 1999; Vol 16(10): 1602-1607.

Guth J, Martino G, Pasapane J et al. Multifunctionality in skin care with a new film forming polymer. HAPPI 1991; May: 80-84.

Guy RH. Current status and future prospects of transdermal drug delivery. Pharm Res 1996; 13 (12): 1765-1769.

Guy RH, Hadgraft J. Selection of drug candidates for transdermal drug delivery. In Transdermal Drug Delivery, eds Hadgraft J, Guy RH, New York; Mercel Dekker, Inc., 1989: 59-81.

Haarmann N, Reimer M. Neo Heliopan sunscreen filters. H&R product information 1993: 2-3.

Hadgraft J. Skin penetration enhancement. In Prediction of Percutaneous Penetration eds Brain KR, James VJ, Walters KA, Cardiff: STS, 1993, Vol. 3b: 138-148.

Hadgraft J, Williams DG. Azone: mechanisms of action and clinical effect. In Pharmaceutical Skin penetration enhancement, eds Walters KA, Hadgraft J, New York: Mercel Dekker, Inc., 1993, Vol. 59: 175-197.

Hagedorn-Leweke U, Lippold BC. Absorption of Sunscreens and other compounds through human skin *in vivo*: derivation of a method to predict maximum fluxes. Pharm Res 1995; 12: 1354-1360.

Harrison JE, Watkinson AC, Green DM et al. The relative effect of Azone and Transcutol on permeant diffusivity and solubility in human stratum corneum. Pharm Res 1996; 13(4): 542-546.

Hayden CGJ, Roberts MS, Benson HAE. Systemic absorption of sunscreen after topical application. The Lancet 1997a; 350(9081): 863-864.

Hayden CGJ, Roberts MS, Benson HAE. Sunscreens-Toxicological aspects. In Dermal Absorption and Toxicity Assessment, eds Roberts MS, Walters KA, New York: Mercel Dekker, Inc., 1997b; 80: 90-95.

Hemker W. Universal oil-in-water polyelectrolyte emulsifiers for advanced cosmetic product formulation. Seifen Ole Fette Washse 1990; 116: 505-508.

Higuchi T. Physical chemical analysis of percutaneous absorption process from creams and ointments. J Soc Cosmet Chem 1960; 11: 85-97.

Houk J, Guy RH. Membrane models for skin penetration studies. Chem Rev 1988; 88(3): 455-470.

Hurks HMH, Out-luiting C, Van-Der-Molen RG et al. Differential suppressions of the human mixed epidermal cell lymphocyte reaction (MECLR) and mixed lymphocyte reaction (MLR) by cis-urocanic acid. Photochem Photobiol 1997; 65 (4): 616-621.

Jass HE. The sunscreen industry in the United States: past, present, and future. In Sunscreens: development, evaluation, and regulatory aspects, eds Lowe NJ, Shaath NA, New York: Mercel Dekker, Inc., 1990: 149-159.

Jellinek JS. Formulation and function of cosmetics. New York: John Wiley&Sons, Inc., 1970: 25-27.

Jiang R, Benson H.A.E et al. *In vitro* Human Epidermal and Polyethylene Membrane Penetration and Retention of the sunscreen Bz-3 from a range of solvents. J Pharm Res 1998a; 15(12): 1863-1868.

Jiang R, Hayden CGJ, Prankerd RJ et al. High performance liquid chromatographic assay for common sunscreening agents in cosmetic products, bovine serum albumin solution and human plasma. J Chromatogr B 1996; 682:137-145.

Jiang R, Roberts MS, Collins DM et al. Absorption of sunscreens across human skin: an evaluation of commercial products for children and adults. Br J Clin Pharmacol 1998b; 48: 635-637.

Jiang R, Roberts MS, Prankerd RJ et al. Percutaneous absorption of sunscreen agents from liquid paraffin: self association of octyl salicylate and effects on skin flux. J Pharm Sci 1997; 86(7): 791-796.

Johnson JA, Fusaro RM. Protection against long ultraviolet radiation: topical browning agents and new outlook. Dermatologica 1987; 175: 53-57.

Kimura K, Katoh T. Photoallergic contact dermatitis from the sunscreen ethylhexyl-p-methoxycinnamate (Parsol MCX) Contact Dermatitis 1995; 32(5): 34-35.

