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A Study of Anionic Drug Transport Processes Governing Passive and Enhanced Transdermal Delivery

A Thesis Submitted to the University of Dublin

by

Dara C. Fitzpatrick, B.Sc.

For the Degree of Doctor of Philosophy

University Physical Chemistry Research Laboratory

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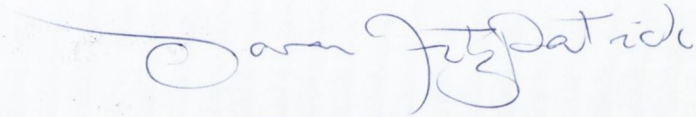
Dublin 2

September 2000

Declaration

This thesis has not been submitted as an exercise for a degree at any other university. Except where otherwise indicated the work herein has been carried out by the author alone.



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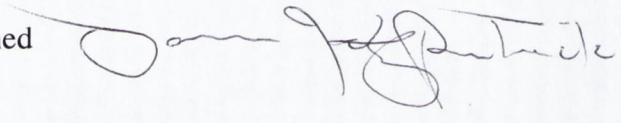
Signed 

To my Partner Jacqui S. Churcher

my Mother Honor-Marie Mills

and my late Father

Kieran D. Fitzpatrick

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Summary

The research contained herein is directed at defining some of the factors which govern the passive and enhanced transdermal transport of anionic drugs and will extend current knowledge which largely pertains to the analogous behaviour of cationic drugs. Measurements have been made of the release profiles of anionic drugs from liquid crystalline gels across artificial and human *stratum corneum* membranes. The effects of the addition of surfactants and fatty acid chemical penetration enhancers, and of the use of electrical assistance and also the synergistic effects of the similtaneous application of both methods to increase drug transport have also been studied.

Sodium diclofenac was used as the model anionic drug compound and was incorporated into the oil phase of the vehicle. From here it diffused into the aqueous phase and across the membrane into the receptor media. The effect of incorporating anionic (oleic acid) and cationic (surfactant, e.g., Benzyldimethyldodecylammonium Bromide) chemical permeation enhancers was evaluated. Direct current iontophoresis was also investigated as a means of increasing the transport of the drug out of the vehicle and across the membrane into receptor mediums. The release profiles of similar drugs, such as sodium indomethacin, sodium benzoate and sodium salicylate, were also characterised in detail.

The passive releases of all drugs investigated across a non-rate limiting membrane were shown to adhere to matrix diffusion control. The incorporation of a cationic enhancer had the effect of forming ion-pairs with the drug and this, in general, resulted in a decrease in the amount of drug which diffused from the vehicle. The extent of the decrease was found to depend on the ratio of drug to enhancer. The addition of anionic oleic acid to the vehicle had no effect on the passive release profile of the drugs across non-rate limiting membranes.

Iontophoresis (electrical assistance) was found to enhance the release of all anionic drugs from both the liquid crystalline and agar-based vehicles. However, detection of the drug in the receptor medium was made difficult by the electrochemical change of the drug anions to the acid form. This was brought about by the electrolysis of water at the anode. The production of hydronium ions led to the formation of the acid form of the drugs, which are less soluble in the aqueous receptor

medium, e.g., sodium diclofenac formed fine precipitate. The addition of oleic acid slightly improved the apparent release profile of sodium diclofenac due to this chemical enhancer acting as a buffer and reducing the formation of diclofenac acid. Iontophoresis also reduced the extent of ion pair formation between drug and surfactant thus increasing the amount of diclofenac detected.

The passive transdermal transport of sodium diclofenac and sodium indomethacin across full thickness human skin was detected over a 24hr period. The presence of oleic acid in the vehicle did not influence the passive delivery of the drug. However, the presence of surfactant inhibited the passive transdermal release of the drug. It could neither be proved nor disproved that the addition of either enhancer acts synergistically with electrical assistance to increase the transdermal transport of sodium indomethacin, however, there was evidence of synergistic enhancement of sodium diclofenac across human *stratum corneum* in the presence of oleic acid and cathodal iontophoretic assistance.

The effect of buffering the system was found to have three effects. Passive release is inhibited by a reduction in the ionic concentration gradient within the vehicle between the oil and water phases. During cathodal iontophoresis the presence of buffer ions reduced the number of hydronium ions after their production, thus increasing the amount of drug detected. Buffer ions were also found to reduce the formation of ion pairs between drug and surfactant.

The research reported here has shown clearly that the transport of anionic drugs is enhanced by iontophoresis and may have been overlooked previously by other researchers due to electrochemical changes in the nature of the drug induced by a continuous direct current. It has also elucidated the manner in which parameters such as pH, vehicle type, ionic compatibility of drug and enhancer, and buffering play a crucial role in the release profiles and transdermal transport of anionic drugs.

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Chapter 1

Introduction

Chapter 1

Introduction

1.1 General Introduction

Transdermal drug delivery (TDD) has for many years been recognized as an alternative method of achieving increased bioavailibility of drugs with less side effects and better patient compliance. Added benefits include increased predictability and reproducibility of drug release kinetics and pharmacokinetics. Theoretically, transdermal drug delivery is more beneficial than all other conventional methods of delivery, such as oral, intravenous or intramuscular administration, due to its more controlled delivery (Smith and Maibach, 1995)

All drugs taken orally undergo "first pass" processes before reaching the systemic blood circulation. The acidity of the stomach is the first problem encountered where the active moieties of a drug can be oxidised. The next problem is adsorption through the lining of the small intestine, where active transport may only take a small proportion of a drug across to the blood stream. Once in the blood stream the drug must go through the liver where the bulk of drug metabolism is carried out by means of redox reactions, dealkylation, deamination, dehalogenation and hydroxylation. These and other processes all go to make the molecule more water soluble, aiding excretion from the body.

The transdermal route evades most of these problems because the administered drug can diffuse directly into the systemic blood system. Other benefits of TDD is the avoidance of side effects, e.g., many Non-Steroidal Anti-Inflammatory Drugs (NSAIDs), cause the patient to suffer nausea. Also, increasingly many modern drug compounds are highly specific in their action and hence require more development leading to increased cost e.g., peptides for the treatment of growth disorders. TDD may in future be able to deliver a higher percentage of drug than conventional methods and thus reduce the amount of drug required to achieve therapeutic levels leading in turn to reduced costs. Figure 1:1:1 shows an ideal

