PERCUTANEOUS ABSORPTION OF CYCLIZINE AND ITS ALKYL ANALOGUES

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"Winning is not the first thing, but it beats anything that comes second."

&

"Who Passed You The Ball when You Scored"

Anonymous

GO MMA LE PAPA

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Abstract

Percutaneous absorption of cyclizine and its alkyl analogues

Percutaneous delivery of drugs promises many advantages over oral or intravenous administration, such as a better control of blood levels, a reduced incidence of systemic toxicity, an absence of hepatic first-pass metabolism, better patient compliance, etc. However, the dermal drug transport is limited by the unsuitable physicochemical properties of most drugs and the efficient barrier function of the skin. Thus, numerous attempts have been reported to improve topical absorption of drugs, concentrating mainly on the barrier function of the stratum corneum by use of penetration enhancers and/or skin warming. An alternative and interesting possibility for improved dermal permeability is the synthesis of derivatives or analogues with the aim of changing the physicochemical properties in favour of skin permeation, efficacy and therapeutic value.

Cyclizine (**I**) is an anti-emetic drug primarily indicated for the prophylaxis and treatment of nausea and vomiting associated with motion sickness, post operation and Menière's disease. It acts both on the emetic trigger zone and by damping the labyrinthine sensitivity. Pharmacologically it has anti-histaminic, antiserotonergic, local anaesthetic and vagolytic actions. It is widely used and also suitable for children from six year of age. Percutaneous absorption of (**I**) can, among others, avoid the "first-pass" effect and the discomfort of injection.

The main objective of this study was to explore the feasibility of percutaneous absorption of (**I**) and its alkyl analogues *via* physicochemical characterization and assessment of their permeation parameters. The intent was also to establish a correlation between the physicochemical properties of these compounds and their percutaneous rate of absorption. To achieve these objectives, the study was undertaken by synthesizing the alkyl analogues and determining the physicochemical parameters relevant to skin transport. Identification and level of purity for the prepared analogues were confirmed by mass spectrometry (MS), nuclear magnetic resonance (NMR) spectrometry and infrared (IR) spectrometry. Experimental aqueous solubility (25 °C & 32 °C) and partition coefficient for each compound were determined. *In vitro* permeation studies were performed at pH 7.4, using Franz diffusion cells with human epidermal

membranes. Diffusion experiments were conducted over a period of 24 hours maintaining a constant temperature (37 °C) by means of water bath. All samples were analysed by high pressure liquid chromatography (HPLC).

Cyclizine (**I**) has a methyl group at N-4. Increasing the alkyl chain length on N-4 of the piperazine ring resulted in compounds with lower melting points and higher water solubility than (**I**). (**II**) exhibited 3-fold increase in water solubility, followed by (**IV**) with about 2.5 fold increase. The water solubility of (**III**) was almost the same as that of (**I**). Log partition coefficients increased linearly with increasing alkyl chain length. The analogues therefore, possessed more favourable physicochemical properties to be delivered percutaneously. Indeed, the *in vitro* skin permeation data proved that these analogues could be delivered more easily than (**I**) itself. The flux of (**I**) was 0.132 $\mu g/cm^2/h$ in a saturated aqueous solution. Compound (**II**) resulted in a 53-fold (6.952 $\mu g/cm^2/h$) increase in permeation compared to (**I**). (**III**) and (**IV**) resulted in a 2- and 5-fold enhancement of permeation respectively.

Based on the results of the study, it seems that increased aqueous solubility and low level of crystallinity play a vital role in optimizing percutaneous absorption of (**I**) and its alkyl analogues. But the importance of the effect of increased lipophilicity cannot be ignored. The low percutaneous absorption of (**I**) might be attributed to its low aqueous solubility and increased crystallinity, as is evident from the higher melting point than the analogues. From all the permeability data using aqueous solutions, it is clear that compound (**II**) is the best permeant of this series and in addition it is known that this compound antagonizes the effects of histamine.

Key Words:	Percutaneous absorption; cyclizine; alkyl analogues; physicochemical properties; aqueous solubility; partition coefficient; melting point

Opsomming

Perkutane absorpsie van siklisien en alkielanaloë daarvan

Die perkutane aflewering van geneesmiddels hou heelwat voordele in bo orale of intraveneuse toediening, waaronder beter beheer oor bloedvlakke, laer voorkoms van sistemiese toksisiteit, geen eerstedeurgangsmetabolisme deur die lewer nie, beter pasiëntmeewerksaamheid, ens. Transdermale deurgang van geneesmiddels word egter deur die ongunstige fisies-chemiese eienskappe van die meeste middels en die effektiewe versperring deur die vel verhinder. Talle pogings is dus aangewend om die topikale absorpsie van geneesmiddels te verbeter en dit het hoofsaaklik gefokus op die versperrende funksie van die startum corneum deur gebruik van penetrasieverbeteraars en/of verwarming van die vel. 'n Alternatiewe en interessante moontlikheid vir die verbetering van dermale deurlaatbaarheid, is die sintese van derivate of analoë met die doel om fisies-chemiese eienskappe gunstig vir deurgang deur die vel, vir effektiwiteit en terapeutiese waarde te kry.

Siklisien (I) is 'n anti-emetiese middel wat hoofsaaklik aangedui is vir die profilakse en behandeling van naarheid en braking vanweë bewegingsiekte, na operasies en vanweë Meniére se siekte. Dit werk op sowel die chemo-emetiese snellerarea en deur die sensitiwiteit van die labirint te onderdruk. Farmakologies het dit antihistamien-, antiserotinergiese, lokaalverdowende en vagolitiese aktiwiteit. Dit word algemeen gebruik en is ook geskik vir kinders vanaf ses jaar oud. Die perkutane absorpsie van (I) kan onder meer die eerstedeurgangeffek en die ongemak van 'n inspuiting voorkom.

Die hoofdoel van hierdie studie was om die geskiktheid van perkutane absorpsie van (I) en sy alkielanaloë te ondersoek *via* karakterisering van fisies-chemiese eienskappe en beoordeling van perkutane deurgang van hierdie verbindings. Die oogmerk was om 'n korrelasie te vind tussen die fisies-chemiese eienskappe en tempo van perkutane absorpsie. Ten einde hierdie doelstellings te bereik, is die alkielanaloë gesintetiseer en die fisies-chemiese eienskappe, soos van belang vir transport deur die vel, bepaal. Die strukture van die gesintetiseerde verbindings asook hulle suiwerheid is met behulp van fisiese tegnieke, waaronder massaspektrometrie (MS), kernmagnetieseresonansie-spektrometrie (KMR) en infrarooispektrometrie (IR), bepaal. Die wateroplosbaarheid

(25 en 32 °C) en verdelingskoëffisiënt van elke verbinding is eksperimenteel bepaal. *In vitro*-bepaling van velpermeasie is gedoen met behulp van Franz-diffusieselle en menslike vel by pH 7.4. Diffusie deur die vel is oor 'n periode van 24 uur by 'n konstante temperatuur (37 °C) gemeet. Alle monsters is met behulp van hoëdrukvloeistofchromatografie (HDVC) ontleed.

Siklisien (**I**) het 'n metielgroep aan N-4. Verlenging van hierdie alkielketting aan N-4 van die piperasiengroep lewer verbindings met laer smeltpunte en hoër wateroplosbaarheid as (**I**). Die wateroplosbaarheid van (**II**) is 3 keer en dié van (**IV**) ongeveer 2.5 keer hoër as die van (**I**). Die wateroplosbaarheid van (**III**) is ongeveer dieselfde as die van (**I**). Die verdelingskoëffisiënte neem lineêr met toename in kettinglengte toe. Die analoë het dus gunstiger fisies-chemiese eienskappe vir aflewering deur die vel. Data van *in vitro*-velpermeasie toon inderdaad dat hierdie verbindings makliker as (**I**) afgelewer word. Die fluks van (**I**) vanuit 'n versadigde oplossing is 0.132 $\mu g/cm^2/h$. Die deurgang van verbinding (**II**) is 53 keer hoër (6.952 $\mu g/cm^2/h$) as dié van (**I**) terwyl die van (**III**) en (**IV**) onderskeidelik 2 en 5 keer hoër is.

Op grond van die resultate van die studie lyk dit asof hoër wateroplosbaarheid en lae kristalliniteit 'n belangrike rol speel in die optimisering van die perkutane absorpsie van (**I**) en sy analoë, hoewel die belang van hoër lipifilisiteit nie onderskat kan word nie. Die lae perkutane absorpsie van (**I**) kan aan die lae wateroplosbaarheid en hoë kristalliniteit toegeskryf word soos uit die hoër smeltpunt blyk. Uit die permeasiedata is dit duidelik dat verbinding (**II**) die beste penetreerder van hierdie reeks is en dit is ook bekend dat hierdie verbinding die effekte van histamien antagoniseer.

Sleutelwoorde:	Perkutane absorpsie; siklisien; alkielanaloë; fisies-chemiese eienskappe; wateroplosbaarheid; verdelingskoëffisiënt; smeltpunt

Table of contents

Table of Figures

Table of Tables

Chapter 1

Introduction and statement of the problem

Recently, the dermal route has vied with oral treatment as the most successful innovative research area in drug delivery. In the USA alone, out of 129 drug delivery candidate products under clinical evaluation, 51 are transdermal or dermal system; 30 % of the 77 candidate products in preclinical development represent such drug delivery. The worldwide transdermal patch market approaches £2 billion, yet is based on only few drugs — scopolamine (hyoscine), nitroglycerine, clonidine, estradiol (with and without norethisterone or levonorgestrel), testosterone, fentanyl and nicotine, with a lidocaine patch soon to be marketed. The fundamental reason for such few transdermal drugs is that highly impermeable human skin limits daily drug dosage delivered (Barry, 2001).

The development of more sophisticated drugs has demanded the need for more sophisticated methods to deliver those drugs. Conventional drug delivery techniques using tablets and injections are often not suitable for new protein-based, DNA-based, and other therapeutic compounds produced by modern biotechnology. An attractive alternative method of delivery involves drug administration across the skin. Percutaneous delivery possesses many potential advantages, however the poor permeability of the human skin severely limits the delivery (Henry *et al.*, 1998).

Nevertheless, pharmaceutical scientists have accepted the challenges of transdermal drug delivery and over the years considerable progress and achievement have been recorded. The skin offers a large (1 – 2 m^2) and very accessible surface for drug delivery. Transdermal applications, relative to other routes, are quite noninvasive, requiring the simple adhesion of a "patch" much like the application of a Band-Aid. As a result, patient compliance is generally very good. Transdermal systems can easily be removed either at the end of the application period, or in the case where continued delivery is contra-indicated – with the exception of intravenous infusion, no other delivery modality offers this advantage.

Cyclizine is a piperazine derivative which belongs to the anti-histamine group of drugs. It is described chemically as a 1-diphenylmethyl-4-methylpiperazine. It acts both on the emetic trigger zones and by damping the labyrinthine sensitivity. Pharmacologically it

has anti-histaminic, antiserotonic, local anaesthetic and vagolytic actions (Susan *et al.*, 1989). Therapeutically it is an anti-emetic agent, the normal dose being 50 mg 4 – 6 hourly. It is recommended for the treatment and prevention of motion sickness, post-operative vomiting and menieres disease (Dundee & Jones, 1968). The synthesis of cyclizine was reported by Baltzly *et al.* (1949) (Wellcome Research Laboratory, Tuckahoe, N.Y.). Its anti-histaminic action was discovered by Castillo *et al.* (1949) and reported to be one-fourth as active as its congener chlorcyclizine in blocking the histamine-induced spasm of the tracheal chain preparation. The use of cyclizine hydrochloride for the prevention of seasickness and airsickness has been described by Chinn *et al.* (1952, 1953). Gutner *et al.* (1954), using microcaloric and galvanic stimulation methods, found that cyclizine notably decreased labyrinthine sensitivity in human subjects and Dent *et al.* (1954) found that cyclizine alleviated postoperative nausea and vomiting. The same investigation also found that the drug partially antagonized vomiting induced by the administration of apomorphine to dogs.

The investigation that demonstrated that anti-emetic activity is a more or less general pharmacological function of anti-histaminic agents was described by Boyd *et al.* (1955), in which thirty-one antihistaminic agents were screened for anti-emetic activity. A majority of these drugs (cyclizine included) were found to be anti-emetic.

As mentioned earlier that cyclizine is recommended for the treatment and prevention of motion sickness, drugs for this disease are used to protect persons from disturbances experienced in travel (*i.e* sea, air, car, and trainsickness). A relatively large portion of travelers experience motion sickness and the frequent opinion expressed by seasickness patients that they would prefer death to severe seasickness is commentary on the need for therapeutic measures in this condition (Burger, 1960). The most commonly used anti-motion sickness drugs are the anticholinergics and antihistamines. One of the most effective of anticholinergics is scopolamine. Despite its efficacy in alleviating the symptoms of motion sickness, it causes significant adverse side effects such as drowsiness and dry mouth. Antihistamines are generally considered less effective against motion sickness but are also widely used and include such drugs as cyclizine and meclizine (Gowans, 2000).

Cyclizine is available as an 'over the counter' preparation. In the UK, its trade names are Valoid and Marzine. Susan *et al.* (1989) undertook a study, as a concern that the abuse of cyclizine has become increasingly common in the clinic population of the Trent Regional Addiction Unit (UK). The study was an attempt to discover from patient's point

of view, why this should be so and to obtain a subjective account of the effects of cyclizine when taken by opiate dependents receiving methadone. The study provided evidence that cyclizine is capable of inducing dependence. Tolerance to the effects of cyclizine occurred in 50% (10 opiates dependence) of the subjects and all described a compulsion to continue taking the drug, despite recognizing that it was harmful, intense craving for cyclizine was common. But the results did not show any readily apparent correlation between the phenomenology of cyclizine abuse and dosage used, nor with the age or sex of the abuser. The discovery of the effects of cyclizine rapidly let to intravenous administration for maximum effect. Since this study, the formulation of Marzine has been changed. Cyclizine has been replaced by 15 mg of the anti-emetic Cinnerizine, which contains 50 mg of cyclizine hydrochloride. Marzine is now out of favour as a drug of abuse (Susan *et al.,* 1989).

The cases of three patients dependent on opiates (prescribed for chronic pain) abusing cyclizine were described, whereby all patients complained of nausea and increased pain when the authors withdrew cyclizine. They were unable to substitute other anti-emetic agents for cyclizine and it was considered that cyclizine dependence occurred in the treatment of chronic pain and could complicate its management (Hughes & Coote, 1986).

Beside this unwanted circumstances of abuse, cyclizine proved to be useful in several investigations in the past. Norton *et al.* (1954) reported that in anesthetized cats, cyclizine blocks the vagus response, relaxes the tone and rhythmic contractions of the ileum and blocks the injected histamine at low doses (0.5 mgm/kgm). It is effective in reducing the mortality in guinea pigs exposed to nebulised histamine in which it was found to be as effective as diphenhydramine (*in vivo*) in preventing or reducing the severity of bronchoconstriction. It is also suggested that cyclizine may specifically block the vagus nerve peripherally and that this block may be central is indicated by the anti-emetic properties of the compound.

Cyclizine in combination with ergotamine and caffeine is a constituent of the anti-migraine drug Migril. It is also combined with morphine in the analgesic cyclimorph. Thirty mg cyclizine is combined with dipipanone in each tablet of the analgesic Diconal (Wellconal) (Susan *et al.,* 1989).

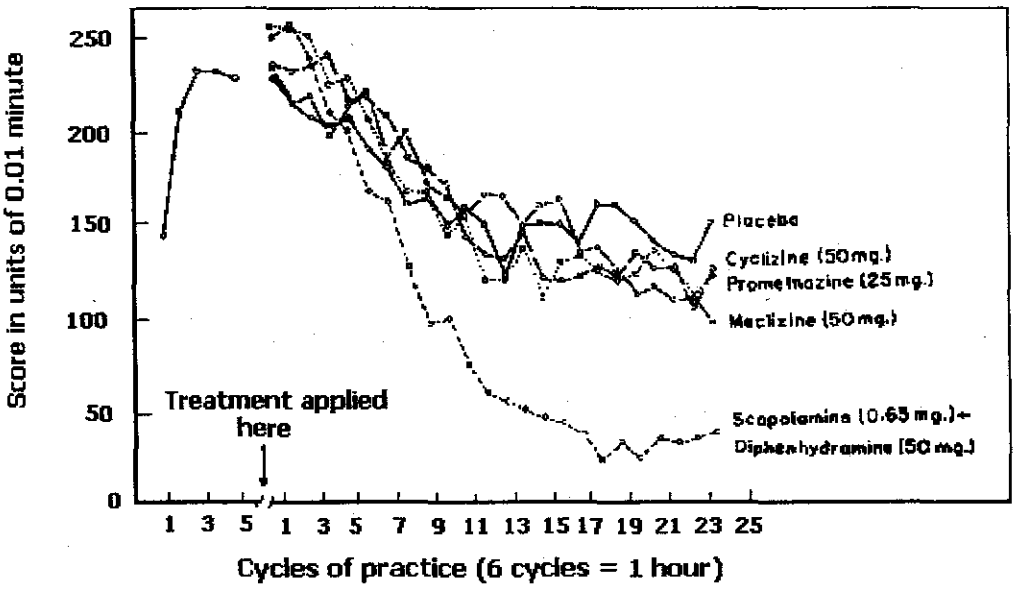

FIGURE 1-1: Effects of motion sickness remedies upon tracking proficiency (Payne & Moore, 1955).

Figure 1-1 above represent the effects of depressant drugs and placebo upon tracking behavior. The tracking task required the operator to monitor the drifts of four accentrically driven aircraft instrument pointers and to keep them within prescribed scale area by timely and coordinated adjustments of multiple aircraft controls. Score accumulated only when all pointers are kept within the prescribed areas concurrently. Results showed that the depressant effect of scopolamine-diphenhydramine mixture becomes and remains highly significant. Conversely, no adverse effects attributable to cyclizine, meclizine and promethazine are detectable. From the perceptual-motor view point there would be no valid reason why operating personnel could not take advantage of the beneficial effects which cyclizine, meclizine and promethazine have upon motion sickness and other disorders (Payne & Moore, 1955).

The administration of cyclizine by subcutaneous route was recently reported by Verma (2001). It is stated that cyclizine is listed in their 'palliative care formulary' as available for subcutaneous injection or infusion. An audit to determine whether there were any local problems with the use of subcutaneous cyclizine was conducted. Over approximately 2 months, 92 patients received an intra-operative injection of cyclizine 50 mg via a subcutaneous cannula. Patients were reviewed for recovery and then at 24 hour for acute pain. The result showed a small incidence of very minor side effects (Table 1-1). It came to the believe that cyclizine lactate is a versatile agent for the

management of postoperative nausea and vomiting. Following the withdrawal of droperidol, the administration of cyclizine via an indwelling subcutaneous cannula provides a useful anti-emetic alternative and could be adopted more widely (Varma, 2001).

TABLE 1-1: Side effects following the subcutaneous administration of cyclizine lactate (n < 92)

	Discomfort	**Pruritis**	**Erythema**	**Skin changes**
Recovery	1 (1.09 %)	0	5 (5.43 %)	0
24h post operative pain	1 (1.09 %)	0	3 (3.26 %)	0

Oral therapy of cyclizine has proven to be effective and have minor side effects, but administration of cyclizine via the dermal route can bypass liver metabolism and provide a high local tissue drug level without systemic complications. There is much well documented evidence to believe that cyclizine's action is on the histamine receptors, therefore, if the drug's delivery can be targeted, it should be possible to deal with histamine receptor nearer or at the targeted area (CNS). Hence cyclizine acts to block histamine receptors in the vomiting center and thus reduce activity along these pathways.

The numbers of drugs that meet the physicochemical criteria and have suitable pharmacological properties and adequate potency to be considered as feasible candidates for dermal delivery is quite limited. The majority of drugs are precluded from consideration for transdermal application because of their limited ability to penetrate the skin at a sufficient rate (Anderson, 1993). Is therefore necessary to have a thorough understanding of a drug's physicochemical properties, particularly its absolute solubilities and related partitioning tendencies (Sloan *et al.,* 1986 and Flynn & Yalkowsky, 1972).

Methods to enhance penetration may be by alteration of the stratum corneum through the use of chemical penetration enhancers, providing an additional driving force for transport (e.g., iontophoresis), or by chemically modifying the drug itself through prodrug formation (Anderson, 1993). The latter approach is the subject in this project whereby the idea of using alkyl analogues of cyclizine was embraced. The most important feature

that an analogue of this compound might contribute is decreased crystallinity and increased lipophilicity. Hamlin *et al.* (1949) synthesized a group of 1,4-disubstituted piperazines for pharmacological investigation and in an effort to obtain a superior antihistaminic agent having lower duration of action and lower incidence of side effects. The prepared compounds included cyclizine (1-diphenylmethyl-4-**methyl**piperazine), 1-diphenylmethyl-4-**ethyl**piperazine, 1-diphenylmethyl-4-**butyl**piperazine and others. The compounds were found to antagonize the effects of histamine.

The objectives of this study were thus to:

- synthesise selected alkyl analogues of cyclizine
- determine the physicochemical properties of the compounds that are relevant to their percutaneous delivery
- determine the permeation of cyclizine and its selected alkyl analogues through the human skin from saturated aqueous solution, and
- develop relationships which exist between the physicochemical properties of cyclizine and its selected alkyl analogues and their percutaneous delivery.

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Chapter 2

Percutaneous absorption

2.1 Introduction

The absorption of chemicals across the skin has attracted considerable interest in the last decade. The advent of transdermal drug delivery, in particular, has led to the reconsideration of many aspects of the percutaneous penetration process. Most notably, the mechanisms by which molecules penetrate the dermal barrier and the dependence of absorption kinetics and extent upon the physicochemical properties of the permeant have required particular attention (Guy & Hadgraft, 1988).

If one consider the number of compounds which daily come into contact with the skin without causing local or even systemic side effects, one could suppose that skin is impermeable to many substances. In fact local application is the basis of dermatological therapy and the concomitant therapeutic or toxic effect elicited systemically are a direct proof of the permeability of the skin (Schalla & Schafer, 1982). Baker (1985) reported that the thought of the skin been impermeable has changed and the progress achieved in this area clearly demonstrates that the skin is a complex organ and allows the passage of chemicals into and across the skin.

Absorption or transport of drugs, toxicants or other chemicals into or through the skin depends on a number of factors: characteristics of the penetrant, condition of the skin, other chemicals present with the penetrant and external conditions such as temperature, humidity and occlusion. Under most conditions, the factor with perhaps the greatest influence on the rate or extent of skin absorption is the character of the penetrant (Smith, 1990).

Studying the different layers of the skin, the nature of their biochemical and physiological activities and the interaction between barrier elements and penetrants, should indicate how to manipulate the structure of drugs and the pharmaceutical formulation to cause selective and effective permeability. With increased understanding of percutaneous absorption, the advent of singularly effective new topical drugs can be expected. It is

thus important to get an overview of the skin itself and particularly physicochemical properties that can influence percutaneous delivery. In this regard a literature study was conducted to investigate the following aspects:

- skin as barrier to percutaneous absorption;
- the process of percutaneous absorption and
- the physicochemical factors influencing percutaneous absorption.