Klein K. Formulating effective yet elegant sunscreen products DCI 1989; August: 22-30; 78-79.

Klein K. Sunscreen formulation: So what's new? Cosmet & Toilet 1990a; 105: 91-94.

Klein K. Formulating sunscreen products. In Sunscreens: development, evaluation, and regulatory aspects, eds Lowe NJ and Shaath, New York: Mercel Dekker, Inc., 1990b: 253-266.

Klein K. Encyclopedia of UV absorbers for sunscreen products. Cosmet&Toiletries 1992; 107: 45-63.

Klein K. Sunscreen Products: Formulation and regulatory considerations. In Sunscreens: development, evaluation, and regulatory aspects, Second Edition, eds Lowe NJ, Shaath NA, Pathak MA, New York: Mercel Dekker, Inc., 1997: 285-311.

Kowaluk EA, Roberts MS, Polack AE. Dynamics of colmethiazoleedisylate interaction with plastic infusion systems. J Pharm Sci 1984; 73(1): 43-47.

Lalor CB, Flynn GL, Weiner N. Formulation factors affecting release of drug from topical formulations 1. Effect of emulsion type upon *in vitro* delivery of ethyl P-aminobenzoate. J Pharm Sci 1994; 83 (11): 1525-1528.

Lalor CB, Flynn GL, Weiner N. Formulation factors affecting release of drug from topical formulations 2. Effect of solubility on *in vitro* delivery of a series of n-alkyl p-aminobenzoates. J Pharm Sci 1995; 84 (6): 673-676.

Lavker RM, Gerberick GF, Veres D et al. Cumulative effects from repeated exposures to suberythmal doses of UVB and UVA in human skin. J Am Acad Dermatol 1995; 32(1): 53-62.

Lazar MG, Naillet A, Fructus AE et al. Evaluation of *in vitro* percutaneous absorption of UV filters used in sunscreen formulations. DCI 1996; May: 50-62.

Levine N, Griego RD. Tanning promoters and sunscreens. In pigmentation and pigmentory disorders, eds Levine N, Raton B, FLA: CRC press, 1993: 467-489.

Lizawa O, Kato T, Tagami H et al. Long term follow up study of changes in liquidperoxide levels and the activity of superoxide dismutase, catalase and glutathione peroxidase in mouse skin after acute and chronic UV irradiation. Arch Dermatol Res 1994; 286 (1): 47-52.

Lopez A, Pellett MA, Llinares F. et al. The enhancer effect of several phenyl alcohols on percutaneous penetration of 5-flurouracil. Pharm Res 1997; 14(5): 681-685.

Lorenzetti OJ. Propylene glycol gel vehicles. Cutis 1979; 23: 747-750.

Louise E, Agrapidis-Paloympis LE. The effect of solvents on the ultraviolet absorbance of sunscreens. J. Soc Cosmet Chem. 1987; 38: 209-221.

Luftman DB, Lowe NJ, Moy RL. Sunscreens: update and review. Dermatol Surg Oncol 1991; 17: 744-746.

Maclennan R, Green AL, Mcleod GRC et al. Increasing incidence of cutaneous melanoma in queensland, Australia. J Natl Can Inst 1992; 84 (18): 1427-1432.

Macleod TM, Frain-Bell W. A study of physical light screening agents. Br J Dermatol 1975; 92: 149-156.

Macpherson SE, Scott RC, Williams FM. Percutaneous absorption and metabolism of aldrin by rat skin in diffusion cells. Arch Toxicol 1991; 65 (7): 599-602.

Makki S, Muret P, Bassignot P et al. Percutaneous absorption of three psoralens commonly used in therapy. Effect of skin occlusion *in vitro* study. In prediction of percutaneous penetration, eds Brain KR, James VJ, Walters KA, Cardiff: STS Publishing Ltd, 1995, Vol 4a: C107.

Marieb E. The integumentory system. In Human anatomy and physiology, Second Edition, California. The Benjamin/ Cummings Publishing Company, Inc., 1992: 138-156.

Meadows T. The effect of various sunscreen combinations on a product's SPF value. J Soc Cosmet Chem 1990; 41: 141-146.