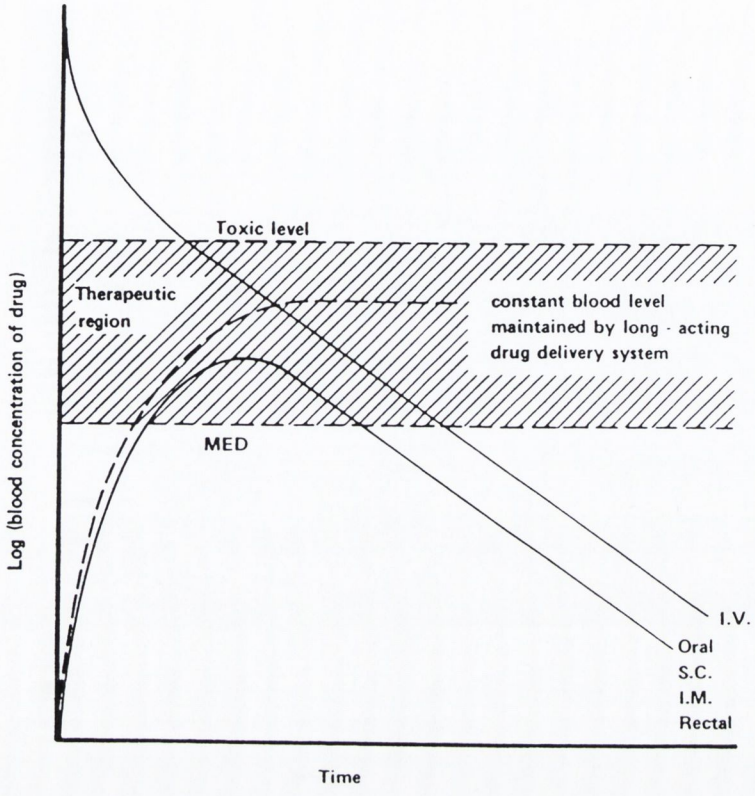


Figure 1:1:1 *Diagram comparing theoretical blood drug concentration profiles of a long acting controlled release drug delivery system with immediate release conventional dosage forms via various routes of administration. (MED – minimum effective dosage level, I.V.-intravenous, I.M.-intramuscular), (Chien, Y.W., 1982)*

profile of a long acting controlled release delivery system (broken line) compared to conventional dosage forms. Probably the most significant benefit of TDD is its ability to maintain a constant blood plasma level of drug systemically i.e., it fits the broken line profile in Fig 1:1:1 above. Using TDD the opportunity also exists to withdraw the dosage form by simply removing the patch. The benefit of TDD compared to multiple dosage forms is shown in Fig 1:1:2 overleaf. The usual range of therapeutic plasma concentrations of commonly used drugs is relatively narrow with the ratio of the upper to lower concentration limit being typically approximately 2. The result of the fluctuations shown in Fig 1:1:2 lead to periods where there is no therapeutic effect when levels drop below the minimum dosage levels. Equally, periods exist where side effects may result from the drug concentration exceeding the toxic dose level. TDD

can be used to maintain drug concentrations within a narrow therapeutic range which can minimize the incidence and severity of adverse side effects, (Levy, 1973). TDD also presents the opportunity to use drugs where the therapeutic range and the toxic ranges lie close together and lessens the need for patient compliance because a strict treatment schedule no longer applies.

TDD systems can be designed to release drugs in the vicinity of the target tissues that require treatment while drug exposure of other non-target tissue is minimized. The therapeutic effectiveness of a TDD system depends on the rate at

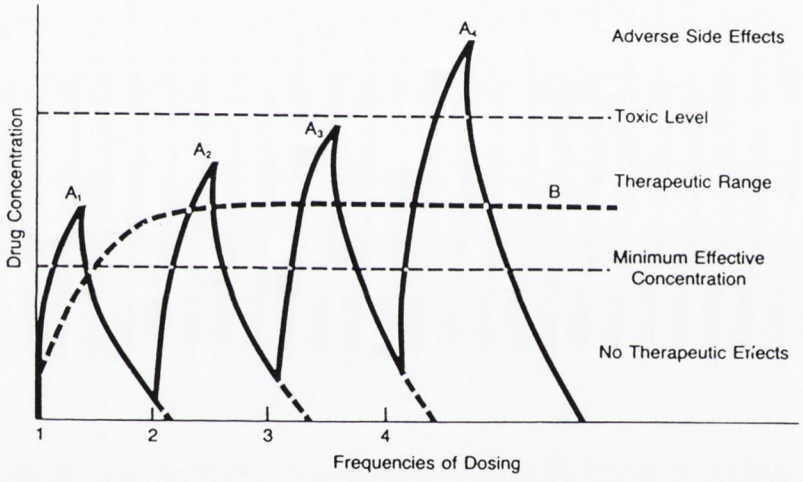


Figure 1:1:2 *Illustration comparing blood drug concentration profiles resulting from administration of multiple doses of a conventional dosage form ($A_1, A_2, A_3, ...$) and a single dose of a long-acting controlled-release drug delivery system (B).(Chien, Y.W., 1982)*

which the drug is delivered by the device. The rate at which a drug diffuses into the *stratum corneum* (SC) depends on the concentration gradient which occurs when the device is applied to the SC, (Corish and Corrigan, 1990) and also on the design features of the device. Some of the more common devices are now described.

Matrix Diffusion Controlled Drug Delivery System (MDCDDS)

The devices to be described all work on similar principles and all have the same objective: to deliver drug content in a controlled fashion.

MDCDDS are generally made from polymeric, hydrophobic gels. The matrix itself is designed to act as the reservoir and control the rate of release. *In-vitro* experiments have shown the difficulties in assessing the rate of release from these matrices due to effects such as hydrodynamic diffusion layer. The diffusion of lipophilic drugs across the hydrodynamic diffusion layer sandwiched between the drug delivery device and the absorption surface of tissue is often the rate-determining step, (Liu *et al.*, 1985). Lui demonstrated that when the hydrodynamic layer is taken into account an "intrinsic drug release" from the matrix shows a linear relationship with loading dose. MDCDDS are fabricated in such a way as to encapsulate the reservoir in a compartment lined with an impermeable membrane leaving only one side exposed to place in contact with the *stratum corneum,* see design (d) in Figure 1:1:4.

Depending on the nature of the reservoir a permeable membrane may also be required to maintain the integrity of the device. A commercial example of this type of TDD System is Niconil® manufactured by Parke, Davis and Company. The patch is prescribed in two dosage forms of 15 and 30 mg, delivering 11 and 22mg respectively, of nicotine *in-vivo* over a twenty-four hour period.

Adhesive Diffusion Controlled Drug Delivery System (ADCDDS)

In this type of TDD system the matrix-containing drug is dispersed in a pressure sensitive adhesive (PSA). Adhesive polymers of this type are permanently tacky at room temperature, hold the TDD system to the skin with gentle pressure and can be easily removed without causing pain or depositing adhesive residue. Compounds 1, 2 and 3 in Figure 1:1:3 illustrate the chemical structure and polymeric reaction of acrylic, polyisobutylene and silicone currently used in TDD systems. Of these silicone is probably the best due to its inherent chemical and thermal stability. An exception to this general rule was observed by Kim *et al.*, (2000), who found that