2.2 The skin as barrier to percutaneous absorption

The skin is the most accessible and probably the most extensive organ of the body. It is well vascularised, elastic and self-regenerating (Lund, 1994). It is a complex organ that serves to protect human from chemical, physical and biological intrusion, while retaining moisture and providing thermal regulation. It consists of three primary regions: the epidermis, the dermis and the hypodermis (Figure 2-1).

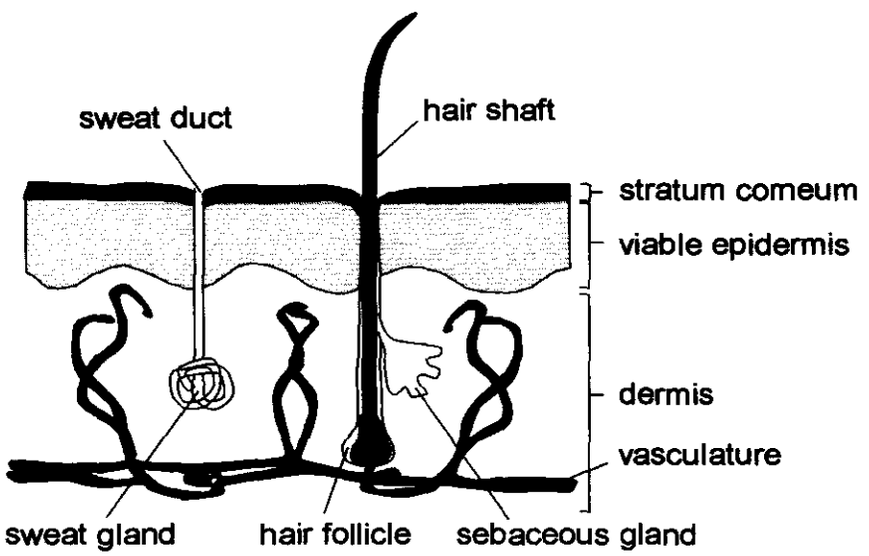

FIGURE 2-1: Skin features relevant to percutaneous absorption of chemicals (Clifford, 2002).

The human surface is known to contain on the average, 10 – 70 hair follicles and 200 – 250 sweat ducts on every square centimeter of the skin area. These skin appendages however, actually occupy grossly only 0.1 % of the total human skin surface (Chien,

1987). Potts *et al.* (1992) reported that one of the principal roles of the skin is to act as a barrier to the outward transport of water and the inward movement of topically contacting substances. In the context of percutaneous absorption, the barrier to the ingress of molecules is of greatest relevance, although the water-barrier properties are essential for survival. Nevertheless, the mechanistic basis of water-barrier function and percutaneous absorption appears similar. Understanding the development of this barrier can facilitate attempts to enhance percutaneous absorption of drug molecules. Conversely, this knowledge can also be beneficially applied to predict the risk associated with the percutaneous absorption of toxic compounds.

2.2.1 Stratum corneum

The stratum corneum is the outermost layer of the skin and is the major source of resistance to the permeation of the skin by drug molecules. This coherent membrane, which is 15-20 μm thick over much of the human body, consists primarily of blocks of cytoplasmic protein matrices (keratins) embedded in extracellular lipids. The keratin-containing cells (corneocytes) are arranged in an interlocking structure somewhat akin to brick and mortar. In human, the extracellular mortar consists of a structured complex containing several groups of lipids (Walters, 1990). Figure 2-2 shows the structure of the stratum corneum.

Lund (1994) documented that the stratum corneum consists of aggregates of closely packed cells, and contains both lipid and aqueous regions. Lipid-soluble drugs can pass readily through lipid regions of the cell membrane whereas water-soluble drugs pass through because of hydrated protein particles within the cell wall. There is some evidence that compounds with both lipophilic and hydrophilic properties, that is with an oil-water partition coefficient close to unity, are best able to pass through the stratum corneum. Water-soluble ions and molecules, unless very small, do not pass through. Gases readily pass through the stratum corneum and this may account for the good penetration found for volatile dugs.

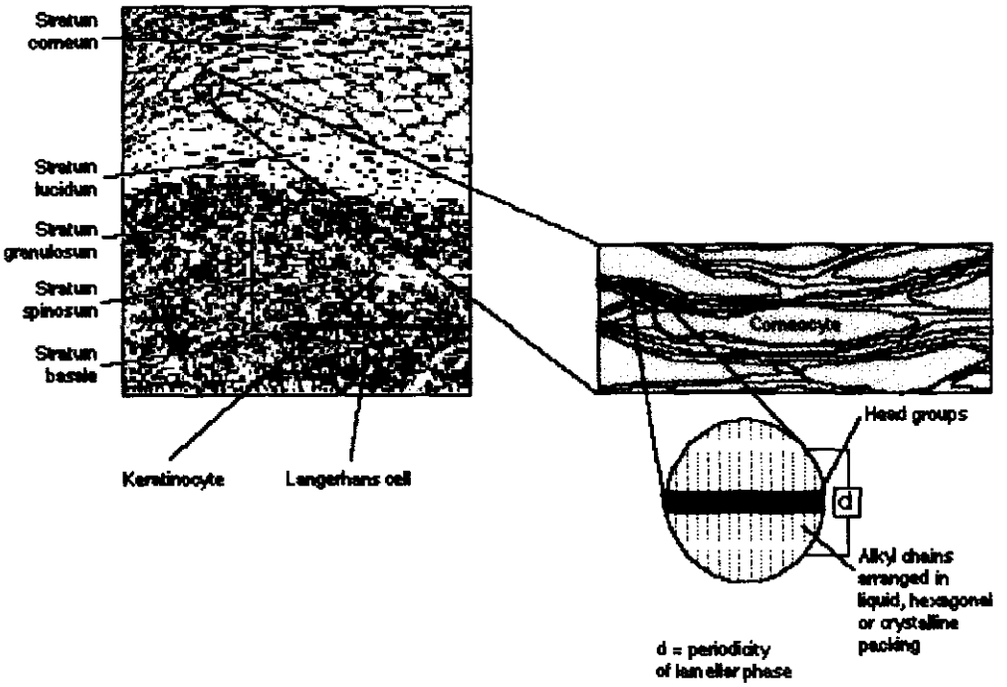

FIGURE 2-2: Schematic representation of the epidermis of the skin. The structure of the stratum corneum is shown in the inset diagrams (Bouwstra, 1994/5).

The results of several experiments have shown that the stratum corneum is the rate-limiting barrier to the penetration of many chemicals through the skin (Potts *et al.*, 1992). Scheuplein (1976) tape-stripped the skin, thereby removing the stratum corneum and compared the resulting permeability with that of unstripped skin. Removal of the stratum corneum increased the permeability of many solutes by orders of magnitude, strongly implicating the stratum corneum as the primary rate-limiting barrier. The stratum corneum is breached by hair follicles and sweat ducts, which could theoretically provide a low resistance rapid diffusion pathway across the skin This shunt pathway may be significant for extremely slow penetrants such as polar steroids, but the relatively small surface area of the follicles suggests that for most penetrants, dermal permeation dominates except during the period immediately after application (Scheuplein, 1976).

Because of stratum corneum's highly organized structure, it is the major permeability barrier to external materials and is regarded as the rate-limiting factor in the penetration of therapeutic agents through the skin (Foldvari, 2000).

2.2.2 Viable epidermis

The living cells of the epidermis are located immediately below the stratum corneum. Lying directly above the dermis is a single layer of cells called the stratum basale, which constantly divides to produce keratinocytes. Mitosing cells from the stratum granulosum where they flatten, their content becomes granular and keratin forms. Ultimately, through oxygen and nutrients deprivation, the cells shrinks and die to become the cells of the stratum corneum. Within the stratum corneum, the cells become more compacted as they proceed towards the surface, until they are eventually lost by abrasion (Lund, 1994).

The viable epidermis is often regarded as having properties of an aqueous gel and, as such, does not present a significant barrier to penetration in most circumstances. If the stratum corneum is damaged or if extremely lipophilic drugs are being used, the viable epidermis can act as a rate-limiting factor in percutaneous absorption (Walters, 1990).

2.2.3 Dermis

Below the epidermis is the dermis or corium. Convolutions in the boundary between the two layers increase the area of contact between the epidermis and the dermis with its numerous blood vessels, nerves and lymphatic, and bring the blood supply closer to the skin surface. The dermis is about 3.2 mm thick and is the largest of the three skin layers. It is predominantly connective tissue and the few cells it contains are principally involved in the secretion of elastin and collagen (Lund, 1994).

Because of the blood vessels approach the interface of the two layers very closely, the dermis cannot be considered as a significant barrier to inward drug permeation *in vivo* (Walters, 1990).

2.2.4 Subcutaneous fat layer and appendages

The final layer of skin, the subcutaneous fat layer or hypodermis, contain adipose cells which serve principally as an energy source and contribute to the temperature regulation of the skin (Lund, 1994). The dermis supports the appendageal structures, specifically the hair follicles and sweat glands. The pilosebaceous unit comprises of the hair follicle, the hair shaft and the sebaceous gland. The hair follicle is an invagination of the

epidermis that extend deeper into the dermis. The lining of the lower portion of the hair follicle is not keratinised and presumably offers a lesser barrier to diffusion than the stratum corneum. With respect to drug delivery, interest in these structures has centered upon the possibility that they may provide "shunt" pathways across the skin, circumventing the need to cross the full stratum corneum. While this is a completely reasonable hypothesis, it is somewhat irrelevant from a practical standpoint because the follicles occupy a relatively insignificant fraction of the total surface area available for transport (~0.1 %). A similar argument can be made with respect to the sweat glands which cover a considerably smaller total area than the follicles. However, appendageal transport may assume a much more important role when specialised transport technology are used to increase dermal delivery (Delgado-Charro & Guy, 2002).

2.3 The process of percutaneous absorption

Percutaneous absorption is the term used to describe the penetration of a substance (drug or chemical) through the skin and subsequently movement into the systemic circulation (Lund, 1994). Percutaneous absorption involves the following sequence of events: (1) Partitioning of the molecule into the stratum corneum from the applied vehicle phase, (2) diffusion through the stratum corneum, (3) partitioning from the stratum corneum into the viable epidermis, (4) diffusion through the epidermis and upper dermis and (5) capillary uptake (Figure 2-3). Molecules traverse membranes either by passive diffusion or by active transport (Potts *et al.*, 1992).

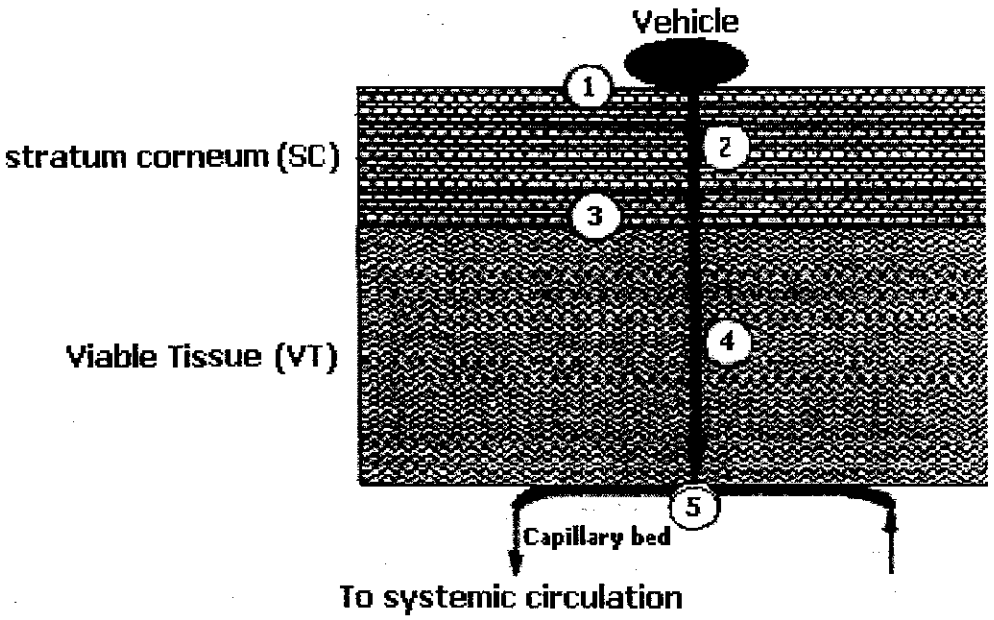

FIGURE 2-3: The sequential steps involved in percutaneous absorption (Potts *et al.*, 1992).

Passive diffusion, the most important mechanism, requires the compound to be of low molecular weight, to be lipophilic and a concentration gradient must exist. The rate of transport across a membrane by passive diffusion is derived from the classical equation for the First Law of Diffusion of Fick. For drugs that do not meet the requirement for passive diffusion, other transfer mechanism may be operative. In active transport, transfer can be against the concentration gradient and the compound can accumulate in high concentrations. There is energy requirement for active transport. Two principal absorption routes identified are the transappendageal route and transepidermal route. In transappendageal route the barrier afforded by the stratum corneum is avoided and there is relatively rapid ingress via sweat glands and hair follicles. The transepidermal route corresponds to the diffusion across the stratum corneum. According to Lund (1994), unbroken epidermis constitutes the larger surface for absorption and is widely regarded as the major, but not the exclusive pathway for the percutaneous absorption of many compounds.

The driving force for absorption or transport of any penetrant is proportional to the concentration gradient of that penetrant within the skin (Smith, 1990).

2.3.1 Routes of penetration

At the skin surface, molecules contact cellular debris, microorganism, sebum and other materials, which negligibly affect permeation. The penetrant has three potential pathways to the viable tissue — through hair follicles with associated sebaceous glands, via sweat ducts, or across contineous stratum corneum between these appendages (Figure 2-4). Fractional appendageal area available for transport is only about 0.1 %; this route usually contributes negligibly to steady state drug flux. The pathway may be important for ions and large polar molecules that struggle to cross intact stratum corneum. Appendages may also provide shunts, important at short times prior to steady state diffusion. Additionally, polymers and colloidal particles can target the follicle. The sebaceous gland cells are more permeable than corneocytes and thus drugs can reach the dermis by entering the follicle (bypassing the invaginated stratum corneum), passing through the sebaceous gland or penetrating the epithelium of the follicular sheath. The rich blood supply aids absorption, even though the shunt route cross-section area is small (Barry, 2001).

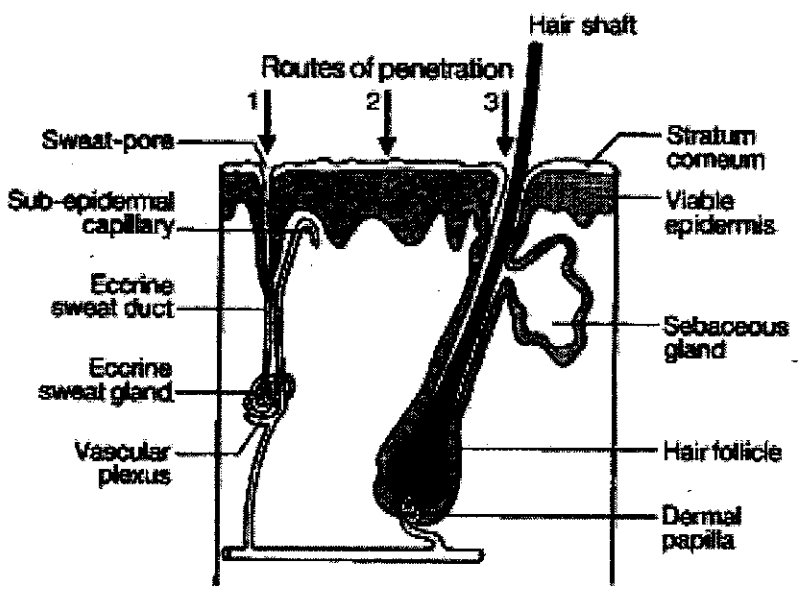

FIGURE 2-4: Simplified diagram of skin structure and macroroutes of drug penetration: (1) via the sweat ducts, (2) across the continuous stratum corneum or (3) through the hair follicles with their associated sebaceous glands (Barry, 2001).

Figure 2-5 illustrates the intercellular and transcellular routes of drug permeation through the intact stratum corneum. Because the intercellular space of the stratum corneum was

originally assumed to comprise a tiny portion of its overall volume, this space traditionally had been discounted as a possible pathway. Freeze-fracture studies showed, however that the intercellular volume may be a factor of 3 – 7 times greater than previously appreciated and now it is believed to be between 5 and 30 % of the total volume (Elias, 1981).

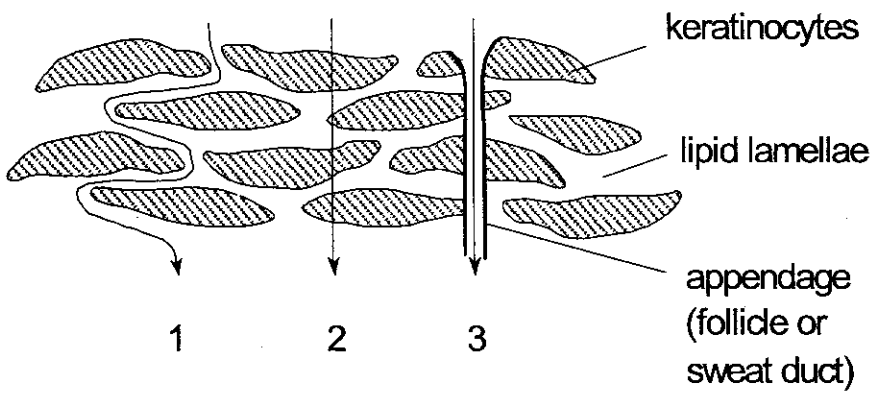

FIGURE 2-5: Skin permeation routes through the stratum corneum: (1) intercellular diffusion (2) transcellular diffusion and (3) diffusion through appendages (Clifford, 2002).

In a study by Albery and Hadgraft (1979), percutaneous absorption of methyl nicotine showed that this molecule penetrates human skin through the intercellular and not via a transcellular pathway. These observations suggest that the intercellular lipid matrix might be the major rate-determining pathway by which many substances traverse the stratum corneum.

The relative importance of these dual routes for any particular penetrant will depend on several factors, including the chemical potential, the partition coefficient within these protein or lipid regions (Barry, 1987).

2.3.2 Advantages of percutaneous absorption

The positive features of delivering drugs across the skin to achieve systemic effect can be summarized as follows: Avoidance of significant presystemic metabolism (for example, that due to degradation in the gastrointestinal tract or by liver), and the need, therefore, for a lower daily dose. Drug level can be maintained in the systemic

circulation, within the therapeutic window for prolonged periods of time. Improved patient compliance and acceptability of the drug therapy (Delgado-Charro & Guy, 2002).

2.4 Physicochemical factors influencing percutaneous absorption

Percutaneous absorption involves the movement of molecules across the epidermal cellular structure. Therefore, factors influencing percutaneous absorption are essentially the same as those influencing gastrointestinal absorption. Additional variables to percutaneous delivery are the condition of the skin, the skin age, the area of skin, the thickness of the barrier phase, species variation and the moisture content of the skin. The primary factors that determine the rate of diffusion through the skin are the physicochemical properties of the drug. Secondary factors are the nature of the vehicle, the pH, the concentration of the drug and the temperature (Idson, 1975).

2.4.1 Solubility in the stratum corneum

The physicochemical properties of drugs, especially their solubilities, are crucial to decisions about and the design of novel system of delivery (Flynn & Yalkowsky, 1972). Generally, the greater a drug's innate tendency to dissolve, the more likely it is that the drug can be delivered at an adequate rate across any membrane, including the skin. The concentration of drug that can build in the stratum corneum, the skin's principal barrier element, bears some relationship to the solubilities of the drug in organic solvents such as hexane because the diffusion conduit through this barrier is itself lipoidal (Flynn, 1995).

In the formulation of preparations for topical application, it is profitable to select or prepare compounds having the required solubility characteristics before attempting to promote their penetration by pharmaceutical manipulation (Idson, 1975).

In order to permeate through the skin, the molecules need to penetrate from the vehicle into the outermost lipophilic tissue – the stratum corneum. Subsequently, the molecule needs to partition out of the stratum corneum into the essentially aqueous viable epidermis. For very lipophilic molecules the rate-determining step is the partition of the drug from the stratum corneum into the epidermis, whereas for hydrophilic molecules, it

is penetration into the stratum corneum. Optimum skin permeation is therefore reached with molecules having "mixed" lipophilic/hydrophilic properties (Surber *et al.*, 1993).

2.4.1.1 Solubility parameters

A low solubility parameter for a solute is synonymous with high lipophilicity (Roy & Flynn, 1989). A number of studies have suggested that following from the Hiledbrand-Scatchard theory for crystalline solids in regular solution, the permeability, and hence the partition coefficient between the skin and the solvent may be related to the solubility parameter for the solute in the system (Liron & Cohen 1984 and Roy & Flynn, 1989). The solubility parameter of the skin has been estimated as ~10 and therefore drugs, which possess similar values, would be expected to dissolve readily in the stratum corneum (Liron & Cohen, 1984). Thus, penetrants with high solubilities in the stratum corneum will tend to exhibit high fluxes, or at least will not be limited by solubility considerations.

The solubility parameter of an organic solute in the stratum corneum can be estimated from equation 2-1. If the solubility of the solute in a non-polar organic solvent (like hexane) is known, as well as the heat of fusion and the melting point, and the solubility parameter of the solvent.

$$\ln X_2 = \frac{-\Delta H_f}{RT}\left(\frac{T_f - T}{T_f}\right) + \frac{\Delta C_p}{R}\left[\frac{T_f - T}{T} - \ln\frac{T_f}{T}\right] - \frac{V_2\phi_1^2}{RT}(\delta_1 - \delta_2)^2 \qquad \text{(Equation 2-1)}$$

Where:

- X_2 is the solute's mole fraction solubility in hexane
- ΔH_f is the heat of fusion
- R is the gas constant
- T_f is the melting point of the solid in degrees Kelvin
- T is any experimental temperature less than T_f
- ΔC_p is the difference in heat capacity between the solid form and the hypothetical supercooled liquid form of the compound, both at the same temperature
- V_2 is the molar volume of the liquid solute
- Φ_1 is the volume fraction of the solvent
- δ_1 is the solubility parameter or square-root of the cohesive energy density of the solvent (hexane)

- δ_2 is the solubility parameter or square-root of the cohesive energy density of the solute.

Roy & Flynn (1988) assessed the solubilities of the narcotics (morphine, hydromorphine, codeine, fentanyl, sufentanil and meperidine) in selected solvents i.e hexane and water. As the prototypical narcotic, morphine's solubilities in solvents of wide-ranging polarity have been characterized and these established an overall picture of the solubility behaviour of the class. The solubility parameter (δ_2) of morphine calculated from its solubility in hexane and its heat of fusion was virtually identical to the best-fit solubility parameter obtained from the solubilities in all London solvents. This was in agreement with the work of Neau and Flynn, which demonstrated that the solubility parameters of alkyl *p*-aminobenzoates can be determined accurately and with a deviation of no more than ± 0.2 $(cal/cm^3)^{1/2}$ from their solubilities in *n*-hexane or *n*-heptane and their heat of fusion and melting points.