Meyer TA, Powell JB. Quantitation of the nitrosamine 2 ethylhexyl-4-(N-Methyl-N-nitrosoamino) benzoate (NPABAO) in sunscreen product. J Assoc Off Anal Chem 1991; 74 (5): 766-771.

Michaels AS, Chandrasekaran SK, Shaw JE. Drug permeation through human skin: theory and *in vitro* experiment measurement. Am Inst Chem Eng 1975; 21: 985-996.

Miselnicky SR, Lichtin L, Sakr A. The influence of solubility, protein binding, and percutaneous absorption on reservoir formation in skin. J Soc Cosmet Chem 1988; 39: 169-177.

Mollgaard B, Hoelgaard A. Vehicle effect on topical drug delivery. I. Influence of glycols and drug concentrations on skin transport. Acta Pharm Suec 1983; 20: 433-450.

Monash S. Location of the superficial epithelial barrier to skin penetration. J Invest Dermatol 1957; 29: 367-376.

Naik A, Guy RH. Infrared spectroscopic and differential scanning calorimetric investigations of the stratum corneum barrier functions. In mechanisms of transdermal drug delivery, eds Potts RO and Guy RH, Redwood City: Cygnus, Inc., 1997, Vol 83: 87-162.

Nannipieri E, Carelli V, Dicolo G et al. Vehicle influence on the permeation of a highly lipophilic molecule. An *in vitro* technique to evaluate skin-vehicle interactions. Int J Cosmet Sci 1990; 12: 21-31.

Natow AJ. Sunscreens. Cutis 1986; 38: 157-158.

Nishi J, Ogura R, Suigiyama M et al. Involvement of active oxygen in liquid peroxide radical reaction of epidermal homogenate following ultraviolet light exposure. J Invest Dermatol 1991; 97: 115-119.

Norval M, Gibbs, NK, Gilmour J. The role of urocanic acid in UV-induced immunosuppresion: recent advances (1992-1994). Photochem Photobiol 1995; 62(2): 209-217.

O'Donoghue MN. Sunscreens: the ultimate cosmetic. Dermatol Clin 1991; 9: 99-104.

Okereke CS, Barat SA, Abdelrahman MS. Safety evaluation of benzophenone-3 after dermal administration in rats. Toxicol Lett 1995 Oct; 80 (1-3): 61-67.

Okereke CS, Kadry AM, Abdelrahman MS et al. Metabolism of benzophenone-3 in rats. Drug Metab Dispos 1993; Sep-Oct; 21 (5): 788-791.

Oteri R, Johnson S, Dastis S. A new waterproofing agent for sunscreen products Cosmet & Toiletries 1987; 102:107-109.

Patel NP, Highton A, RL Moy. Properties of topical sunscreen formulations: a review. J Dermatol Surg Oncol 1992; 18: 316-320.

Pathak MA. Sunscreens and their use in the preventive treatment of sunlight induced skin damage. J Dermatol Surg Oncol 1987; 13(7): 739-750.

Pathak MA. Intrinsic photoprotection in human skin. In sunscreens: development, evaluation, and regulatory aspects, eds Lowe NJ, Shaath NA, New York: Mercel Dekker, Inc., 1990: 73-83.

Pathak MA. Photoprotection against harmful effects of solar UVB and UVA radiation: an update. In sunscreens: development, evaluation, and regulatory aspects. Second edition, eds Lowe NJ, Shaath NA, Pathak MA, New York: Mercel Dekker, Inc., 1997: 59-79.

Pathak MA, Robins P. A response to concerns about sunscreens: a report from the skin cancer foundation. J Dermatol Surg Oncol 1989; 15(5): 486-487.

Pathak MA, Stratton K. Free radicals in human skin before and after exposure to light Arch Biochem Biophys 1968; 123: 468-476.

Portnoy B. The effect of formulation on the clinical response to topical fluocinolone acetonide. Br J. Dermatol 1965; 77: 579-581.

Potts RD. Physical characterization of the stratum corneum: the relationship of mechanical and barrier properties to lipid and protein structure. In Transdermal drug delivery, eds Hadgraft J, Guy RH, New York: Mercel Dekker, Inc., 1989, Vol, 35: 23-57.