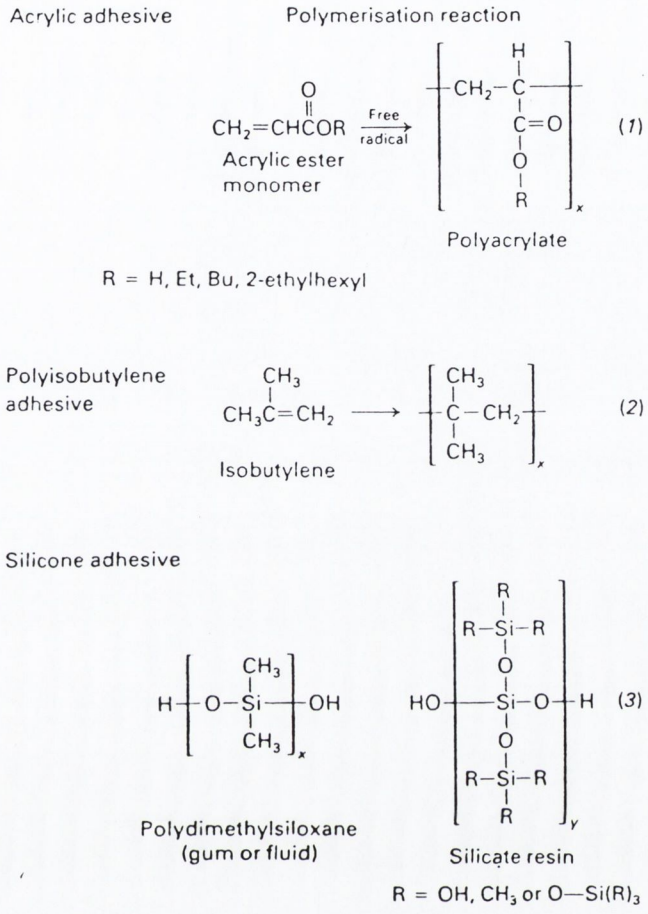


Figure 1:1:3 *Chemical structures and polymerization reactions of pressure sensitive adhesives used in TDD systems, (Pfister et al., 1991).*

tacrine, used in the treatment of Alzheimer's disease, diffused best from acrylic PSA's.

The diffusion co-efficient from PSA's is influenced in 2 ways. Pfister *et al.* (1991) and Kokubo *et al.*, (1994) both observed the link between different functional groups and diffusion co-efficient. They found that basic amine functional drugs (85% of all TDD drugs) can react with acidic moieties of acrylic adhesives causing a loss of adhesion and tack before the device is applied to the SC. Silicone PSA's with a high silanol content also have less than desirable compatibility with certain amine functional groups, resulting in a similar adhesion degradation. Extractable ingredients

that are added to stabilize, plasticise, increase tack, prevent oxidation or catalyse organic adhesives for further cross-linking also put limits on acrylic and polyisobutylene PSA's causing skin irritation on contact.

Kokubo *et al.,* (1990) also evaluated the diffusion co-efficient from several PSA matrices by the microscopic viscosity of the matrices. The microscopic viscosity of the matrices can be derived from the free volume (f) calculated at glass transition temperature (T_g) of the polymer in the matrices. The free volume can be calculated using equation 1:1:1 below.

$$f = f_g + \alpha \, . \, v \, (T - T_g) \qquad \textit{Equation 1:1}$$

Where f_g is the free volume at the glass transition temperature (T_g), α is the thermal expansion co-efficient of the PSA polymer, and v is its molar volume at T_g.

Rajesh *et al.,* (1994) and Roy *et al.,* (1996) found the rate of drug permeation through SC is influenced by the drug to adhesive ratio; first increasing with increase of drug to adhesive ratio and then showing no significant change at a high drug to adhesive ratio. Rajesh suggested that nitroglycerin, at a high ratio, exists in part dispersed as droplets in the adhesive and in part dissolved to give an apparent emulsion. Roy found the skin flux of fentanyl increased proportionately as the drug loading in a polyisobutylene (PIB) adhesive was increased from 1 to 4%, and a plateau was reached beyond 4% drug loading. This result suggests that fentanyl concentration in the PIB adhesive may have reached saturation above 4% drug loading and the optimum skin flux was accomplished from the system because of attainment of maximum thermodynamic activity.

The literature provides information on the diffusivity of various drugs in several PSA matrices predicted by molar volume, functional group of the drugs and the concept of free volume of the polymer matrices. Designs (a) and (b) in Figure 1:1:4 represent an adhesive type TDD system. A commercial example of this type of TDD system is Evorel®, registered to the Ortho Division of Cilag Limited. Each patch contains 3.2 mg of oestradiol corresponding to a release rate of 50 mg in 24 hours. Figure 1:1:4 shows typical designs of current second generation TDD systems which represent the devices outlined or which may have an amalgam of design features.

Membrane Diffusion Controlled Drug Delivery System (MDCDDS)

This type of TDD system is similar to the matrix diffusion controlled device. The need for a membrane in addition to the matrix arises in several instances. If the viscosity of the matrix is too low, a membrane is required to maintain the integrity of the device. Secondly, if the matrix of choice would deliver the drug at too fast a rate, a membrane may be required to control the rate at which the drug may diffuse out of the device.

These types of TDD systems are composed of a drug reservoir in which the drug is dispersed either in a finely divided liquid suspension or as pure drug particles. A peel strip protects the microporous or non-porous membrane, which is placed in contact with the skin. A commercial example of a membrane diffusion controlled TDD system is Transiderm-Nitro® made by Ciba Laboratories. It can be prescribed in several dosage forms of 25, 50 and 75 mg, corresponding to an average delivery of 5, 10 and 15mg of glyceryl trinitrate, respectively.

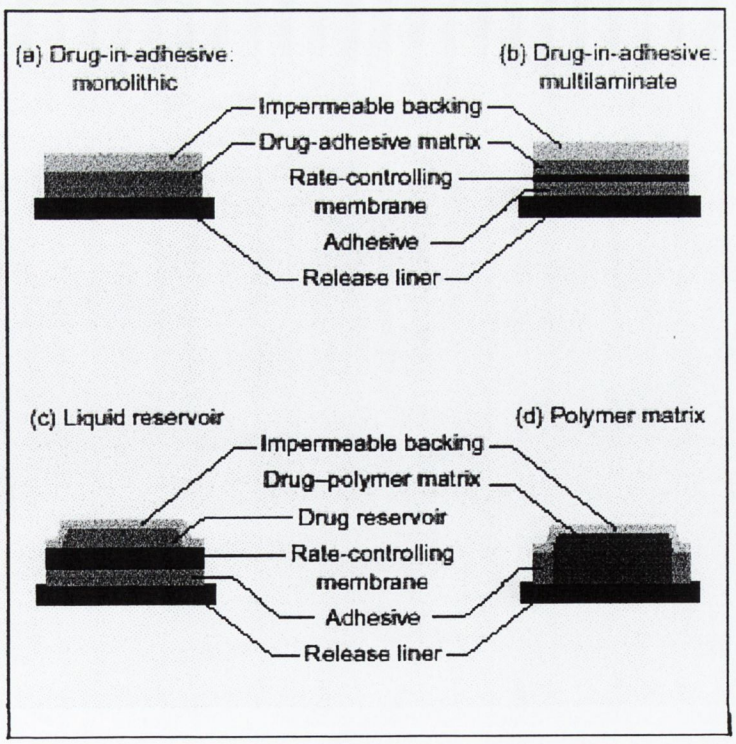


Figure 1:1:4 *Schematic diagrams of typical designs of currently available TDD systems, (Tan, H.S., Pfister, W.R., 1999)*