Roy & Flynn (1988) found a monotonic relationship with δ_2 and octanol-water partition coefficient (K_{oct}) for six narcotic alkaloids. For solutes with log K_{oct} less than –1, the δ_2 was constant at about 9.5. Above this value of log K_{oct}, δ_2 increased sharply. Maximum permeability coincided with a δ_2 of 9.6 – 9.8 in a plot of log K_{oct} against the square root of δ_2. The observed decrease in permeability coefficient with further increase in δ_2 corresponds to a dependence of permeability on lipophilicity.

2.4.1.2 Aqueous solubility

The solubility of the penetrant in the various phases present in the skin and its surrounding plays a large part in determining the rate of penetration. In a typical case, the penetrant will be present on the skin surface either dissolved in or dispersed in a vehicle of some sort. While it is the concentration of penetrant within the skin that controls the rate of transport, that concentration is dependent on the concentration and solubility of the penetrant in the vehicle on the skin surface. It should be pointed out that once the penetrant has crossed the stratum corneum, it must partition into the underlying layers of epidermis, dermis and circulatory system. These tissues are typically more hydrophilic than is the stratum corneum and can present a barrier to transport of extremely hydrophobic penetrants (Smith, 1990).

The stratum corneum, which is the rate-limiting biological barrier to percutaneous absorption, is considered to be lipophilic in nature, dermal delivery of a drug requires

that the drug exhibit significant lipid solubility. However the drug must also exhibit some appropriate degree of water solubility in addition to its lipid solubility, in order to partition into and through at least the initial hydrophilic macrophages of the lipophilic stratum corneum and in addition, solubility of drug in the internal aqueous phases is essential for it to express its systemic potency (Beall, 1993).

Modification of molecular structure to change the partitioning and solubility characteristics of the compound can alter the rate of penetration through the epidermis has been used extensively for corticosteroids. As a general rule, for good absorption the drug should have an oil-water partition coefficient close to unity and a moderately high water solubility. Among many esters of betamethasone tested, the highest topical activity was found for the 17-valerate esters, which exhibited this properties (Lund, 1994).

The study of Fourie (2001), found that N-methyl and N-ethyl analogues of carbamazepine have higher aqueous solubility (25°C & 32°C) than carbamazepine itself and this parameter was in agreement with the partition coefficients and steady state flux of these analoques. Cordero *et al.* (1997) investigated the *in vitro* penetration of a series of nonsteroidal anti-inflammatory drugs (NSAID) across excised human skin. Selected physicochemical parameters were experimentally determined; this includes the experimental solubilities in buffer at pH 6.6 and in water. Significant differences were found in flux values of the various NSAIDs. Ketoproven exhibited the highest flux, followed by ketorolac and aceclofenac. The high trandsdermal fluxes of ketoproven and ketorolac are associated with their intrinsic solubilities together with their moderately high k_p values.

Beall (1994) studied the dermal delivery of 5-fluorouracil (5-FU) by 1-alkyloxycarbonnyl-5-FU prodrugs. The results from the diffusion cell experiments show that changes in the promoiety that result in increase water solubilities of more lipid soluble derivatives lead to increased rates of delivery of the total 5-FU species through the skin. There is a good correlation between water solubility and flux within the series of more lipid soluble prodrugs. The most water-soluble member of the series is the most effective member at enhancing flux (Beall, 1994).

The activities of highly water-soluble and highly oil-soluble molecules are less than those of drugs with a more evenly balanced solubility behaviour (Lund, 1994).

2.4.2 Diffusion coefficient

Barry (1988) defined "diffusion coefficient" (D) as the measure of how easily a molecule diffuse through the stratum corneum. The diffusion coefficient of a drug in the skin is dependent on properties of the drug and the medium through which it diffuses. One drug property which is well documented as having a major influence on the diffusion coefficient is that of molecular size and mass. Another important parameter which has been documented is the drug state, e.g. ionised or nonoinsed, with nonionised forms diffusing more freely than ionised forms.

Chemicals are transported into and through the skin by a solution-diffusion process. The penetrant must dissolve in the skin, diffuse across the skin and partition into the body fluids or tissues beneath the skin. Due to the extraordinary barrier that skin represents to most penetrants, diffusion across the skin is typically the slowest and therefore rate-controlling step in this process. In this case, the rate of transport across the skin can be described by the following approximation of Fick's first law (Smith, 1990):

$$J = \frac{D\Delta C_m}{\ell} \qquad \text{(Equation 2-2)}$$

Where:

J is the flux of penetrant across the skin ($g/cm^2/s$)
D is the diffusivity of the penetrant in the skin in cm^2/s
ΔC_m is the difference in penetrant concentration within the skin in g/cm^3
ℓ is the thickness of the skin in centimeters.

In the simplest sense, the skin can be considered as a bilaminate membrane consisting of adjacent lipoidal and aqueous layer. Transport through this structure, assuming that the permeant exists at unit activity on the stratum coneum surface, is governed by two diffusion coefficients (D_s, D_v), two associated diffusion path lengths (l_s, l_v), and a partition coefficient (K) of the penetrant between stratum corneum and viable tissue (Figure 2-6).

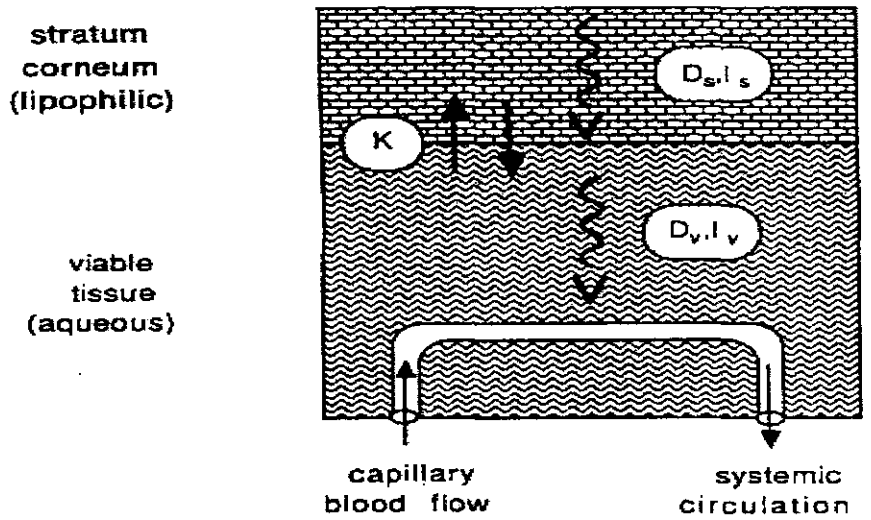

FIGURE 2-6: Schematic representation of skin as a bilaminate membrane. Diffusion coefficient and diffusion path lengths through the adjacent layers are indicated (Guy & Hadgraft, 1988).

The value of l_s reflects the distance from the top to the base of the stratum corneum, l_v is the distance from the base of the stratum corneum to the upper dermal capillaries, D_s is the characteristic diffusion through the stratum corneum and D_v is the characteristic diffusion through an aqueous protein gel (Guy & Hadgraft, 1988).

For typical small polar molecules such as water and the alcohols, the reported diffusion coefficient in stratum is of the order of 10^{-10} to 10^{-9} cm^2sec^{-1} (Table 2-1).

TABLE 2-1: Approximate permeability data for various homologous series penetrating through skin (Barry, 1983).

Penetrant	Diffusion coefficient (cm^2sec^{-1})
C4 Series:	
Ethyl ether	10^{-9}
2-Butanone	10^{-9}
1-Butanol	10^{-9}
2-Ethoxyethanol	10^{-10}
2-3-Butanedoil	10^{-9}
Alcohols:	
Ethanol	10^{-9}
Pentanol	10^{-9}
Octanol	10^{-9}
Steroids:	
Progesterone	2×10^{-11}
Cortexone	2×10^{-11}
Cortexolone	4×10^{-12}
Cortisone	1×10^{-12}
Cortisol	3×10^{-3}

The speed with which materials diffuse depends first and foremost on the state of matter of the diffusing medium. In gases and air, typical diffusion coefficients are large (on the order of 0.05 – 1.00 cm^2sec^{-1}) because the free volume or void space available to the molecule is large compared to their size and the mean free path between molecular collisions is great. In liquids, the void space is much smaller, mean free paths are decreased, and diffusivities are much reduced. Thus, for an aqueous lotion on the skin, diffusion coefficients within the vehicle would be in the region of 10^{-5} to $10^{-6}cm^2sec^{-1}$. Diffusivities progressively drop as the consistency of the material increases until, for a true crystalline solid with no free volume, molecules other than small gas molecules are stopped completely. The diffusion coefficient of a drug, either in a topical vehicle or in the skin, depends on the properties of the drug and the diffusion medium and on the extent of interaction between them.

In general, during any skin permeation process, the apparent diffusion coefficient determined may reflect influences other than the intrinsic mobility of the penetrant

molecules. Such influences could include changes in drug mobility through the stratum corneum arising from plasticization by vehicle or penetrant, or deviation from ideal solution behavior. Internal chemical reactions within the tissue could immobilize a fraction of the penetrant molecules. All those factors may produce concentration dependent changes. However, regardless of the mechanism which affects the magnitude of the diffusion coefficient, its value reflects the rate of penetration of a specific drug under specified condition, and diffusion coefficient is therefore a very useful parameter to know (Barry, 1983).

2.4.3 Partition coefficient

The single most important characteristic influencing skin penetration is distribution into the horny layer. The horny layer has for many years been identified as a non-polar membrane. Its solvent properties have therefore been mimicked by various non-polar liquids including ether, octanol and isopropyl myristate, usually expressed through an organic solvent (or "oil")/aqueous solution partition coefficient (Zatz, 1993).

The lipid/water partition coefficient denotes the ratio of the concentration of drug in two immiscible phases and is an important factor controlling the rate of transmembrane movement (Ritsche, 1988). Essentially, the stratum corneum barrier is lipophilic, with the intercellular lipid lamellae forming a conduit through which drugs must diffuse in order to reach the underlying vascular infrastructure and to ultimately access the systemic circulation. For this reason, lipophilic drugs are better accepted by the stratum corneum. A molecule must first be liberated from the formulation and partition into the uppermost stratum corneum layer, before diffusing through the entire thickness, and must then repartition into the more aqueous viable epidermis beneath. Ideally, a drug must possess both lipoidal and aqueous solubilities: if it is too hydrophilic, the molecule will be unable to transfer into the stratum corneum; if it too lipophilic, the drug will tend to remain in the stratum corneum layers (Naik, 200).

The important role that the partition coefficient may play in establishing the flux rate has been emphasized. In particular, when the membrane provide the sole or by far the major source of diffusional resistance, then the magnitude of the partition coefficient is very important. This is often the situation with percutaneous absorption, in which the resistance of the stratum corneum to the passage of the diffusant is usually the rate-limiting step in the overall absorption process. The stratum corneum to vehicle partition

coefficient is crucially important in establishing a high initial concentration in the first layer of the tissue. The relationship between partitioning behavior, chemical structure and biological activity is a pervasive theme in modern pharmaceutical literature (Barry, 1993).

Physicochemical parameters such as aqueous solubility and lipophilicity, have been shown to influence membrane flux, therapeutic activity and pharmacokinetic profiles of medicine. The use of partition coefficient in predicting the transdermal absorption of nonsteroidal anti-inflammatory drugs was found to be useful, but is advisable to include other indicative parameters (Goosen *et al.*, 1998).

A homologous series of hair dyes was selected for percutaneous absorption studies with excised human skin. The permeability constants obtained for the dyes were compared with octanol/water and skin membrane/water partition coefficient. The compounds examined were: p-phenylenediamine, o-phenylenediamine, 2-nitro-p-phenylenediamine, 2-amino-4-nitriphenol, 4-chloro-m-phenylenediamine and 4-amino-2-nitrophenol. Skin absorption of the dyes was observed when they were applied in aqueous solution. With one exception, the octanol/water partition coefficients were in the same rank order as the permeability constants. The determination of the partition of the hair dyes between water and either stratum corneum or epidermis was more complex. Preliminary stratum corneum/water partition studies results in values that were in the reverse order of the skin permeatition. When binding of the compounds to components of the membrane was saturated, the partition values more closely duplicated the rank order of permeability of the dyes. Prediction of percutaneous absorption of substances based on their partition coefficients may be confounded if these compounds are capable of binding to skin (Bronaugh & Congdon, 1984).

Figure 2-7 below represents the permeability data of hydrocortisone 21-esters. The best correlation of partition coefficient was found with permeability coefficient.

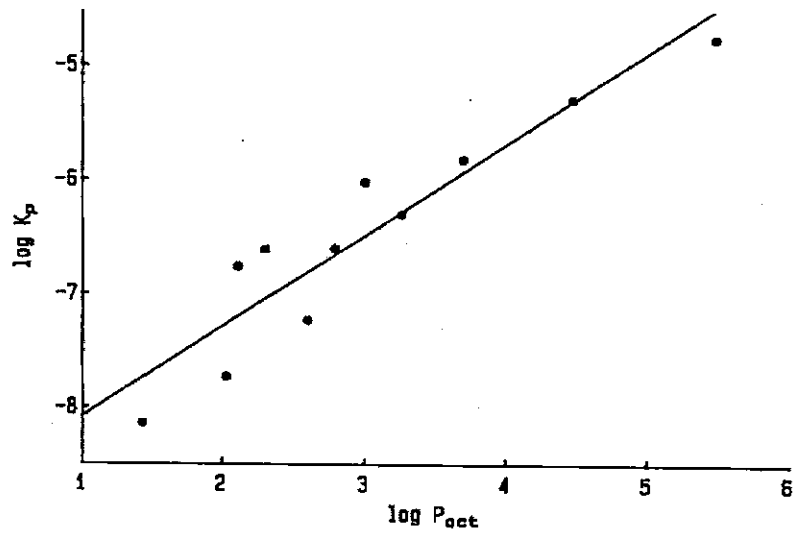

FIGURE 2-7: Log-log plot of permeability coefficients ($cm.s^{-1}$) versus 1-octanol-water partition coefficients for hydrocortisone 21-esters.

The octanol/buffer partition coefficients of buprenorphine and four prodrugs were determined by Stinchcomb *et al.* (1996). The results showed that, even though the flux of the prodrugs was not higher than that of parent drug, partition coefficient increase logarithmically with the increasing alkyl chain length (Table 2-2). The failure of the prodrug to deliver greater levels of buprenorphine under these circumstances is rooted in the permeation mechanism (Stinchcomb *et al.,* 1996).

TABLE 2-2: Partition coefficients of buprenorphine and four prodrugs.

Drug	Log K_{oct} at 25 °C
Buprenorphine	2.9
Acetyl prodrug	3.5
Propyl prodrug	3.7
Butyl prodrug	4.1
Isobutyl prodrug	4.2

It is probable that compounds which have a log K_{oct} of less than –1 will have difficulty in distributing from the vehicle into the stratum corneum and therefore only compounds with log K_{oct} > -1 can be considered as potential candidates for dermal delivery. For

compounds with log K_{oct} > 2, there are potential problems in achieving steady plasma concentrations in a reasonable time span. This is due to the drug being delayed in the stratum corneum where a reservoir can be established (Guy & Hadgraft, 1989). One classic example demonstrating this is the relationship between the *in vivo* dose absorbed and the partition coefficients of a range of non-steroidal anti-inflammatory agents and salicylates. Below the optimum log K_{oct} ~ 2.5 value, the absorption rate increases with K_{oct} as a result of the larger partition coefficient providing a larger concentration gradient across the stratum corneum (Hadgraft & Wolff, 1993).

2.4.4 Hydrogen bonding

The most powerful determinant of diffusion across the stratum corneum is the hydrogen bonding (H-bonding) capacity of the penetrant, measured in by α (H-donor) and β (H-acceptor). The hydrogen bonding ability of a compound might be related to its relative affinities for strongly H-bonding (water) and non-H-bonding (Hexane) liquids. The strength of H-bonding of a penetrant to stratum corneum depends on its own α and β values and those of the stratum corneum. For example, in the extreme case, if the stratum corneum had a β value of zero then α of the penetrant would be irrelevant. It is likely therefore, that a partitioning solvent used to model H-bonding must have similar α and β properties to stratum corneum (Pugh *et al.*, 1996).

Anderson & Raykar (1989) suggested that the stratum corneum barrier microenvironment resembled a H-bonding organic solvent and El Tayar *et al.* (1991a) suggested that the H-bond donor potential of the solute was the dominant feature in epidermal penetration transport. In contrast, Roberts (1976) suggested that both the H-bonding donor and acceptor potential of a solute governed its transport through the epidermis.

Potts & Guy (1995) related partitioning to the drug's molecular volume (MV) and H- bond donor and acceptor activity. The H-bonding terms shows that increased solute H-bond acceptor and donor activity resulted in decreased partitioning into the organic phase due to the free energy cost associated with the disruption of H-bonds in the aqueous phase. In each case, however, a smaller decrease was seen for octanol due to the finite H-bonding ability and water solubility in this solvent. In other words, H-bonding solutes are better accommodated in octanol than in alkanes. The H-bond donor regression coefficients show that solutes with H-bond donating ability, partition least well into

alkanes. Potts & Guy (1995) also documented the results which imply that the stratum corneum lipids accept H-bond better than they donate but that, like octanol, polar species can be accommodated more easily in the stratum corneum than in alkane solvents.

Solutes containing substituents which could both donate and accept H-bond (e.g $-CONH_2$, -COOH and –OH), partition similarly into stratum corneum and octanol, but less favourably into heptane. Conversely, when these substituents were replaced by groups which could only accept H-bond (e.g. $-CON(CH_3)_2$ and $-COOCH_3$), the free energies of partitioning into the stratum corneum were more similar to those into heptane than those into octanol. It follows, then that the most appropriate partitioning model for stratum corneum lipids is neither octanol-water nor hydrocarbon-water; rather, the "correct" model depends upon the properties of the solute (Potts & Guy, 1995).

Pugh *et al.* (1996) examined the effect of specific H-bonding groups on diffusion across the stratum corneum, estimated the H-bonding potential of the stratum corneum and examined how far the technique is applicable to polyfunctional compounds. Calculated diffusion coefficient was obtained using equation 2-3:

$$\log (D/h) = -1.32 - 1.30\alpha - 2.57\beta \qquad \text{(Equation 2-3)}$$

and regressed against the number (1 or 0) of each functional group responsible for H-bonding.

$$\log (D/h) = -1.36 - 1.67\text{acid} - 1.41\text{alcohol} - 1.17\text{phenol} - 0.986\text{carbonyl} - 0.759\text{ether} - 0.0502C^* \qquad \text{(Equation 2-4)}$$

Where "log (D/h) is the diffusion across the stratum corneum, "acid" is the interger number of acid groups present and C* is the number of carbon atoms not involved in H-binding. Table 2-3 present the α and β of the functional groups.

TABLE 2-3: Hydrogen bonding values of different functional groups (Pugh *et al.*, 1996).

Group	α	β
Alcohols	0.37	0.48
Phenols	0.57	0.32
Acids	0.60	0.45
Ether	0.00	0.45
Ketone	0.00	0.51
C*	0.00	0.00

The stratum corneum lipids was calculated to have 14.33 total α effect and the β effect as 21.54 and reported that the maximum rate of diffusion of an infinitely small, non-bonding molecule is about 0.03 cm/h (Pugh *et al.*, 1996).

In a study by Beall *et al.* (1994), the solubility of the 1-akyloxycarbonyl series of 5-flourouracil prodrugs showed that removing the amide-like NH group from the promoiety and replacing it with an oxygen group significantly increases the water solubilities of at least the first four members of the series. The change also improved their lipid solubilties as well.

2.4.5 Melting point

The melting point of substances reflects their relative hydrophobia associated with a low level of crystalline interactions. Drug crystallinity, or melting point, influences permeability and was found to be inversely proportional to lipophilicity (log K_{oct}). The melting point of a substance is often considered to be an indicative of the maximum flux attainable through the skin. The lower the melting point, the greater is the drugs ability to permeate the skin. It is assumed that there should be an exponential increase in dermal flux with a decreasing melting point (Calpena *et al.*, 1994, Cleary, 1993 and Guy & Hadgraft, 1989).

Calpena *et al.* (1994) conducted a study to determine the permeation parameters (transdermal permeability rate (k_p), lag time and flux) as a measure of the intrinsic permeability of various anti-emetic drugs across the skin. The k_p varied inversely with melting point, compounds with lower melting point had higher k_p values (Figure 2-8).

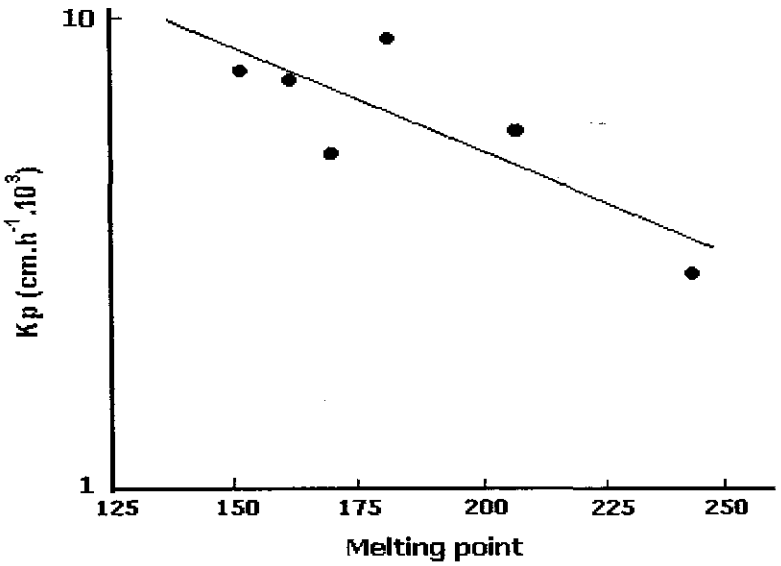

FIGURE 2-8: Linear relationship between log k_p values and melting point (mp) for anti-emetic drugs assayed (Calpena *et al.*, 1994).

Linear correlation was found between logarithm of k_p and the melting point as expressed by equation 2-5.

$$\text{Log } k_p = 1.6018 - 0.004461mp \qquad \text{(Equation 2-5)}$$
$$(r = 0.8120;\ p > 0.05)$$

Kerr and colleagues (1998) determined the melting points and other physicochemical properties (i.e lipid solubility, partition coefficient (K_{oct})) of alkylcarbonylmethyl prodrugs of theophylline. The data presented in Table 2-4 reflects the correlation between melting point, lipid solubility and partition coefficient. Lipid solubility and partition coefficient increase with decrease in melting point. The first several members of a series are usually the members that give the greatest increase in delivery of the parent drug.

TABLE 2-4: Melting point (mp), lipid solubility (S_{IMP}) and partition coeffients for 1-alkylcarbonyloxymethyl prodrugs of theophilline (Kerr *et al.*, 1998).