Potard G, Laugel C. Quantitative HPLC analysis of sunscreens and caffeine during *in vitro* percutaneous penetration studies. Inter Journ of Pharm.1999; 189: 249-260.

Potard G, Laugel C. The Stripping Technique: *In Vitro* Absorption and Penetration of Five UV Filters on Excised Fresh Human skin. Skin Pharm Appl skin Physiol. 2000; 13: 336-344.

Ramsey JD, Woolen BH, Auton TR et al. The predictive accuracy of *in vitro* measurements for the dermal absorption of a lipophilic penetrant (fluazifop butyl) through rat and human skin. Fundam Appl Toxicol 1994; 23(2): 230-236.

Reed BL, Finnin BC. Discovery, evaluation and development of a new class of safe penetration enhancers. In Perspectives of Percutaneous Penetration, eds Brain KR, James VJ, Walters KA, Cardiff: STS Publishing, 1997, Vol 5a: 74.

Roberts MS, Anderson RA. The percutaneous absorption of phenolic compounds: the effect of vehicles on the penetration of phenol. J. Pharm Pharmac 1975; 27: 599-605.

Roberts MS, Anderson RA, Swarbrick J. Permeability of human epidermis to phenolic compounds. J Pharm Pharmac 1977; 29:677-683.

Roberts MS, Horlock E. Effect of repeated skin application on percutaneous absorption of salicylic acid. J Pharm Sci 1978; 67(12): 1685-1687.

Roberts MS, Walker M. The most natural penetration enhancer. In Pharmaceutical skin penetration enhancement, eds Walters KA, Hadgraft J, New York: Mercel Dekker, Inc., 1993, Vol 59: 1-30.

Roelandts R, Vanhee J, Bonamie A et al. A survey of ultraviolet absorbers in commercially available sun products. Int J Dermatol 1983; 22: 247-255.

Roy SD, Ross E, Sharma K. Transdermal delivery of bupernophine through cadaver skin. J Pharm Sci. 1994; 83(2): 126-130.

Ruddy SB. Surfactants. In Percutaneous Penetration Enhancers, eds Smith EW, Maibach HI, BocaRaton: CRC Press, Inc., 1995: 245-257.

Rundel RD, Nachtwey DS. Skin cancer and ultraviolet radiation. Photochem Photobiol. 1978; 28: 345-356.

Runger TM, Epe B, Moller K. Repair of ultraviolet A and singlet oxygen-induced DNA damage in xeroderma pigmentosum cells. J invest Dermatol 1995; 104(1): 68-73.

Sato K, Sugibayashi K, Morimoto Y. Effect and mode of action of aliphatic esters on *in vitro* skin permeation of nicroandil. Int J Pharm 1988; 43: 31-40.

Saunal K, Illel B, Mary F. Stratum corneum reservoir methodology: a predictive model for penetration enhancer evaluation. In prediction of percutaneous penetration: methods measurements, modeling, eds scott RC. Guy RH, Hadgraft J, Bodde HE, London: IBC Technical Services, 1991, Vol 2: 288-300.

Sayre RM. Physical sunscreens. J Soc Cosmet Chem 1990; 41: 103-109.

Schaefer H, Redelmeier TE. Skin barrier: principles of percutaneous absorption. Basel: S Karger AG, 1996.

Scheuplein RJ. Mechanism of percutaneous absorption 1. Route of penetration and influence of solubility. J Invest Dermatol 1965; 45: 334-345.

Scheuplein RJ. Analysis of permeability data for the case of parallel diffusion pathways. Biophys J 1966; 6 (1): 1-17.

Scheuplein RJ. Mechanism of percutaneous absorption 11. Transient diffusion and the relative importance of various routes of skin penetration. J Invest Dermatol 1967; 48 (1): 79-88.

Scheuplein RJ, Blank IH. Permeability of the skin. Physiol Rev 1971; 51: 702-747.

Scheuplein RJ, Blank IH. Mechanism of percutaneous absorption IV. Penetration of nonelectrolytes (alcohols) from aqueous solutions and from pure liquids. J Invest Dermatol 1973; 60(5): 286-296.