Drug	Product	Developer/marketer	TDD system design[a]	Pressure-sensitive adhesive type[b]
Clonidine	Catapres-TTS®	Alza/Boehringer Ingelheim	DIA-multi-laminate	PIB
Estradiol	Climara®	3M Pharmaceuticals/Berlex/Schering AG	DIA-monolithic	Acrylate
Estradiol	Estraderm®	Alza/Novartis	Reservoir	PIB
Estradiol	Vivelle®	Noven/Novartis	DIA-monolithic	Acrylate
Estradiol	FemPatch®	Cygnus/Parke-Davis	DIA-monolithic	Silicone
Estradiol/ Norethindrone Acetate	CombiPatch®	Noven/Rhone-Poulenc Rorer	DIA-monolithic	Acrylate
Fentanyl	Duragesic®	Alza/Janssen Pharmaceutical	Reservoir	Silicone
Nicotine	Nicoderm®	Alza/SmithKline Beecham	DIA-multi-laminate	PIB
Nicotine	Harbitrol®	Lohmann/Novartis	Polymer matrix	Acrylate
Nicotine	Nicotrol®	Cygnus/McNeil Consumer Products	DIA-monolithic	PIB
Nicotine	Prostep®	Elan/Lederle	Polymer matrix	Acrylate
Nitroglycerin	Transderm-Nitro®	Alza/Novartis	Reservoir	Silicone
Nitroglycerin	Minitran™	3M Pharmaceuticals	DIA-monolithic	Acrylate
Nitroglycerin	Nitro-Dur®	Key Pharmaceuticals/Schering Plough	DIA-monolithic	Acrylate
Nitroglycerin	Nitrodisc®	G.D. Searle/Roberts	Polymer matrix	Acrylate
Nitroglycerin	Deponit®	Lohmann/Schwarz Pharma	DIA-multi-laminate	PIB
Scopolamine	Transdermal-Scop®	Alza/Novartis	DIA-multi-laminate	PIB
Testosterone	Androderm®	TheraTech/SmithKline Beecham	Reservoir	Acrylate
Testosterone	Testoderm®	Alza	DIA-monolithic	EVA

[a]TDD system designs: drug-in-adhesive (DIA), liquid reservoir (reservoir), multi-laminate drug-in-adhesive (DIA)/control membrane composite (multi-laminate)

[b]PSA Types: polyisobutylene (PIB), polyacrylate copolymer (acrylate), polysiloxane-based (silicone), ethylene-vinyl acetate copolymer (EVA).

Table 1:1:1 *TDD systems currently available on the market. (Tan and Pfister, 1999) For a more comprehensive listing and release rates, see appendix I. (Venkatraman and Gale, 1999)*

Microsealed Drug Delivery System (MDDS)

This type of TDD system is a combination of membrane and matrix diffusion controlled devices. A drug reservoir is prepared by dispersing a suspension of solid drug particles in a water-soluble polymer, a silicone elastomer. The elastomer is cross-linked to form a stable dispersion of liquid droplets in a solid polymer matrix. The drug particles are suspended within each liquid droplet. The matrix can then be moulded or shaped to achieve the desired release rate for a specific drug substance. A membrane is also incorporated to control the rate of release and to add strength to the device. A commercial example of a microsealed drug delivery system is Nitro-Dur® which comes in four dosage forms, 2.5, 5.0, 10.0, and 15mg / 24 hour

Many of the commercial applications cited above make use of drugs which are known to have a significant penetration of the SC. A desired aim of all transdermal

research is to assist in the delivery of a wider range of drugs. The approaches taken to achieve this aim have been many and varied. The fundamental problem encountered by all researchers is the excellent barrier properties of the skin. Most approaches have been to find a way to temporarily reduce these properties so as to allow the drug to diffuse into the systemic blood circulation. Others have sought to modify or derivitise the drug molecule to make it more suitable. In more recent times a combination of approaches have shown promising results.

In the approach taken to the work carried out in this thesis, a simplified yet practical overview of the problems to hand has been formulated. Drug molecules which are suitable for TDD or drugs where there are specific benefits in using TDD, e.g., avoidance of side effects, have been classified according to their chemical natures i.e., cationic, anionic and neutral. Previous approaches tended to classify drugs according to their therapeutic effect e.g., analgesics. Charged drug molecules are, in general, considered to be unsuitable for TDD. However, research continues into possible methods of enhancing the TDD of these particular species. Research previously carried out in this laboratory, (Nolan, 1995), found that a combination of physical and chemical enhancement significantly improved the TDD of salbutamol base, a cationic drug. Oleic acid was used to reduce the barrier function of the SC – chemical enhancement, and an electrical potential was used to attract the drug across to an electrode of opposite polarity – physical enhancement. The use of the resulting current in this instance is known as iontophoresis. These and other techniques will be expanded upon in subsequent chapters.

A similar approach has been adopted for the research presented in this thesis with respect to anionic drug species. Coincidentally, the anionic drugs under investigation are classed as non-steroidal anti-inflammatories. Combined enhancement of this type of drug has been infrequently reported in the literature. Given the side effect of gastro-intestinal (GIT) irritation, the benefits of a TDD system would be significant. It could increase the localisation of treatment to an inflamed area by direct application or by application to an area where the systemic blood flow will take the drug easily to the inflamed area.

1.2 Barrier Function of the Skin

Shall I compare thee to a rubber sheet
Protecting man from toxins, drought and heat,
Where mortal bricks and lipid mortar meet
To build a multilayered wall so neat,
That with thou living membranes can't compete
Or rather – to a horny piece of meat
That makes Saran wrap look quite obsolete

- H. Boddé †

Much of the recent success in the field of TDD is attributable to the rapidly expanding knowledge in the field of *stratum corneum* (SC) barrier function. Modern concepts that have evolved in this discipline have dramatically changed the way scientists view the SC, though some researchers still consider the understanding of the physiology of the SC to remain immature, Rees, J., (1999).

The skin is a multilayered organ and has, anatomically, many histological layers. Its primary function is to protect the body against uncontrolled loss of water and in addition prevents water and matter from the environment from indiscriminately entering the living system. It is generally described in terms of three distinct tissue layers: the epidermis, the dermis and the subcutaneous fat layer. The epidermis, the outermost layer of the SC is composed of stratified epithelial cells. These cells are held together mainly by complex interlocking bridges, which are responsible for the unique integrity of the SC. Investigations have shown the epidermis to consist of two main parts: the *stratum corneum* and the *stratum germinativum*. The *stratum corneum* forms the outermost layer of the epidermis and consists of many stratified layers of compacted, flattened, keratinised cells which have lost their nuclei. They are formed and continuously replenished over every two week period by the slow upward migration of cells produced by cell layer of the *stratum germinativum*, Chien, (1982).