Alkyl =	Mp (°C)	S_{IPM} (±SD) (µmol/ml)	K_{oct} ± SD
Th	270 – 274	0.34	0
CH_3	163.5 – 165	2.75 (0.099)	0.141 (0.006)
C_2H_5	146 – 147	2.93 (0.11)	0.634 (0.05)
C_3H_7	104 –105	25.4 (0.32)	2.41 (0.27)
C_4H_9	86 – 87	44.0 (1.5)	8.42 (0.18)
C_5H_{11}	58 – 60	77.8 (0.45)	28.0 (2.5)

2.4.6 Ionisation

The pH partition theory is well documented for general absorption of ionisable drugs across the gastro-intestinal tract, but it is less well described in the dermal and transdermal delivery of drugs. This is perhaps surprising given the number of medicines that are delivered to the skin and which would be ionised over the normal physiological pH range of the dermal tissues (4-7.4). It is generally accepted that, where possible, the free acid or free base should be used, however perhaps this premise should be questioned (Hadgraft & Valenta, 2000). Lund (1990) also documented that, where a drug molecule is capable of dissociating into ions, equilibrium exists between the ionized and unionized species. However is only the unionized form that is appreciably absorbed. Because the proportion of ionized and unionized forms of the weak acids and bases varies with the hydrogen ion concentration, pH changes can alter markedly their rate of absorption.

A strong acid or strong base will exist in the ionized form at all pH values and will be poorly absorbed through membranes. For weak acids and bases the proportion of unionized absorbable species and ionized non-absorbable species can be calculated at any pH if the dissociation constant or (ionization constant, K_a) for the acid or base is known. The dissociation constant of a weak acid (HA) ionizing to give hydrogen ions (H^+) and ions of the conjugate base (A^-) is defined by:

$$K_a = \frac{[H^+][A^-]}{[HA]} \qquad \text{(Equation 2-6)}$$

where $[H^+]$, $[A^-]$ and [HA] are the concentrations of the hydrogen ions, conjugate base and unionized acid respectively. The pK_a value for an acid is the common logarithm of the reciprocal of the dissociation constant, and is a measure of acid strength. A pK_a value below 2.5 indicates a strong acid. Weak acids have pK_a value in the range 2.5 to 8 and very weak acids a pK_a value greater than 8. The relative proportions of ionized and unionized species can be calculated from the Henderson-Hasselbalch equation:

$$\log_{10}\frac{[A^-]}{[HA]} = pH - pK_a \qquad \text{(Equation 2-7)}$$

Ionization of weak base (B) may be represented as producing hydroxyl ions (OH^-) and ions of the conjugate acid (BH^+) (Lund, 1994):

$$K_b = \frac{[BH^+][OH^-]}{[B]} \qquad \text{(Equation 2-8)}$$

A high pK_a value indicates a strong base and equation 2-9 predicts that the proportion of ionized species is increased by a decrease in pH.

$$\log_{10}\frac{[BH^+]}{[B]} = pK_a - PH \qquad \text{(Equation 2-9)}$$

According to Hadgraft & Valenta (2000), the transport properties of a permeant can be described by the permeabilities of the ionized and unionized species and the respective concentrations k_{ion}, k_{punion}, c_{ion} and c_{union} respectively.

$$J_{tot} = k_{punion} * c_{union} + k_{pion} * c_{ion} \qquad \text{(Equation 2-10)}$$

(J_{tot}) = total flux of the permeant

For any given pH, pK_a and total applied concentration, it is therefore possible to calculate the amounts of c_{ion} and c_{union}.

If passive diffusion is the predominant mechanism for percutaneous penetration of methotrexate, then changes in vehicle pH would be expected to influence the *in vivo* percutaneous absorption of methotrexate through human cadaver skin, since transport by passive diffusion is generally maximized when the drug is present in the unionized

form. Table 2-5 presents the effect of pH on *in vitro* percutaneous penetration of methotrexate (Vaidyanathan, 1985).

TABLE 2-5: Effect of pH on *in vitro* percutaneous penetration of methotrexate (Vaidayanathan, 1985).

pH	Steady-state penetration rate ($ug.cm^{-2}h^{-1}$)
1.98	0.1588
2.98	0.1995
3.87	0.4064
4.12	0.3182
5.29	1.6687
6.34	2.5819

Smith (1990) documented that the transdermal absorption of scopolamine was shown to be substantially higher at pHs above the pK_a of the weak base, in general higher fluxes will be obtained by maintaining the pH such that the penetrant is unionized. It should be noted that nonphysiological pHs may also change properties of the skin, that could affect solubility, partitioning or binding, resulting in changes in dermal penetration. Swabrick *et al.* (1984) found that the permeability coefficient of the unionized chromone-2-carboxylic acids were approximately 10 000 larger than those of the corresponding ionized species.

2.4.7 Molecular size

It is well documented that the molecular size of a substance directly affects its diffusion across simple or complex membranes. The diffusion of molecules through liquids is inversely proportional to the molecular weight of the molecules or to the square or cube root thereof. This phenomenon may also be expected in the case of diffusion across the skin (Calpena *et al.,* 1994). Considering that the horny layer is a compact membrane and that diffusing molecules follow a tortuous path through it, it might seem obvious that the diffusion coefficient would be inversely related to molecular weight (MW) or some measure of molecular size (Zats, 1993). Diffusivity is a kinetic term, and is a rough measure of the ease with which a molecule can move about within a medium (in this case, the skin). The larger the molecule, the more difficult it is to move about, and the lower the diffusivity (Smith, 1993).

Lund (1994) documented that, molecules of small sizes in high concentration tend to penetrate more readily than large molecules. However, for a range of chemically equivalent molecules with similar molecular weight, there is little correlation between their size and absorption potential.

Several authors used the following equation to relate diffusion (D) to size:

$$D = D_o (MW)^b \qquad \text{(Equation 2-11)}$$

D_o is the diffusion coefficient and b refers to the mass selectivity coefficient. For diffusion across membranes, apparent values of b from –3 to –5 indicate a strong dependence of diffusion on molecular weight (Pugh *et al.*, 1996). Anderson and Raykar (1989) using cresol and hydrocortisone esters (N = 16), used other penetration parameter such as k_p rather than D (Equation 2-12, where b = -4.6)

$$k_p = \text{constant}.(K_{oct})^a.\ (MW)^b \qquad \text{(Equation 2-12)}$$

In the study by Calpena *et al.* (1994), no relation was observed between the molecular weight and the transdermal permeability rate constant (k_p) of the antiemetics studied (Table 2-6). Alizapride, bromopride and metoclopramide on the one hand and clebopride, domperidone and metopimazine on the other have similar MWs but show clear differences in their k_p values. Other physicochemical characteristics may be more directly involved in dermal permeability, whereas MW may be a secondary factor when there are only small differences in the MWs.

TABLE 2-6: Molecular weights, transdermal permeability rate constants (k_p) and estimated fluxes (J) (Calpena *et al.*, 1994).

Drug	k_p (cm/h) x 10^3	J (mg/cm^{-2}/h) x 10^2
Alizapride	5.7 ± 3.2	2.82 ± 1.58
Bromopride	7.8 ± 2.1	3.89 ± 1.05
Clebopride	7.4 ± 4.2	3.71 ± 2.09
Domperidone	2.8 ± 0.9	1.40 ± 0.44
Metoclopramide	9.1 ± 2.5	4.54 ± 1.27
Metopimazine	5.1 ± 1.4	2.53 ± 0.78
Scopolamine	4.1 ± 1.8	2.03 ± 0.90

2.5 The influence of alkyl chain length on percutaneous absorption

Molecular modification of active substance can have marked effects on their activity. Changes in functional groups that alter the solubility and the partition coefficient of the substance between the vehicle and the skin barrier may retard or enhance skin penetration (Lund, 1994). Improvement in delivery of a drug frequently requires the design of transient derivatives of the drug which are called prodrugs. The design of prodrugs that exhibit the desired hydrophilic/lipophilic balance in their solubility which is necessary for the efficient dermal or transdermal of their parent drug, requires precise knowledge of the relative aqueous and lipid solubilities of the members of the proposed series of prodrugs (Beall, 1993a). An understanding of the manner in which these physicochemical properties (aqueous and lipid solubilities, partition coefficient and others) change within a homologous series i.e incremental addition of methylene units, can be of use in choosing a derivative having optimum properties (Yalkowsky *et al.,* 1972).

For a homologous series of chemicals, lipid/water partition coefficient increases exponentially with increasing chain length. Thus for a membrane of fixed or normalized thickness, the permeability coefficient through a lipid pathway will directly reflect partitioning and will follow:

$$P_n = P_{(n=10)}\, 10^{\pi n} \qquad \text{(Equation 2-13)}$$

In this equation, n is the alkyl chain length, $P_{(n=10)}$ is an intercept value equating to the homologue with no alkyl chain length, and π is a positive constant related to the free energy of partitioning of a methylene unit. This relationship holds as long as the rate-determining step in crossing the membrane is passage through a lipid region; the equation indicates that, for a pure lipid membrane, a plot of the logarithm of the permeability coefficient versus the alkyl chain length of the permeant will be a straight line with an intercept at $P_{9n=100}$ and a slope equal to π (Walters, 1990).

According to Yalkowsky & Flynn (1973), equation 2-14 is a useful relationship which allows chain length (n) to be used in lieu of partition coefficients (log K_n) in theoretical analysis.

$$\text{Log}K_n = \text{log}K_o + \Pi_n \qquad \text{(Equation 2-14)}$$

Depending on the organic phase chosen, the value of Π, the slope of the $\log K_n$ versus *n* plot, can be as small or smaller than 0.3 and as large as 0.7. Values of 0.3 to 0.5 are typical for biological membranes. At the lower end of the range it takes an addition of three methylene units to the alkyl chain length to produce a 10-fold increase in partition coefficient. Two methylene units produce this effect when the Π value hovers around 0.5.

The permeation of simple hydrophobic membranes by alkyl homologue applied to the membrane aqueous solution often evidences a dominating influence of o/w partitioning on the permeability coefficient through the short to middle chain length of the series (Flynn, 1989). This is illustrated in Figure 2-9, in which permeability coefficient profiles for three homologous series permeating silicone rubber membranes are described (Flynn & Yalkowsky, 1972).

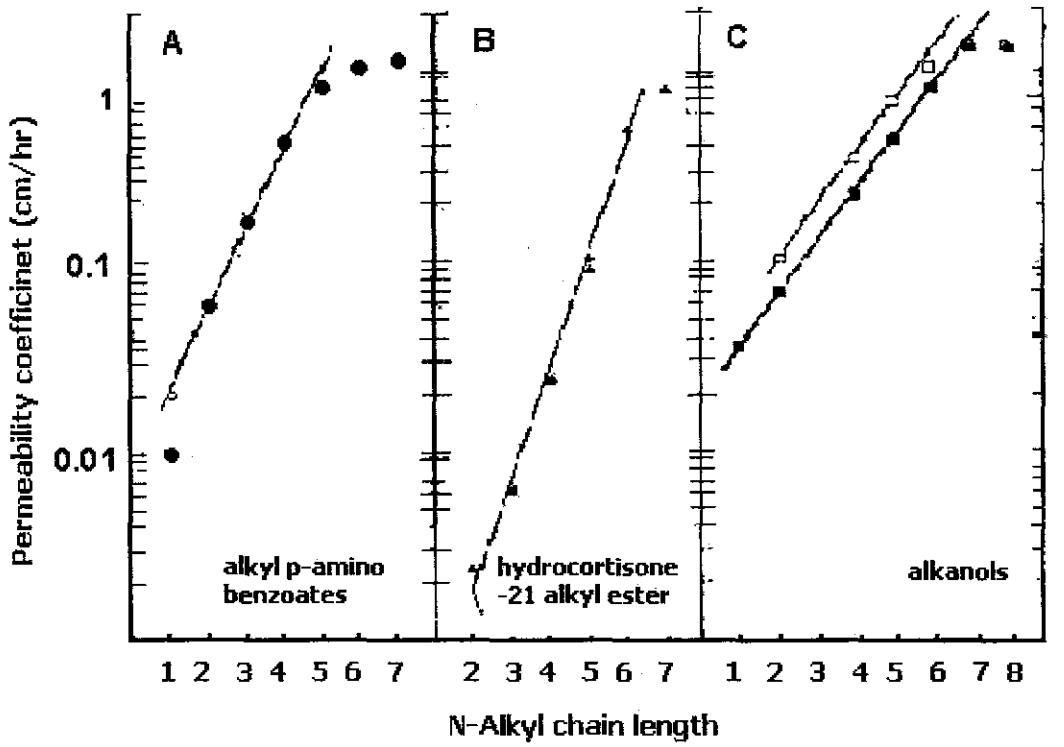

FIGURE 2-9: Series of plots of 37 °C permeability coefficients as a function of alkyl chain length. (A) Data for the alkyl-*p*-amino benzoates, (B) the hydrocortisone-21-alkyl ester permeability coefficients and (C) data for the homologous *n*-alkanols through 74μm (□) and 100μm (■) homemade silicone rubber membranes (Flynn & Yalkowsky, 1972).

It is seen that $\log K_{oct}$ for short-chain membrane of each of these very different homologous series rises sharply and linearly with increased length of the alkyl chain. It will also be noticed that for each series the profile levels out at some long-chain length. Partitioning into the membrane follows the $\log K_n$ versus *n* homologous relationship expressed in equation 2-14. At the shorter chain length the o/w partition coefficient is very small and consequently the membrane resistance is high enough to totally control the rate of the permeation process. However, as the chain is lengthened, the resistance of the membrane is exponentially decreasing owing to its reciprocal dependency on the o/w partition coefficient. The summed resistance of the boundary layers at the same time remains unchanged or even increases gradually because of the effect of increasing molecular size on diffusivity. Consequently, a point is reached at which the low, but unchanging, resistance of the boundary layers assumes rate-controlling proportions (Flynn, 1989).

Yalkowsky *et al.* (1972) investigated the importance of alkyl chain length on physicochemical properties (i.e., melting points, solubilities and partition coefficients) of an organic homologous series of alkyl *p*-aminobenzoates. The melting points of the alkyl *p*-aminobenzoic acid esters determined by DSC are shown in Figure 2-10. As chain length is increased, the melting point decreases almost linearly to the butyl ester and then increases gradually and irregularly. The solubilities of each ester studied in water, silicone oil and hexane provide estimates of the partition coefficients of each homolog between the immiscible phases. The logarithm of the partition coefficient is linearly dependent on chain length. Figure 2-11 shows this linearity and the absence of an inflection point at four carbons for both the silicone oil-water and the hexane-water partition coefficients.

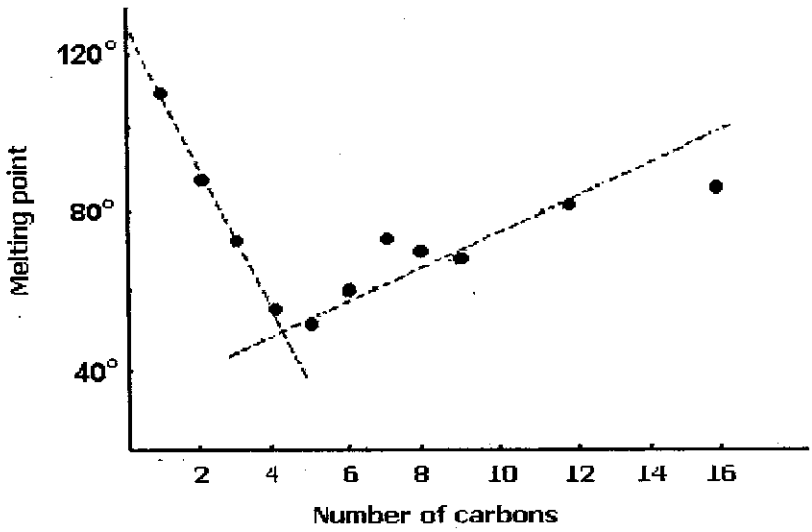

FIGURE 2-10: Melting points of alkyl *p*-aminobenzoates. (Yalkowsky *et al.*, 1972).

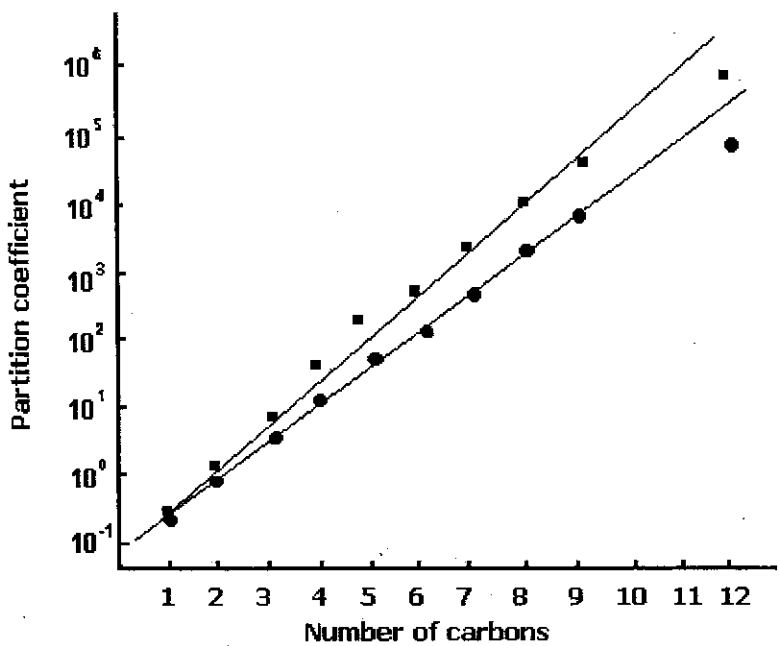

FIGURE 2-11: Partition coefficients of alkyl *p*-aminobenzoates. ●, silicone oil-water; and ■, hexane-water. (Yalkowsky *et al.*, 1972).

The curve of melting points (Figure 2-10) shows a sharp change in slope at the butyl derivative. This is because melting points of crystalline materials are heavily dependent upon crystal energies. It is proposed that the nonlinearity of the curve is due to a change

in crystal structure with chain length. The crystal structure of the lower homologs is probably determined primarily by the aromatic ring and the dipolar nature of the moiety. If the chain is increased beyond four carbons, the linear aliphatic chains begin to exert a dominating effect. This is also true for the solubilties. Partition coefficient is a property of the solute and therefore is not dependent on crystal structure, is only dependent upon the interactions occurring in solution (Yalkowsky *et al.,* 1972).

Flynn & Yalkowsky (1972) on their study of the effect of alkyl chain length on the flux across a synthetic membrane found that a plot of the logarithm of steady-state flux from saturated solution against chain length gave a parabolic curve. The maximum flux is attained between C-3 and C-4. For chain length increasing beyond C-4, the flux decreased due to diminished aqueous solubility. These results indicate that the mechanism of diffusion passes from regulation by the membrane to being governed by the diffusion layer at about C-4. Sasaki *et al.* (1990) obtained similar results, whereby the butyl derivative (C-4) showed the highest flux. The flux decreased with greater alkyl chain length of the derivative.

2.6 Conclusion

Skin permeation and uptake measurements are useful in product development and toxicologic evaluation. The target site of action and intended use of a product determine the type of absorption behaviour that is most desirable. For most compounds, the horny layer (stratum corneum), the outermost skin section consisting of a compressed amalgam of dead cells separated by oriental layers of neutral lipids, represents the principal barrier to transport. This makes possible the use of excised skin in *in vitro* diffusion experiments. Shunt diffusion via follicles and glands may contribute significantly to the absorption of many drugs (Zats, 1993).

The major advantage claimed for percutaneous absorption is that it avoids the vagaries of the gastrointestinal milieu and does not shunt the drug directly through the liver, thereby avoiding the "first-pass" effect. The major disadvantages for percutaneous absorption are related to the barrier properties of the skin.

The major route of penetration through the stratum corneum is via the lipid-rich intercellular channels. Thus, the first physicochemical constant that can be identified as being significant is the lipid/water partition coefficient of the drug. Another parameter that

should be taken into account is the solubility of the drug in the skin lipids. Ideally it should be possible to predict the rate and extent of percutaneous absorption from the knowledge of the simple physicochemical properties of the drug.

As the full metabolic potential of the skin is gradually being unravelled along with the physicochemical processes involved in the dermal diffusion process, it can be anticipated that more prodrugs will be designed for dermal delivery to optimize the bioavailability of each drug delivered via the percutaneous route, for either local or systemic action (Chan & Li Wa Po, 1989).

Present research is aimed at determining the physicochemical properties of cyclizine and its alkyl analogues relevant to skin transport and their percutaneos delivery through human skin *in vitro.*

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Chapter 3

Synthesis and characterization of cyclizine alkyl analogues

3.1 Introduction

Cyclizine (**I**) is an anti-emetic drug primarily indicated for the prophylaxis and treatment of nausea and vomiting associated with motion sickness. It is widely used and also suitable for children from six years of age. It also exhibits slow onset of action and long duration of action (Korolkovas, 1988). (**I**) is conventionally administered orally in the form of tablets and syrups, rectally as suppositories and as intramuscular injections. Percutaneous delivery of (**I**) can avoid the "first-pass" effect, minimize the discomfort of injections and improve patient complience. Walker & Kanfer (1995) reported that, despite widespread use of the drug, particularly for paediatric application, not much is known about the pharmacokinetics of this compound.

In the development and design of topical and transdermal formulation it is important to take into account the physicochemical properties of the penetrant. They will provide indications on the feasibility of efficient delivery of the drug using this route of administration and suggest design strategies that will be required in either formulation or reformulation of the product (Hadgraft & Wolff, 1993). The delivery problems can be attributed to the low water and lipid solubility of the compound, thus it is likely that the delivery characteristics can be improved by using the prodrug approach, i.e. development of derivatives (analogues) possessing both a high water-solubility and lipophilicity at physiological pH (pH 7-8). The most important feature that an analogue of cyclizine might contribute is decreased crystallinity and increased lipophilicity.

Calpena *et al.* (1994) documented the physicochemical properties of some anti-emetic drugs (Table 3-1) and highlighted the possibility of their delivery through the skin of hairless rats as the membrane.

TABLE 3-1: Physicochemical properties for the assayed anti-emetics (Calpena *et al.*, 1994).

Drug	MW, g/mol	mp, (°C)	pK_a	K_{oct}
Aliprapride	339.90	207.0	9.15	6.9 ± 0.4
Bromopride	344.26	152.5	9.35	21.8 ± 0.8
Clebopride	507.90	162.0	8.59	0.2 ± 0.0
Domperidone	425.92	242.5	8.06	1034.7 ± 202
Metoclopramide	354.30	182.0	8.77	1.4 ± 0.1
Metopimazine	445.61	170.5	8.64	65.5 ± 25.1
Scopolamine	303.35	59.00	7.55	4.7 ± 0.7

There have been numerous reports on the analysis of cyclizine (I) with thin layer chromatography (TLC), high-pressure liquid chromatography (HPLC), gas chromatography (GC) and other techniques. Musumarra (1984) identified (I) and many other compounds by the application of TLC in forty different eluent mixtures. The R_f values of (I) found ranged from 0.02 – 0.85 depending on the eluent used. Dutt and Poh (1980) used ninhydrin as a spray reagent for the detection of (I) on TLC (R_f = 0.76).

Jalal *et al.* (1988) described a reverse-phase HPLC method for the determination of (I) in commercial tablets. Walker & Kanfer (1987, 1995) reported the sensitive HPLC determination of (I) and its demethylated metabolite (norcyclizine) in biological fluids using solid phase extraction and coulometric detection using a reverse-phase C_{18} analytical column and phosphate buffer (0.05M, pH 3):acetonitrile (6:4 & 7:3) as mobile phase. Determination of (I) and norcyclizine in plasma and urine using gas-liquid chromatography with nitrogen selective detection was described by Land *et al.* (1981).