Scheuplein RJ, Morgan LJ. "Bound water" in keratin membranes measured by a microbalance technique. Nature 1967; 214 (29): 456-458.

Scheuplein RJ, Ross LW. Effects of surfactants and solvents on the permeability of the epidermis. J Soc Cosmet Chem 1970; 22: 853-873.

Scheuplein RJ, Ross LW. Mechanism of percutaneous absorption V. Percutaneous absorption of solvent deposited solids. J Invest Dermatol. 1974; 62: 353-360.

Schneider IM, Dobner B, Neubert R et al. Evaluation of drug penetration in to human skin *ex vivo* using branched fatty acids and propylene glycol. Int J Pharm 1996; 145: 187-196.

Schwack W, Rudolph T. Photochemistry of dibenzoyl methane UVA filters: part-1. J Photochem Photobiol B 1995; 28(3): 229-234.

Scott RC, Ramsey JD. Comparison of the *in vivo* and *in vitro* percutaneous absorption of a lipophilic molecule (Cypermethrin, a pyrethroid insecticide). J Invest Dermatol 1987; 89 (2): 142-146.

Secard DL. Acrylic emulsifying and stabilizing polymers. Cosmet & Toiletries 1984; 99: 73-76.

Shaath NA. On theory of ultraviolet absorption by sunscreen chemicals. J Soc Cosmetic Chem 1987; 82: 193-207.

Shaath NA. Stability and efficacy of UV filters. Seifen Ole Fette Wachse 1991; 117: 45-46.

Shaath NA. Evolution of modern sunscreen chemicals. In Sunscreens; development evaluation, and regulatory aspects, Second Edition, eds, Lowe NJ, Shaath NA, Pathak MA, New York: Mercel Dekker, Inc., 1997: 3-33.

Shaath NA, Worldwide K, Vernon M et al. Photodegradation of sunscreen chemicals: solvent considerations. Cosmetic & Toiletries 1990; 105: 41-44.

Shah VP, Elkins JS, Williams RL et al. Evaluation of the test system Used for *In Vitro* Release of Drugs for Topical Dermatological Drug Products. Pharm Dev and Tech 1998; 4(3): 377-385.

Sheretz EF, Sloan KB, Mc Tieman RG. Use of theoretical partition coefficients determined from solubility parameters to predict permeability coefficients for 5-flurouracil. J Invest Dermatol 1987; 89(2): 147-151.

Sheth NV, Freeman DJ, Higuchi WI et al. The influence of azone, propylene glycol and polyethylene glycol on in vitro skin penetration of triflurothymidine. Int J Pharm 1986; 28: 201-209.

Sloan KB. Use of solubility parameters from regular solution theory to describe partitioning-driven processes. In Prodrugs, ed KB Sloan, New York; Mercel Dekker Inc., 1992, Vol. 53: 179-204.

Sloan KB, Koch SAM, Siver KG, Flowers FP. Use of solubility parameters of drug and vehicle to predict flux through skin. J Invest Dermatol 1986; 87 (2): 244-252.

Sober AJ. Solar exposure in the etiology of cutaneous melanoma. Photo Dermatol 1987; 4: 23-31.

Stoughton RB. Dimethylsulfoxide (DMSO) induction of a steroid reservoir in human skin. Arch Dermatol 1965; 91: 657-660.

Stoughton RB, Fritsch W. Influence of dimethylsulfoxide (DMSO) on human percutaneous absorption. Arch Dermatol 1964; 90: 512-517.

Stoughton RB, Mcclure WO. Azone: a new non toxic enhancer of cutaneous penetration. Drug Dev Ind Pharm 1983; 9: 512-517.

Sundaram C, Koster W, Schallreuter KU. The effect of UV radiation and sun blockers on free radical defense in human and guinea pig epidermis. Arch Dermatol Res 1990; 282 (8): 526-531.

Suzuki T. Tsutsumi H, Ishidai A. Secondary droplet emulsion: mechanism and effects of liquid crystal formation in o/w emulsions. Dispersion Sci Technol 1984; 5(2): 119-141.

Szczurko C, Dompmartin A, Michael M et al. Photocontact allergy to Benzophenone-3: ten years of experience. Photodermatol Photoimmunol Photomed 1994; Aug10 (4): 144-147.