Originally the SC was thought to be just an inert barrier of dead cells. Then it was visualised as a bricks-and-mortar structure, in which the proteinaceous dead corneocytes were the bricks and lipids provided the cement. Now it is viewed as the

(† - *deceased*)

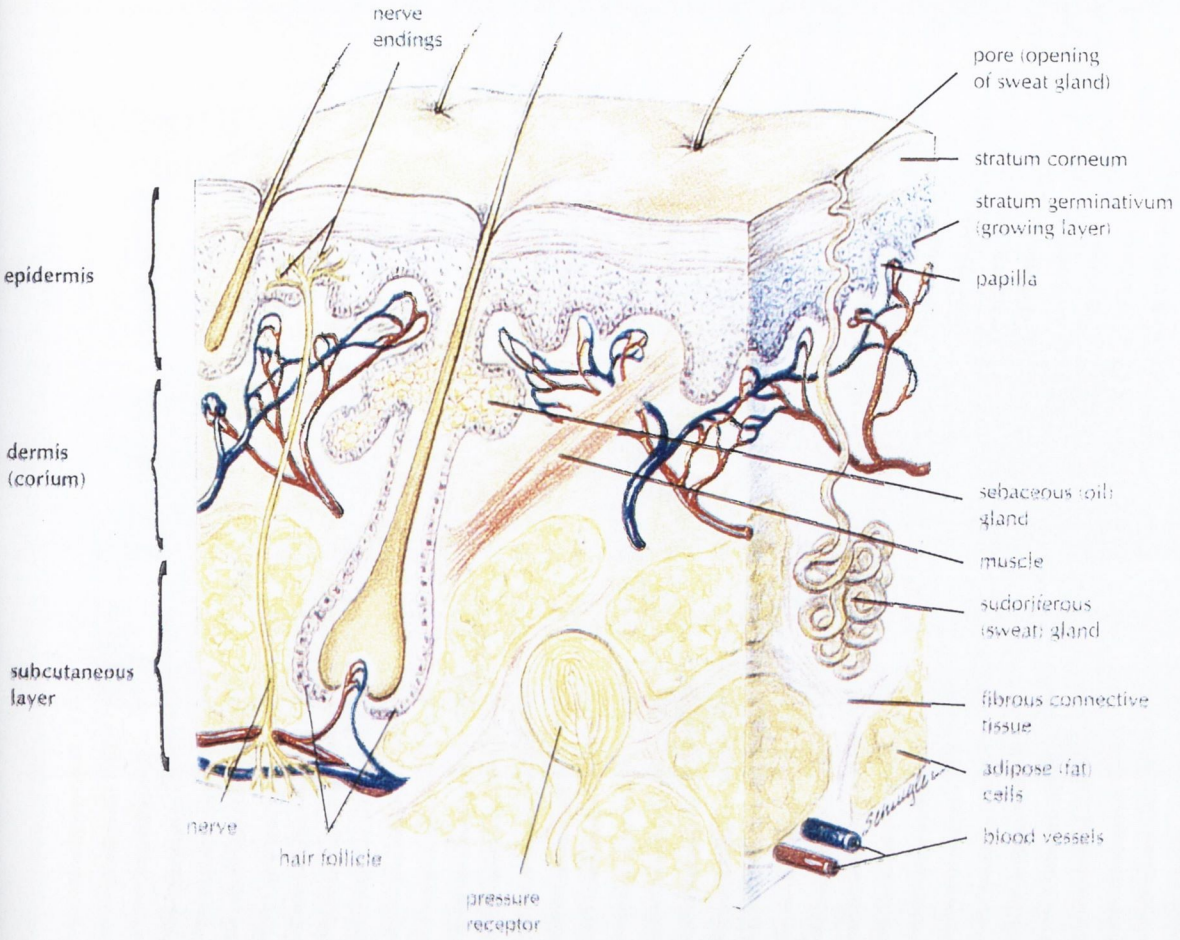


Figure 1:2:1 *Cross section of the skin showing different layers and appendages, (Memmler, Cohen and Wood, 1996)*

culmination of a tightly regulated form of programmed cell death within a complex, metabolically active, and structured lamellar lipid layer, Schaefer, H., (1996) and Williams, M., (1998). The current concept of the skin barrier suggests that permeability is governed by a hydrophilic and hydrophobic "channel". To account both for the barrier function and the hydrophilic and hydrophobic pathways through the SC barrier, Forslind (1994), proposed "the domain mosaic model of the skin barrier". The model suggests that the bulk of lipids are segregated into crystalline/gel domains bordered by "grain borders" where lipids are in a fluid crystalline state. This arrangement provides a "water-tight" barrier that allows a minute and controlled loss of water to keep the corneocytes moistened (giving them plastic properties without which the skin would be brittle). Thus, the model provides for the necessary mechanical properties permitting bending and stress imposed on the skin surface. The

fluid character of the "grain borders" represents areas where lipid and hydrophobic molecules may diffuse through the system on down-hill gradients.

Elias, P.M., (1991), looked on the organization of the *stratum corneum* as a dual-compartment system of lipid-depleted corneocytes embedded in an extracellular matrix of non-polar, lipid-enriched lamellar bilayers. The estimated intercellular space between corneocytes is approximately 20% of the total SC volume, Elias, P.M., (1979). The functions of these intercellular domains are listed in Table 1:2:1 below.

Table 1:2:1 *Functions of stratum corneum intercellular domains, (Elias, P.M., 1991)*

Classic Regulatory Functions	*Newly Appreciated Functions*
Transcutaneous water loss	*Antimicrobial defence*
Cohesion and desquamation	*Balance of free radicals*
Water content	*Balance of antioxidants*
Percutaneous absorption	*Natural sunscreen*

It is these intercellular domains that will become the focus of attention in further discussion on the route, or pathway that drug molecules take through the skin. It is not surprising that the *stratum corneum* has been considered the "black box" with regard to TDD (Potts, 1987). To propose a model for the SC is a complex task given the conflicting requirements to be met, e.g., the ability of the skin to act as a completely water tight barrier whilst at the same time allowing ~ 350ml of water to be lost a day through the skin surface of an average male. A battery of physical chemical methods, including diffraction, X-ray diffraction, electron spin resonance, polarising spectroscopy and microcalorimetry have provided useful information about lipid-lipid and lipid-protein interactions in intercellular domains (Rehfeld, 1982; Knutson, 1985; Golden, 1986; and White, 1988). Newer methods such as freeze-fracture, fixation and staining should combine in the future to elucidate a fuller understanding of the morphology of the SC to aid in the design and selection of percutaneous penetration enhancers.

1.3 Principles of Percutaneous Absorption

Transdermal permeation can be visualised as a composite of a series of steps in sequence, that is, adsorption of a penetrant molecule onto the surface layers of the *stratum corneum,* diffusion through it and through the viable epidermis and finally through the papillary dermis and into the microcirculation. The viable tissue layers and the capillaries are relatively permeable and the peripheral circulation is sufficiently rapid (Perl, 1963), to make partitioning and diffusion through the SC the rate-limiting step for the great majority of substances.

The many and varied parameters which pertain to percutaneous absorption will be further discussed in this section. These parameters are tabulated in Table 1:3 which illustrates their participation in the main sequential steps to percutaneous absorption, i.e., partitioning into and diffusion through the SC.