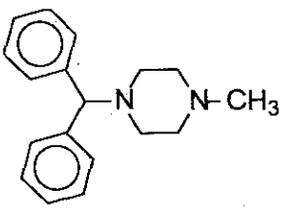
The objectives of this part of the study were to synthesis and characterize alkyl analogues of (I) and to develop analytical methods that are easy to use and sensitive enough for the quantitative determination of (I) and its alkyl analogues.

3.2 Description of cyclizine

3.2.1 Name, formula and molecular mass

Cyclizine is 1-(diphenylmethyl)-4-methylpiperazine.

Other names: Compound 47-83, Marezine, N-benzhydryl-N'-methylpiperazine, Nautazine, Neo-Devomit, Valoid and Wellcome preparation 47-83.



Formula: $C_{18}H_{22}N_2$

Molecular Mass: 266.4 (Benezra & Yang, 1977)

3.2.2 Appearance, colour and odour

Cyclizine is a crystalline, white and odourless powder.

3.2.3 Melting point, stability and solubility

The melting range of cyclizine is 106 °C to 109 °C. Cyclizine is stable up to 5 years at room temperature. At 105 °C cyclizine suspensions at pH 11.5 decompose to N-methylpiperazine, benzhydrol and benzophenone. The solubility of cyclizine at 25 °C is as follows:

Solvent	Solubility gm/ml
Water	< 0.1
Ethanol	0.17
Chloroform	1.1
Ether	0.17 (Benezra & Yang, 1977)

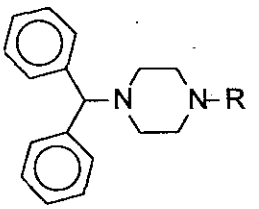
3.3 Experimental

Synthesis of cyclizine alkyl analogues was done according to the literature methods described by (Zikolova & Ninov, 1972 and Zikolova *et al.*, 1984). 1-

(Diphenylmethyl)piperazine, ethyliodide, propyliodide and butyliodide were obtained from Sigma-Aldrich, (UK).

3.3.1 Synthesis of cyclizine analogues

- **General method**



$R = CH_2CH_3$	1-(Diphenylmethyl)-4-ethylpiperazine (**II**)
$R = (CH_2)_2CH_3$	1-(Diphenylmethyl)-4-propylpiperazine (**III**)
$R = (CH_2)_3CH_3$	1-(Diphenylmethyl)-4-butylpiperazine (**IV**)

To 0.12 mole (30.28 g) of 1-(diphenylmethyl)piperazine in 20 ml dry benzene, 20.6 g anhydrous sodium bicarbonate was added. The reaction was stirred and heated under reflux. To the suspension, 0.12 moles of ethyliodide or propyliodide or butyliodide dissolved in 20 ml dry benzene was added dropwise over a period of 20 minutes. The reaction was refluxed till completion as followed on TLC. It was filtered, washed with dry benzene and the solvent was removed under vacuum. In each instance an almost white powder (compounds **II**, **III** or **IV**) was purified by column chromatography (ethyl acetate:dichloromethane:methanol, 7:2:1) and recrystallised at room temperature.

3.3.2 Chromatographic techniques

3.3.2.1 Thin layer chromatography (TLC)

Analytical thin layer chromatography was performed on precoated Merck silica gel backed plates (thickness 0.25 mm) with eluant system consisting of ethyl acetate:dichloromethane:methanol. Mobile phase was prepared by mixing solvents in a volume to volume ratio (7:2:1).

3.3.2.2 Column chromatography

Column chromatography was performed using Merck Silica 60 (0.063-0.200 mm) silica gel, with eluent system consisting of ethyl acetate:dichloromethane:methanol. Mobile phase was prepared by mixing solvents in a volume to volume ratio (7:2:1).

3.3.3 Instrumentation

3.3.3.1 Mass spectroscopy (MS)

Electron impact (EI+) mass spectra were recorded on a micromass autospec ETOF mass spectrometer.

3.3.3.2 Nuclear magnetic resonance spectroscopy (NMR)

^{13}C and ^{1}H nuclear magnetic resonance spectra were recorded on a Varian Gemini-300 spectrometer. ^{13}C spectra were recorded at a frequency of 75 MHz, while ^{1}H spectra were recorded at a frequency of 300 MHz. The following abbreviations were used to describe the multiplicity of ^{1}H signals: s = singlet, t = triplet, st = sextet, bs = broad singlet and m = multiplet.

3.3.3.3 Infrared absorption spectra

Infrared spectra were recorded on a Nicolet 550 series II spectrometer using KBr pellets.

3.3.3.4 Melting point

Melting points were taken in capillary tubes on an electrothermal digital Büchi B-540 melting point apparatus and are uncorrected.

3.3.4 High-pressure liquid chromatography (HPLC) analytical procedure

3.3.4.1 Reagents and materials

HPLC analytical grade acetonitrile (Across Organics, New Jersey, USA), potassium dihydrogen phosphate and phosphoric acid (Merck, Johannesburg, SA) were used. HPLC grade, double deionised water from a Milli-Q 50 purification system was used throughout the experiment. n-Octanol was obtained from BDH Laboratory Supplies (Poole, England).

3.3.4.2 The HPLC system

HPLC system used during the study comprised of Shimadzu LC-6A delivery system, Shimadzu SPA-6A UV detector, Shimadzu SCL-6B system controller with SIL-6B autosampler and Shimadzu CR6A chromatopac (integrator). A Machery-Nagel Lichrospher (4 x 250 mm), 100 Å pores - 5 µm, RP – 18 endcapped column (Machery-Nagel, Düren, Germany) was used. Compounds were analysed with a mobile phase comprising of acetonitrile:0.05M (pH 3) phosphate buffer (6:4) with UV detection at 200 nm. The pH was adjusted to 3 with 15% phosphoric acid solution. The flow rate was 1.0 ml/min and column temperature was maintained at 25 °C. The retention times for (**I**), (**II**), (**III**) and (**IV**) were 8, 10, 10 and 12 minutes repectively.

3.3.4.3 Preparation of solutions

Ten milligrams of (**I**) and its alkyl analogues were separately dissolved in 50 ml volumetric flasks with HPLC grade water:methanol (9:1) solution to produce 200 µg/ml solutions. Ten milliliters of each solution were transferred to 100 ml volumetric flasks and filled to volume with HPLC grade water to produce 20 µg/ml stock solutions. Standard solutions with 0.2, 0.4, 0.6, 1.0, 3.0, 5.0, 8.0 and 10.0 µg/ml were prepared.

3.3.4.4 Calibration curve

Calibration curves for (**I**) and its alkyl analogues were established using the standard solutions prepared (0.2, 0.4, 0.6, 1.0, 3.0, 5.0, 8.0 and 10.0 µg/ml).

3.3.4.5 Linearity

The assay linearity for (I) and its alkyl analogues was determined by performing a linear regression analyses on the peak area ratios versus concentrations in the range 0.2 – 10.0 µg/ml. The regression values were all in the region of 0.9997 and 0.9999.

3.3.4.6 Precision

The precision of the method was investigated in terms of inter-day (reproducibility) and intra-day (repeatability) variations.

- **Inter-day precision**

Precision was determined by performing HPLC analyses (n = 3) of three samples containing known amounts of each compound (0.2, 0.6 and 10.0) on three different days. The inter-day precision for all the compounds was within the acceptable limits, as the % recovery was found to be ranging between 98.5 % and 99.5 %.

- **Intra-day precision**

Precision was determined by performing HPLC analyses (n = 3) of three samples containing known amounts of each compound (0.2, 0.6 and 10.0) on the same day. The intra-day precision for all the compounds was within the acceptable limits, as the % recovery was found to be ranging between 99.0 % and 100.0 %.

3.3.4.7 Sensitivity

Determining the lowest limit of quantification and limit of detection can assess the sensitivity of an analytical method. The limit of quantification is defined as the lowest concentration of an analyte in a sample that can be determined with acceptable precision and accuracy. The limit of detection is the lowest concentration of an analyte in a sample that can be detected, but not necessarily quantified. The limit of detection for (I) was less than 100 ng/ml and for the analogues was ≤ 0.01 µg/ml.

3.3.4.8 Solubility determination

3.3.4.8a Aqueous solubility

The aqueous solubilities of (I) and its alkyl analogues were determined by equilibrating large excess of each compound with HPLC water. The temperature (25 °C) was held constant by means of a water bath. To hasten the attainment of equilibrium, magnetic bars continuously stirred each slurry. Equilibrium was attained within 24 hours. Samples were then filtered through a Millipore filter (0.45 µm), measured with respect to volume and assayed by HPLC.

3.3.4.8b Partition coefficient (K_{oct})

Equal volumes of n-octanol and phosphate buffer (pH 7.4) were saturated with each other for at least 24 hours before the experiment. Solutions of (I) and its alkyl analogues were prepared with the pre-saturated n-octanol phase as a solvent. 5 ml of these solutions were transferred to 10 ml test tube, each containing equal volumes (5 ml) of phosphate buffer. Three tubes of each compound were stoppered and agitated for 1 hour and another set for 2 hours. After centrifugation, the n-octanol and buffer phases were separated and appropriately diluted with methanol and mobile phase respectively before injecting onto the HPLC column. Partition coefficients were calculated as the ratio of drug concentration in the n-octanol phase to that in the buffer phase. There was no difference in the values of K_{oct} when the tubes were agitated for 1 or 2 hours.

3.4 Results

The analogues were successfully synthesized and their MS, NMR and IR spectra are given in Annexure A, B and C respectively. The melting point of (I) was the same as reported in the literature. Molecular mass of each compound was determined experimentally with mass spectroscopy and by empirical calculation. The value of molecular mass was the same in both cases. The ACD-Labs Log D-suite software was used to determine the molar volume.

3.4.1 Spectral data of cyclizine alkyl analogues

Compound (**II**)

6.71g (44.73 %) of almost white product were produced following the analysis by TLC and column chromatography (CC) (ethyl acetate:dichloromethane:methanol, 7:2:1). R_f value: 0.64; mp: 51 °C; m/z (EI+, %) (spectrum 2): M^+ 280 (91), 113 (100), 56 (58), 165 (75), 167 (89), 194 (84), 195 (68), 208 (56), 70 (67); δ_H (spectrum 7, 300 MHz, $CDCl_3$): 1.1 (t, 3H, CH_3), 2.4 ((t, 2H, CH_2), 2.5 (bs, 8H, piperazine protons), 4.21 (s, 1H, CH), 7.3 (m, 10H, aromatic protons); δ_C (spectrum 8, 75 MHz, $CDCl_3$): 11.7 (CH_3), 51.6 (CH_2), 52.2 (CH_2), 52.9 (CH_2), 76.2 (CH), 126.9 (CH), 127.9 (CH), 128.5 (CH), 142.8 (C). ν_{max} (spectrum 14, KBr, cm^{-1}): 725, 960, 1160, 1250, 1460, 2800, 2960, 3460.

Compound (**III**)

8.00g (53.3 %) of almost white product were produced following the analysis by TLC and column chromatography (CC) (ethyl acetate:dichloromethane:methanol, 7:2:1). R_f value: 0.71; mp: 70 °C; m/z (EI+, %) (spectrum 3): M^+ 294 (89), 127 (100), 167 (97), 194 (78), 195 (78) 208 (54), 165 (71), 56 (55); δ_H (spectrum 9, 300 MHz, $CDCl_3$): 0.9 (t, 3H, CH_3), 1.5 (st, 2H, CH_2), 2.31 (t, 2H,CH_2), 2.49 (bs, 8H, piperazine protons), 4.21 (s, 1H, CH), 7.3 (m, 10H, aromatic protons); δ_C (spectrum 10, 75 MHz, $CDCl_3$): 11.8 (CH_3), 19.9 (CH_2), 51.8 (CH_2), 53.4 (CH_2), 60.6 (CH_2), 76.2 (CH), 126.9 (CH), 128 (CH), 128.5 (CH), 142.9 (C). ν_{max} (spectrum 15, KBr, cm^{-1}): 725, 1000, 1160, 1250, 1460, 2800, 2960, 3460.

Compound (**IV**)

5.12g (34.1 %) of almost white product were produced following the analysis by TLC and column chromatography (CC) (ethyl acetate:dichloromethane:methanol, 7:2:1). R_f value: 0.67; mp: 52 °C; m/z (EI+, %) (spectrum 4): M^+ 308 (90), 141 (98), 167 (99), 194 (89), 195 (85), 208 (62), 165 (76), 56 (60); δ_H (spectrum 11, 300 MHz, $CDCl_3$): 0.9 (t, 3H, CH_3), 1.28 (m, 2H, CH_2), 1.42 (m, 2H, CH_2), 2.3 (t, 2H,CH_2), 2.41 (bs, 8H, piperazine protons), 4.2 (s, 1H, CH), 7.3 (m, 10H, aromatic protons); δ_C (spectrum 12, 75 MHz, $CDCl_3$): 13.9 (CH_3), 20.7 (CH_2), 28.9 (CH_2) 51.9 (CH_2), 53.5

(CH_2), 58.5 (CH_2), 76.3 (CH), 126.9 (CH), 128 (CH), 128.5 (CH), 142.9 (C). ν_{max} (spectrum 16, KBr, cm^{-1}): 725, 1000, 1160, 1250, 1460, 2800, 2960, 3460.

3.4.2 Physicochemical properties

The physicochemical properties of (**I**) and its three alkyl analogues are summarized in Table 3-2. All analogues exhibited lower melting points compared to (**I**). The molecular volumes were determined with ACD-Labs LogD-software.

TABLE 3-2: Physicochemical properties of (I) and its alkyl analogues.

Compound	Molecular weight (g/mol)	Molar volume (cm^3)	Melting point (°C) ± SD
I	266.4	250.7	107 ± 0.82
II	280.4	268.2	51.0 ± 1.33
III	294.4	284.7	70.1 ± 0.14
IV	308.5	301.3	52.1 ± 0.75

Aqueous solubilities ± standard deviation (SD) and n-octanol/water (K_{oct}) partition coefficients ± standard deviation (SD) of (**I**) and its alkyl analogues are listed in Table 3-3. (**I**) was found to have aqueous solubility of 0.186 mg/ml at 25 °C, which is relatively low. However, the alkyl analogues exhibited higher aqueous solubilities than (**I**). Increasing alkyl chain on the piperazine ring results in compounds (**II**, **III**, and **IV**) that are more lipophilic.

TABLE 3-3: Aqueous solubility and n-octanol/water partition coefficient for (I) and its alkyl analogues.

Compound	Aqueous solubility (25 °C) (µg/ml ± SD)	Log K_{oct} ± SD
I	185.5 ± 0.20	3.11 ± 0.4
II	569.6 ± 5.39	3.64 ± 0.4
III	189.9 ± 3.30	4.18 ± 0.2
IV	481.8 ± 11.8	4.71 ± 0.5

3.5 Discussion

Physical data

The column chromatographic separation of the analogues appeared to be successful as followed by TLC. NMR analysis as well indicated that there were no mixtures. Further analysis were conducted i.e. MS and IR and proved to be fruitful.

Compound (II), (III) and (IV)

The MS data for the compounds confirmed the presence of the molecular ions (M^+) at m/z 280, 294 and 308, corresponding to the molecular formula $C_{19}H_{24}H_2$ (II), $C_{20}H_{26}N_2$ (III) and $C_{21}H_{28}N_2$ (IV), respectively. M^+ 113, 127 and 141 correspond to the N-alkyl piperazine fragment for (II), (III) and (IV) respectively and the signal at 167 represents the aromatic portion without the alkyl piperazine radical. Signals at 194, 195 and 208 represent the rearrangement and fragmentation of the alkyl piperazine moiety.

In addition to the ^1H NMR and ^{13}CNMR signals of cyclizine (I), compound (II) has a triplet from the methyl group at δ 1.1 in the ^1H NMR spectrum and at δ 11.7 in the ^{13}C NMR spectrum. The ^1H NMR spectrum of (III) shows in addition to signals of (I) a sixtet at δ 1.5 due to the methylene protons and a triplet at δ 0.9 due to the methyl group of the propyl moiety. ^{13}C NMR spectrum of (III) shows these signals due to

methylene carbon atom at δ 19.9 and at 11.8 arising from the methyl group of the propyl moiety. The ^{1}H NMR spectrum of (**IV**) shows in addition to signals of (**I**) a multiplet at δ 1.28 and 1.42 due to the methylene protons and a triplet at δ 0.9 due to the methyl group of the butyl moiety. ^{13}C NMR spectrum of (**IV**) shows these signals due to methylene carbon atoms at δ 20.7 and at 28.9 whereas the methyl group of the butyl moiety resonates at δ 13.9.

Physicochemical properties

The trend in melting points as a function of increasing alkyl chain length can be seen in Table 3-2. Although there is irregularity in the pattern, overall the melting points decrease as the alkyl chain is extended. Compound (**I**) had a melting point of 107 °C. Increasing chain length to $-CH_2CH_3$ and $-CH_2CH_2CH_2CH_3$ resulted in compounds (**II**) and (**IV**) with melting points of about 55 °C lower than (**I**). Increasing the chain to $-CH_2CH_2CH_3$ resulted in a melting point higher than that of (**II**) and (**IV**), however the melting point was lower than that of (**I**). Yalkowsky *et al.* (1972) studied alkyl chain length series and found that melting points decreased overall, but not linearly. This indicates that extra alkyl functionalities disrupt the intracrystalline cohesion of the drug.

The aqueous solubilities and the partition coefficients of (**I**) and alkyl analogues are listed in Table 3-3. Generally the analogues studied are more water soluble than compound (**I**). Although the first member of the analogues (**II**) was about three times more soluble than (**I**), compound (**III**) had aqueous solubility almost equal (about 1.024 times) to that of (**I**). Compound (**IV**) was found to be more than 2.5 times more soluble than (**I**). It clearly appears that compound (**II**) and (**IV**) are more soluble than compound (**III**) despite of the fact that the aqueous solubility of (**IV**) is limited by its extra methylene group.

The changes in aqueous solubilities of these analogues mirror the changes in crystallinity seen through the melting points. The low solubility of (**III**) can be attributed to the higher melting point compared to (**II**) and (**IV**).

Comparing the n-octanol/water partition coefficient as a function of alkyl chain length in Table 3-3, it can be seen that longer chain analogues are more lipophilic than (**I**). The increase in lipophilicity is generally accompanied by a decrease in water-solubility (Bundgaard & Falch, 1985). Inspection of the data in Table 3-3 reveals,

however, that the aqueous solubility increased relative to (**I**) despite the higher log K_{oct} value of the compounds.

Based on the physicochemical properties of the analogues (Table 3-2 and 3-3), it can be seen that compound (**II**) and (**IV**) have reasonably higher aqueous solubilities and higher lipophilicity compared to (**I**). It can therefore be predicted that this analogues should have the highest skin flux. Compound (**I**) and (**III**) have lower aqueous solubilities and higher melting points. This might have a less favourable effect on their skin permeability. Compound (**III**) possess higher log K_{oct} that could result in the compound having slide edge on permeation to (**I**). A calculated prediction of the order of penetration through the skin would be in the order: compound (**II**) > compound (**IV**) > compound (**III**) > compound (**I**) as the result of the fact that the flux determining physicochemical properties, partitioning and solubility, both are more in the same direction. The next chapter will focus specifically on the relative permeabilities of these compounds through the skin.

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Chapter 4

Percutaneous delivery of cyclizine and its alkyl analogues

4.1 Introduction

Dermal drug delivery has been gaining increasing popularity since it offers some advantages over more conventional treatments. Topical therapy can, for example, deliver therapeutic levels of drugs to tissue close to the site of application in a safer and more effective manner than those obtainable with oral delivery (Mikulak *et al.*, 1998). However, the dermal drug transport is greatly limited by the unsuitable physicochemical properties of most drugs and the efficient barrier function of the skin, and is frequently insufficient for medical uses. Thus, many attempts of improving topical absorption have been performed, such as the prodrug approach with the aim of changing pharmaceutical and/ or pharmacokinetic character of the drug and thereby enhancing its permeation, efficacy and therapeutic value (Chan and Li Wan Po, 1989).

Calpena *et al.* (1994) highlighted the possibility of delivering some anti-emetic drugs via the dermal route. They conducted permeability studies with these anti-emetics (cyclizine excluded) using a hairless rat skin as the membrane. Hadgraft and colleagues (1995) examined the same compounds using theoretical approach to evaluate their permeability coefficients. Several of the anti-emetics were found to be likely candidates for dermal anti-emetic activity. Thus cyclizine (I) is one of the anti-emetic drug entities which could be considered for possible percutaneous delivery. This possibility, however, may be hindered by its low aqueous solubility and higher melting point. It therefore appear likely that the delivery characteristics of (I) can be improved by using its alkyl analogues, derivatives possessing both a high aqueous solubility and lipophilicity at physiological pH (pH 7-8) and also possessing a lower melting point than (I).

The objective of this study was to determine the percutaneous delivery of (I) and its alkyl analogues from a saturated aqueous solution and thus determine whether a correlation exists between the physicochemical properties of these compounds and their percutaneous absorption.

4.2 Experimental

4.2.1 Materials and methods

4.2.1.1 Materials

Three cyclizine alkyl analogues (**II**, **III** and **IV**) were synthesized, purified and identified according to previously described methodology (chapter 3). Cyclizine (**I**) was obtained from Sigma-Aldrich (UK). All materials used in the chromatographic procedures and in permeability studies were described in section 3.3.4.1.

4.2.1.2 Chromatography

The amount of (**I**) and its alkyl analogues, which penetrated through the human skin was quantitatively determined by HPLC as described in section 3.3.4.2.

4.2.1.3 Skin preparation

Full thickness female human abdominal skin tissue from cosmetic surgery was sealed in evacuated plastic bags and frozen at –20 °C. The adipose tissue was removed by blunt dissection and immersed the skin in 60 °C water for 1 minute to separated the epidermis. The epidermis was then peeled away from the dermis using forcepts (Harrison *et al.*, 1984). The skin sections were cut into squares, wrapped in aluminium foil, sealed in plastic bags and stored in a freezer at –20 °C until utilized. The frozen skin pieces were thawed at room temperature and examined for defects before mounting them onto the Franz diffusion cells.

4.2.1.4 Skin permeation method

Vertical Franz diffusion cells with a 4 ml capacity receptor compartment and a 1.07 cm^2 diffusion area were used. The epidermal layer of the skin was mounted carefully onto the lower half of the diffusion cells with the stratum corneum facing up in the direction of the donor compartment. The donor compartments were fastened to the receptor compartments with clamps, with the skin acting as a seal between the half-cells. The receptor compartments were filled with phosphate buffer (pH 7.4), taking care that there were no consequential air bubbles left in the compartments. The diffusion cells were placed in a water bath with a constant temperature of 37 °C on a submersible magnetic stirring bed, a small magnetic stirring bar was placed at

the bottom of the lower compartment and the system was allowed to equilibrate for 1 h before adding the saturated drug containing solution to the donor compartment. The experiment was initiated by charging the donor compartment with 700 µl of a freshly prepared saturated solution of the drug and immediately covering it with Parafilm® to prevent any significant evaporation from the donor compartment. At predetermined intervals (2, 4, 6, 8, 10, 12 and 24 h) the entire content of the receptor compartment was withdrawn and replaced with fresh buffer (37 °C). This was done to ensure that sink conditions existed throughout the experiment. Two hundred microlitres of each sample was directly assayed by HPLC to determine the drug concentration in the receptor fluid. The duration of the skin permeation experiment was ≤ 24 h.