Tan HSI, Moseley SE, Leon J et al. Assay of mixture of padimate -O and oxybenzone (Bz-3) in sunscreen formulation by HPLC. J Chromatogr A 1984; 291 (18): 275-282.

Tanaka H, Okada T, Konishi H et al. The effect of reactive oxygen species on the biosythesis of collagen and glycosaminoglycans in cultured human dermal fibroblasts. Arch Dermatol Res 1993; 285 (6): 352-355.

Thune P. Contact and photocontact allergy to sunscreens. Photodermatol 1984; 1: 5-9.

Treffel P, Gabard B. Skin penetration and sun protection factor of ultraviolet filters from two vehicles. Pharm Res 1996; 13(5): 770-774.

Treherne JE. Permeability of skin to some non - electrolytes. J Physiol 1956; 133: 171-180.

Turner NG, Nonato LB. Visualization of stratum corneum and transdermal permeation pathways. In Mechanisms of transdermal drug delivery, eds Potts RO, Guy RH, Redwood City: Cygnus, Inc., 1997, Vol.83: 1-40.

Van Henegouwen GMJB, Junginger EH, De Vries. Hydrolysis of RRR-a-tocopheryl acetate (Vitamin E acetate) in the skin and its UV protecting activity (an *in vivo* study with the rat). J Photochem Photobiol B 1995; 29: 45-51.

Vaughan CD. Using solubility paramters in cosmetics formulation. J Soc Cosmet Chem 1985; 36: 319-333.

Vickers CFH. Existence of reservoir in the stratum corneum . Arch Dermatol 1963; 88: 21-23.

Walters KA. Penetration enhancers and their use in transdermal therapeutic systems. In transdermal drug delivery, eds Hadgraft J, Guy RH. New York: Mercel Dekker, Inc., 1989, Vol 35: 197-246.

Walters KA, Bailik W, Brain KR. Effects of surfactants on penetration across the skin. Int J Cosmet Sci 1993; 15(6): 260-270.

Walters KA, Brain KR, Howes D. Percutaneous Penetration of Octyl salicylate from representative Sunscreen Formulations through Human Skin *In Vitro*. Food and Chem Toxi 1997; 35: 1219-1255.

Waranis RP, Siver KG, Sloan KB. The solubility parameter of vehicles as a predictor of relative vehicle effect on the diffusion of 6-mercaptopurine. Int J Pharm 1987; 36(2-3): 211-222.

Watkinson AC, Brian KR, Walters KA et al. Prediction of the percutaneous penetration

of ultra-violet filters used in sunscreen formulations. Int J Cosmetic Sci 1992; 14: 265-275.

Wertz PW, Downing DT. Stratum corneum: biological and biochemical considerations. In transdermal drug delivery, eds Hadgraft J, Guy RH, New York: Mercel Dekker, Inc., 1989, Vol 35: 1-22.

Wester RC, Maibach HI. Penetration enhancement by skin hydration. In Percutaneous penetration enhancers, eds Smith EW, Maibach HI, Boca Raton: CRC Press, Inc., 1995: 21-28.

Wiechers JW, de Zeeuw RA. Prodrugs and penetration enhancement: implications for dermal metabolism. In prediction of percutaneous penetration: methods, measurements, modelling, eds Scott RC, Guy RH, Hadgraft J, Bodde HE, London: IBC Technical Services, 1991, Vol.2b: 2259-2269.

Wurster DE, Kramer SF. Investigations of some factors influencing percutaneous absorption. J Pharm Sci 1961; 50: 288-293.

Zatz JL. Modification of skin permeation by solvents. Cosmet & Toiletries 1991a; 106: 91-98.

Zatz JL. Assessment of vehicle factors influencing percutaneous absorption. In *in vitro* percutaneous absorption: principles, fundamentals, and applications, eds Bronaugh RL, Maibach HI, Boca Raton: CRC press, Inc., 1991b: 51-66.

Zatz JL. Simulation studies of skin permeation. J Soc Cosmet Chem 1992; 43: 37-48.

Zesh A, Schaefer H. *In vitro*-penetration of radiolabelled hydrocortisone in various vehicles in human skin. Arch Derm Forsch 1973; 246: 335-354.