Table 1:3 *Factors pertaining to percutaneous absorption of drugs*

Partitioning	*Diffusion*
Physicochemical Properties	*Physicochemical Properties*
Vehicle Effects	*Vehicle Effects*
SC Hydration	*SC Hydration*
Age/Race	*SC Metabolism*
Route	*Route*
-	*Reservoir Effect*

1:3:1 Partitioning into the Stratum Corneum

The permeability of biological tissues has long been thought to depend on the lipoidal character of cell membranes, (Davson, 1952; Overton, 1924). Early reviewers of the principles of percutaneous absorption concluded SC had a lipid-like character by inference from mainly clinical observations of membranes other than skin, (Blank, 1960; Calvery *et al.,* 1946; Rothman, 1943, 1954; Tregear, 1964). It was not until relatively recently that conclusive evidence was provided of the relationship between

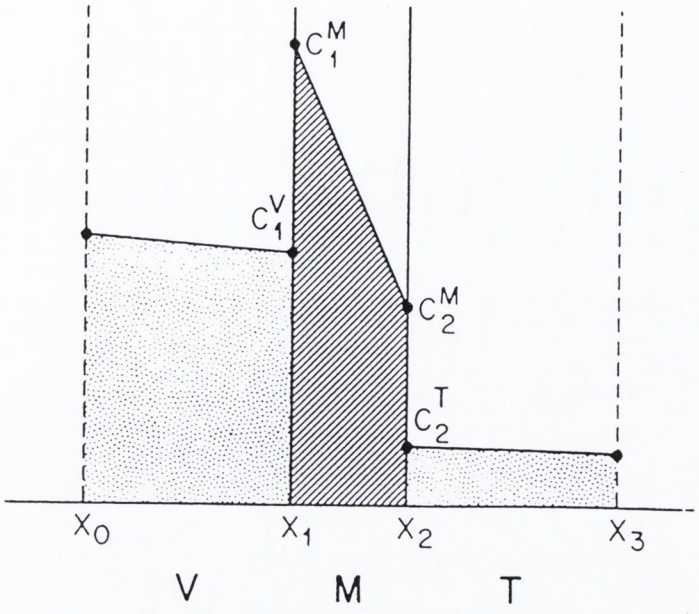


Figure 1:3:1 *A steady-state concentration profile across the SC. The vehicle* (V) *at concentration* C^V, (M) = *Stratum Corneum and* (T) = *viable tissue. The diagram shows that concentrations across adjoining phase boundaries are not the same. (Scheuplein, 1978)*

oil/water partitioning and skin penetration rate, (Treherne, 1956; Nogami, 1958; Loveday, 1961; Blank, 1964).

Before bulk diffusion of a drug substantially advances across the SC, an equilibrium is established in the narrow interfacial region between the vehicle and the SC. This means that the chemical potentials of each component in the solution and at the SC surface rapidly become equal, i.e.,

$$\mu_i^v = \mu_i^m \qquad \textit{Equation 1:2}$$

(where v = vehicle and m = SC). Therefore, it is the chemical potentials μ_i, not the concentrations C_i that become equal across the interface, (see Figure 1:3:1 above). Chemical potentials for non-ideal solutions can be related to concentrations by equations of the form

$$\mu_i = \mu_i^0 + RT\,ln\,\gamma_i\,C_i \qquad \textit{Equation 1:3}$$

(where γ_i is the activity coefficient and C_i is the concentration). The boundary condition (Equation 1:2) assures that the concentrations of both solute (1) and solvent (2) across the interface will be related to each other by the distribution coefficient K_{MV} where

$$K_{MV} = \frac{C_2^V C_1^M}{C_2^M C_1^V}$$

Equation 1:4

and where K_{MV} is constant at constant temperature, (Kipling, 1965). For a dilute solution this is approximately

$$K_{MV} = \frac{C_1^M}{C_1^V}$$

Equation 1:5

The chemical potential of the adsorbed components at the interface are initially not equal to their values deeper within the SC. It is this difference in the chemical potential across the SC that is the principal driving force for skin permeation.

Treherne (1956), was one of the first to employ excised skin (rabbit) in a diffusion cell for the purposes of defining its barrier characteristics but it was Blank (1960), who was first to take a systematic approach to discovering partitioning phenomena. He choose a homologous series of alkanols as permeants. Partition coefficients of these increase systematically and exponentially, which provided a wide range of partitioning values to test the fundamentally important idea that increasing lipophilicity enhances permeability. This idea was reinforced by Higuchi (1960), in which thermodynamic concepts of activity and activity coefficients were introduced. These ideas lead the way for Sheuplein (1965), and Blank *et al.*, (1967), to accurately describe in physicochemical terms a reasonable model of the skin barrier.

Scheuplein *et al.* (1956, 1967, 1976) studied the permeation of alkanols through membranes made of human epidermal sheets, proving that the epidermal barrier had lipoidal qualities by way of the shape of the permeability pattern, see Figure 1:3:2 (A). They also studied skin strata form beneath the epidermis,

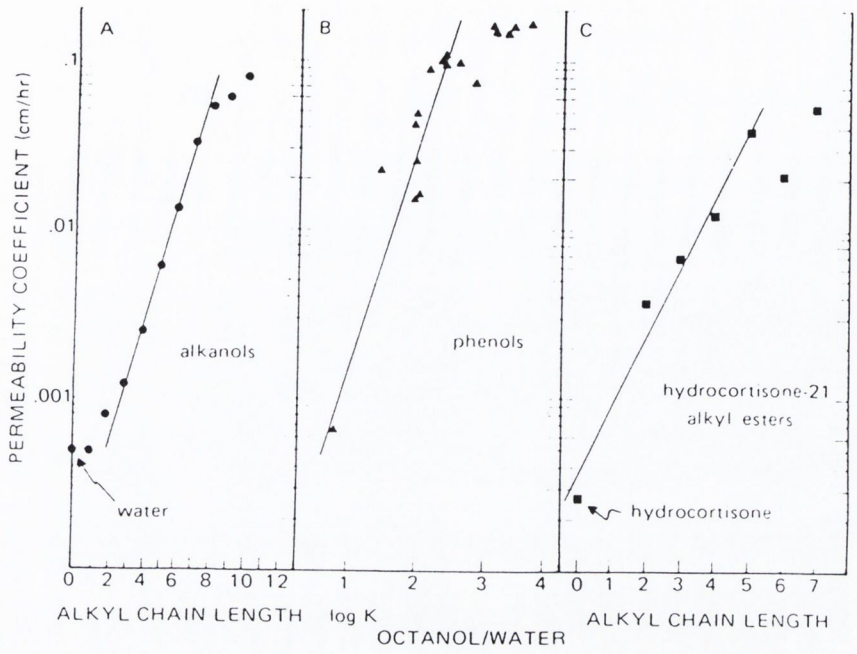


Figure 1:3:2 *Permeability coefficients of water and n-alkanols* (A), *phenols* (B) *and hydrocortisone and its esters* (C) *as functions of increasing hydrophobicity. (Flynn, 1987)*

coming to the opposite conclusion that this diffusion zone of the skin behaves as a watery barrier to the alkanols. Note the similar permeability coefficient of methanol and water in Figure 1:3:2 (A): this will be of significance when route of delivery is discussed later in this chapter.

In vitro experiments with alkyl-substituted phenols (Roberts *et al.*, 1977, 1978) shown in Figure 1:3:2 (B) also provide evidence of an oil/water partitioning dependent route. Smith (1982), obtained data for hydrocortisone and its homologous 21 esters (C) which also suggest lipoidal permeation. While partition coefficients grow exponentially with increased lipophilicity, there is little known concurrent effect on diffusion coefficients. There is evidence that the dependency is not much greater than this in non-crystalline polymers, (Flynn *et al.*, 1974). Therefore, diffusion will be discussed in the next section as a separate phenomenon to partitioning.