4.2.1.5 Solubility determination

The aqueous solubilities of (I) and its alkyl analogues were determined by equilibrating a large excess of each compound with HPLC water. The temperature (32 °C) was held constant by means of a water bath. To hasten the attainment of equilibrium, magnetic bars continuously stirred each slurry. Equilibrium was attained within 24 hours. Samples were then filtered through a Millipore filter (0.45 µm) and assayed by HPLC.

4.3 Data analysis

The permeability coefficient for (I) and its alkyl analogues was calculated using Fick's law of diffusion (Equation 4-1)

$$k_p = \frac{V_R(dC/dt)}{A(\Delta C)} \qquad \text{(Equation 4-1)}$$

Where:

- dC/dt is the steady-state slope of a plot of the cumulative amount of substance which had penetrated the skin as a function of time (µg/h). It was determined by taking the ratio of the total amount permeated in an interval of time to the length of the time interval.
- k_p is the permeability coefficient (cm/h).
- A is the diffusional area, which was 1.075 cm^2 in this study.

- ΔC is the concentration differential existing across the membrane. This was affectively equal to the saturation concentration in the donor phase (μg/ml).
- V_R is the volume of the receptor compartment (4ml).

The flux (μg/cm^2/h) for each compound through the skin was calculated using equation 4-2.

$$\text{Flux } (\mu g/cm^2/h) = k_p \times \text{saturated solubility} \qquad \text{(Equation 4-2)}$$

Where:

- k_p is the permeability coefficient (cm/h).

The lag time (T_L) was calculated from the cumulative amount permeated against time profile, by extrapolating the straight line obtained at steady state through the time axis.

The diffusion coefficient (D) was calculated using the following equation.

$$D(cm^2/h) = \frac{h^2}{6T_L} \qquad \text{(Equation 4-3)}$$

Where:

- D is the diffusion coefficient
- h is the average thickness of the stratum corneum (15 μm)
- T_L is the lag time.

4.4 Results

The *in vitro* penetration of (I) and its alkyl analogues across the human skin were investigated at pH 7.4. Selected physicochemical parameters were determined. Table 4-1 lists the melting points, aqueous solubilities ± standard deviation and the octanol/water partition coefficients for these compounds. Figure 4-1 is a bar chart representing the melting points of (I) and its alkyl analogues. The analogues exhibited lower melting points than (I). The aqueous solubilities presented in Table 4-1, generally show that increasing the alkyl chain length results in more soluble

molecules and exhibiting higher lipophilicity, evident from their higher octanol/water partition coefficients. The aqueous solubility data of (I) and its alkyl analogues are presented as the solubility in Figure 4-2, while Figure 4-3 is a logarithmic plot of experimentally derived permeability coefficients and octanol/water partition coefficients for (I) and its alkyl analogues.

TABLE 4-1: Physicochemical properties of (I) and its alkyl analogues.

Compound	Melting point (°C)	Aqueous solubility (32 °C) (µg/ml ± SD)	Log K_{oct}
I	107	198.6 ± 13.0	3.11
II	51	591.4 ± 9.22	3.64
III	70	210.4 ± 10.4	4.18
IV	52	500.8 ± 15.1	4.71

The permeation parameters (flux, J; lag time, T_L; permeability coefficient, k_p and diffusion coefficient, D) of (I) and its alkyl analogues from their saturated aqueous solutions (pH 7.4) are summarized in Table 4-2. The steady-state flux of each compound was determined from the slope of the linear portion of the cumulative amount versus time plot and their mean steady-state flux is presented as a bar chat in Figure 4-4. The permeation data are plotted as the cumulative amount of drug penetrated through skin as a function of time (Figure 4-5). The lag time was determined by extrapolating the linear portion of the curve to its intersection with the x-axis.

TABLE 4-2: Permeation parameters of (I) and its alkyl analogues through human skin.

Compound	J ± SD ($\mu g/cm^2/h$)	T_L (h)	k_p ± SD (cm/h) 32 °C	D (cm^2/h) x 10^{-3}
I	0.132 ± 0.04	0.15	0.003 ± 0.01	25.0
II	6.952 ± 0.38	0.21	0.044 ± 0.18	17.9
III	0.250 ± 0.02	0.28	0.0044 ± 0.09	13.4
IV	0.686 ± 0.06	0.40	0.005 ± 1.05	9.38

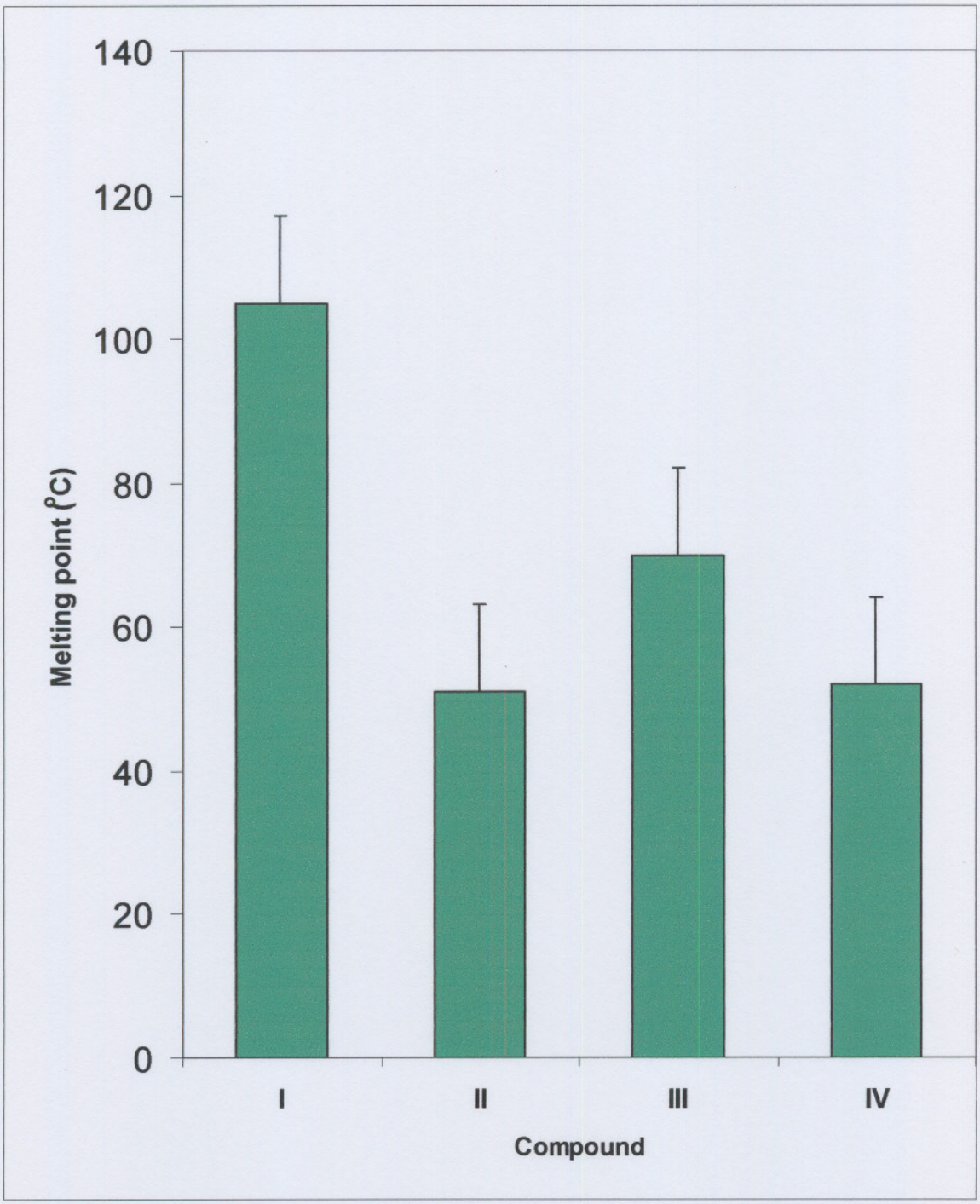

FIGURE 4-1: Melting points of (I) and its alkyl analogues.

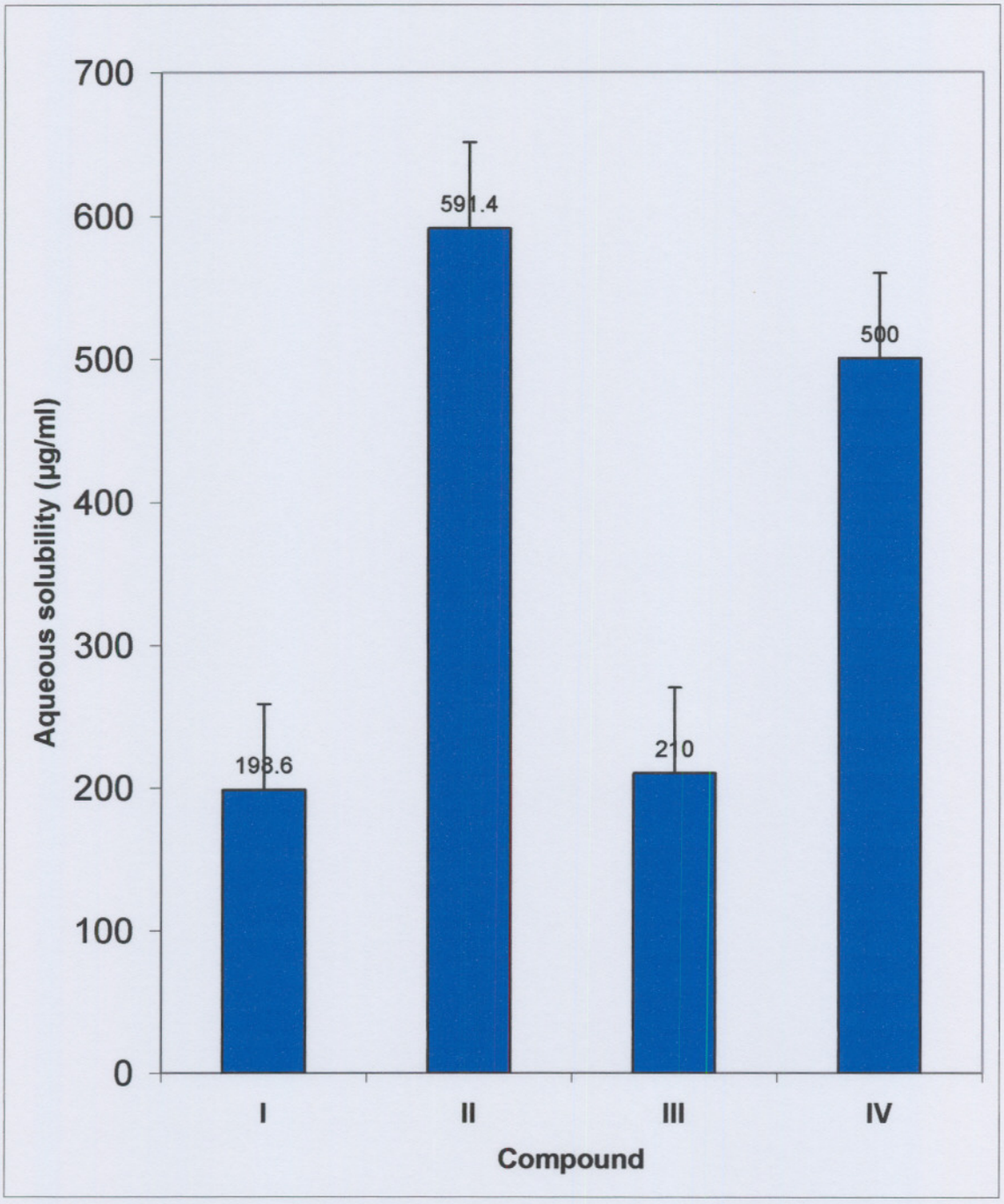

FIGURE 4-2: Aqueous solubilities of (I) and its alkyl analogues at 32 °C.

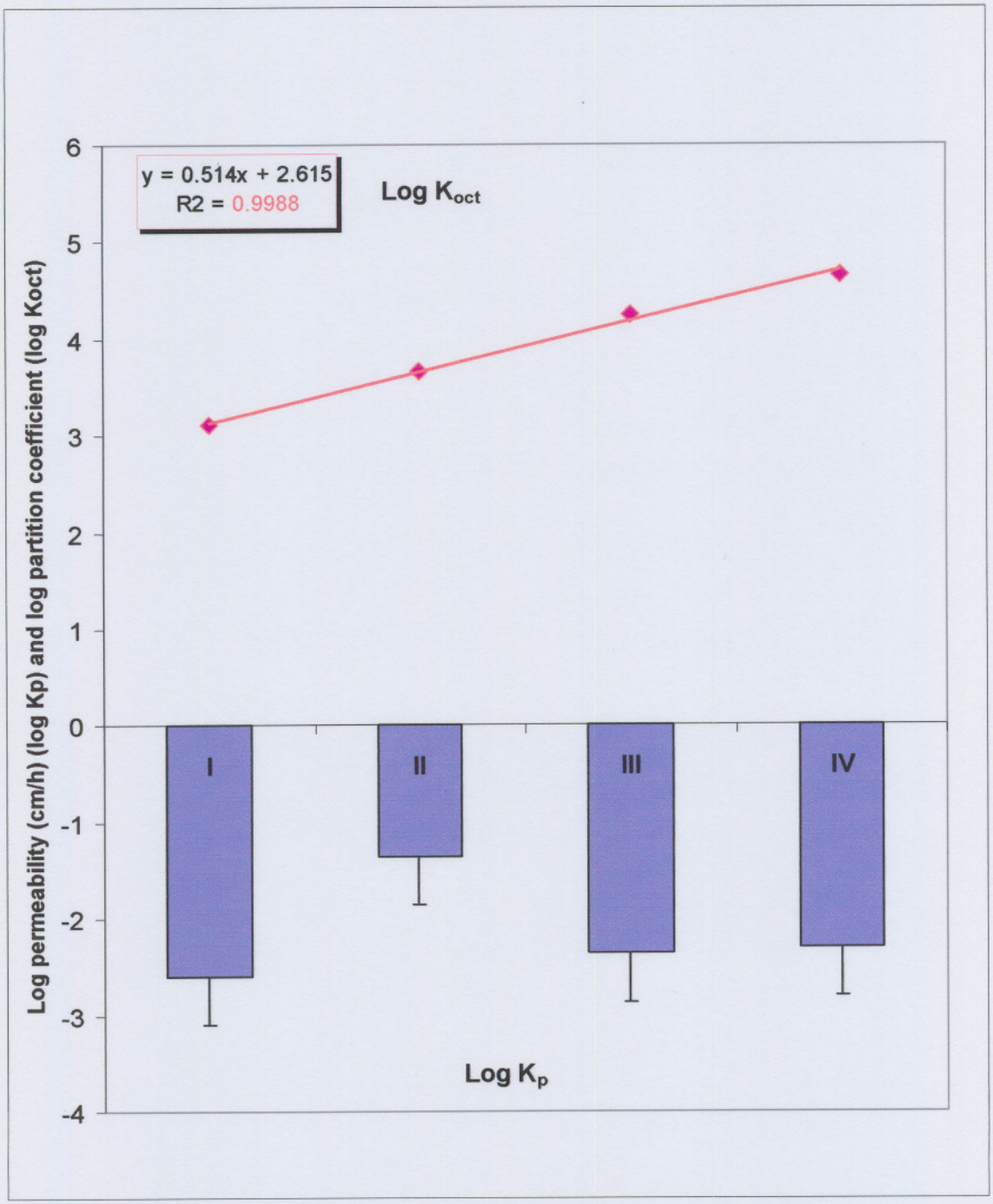

FIGURE 4-3: Permeability (log k_p) and partition coefficients (log K_{oct}) of (I) and its alkyl analogues.

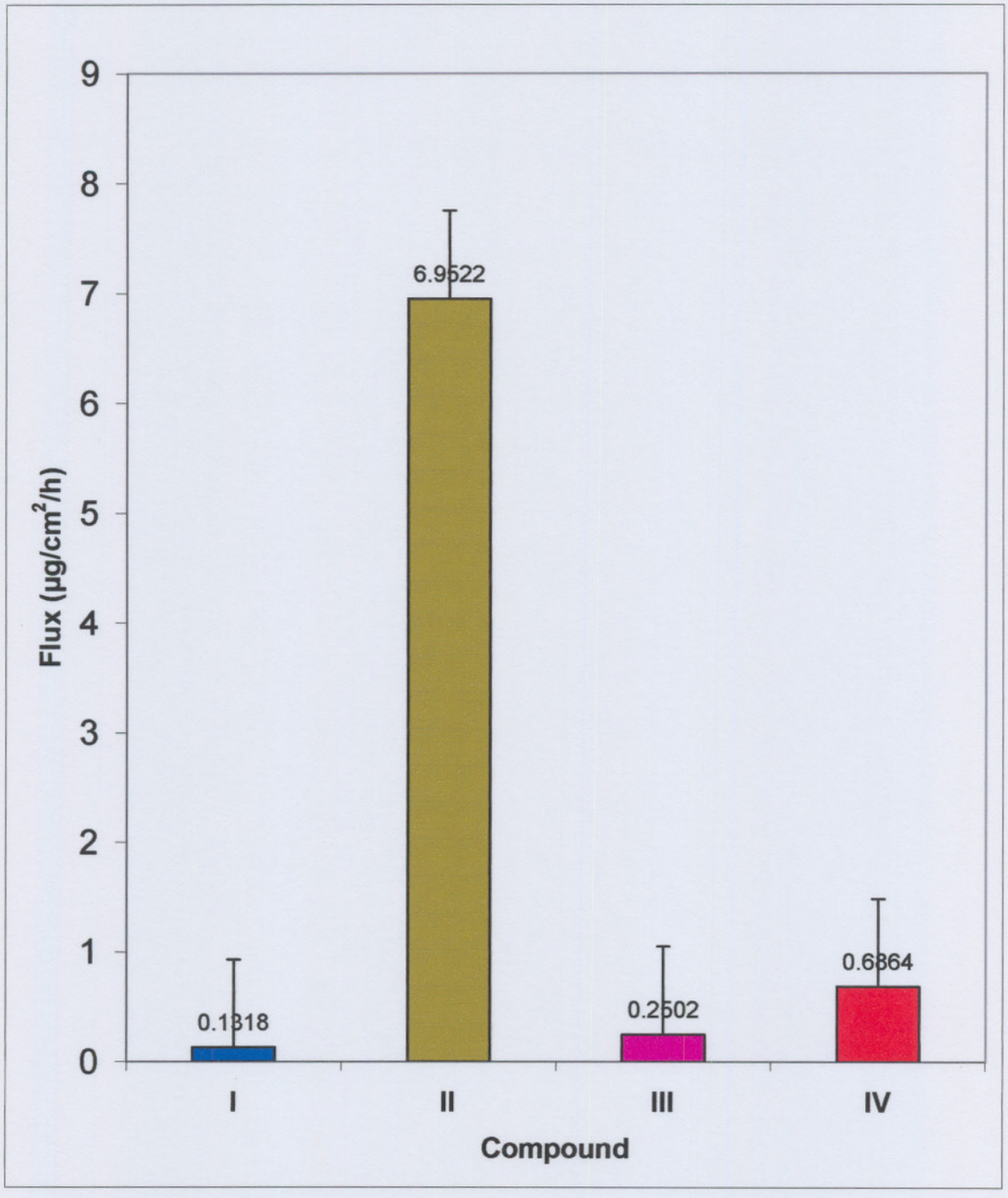

FIGURE4-4: Mean steady-state flux ± SD of (**I**) and its alkyl analogues from saturated aqueous solutions (n = 6).

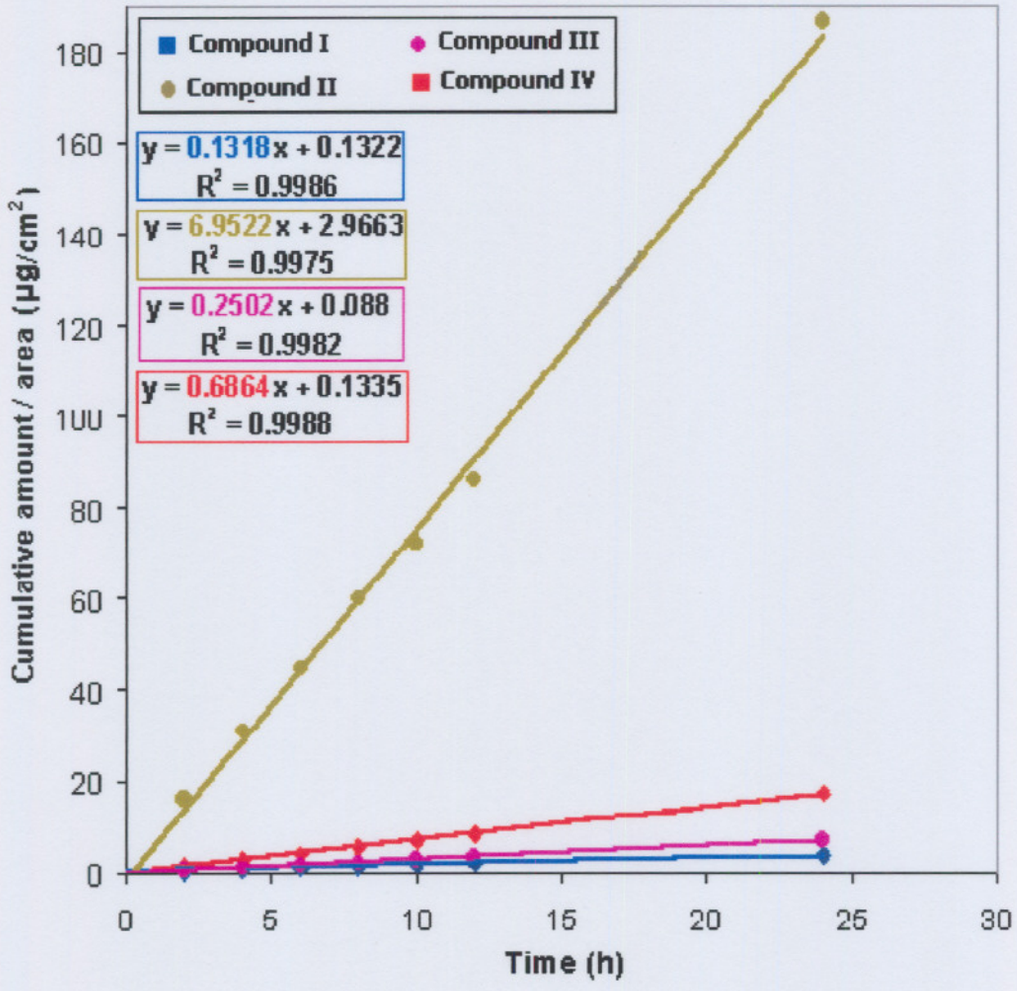

FIGURE 4-5: Permeation profiles of (I) and its alkyl analogues from the saturated aqueous solutions.

4.5 Statistical analysis

The mean steady-state flux of (I) and its alkyl analogues were calculated and statistical comparison was carried out to establish the degree of difference. The statistically significant differences in the flux values ($\mu g/cm^2/h$) of these compounds were obtained through ANOVA (analysis of variation). Statistically significant differences ($p < 0.05$) were indicated for the parameter flux between (I) and all the analogues. There is no statistical difference ($p < 0.05$) between the fluxes of (I) and (III).

4.6 Discussion

The primary aim of this work was to determine the permeation parameters of cyclizine (I) and its alkyl analogues from aqueous solution, as a step towards considering these compounds as potential drugs for percutaneous therapeutic systems. The intent was also to establish a correlation between the physicochemical properties of these compounds and their percutaneous absorption. The study by Goosen and colleagues (1998) showed that physicochemical parameters such as aqueous solubility and lipophilicity influence membrane flux, therapeutic activity and pharmacokinetic profiles of medicines.

Aqueous solubilities and lipophilicities of (I) and its alkyl analogues are shown in Table 4-1. (I) possessed poor solubility in water than its alkyl analogues. The poor solubility of (I) may largely be a result of the high crystalline lattice energy in the molecule as reflected in its higher melting point (107 °C). The replacement of the N-4 proton by alkyl groups, lead to derivatives with decreased crystal lattice energy as manifested in a pronounced melting point decrease and thus higher solubilities. Figure 4-1 and 4-2 are the bar charts representing the melting points and aqueous solubilities of (I) and its alkyl analogues. Increasing the alkyl chain length to $-CH_2CH_3$ resulted in a molecule (II) with almost 3 fold increased aqueous solubility than (I). Amidon (1981) reported that any modification that could cause a reduction in the crystal lattice energy would have a tendency to increase solubility, hence lower melting point. Further increase to $-CH_2CH_2CH_3$ resulted in a molecule (III) with slightly higher solubility (about 6 % increase) than (I), but lower than that of (II) and compound (IV) was about 2.5 times more soluble than (I).

Lipophilicity is very important for dermal permeation because the stratum corneum, the major barrier to drug permeation, is lipid in nature and generally favours permeation of lipophilic drugs. However, it has also been reported that an effective dermal prodrug should possess not only high lipophilicity, but also adequate aqueous solubility (Taylor & Sloan, 1998 and Kerr *et al.*, 1998). In the study by Stinchcomb *et al.* (1996), the results showed that, even though the flux of the prodrugs was not higher than that of parent drug, partition coefficient increased logarithmically with the increase in alkyl chain length.

Permeability (log k_p) and partition coefficients (log K_{oct}) of (**I**) and its alkyl analogues are presented in Figure 4-3. In all the analogues studied, an increase in lipophilicity was observed. From the solubility properties (aqueous and lipid), (**II**) seems the most promising analogue for percutaneous drug delivery.

The percutaneous permeation of (**I**) and its alkyl analogues was studied by an *in vitro* technique using a Franz diffusion cell mounted with the epidermal layer of human skin as the diffusion membrane. The permeation parameters for these compounds are presented in Table 4-2 and in figure 4-4 is the bar chat representing their mean steady-state flux. The compounds attained steady-state diffusion in the skin within 0.4 h and the apparent lag times increased with the increase in alkyl chain length. This was in agreement with the study of transdermal delivery of 5-fluorouracil and its alkylcarbomoyl derivatives by Sasaki and colleagues (1990) where lag times were prolonged with increasing alkyl chain length.

The diffusion experiment showed that there is *in vitro* varying degree of permeation between (**I**) and its alkyl analogues. Generally, the analogues resulted in higher permeation than (**I**). In Figure 4-5 are the permeation profiles of (**I**) and its alkyl analogues. It clearly indicates that, relative to (**I**), there is an increase in the steady-state flux of the analogues. The highest flux is observed with (**II**) followed by (**IV**). This could be attributed to their high aqueous solubility and low level of crystallinity, as is evident from their low melting points. (**I**) and (**III**) exhibited lower flux, hence their higher melting points and low aqueous solubility. Flynn and Yalkowsky (1972) studied the effects of alkyl chain length on the flux across a synthetic membrane. They found that a plot of the logarithm of the steady state flux from saturated solution against chain length gave a parabolic curve. But from the results obtained in this study, one can see that the series is capable of odd-even alternation in aqueous solubility, melting point and in percutaneous permeation. This kind of behaviour is

attributed to the dependence of these physicochemical properties upon the sum of the energy required to disrupt the crystal and the intermolecular interactions between like and unlike species in solution. The linearity of partition coefficient against alkyl chain length is because the partition coefficient is a property of the solute and therefore is not dependent on the crystal structure. It is only dependent upon the interactions occurring in solution. There is a correlation between aqueous solubility, melting point and percutaneous permeation of the compounds. (**II**) and (**IV**) showed high percutaneous permeation hence increased aqueous solubility and low melting point. Low percutaneous permeation of (**I**) and (**III**) is attributed to their low aqueous solubility and high melting point. Thus, the more water-soluble member of the series is the most penetrating member through the human epidermis.

The results of this study suggest that alkylation is a potential useful approach to enhance percutaneous penetration. The following rank order of penetration was established: compound (**II**) > compound (**IV**) > compound (**III**) > compound (**I**).

4.7 Conclusion

The physicochemical properties of derivatives are among the most important factors affecting the percutaneous absorption of drugs. It is suggested from the results of this study that increased aqueous solubility and thermodynamic activity of the alkyl analogues, as expected from low melting point compared to (**I**), enhance their penetration into the hydrophobic stratum corneum. Based on experimental data, there was a varying degree of permeation between the compounds. Although permeation properties of compound (**I**), (**III**) and (**IV**) may not be ruled out, it was clear that compound (**II**) showed the best permeation results. It can be concluded that, the effects of water solubility and melting point seem to be the major factors in optimizing percutaneous delivery within this series, but the importance of the effect of enhanced lipid solubility cannot be ignored.

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Chapter 5

Summary and final conclusion

The skin has evolved primarily as a barrier to water loss from inside the body to the surrounding environment. This barrier also inhibits the entry of substances into the body, thereby providing protection from harmful organisms and molecules. But without any doubt, the skin is the most accessible and probably the most extensive organ of the body. Understanding the development of this barrier can facilitate attempts to enhance percutaneous absorption of drug molecules.

A number of drugs readily passes through the skin and the rate and extend to which this happens are influenced by the physicochemical properties of the drug (Beckett, 1982). Other factors may also have an influence, but if kept constant, it is possible to determine which physicochemical properties are most important in determining the rate and extent of absorption through or into the skin. Recent studies revealed that alkylation approach to improve the dermal delivery of drugs offers several advantages, since it changes the physicochemical properties of the drug (i.e. aqueous solubility, lipophilicity and level of crystallinity).

Cyclizine (I) has been found to be a good anti-emetic for patients having minor surgery. It is also a satisfactory drug against motion sickness and it appeared to have no severe side effects (Marcus & Sheehan, 1965 & Dundee *et al.,* 1966). Results of several trials showed that (I) is undoubtedly effective in preventing motion sickness (Brand & Perry, 1966) and some of its alkyl analogues were found to prevent the actions of histamine. Thus the most important goal of the research was to investigate and gain more about (I) and selected alkyl analogues to eventually consider these compounds for percutaneous delivery system.

The specific aims of the study were to:

- synthesise cyclizine alkyl analogues;
- determine selected physicochemical properties of (I) and its alkyl analogues relevant to their percutaneous delivery;

- ❖ determine percutaneous delivery of these compounds through the human skin in saturated aqueous solutions and
- ❖ establish any relationship between selected physicochemical properties, most importantly aqueous solubility and percutaneous absorption.

The alkyl analogues (**II**, **III**, **IV**) were synthesized and purified (Chapter 3). Their structures and level of purities were verified by spectrophotometric (NMR, MS, IR) and thermal (DSC) techniques. It became evident that when increasing the alkyl chain length on the piperazine ring, the results are compounds with physicochemical properties that appeared to be favourable for percutaneous delivery than (**I**) (Table 3-3). The analogues exhibited lower melting temperatures and higher lipophilicities as demonstrated by their higher octanol/water partition coefficients. They also possessed higher aqueous solubility, with (**II**) toping the series. The overall behaviour of these analogues is in the direction of improving percutaneous delivery.

The *in vitro* skin permeation performed using water as a solvent showed that alkyl analogues were better penetrants than (**I**). It was found that (**II**) proved to be the best penetrant followed by (**IV**) (Chapter 4). This made clear that aqueous solubility and level of crystallinity played important roles in the percutaneous absorption of these compounds. Both these compounds exhibited lower melting points and higher aqueous solubilities than (**I**) and (**III**). The rank order of skin permeation observed in this study was: compound (**II**) > (**IV**) > (**III**) > (**I**). Based on the overall results of this study, not taking away the enhancing effect by lipophilicity, a correlation exists between aqueous solubility of the compounds and their percutaneous absorption. Goosen (1998) and Fourie (2001) observed that aqueous solubilities of alkyl analogues might in same way have influence on their skin permeation. It is established in chapter 4 that one could markedly improve the ability of cyclizine-like compounds to penetrate skin by modulating their structures such that their solubilities, lipophilicities and crystallinities are modified.

<u>In this study:</u>

- ❖ three alkyl analogues of (**I**) were successfully synthesised, purified and identified;
- ❖ the physicochemical properties of (**I**) and its alkyl analogues relevant to their percutaneous delivery were determined;
- ❖ the permeation parameters for these compound were determined in human skin from saturated aqueous solutions and

- a correlation between selected physicochemical properties of these compounds and their percutaneous delivery was established.

In continuation of the study the following should be investigated:

- the effect of longer alkyl chains (above C-4) on the flux properties of the drug;
- the behaviour of the odd alkyl chains versus the even chains and
- the efficacy of the compounds with the increase in alkyl chain length.

5.1 References

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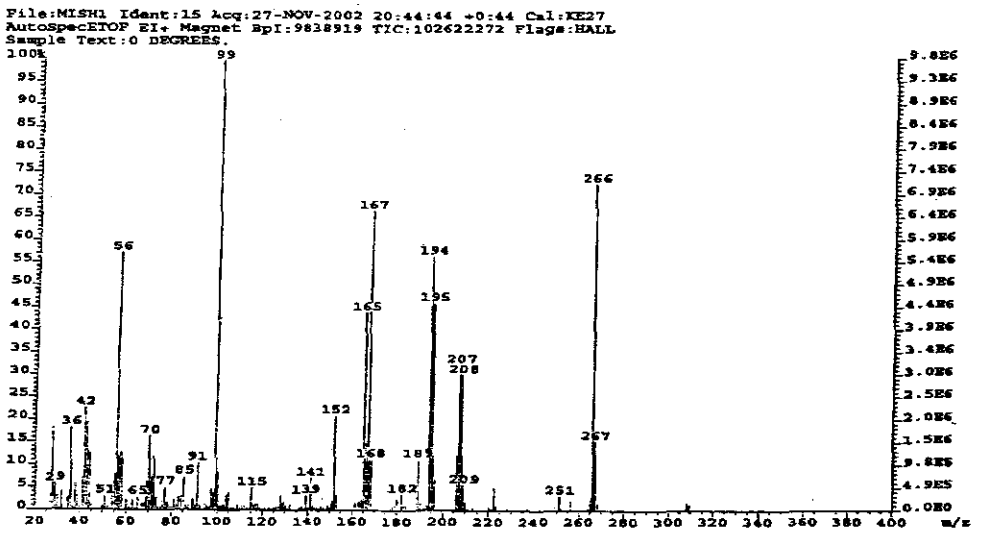
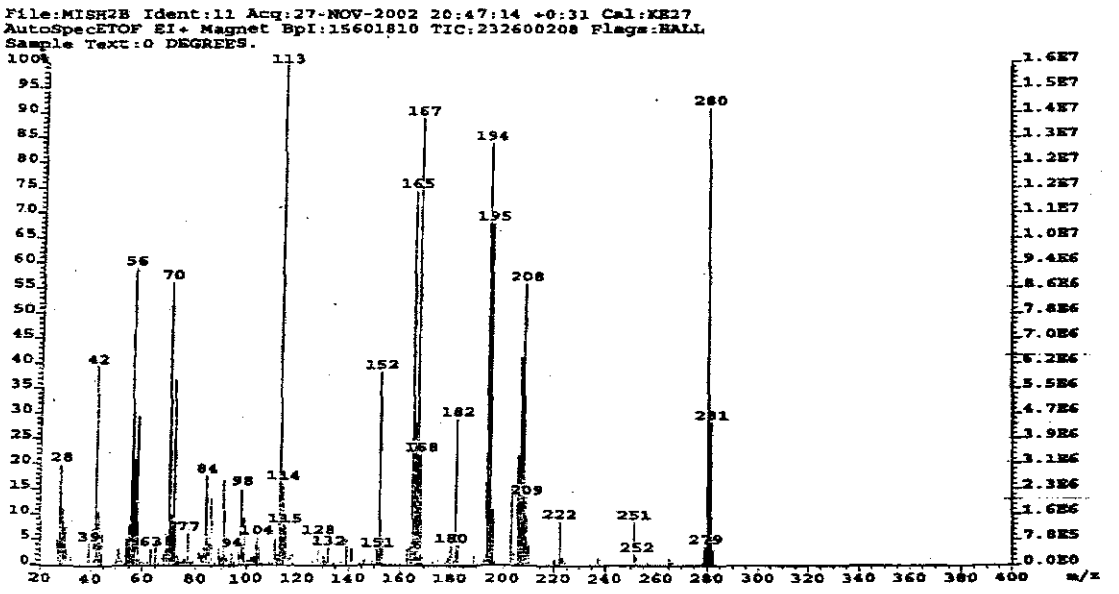
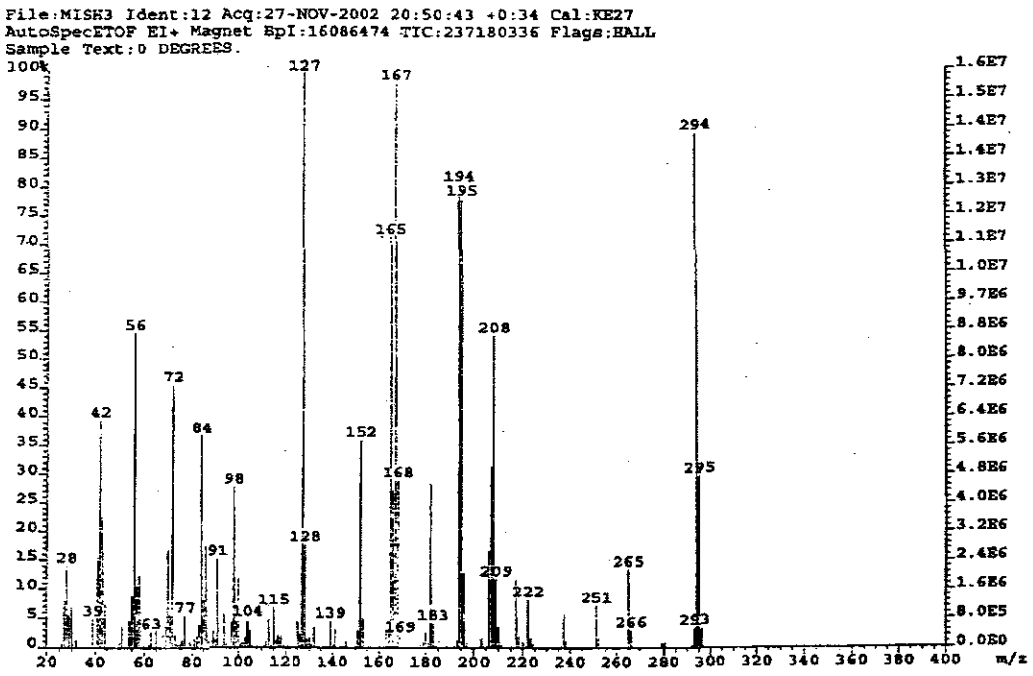
Mass spectrometry



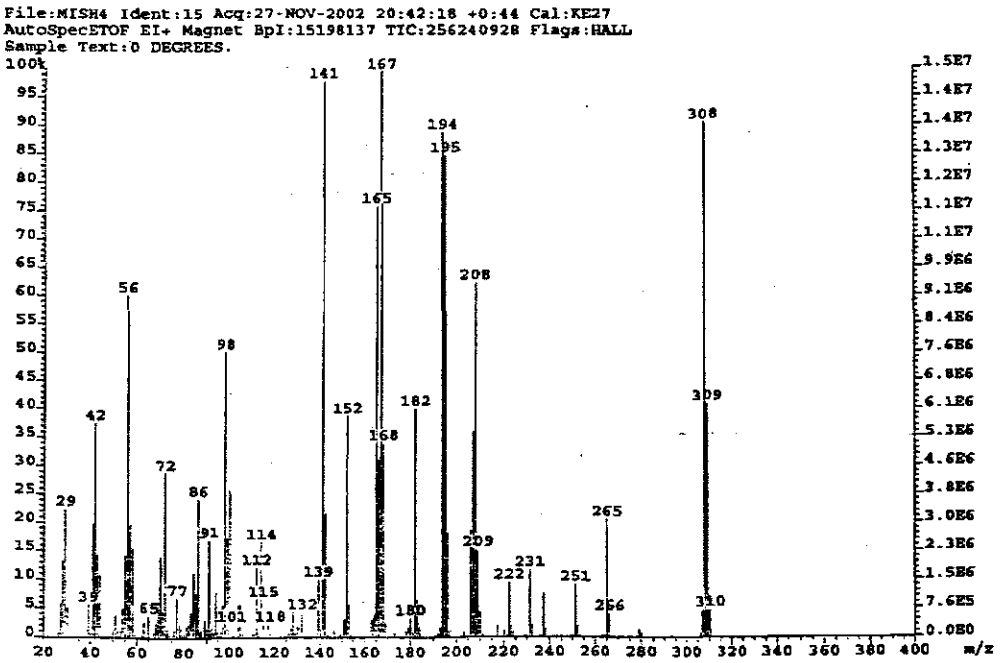
SPECTRUM 1: Mass spectrum of cyclizine (**I**)



SPECTRUM 2: Mass spectrum of compound (**II**)

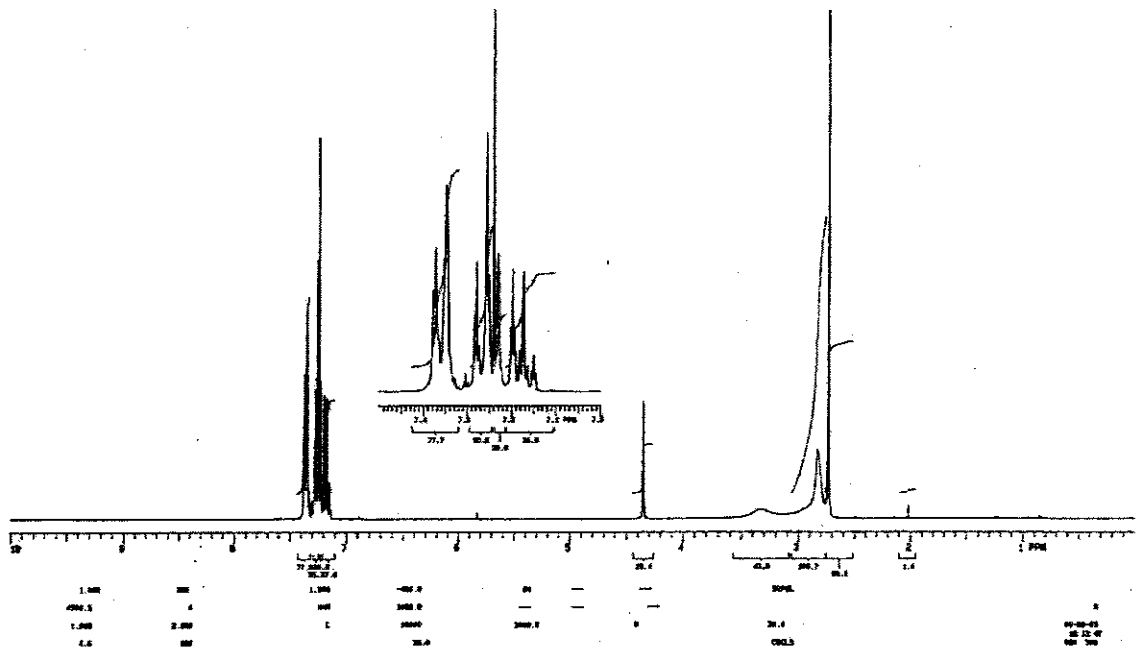


SPECTRUM 3: Mass spectrum of compound (**III**)

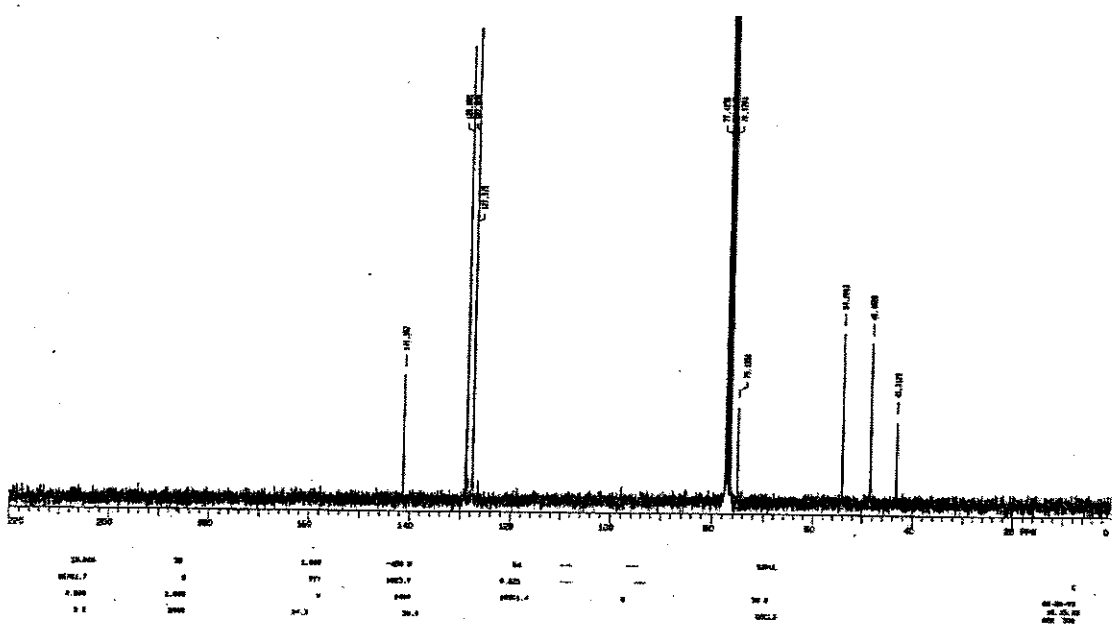


SPECTRUM 4: Mass spectrum of compound (**IV**)