1:3:2 Diffusion through the Stratum Corneum

The most useful model in approximating the passive and relatively simple process of skin permeation is Fick's law, (Equation 1:6). Kedem *et al., (1961)* were one of the first to apply the theory of irreversible thermodynamics to membrane transport thus were able to justify the use of Fick's law. For passive membrane processes at constant temperature, forces exist which tend to produce entropy from the transfer of energy or matter. Typical forces are pressure differences, osmotic gradients and, in the case of electrolytes, electrical potential differences. With skin permeation, however, pressure differences are negligible. Therefore, skin permeation for non-electrolytes can be accurately described as being driven by osmotic gradients, (Scheuplein & Blank, 1971). Fick's first law may be written as

$$J = -D\frac{dc}{dx}$$

Equation 1:6

where J is the rate of flow of the diffusing substance with the concentration gradient dc/dx and D is the diffusion coefficient. Because the SC is not simply an inert diffusion medium but can react with diffusing molecules in ways not consistent with the laws of ideal chemical solutions, there is a need for concentration dependant corrections to the simple form of Fick' law, (Scheuplein, 1977). In light of this, if the diffusion coefficient D is to be determined by means of equation 1.6, it must be in an arrangement in which both J and dc/dx are constant. This is achieved by derivation of equation 1.6 to obtain Fick's second law.

$$\frac{dc}{dx} = -D\frac{d^2C}{dx^2}$$

Equation 1.7

A mathematical model of percutaneous absorption can be obtained in principle by solving equation 1.7. Many attempts have been made to derive models taking into account such parameters as geometry, initial conditions and boundary conditions appropriate to SC diffusion. However, because of the complexity of the SC, an exact

mathematical solution of the diffusion problem is impractical. Each layer of the SC would have a different value for *D*. The complications introduced by the geometry of cells and blood vessels eventually overwhelms any mathematical model attempting to embrace all conditions.

However, working within these constraints many useful models have been proposed. Potts and Guy (1995), put forward "A Predictive Algorithm for Skin Permeability" in which they described drug permeability by a partitioning-diffusion equation. Through free energy relationships, partitioning is related to the molecular volume (MV) of the drug, and hydrogen bond donor (H_D) and acceptor (H_A) activity. Diffusion was related to MV values using a theory of diffusion through lipid lamellae based on free volume fluctuations within the lipid domain. These two explicit descriptions were combined to give an equation describing permeability in terms of the physical properties of the permeant. The aqueous permeability properties of thirty-seven non-electrolytes through human SC were evaluated as a function of these physical properties using a multiple regression analysis. The results showed that 94% of variability in the data could be explained by the model which includes only the values of MV, H_D and H_A. The results of their model provided an algorithm to predict skin permeability based on the values of these parameters. In addition, the relative contribution of various functional groups, (e.g., -COOH) is derived, and can be used to predict SC transport from drug structure alone. The researchers also concluded that the model is applicable to a number of lipid barrier membranes, suggesting a common transport mechanism in all of these.

Similar models to that of Potts and Guy have been proposed by Nara *et al.*, (1992), where molecular size was used as the main parameter and Ando *et al.*, (1984). Another approach to modeling is a two layer diffusion model with polar and non-polar routes in the SC designed by Yamashita *et al.*, (1994) and by Kamato *et al.*, (1989). Heisig's *et al.*, (1996) study represents a crossover between a bi-phasic "brick and mortar" model and modeling based on lag times (Kubota *et al.*, 1986). Irrespective of which model is the most suitable, all proposed models have taken one or more of the factors and parameters that will be discussed in the following section into account.

1:3:3 Physicochemical Properties and Structure-Activity Correlations

A physicochemical property of a molecule, already discussed, is its partition coefficient. The lipid solubility and the water solubility characteristics of the molecule will allow it to proportionately partition between organic and water phases. It is this chemical characteristic that is likened to the partition of a chemical between lipid and water phases of the skin.

Its partition coefficient is probably the single most significant factor in predicting the permeability of a drug molecule. As seen in Figure 1:3:2 many models have been based around partitioning phenomena and with varying degrees of success. Most predictive models will have at least one or two unexpected exceptions cropping up in data analysis. Table 1:3:2 shows data gathered by Bartek and LaBudde, (1975), of partition coefficients and percutaneous absorption data in humans.

Table 1:3:2 *Partition Coefficient (heptane / buffer)* and Percutaneous Absorption in Man. (Bartek and LaBudde, 1975)*

Chemical	*Partition Coefficient*	*Dose Absorbed in Man %*
N-Acetylcisteine	*0.0004*	*2.43*
Cortisone	*0.002*	*3.38*
Caffeine	*0.005*	*47.6*
Testosterone	*1.81*	*13.2*
Parathion	*14.4*	*9.7*
DDT	*28.5*	*10.4*
Lindane	*32.9*	*9.3*
Malathion	*33.2*	*8.2*
Haloprogin	*45.4*	*11.0*
Butter Yellow	*100.8*	*21.6*

**Buffer = 0.1M phosphate buffer, pH 7.4*

The chemicals are arranged by increasing partition coefficient and the table also shows the percentage dose of each absorbed in human SC. The lower the partition coefficient,

the more water soluble the chemical. The one obvious exception in Table 1:3:2 is caffeine. *N*-Acetylcysteine has the lowest partition coefficient and its percentage absorbed dose in humans is also the lowest. The compound with the highest absorption, (apart from caffeine) is butter yellow. It also has the highest partition coefficient. All the other compounds in the series follow a general trend and show some correlation between increased partition coefficient and increased percutaneous absorption in humans. Looking at the values for testosterone and haloprogin can highlight another anomaly in the data. Their partition coefficients are 1.81 and 45.4 respectively, yet they exhibit little difference in percentage dose absorbed. A general conclusion coming from the data is that partition coefficient in itself will only give a limited indication as to whether a compound will be absorbed and that other factors must also be taken into consideration.

Roberts *et al.*, (1977), carried out a similar study with various phenolic compounds. Generally as the octanol/water partition coefficient increased the permeability coefficient also increased. Although the correlation was significant, the partition coefficient taken on its own could not explain all the variation in the data.

An investigation of the percutaneous absorption of steroids by Scheuplein *et al.*, (1969), most obviously outlines the advantages and disadvantages of using only partition coefficient values to predict absorption. A good correlation was found to exist for steroids between *in vitro* determined permeability constants and partition coefficients, especially between SC and water. However, data collected for three similarly structured estrogens failed to show any correlation between their permeability constants and partition coefficients, (see Table 1:3:3).

Table 1:3:3 *In Vitro Permeability Constants and Partition Coefficients for Estrogens, (Scheuplein, 1969).*

Estrogen	K_p *(cm/h x* 10^{-6}*)*	K_m	K_{hex}
Estrone	*3600*	*46*	*3.0*
Estradiol	*300*	*46*	*0.6*
Estriol	*40*	*23*	*0.2*

K_p = permeability constant, K_m = SC / water partition coefficient, K_{hex} = hexadecane / water partition coefficient.

The data in Table 1:3:3 provide evidence that the relationship between partition coefficient and permeability constants can only be described as general and that exceptions such as estrogens do exist.