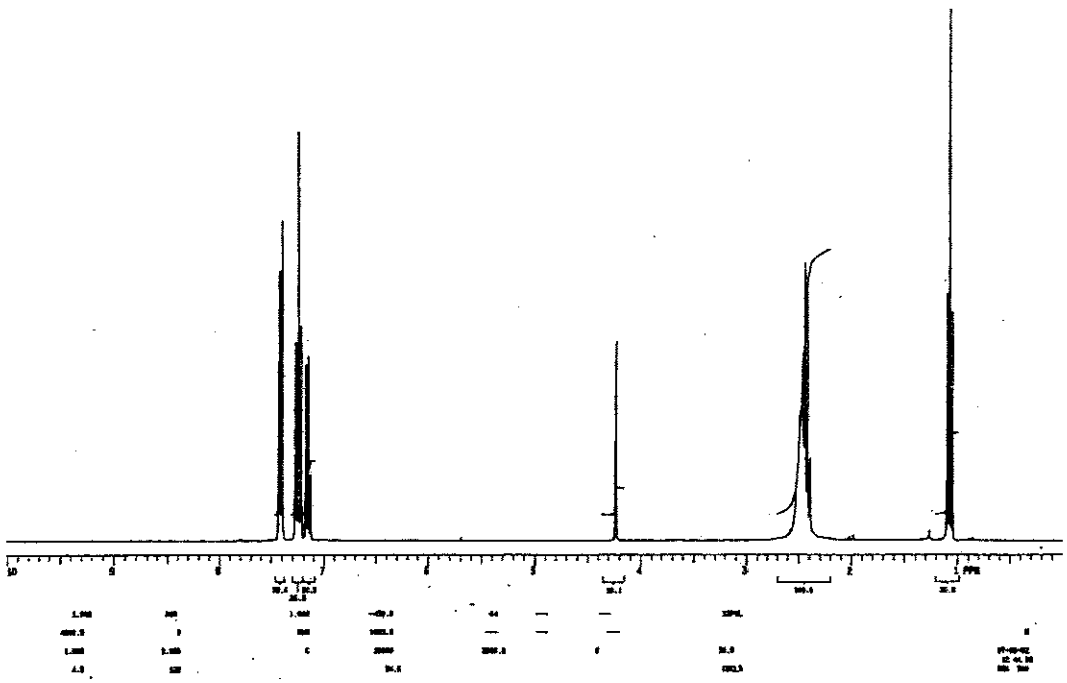
Nuclear magnetic resonance spectra



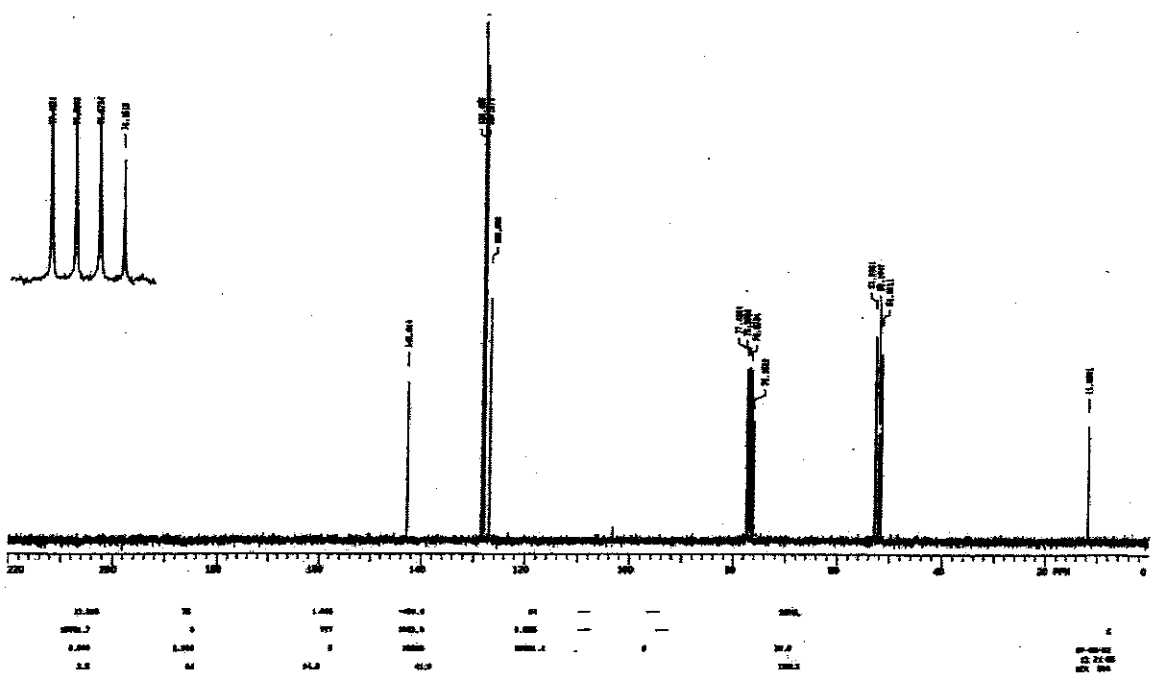
SPECTRUM 5: ^{1}H NMR spectrum of cyclizine (I) in $CDCl_3$



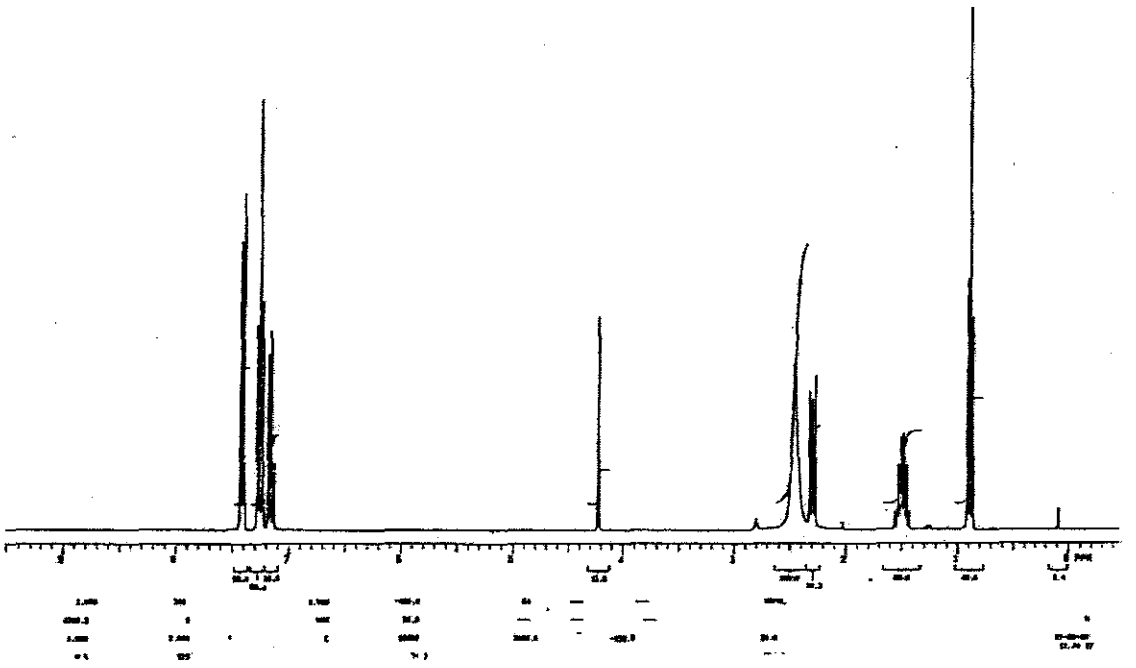
SPECTRUM 6: ^{13}C decoupled spectrum of cyclizine (I)



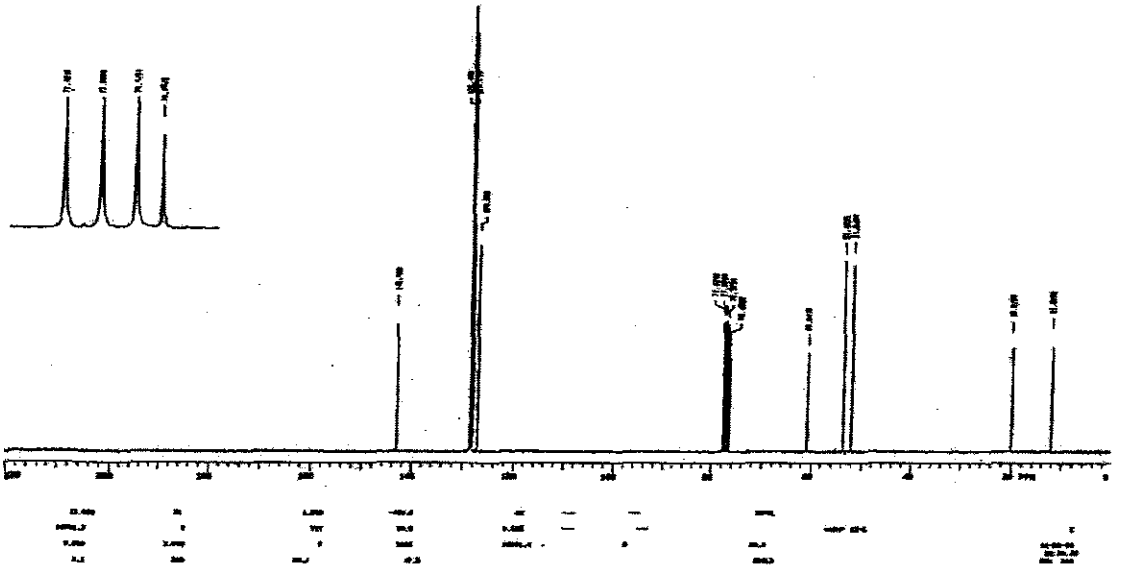
SPECTRUM 7: ^{1}H NMR spectrum of compound (**II**) in $CDCl_3$



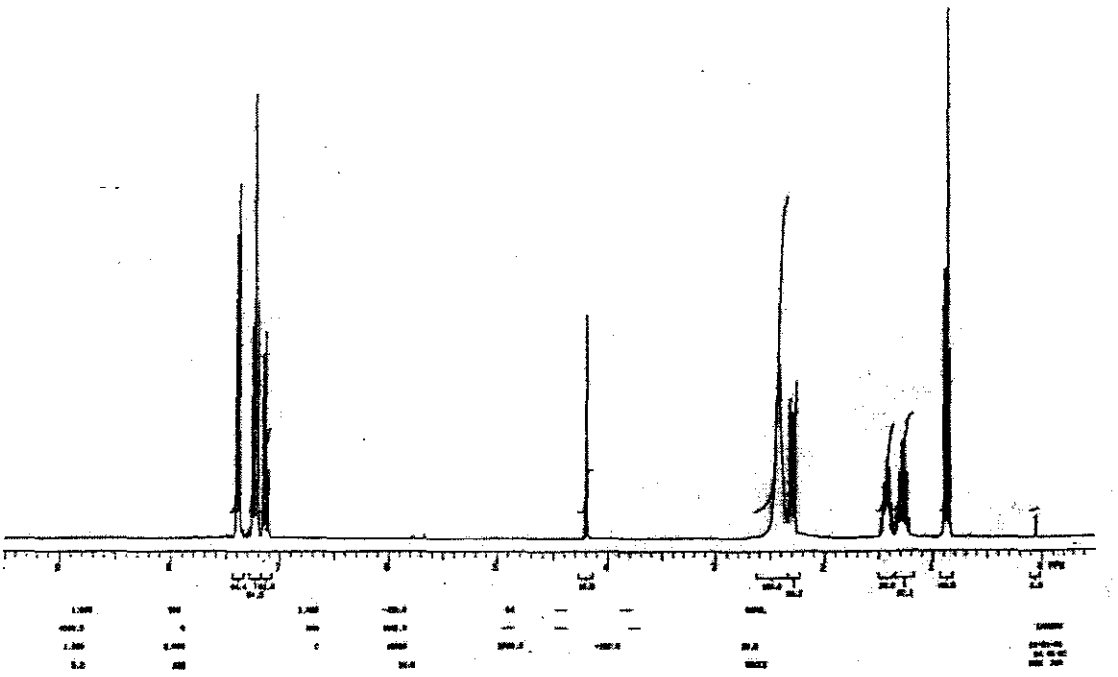
SPECTRUM 8: ^{13}C decoupled spectrum of compound (**II**)



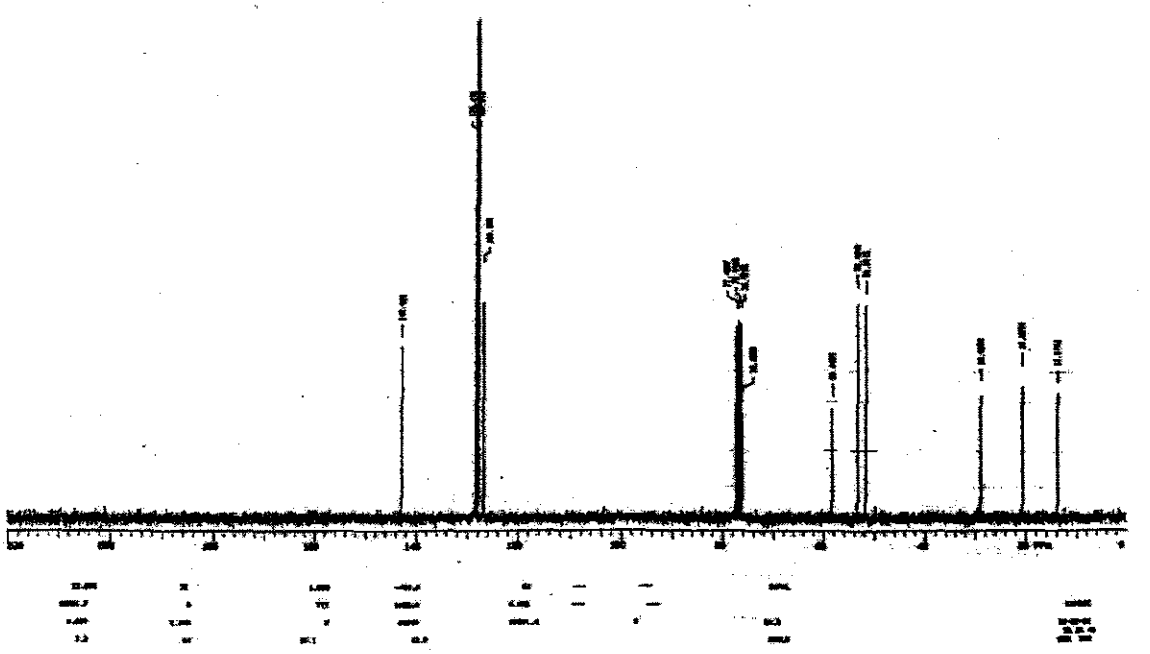
SPECTRUM 9: ^{1}H NMR spectrum of compound (**III**) in $CDCl_3$



SPECTRUM 10: ^{13}C decoupled spectrum of compound (**III**)

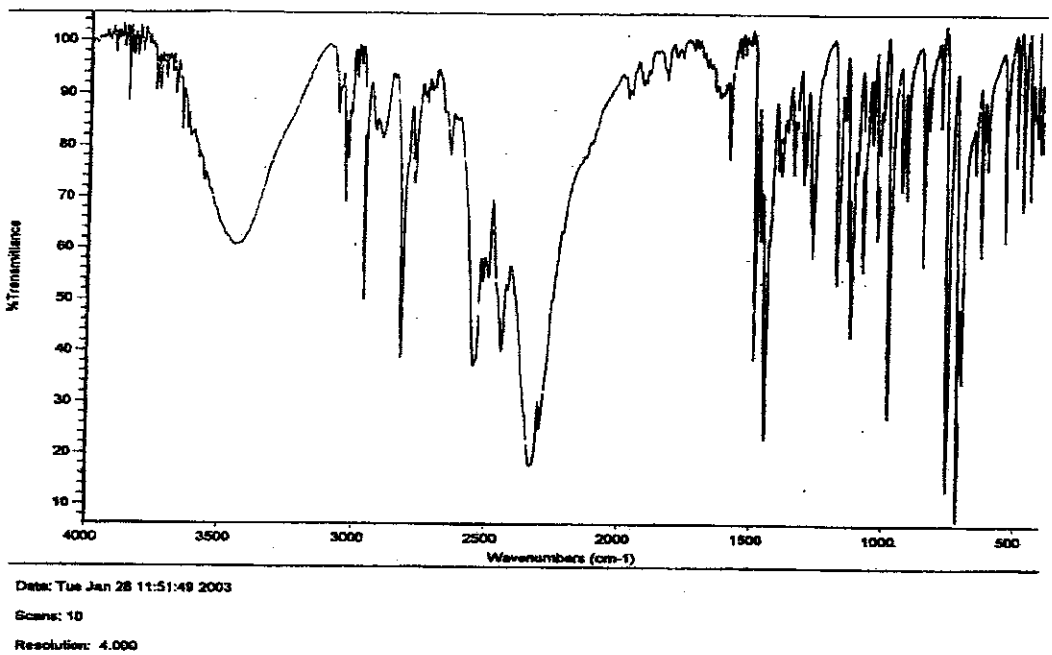


SPECTRUM 11: ^{1}H NMR spectrum of compound (**IV**) in $CDCl_3$

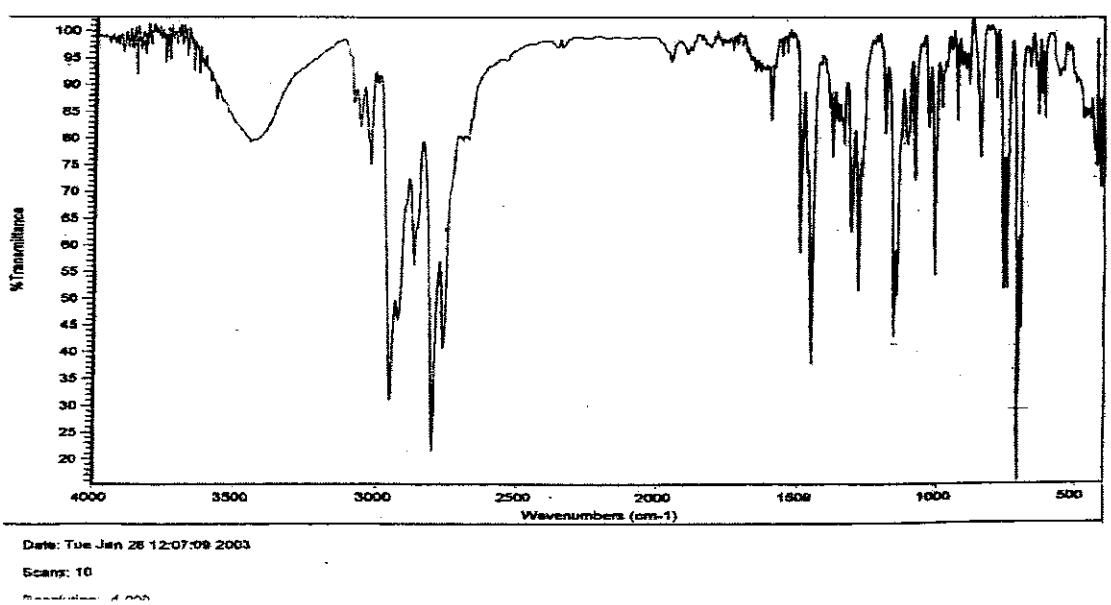


SPECTRUM 12: ^{13}C decoupled spectrum of compound (**IV**)

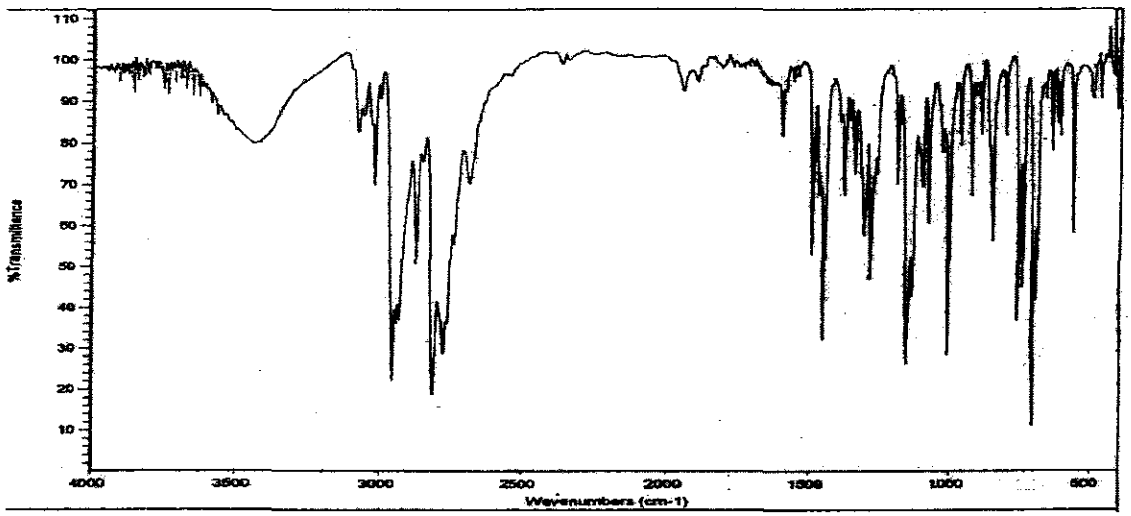
Infrared spectrometry



SPECTRUM 13: Infrared spectrum of cyclizine (I)

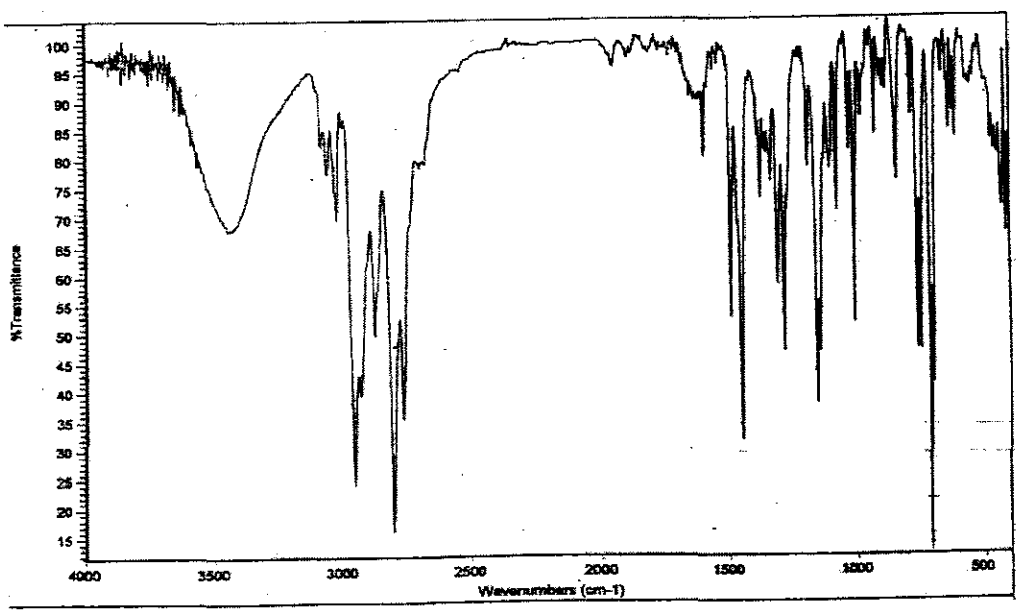


SPECTRUM 14: Infrared spectrum of compound (II)



Date: Tue Jan 28 11:46:02 2003
Scans: 16
Resolution: 4.000

SPECTRUM 15: Infrared spectrum of compound (**III**)



Date: Tue Jan 28 12:13:30 2003
Scans: 16
Resolution: 4.000

SPECTRUM 16: Infrared spectrum of compound (**IV**)