Scheafer *et al.*, (1981), examined the structure-activity relationships of the steroids in Table 1:3:3 and found Estrone had the highest permeability constant. The permeability of Estriol decreased by a factor of 12, with the chemical modification of the reducing ketone on the sixteenth position on the molecule, (see Figure 1:3:3 below).

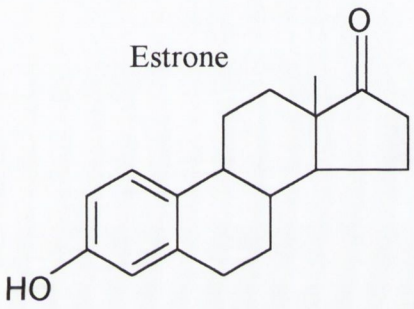
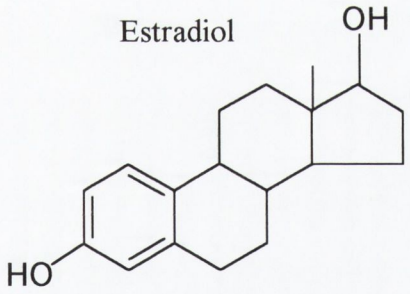
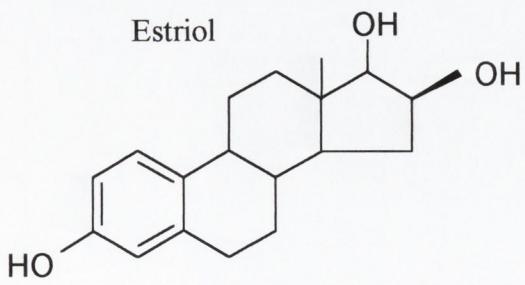
Estrogen	*Permeability Constant*
Estrone 	3600
Estradiol 	300
Estriol 	40

Figure 1:3:3 *Steriod structures of closely related Estrogens*

The addition of a second hydroxyl group to Estriol further decreased the permeability constant by a factor of 7.5. These results demonstrate that with no modification to the backbone of the molecule and only slight modification of functional groups a significant effect is shown in permeability constants.

Scheafer *et al.*, (1981), also studied steroid structures of closely related androgens – Testosterone, Dehydroepiandrosterone (DHT) and Dihydrotestosterone (DTT). The percutaneous absorption of Testosterone was 30% of applied dose, while that of DTT was only 1%, a 30-fold decrease. In this case, the addition of two Hydrogens across a double bond in the A ring of the steroid caused this dramatic effect. Similar examples exist among related Progestins and also for Corticosteroids. The addition of a hydroxy group to Progesterone decreased its permeability constant from 1500 to 600. The addition of a hydroxyl group to Cortexolone decreases its permeablility constant by a factor of 3.

Scheuplein, (1980), noted that the decrease in Steroid permeability with the addition of polar groups was due to a decrease in diffusion coefficient not partition coefficient. The decrease in diffusion coefficient was attributed to an increase in the degree of chemical interaction between polar steroids and lipid-protein / water matrix within the SC. Table 1:3:4 below details the structure of several solutes with the same number of carbon atoms.

Table 1:3:4 *Structure and In Vitro Permeability of solutes with the Molecular Formulae. (Scheuplein and Blank, 1971)*

Solute	*Formula*	K_p *(cm/h)*
Ethyl Ether	C-C-O-C-C	15-17
2-Butanone	O || C-C-C-C	4-5
1-Butanol	C-C-C-C-OH	2-4
2-Ethoxy Ethanol	C-C-O-C-C-OH	0.2-0.3
1,3-Butandiol	OH OH | | C-C-C-C	0.05

The difference in the solutes in Table 1:3:4 is the position of an introduced Oxygen atom and addition of hydroxyl groups. As with Androgen and Estrogen examples these additions have a drastic effect on respective permeability constants.

Another physicochemical property of a molecule is its molecular mass. Intuitively it would be expected that molecular mass would have significant and perhaps predictive influence on permeability constants, yet this is not the case. The addition of a single Hydrogen to a molecule can have a greater effect on permeability than the magnitude of its molecular mass. Establishing a general upper maximum level of molecular weight for percutaneous absorption has not been demonstrated by research. A general consensus was thought to be 5000 a.m.u., but an observation by Scheaffer *et al.*, (1981), of the penetration of Herparin (molecular weight = 17000) made this generalisation invalid.

Alternatively, increasing molecular weight can be indicative of a decrease in molar flux. As the mass of individual molecules increases with increasing molecular weight there follows a decrease in the number of molecules that can be applied to each cm^2 of skin (Wester and Maibach, 1985).

As with partition coefficeints, the use of molecular weight alone to predict percutaneous absorption is not reliable, (Beetge *et al.*, 2000).

1:3:4 Route of Percutaneous Absorption

There are potentially three distinct routes of penetration through the SC: (1) the follicular regions, (2) the sweat ducts and (3) the unbroken SC in between these appendages, (see Figure 1:3:4). Route three can be further subdivided into intercellular and intracellular routes, (see Figure 1:3:5).

A frequently posed question is which of the above represents the principal route of penetration. In general the question has resulted in a division of opinion, one view favouring percutaneous absorption primarily by diffusion via sweat ducts and hair follicles and the other view holding diffusion through the unbroken SC as the principal route. Interestingly, a literature review provides adequate data to support both views under different conditions. Those supporting the former view cite the preferential staining of hair follicles and the rapid diffusion of charged dyes through

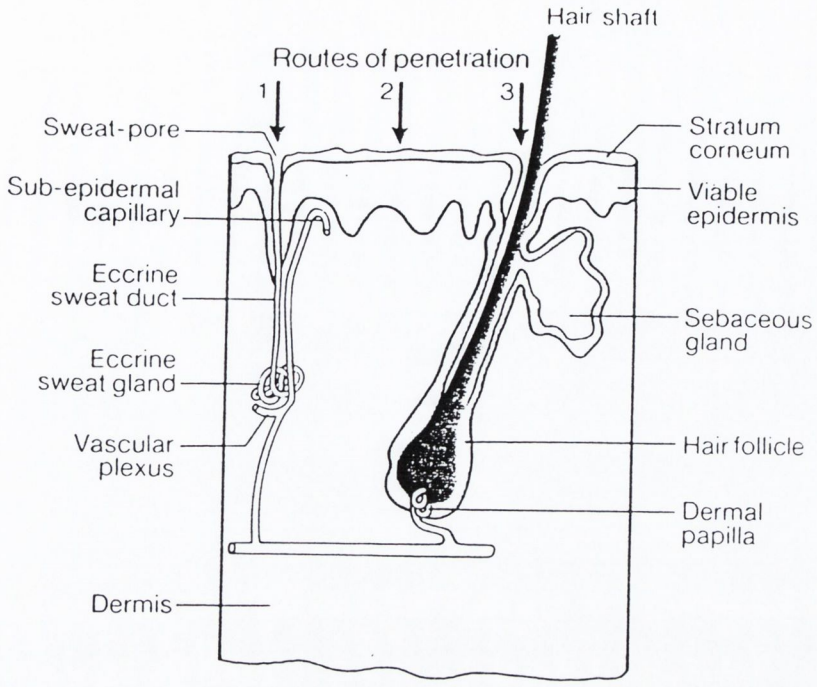
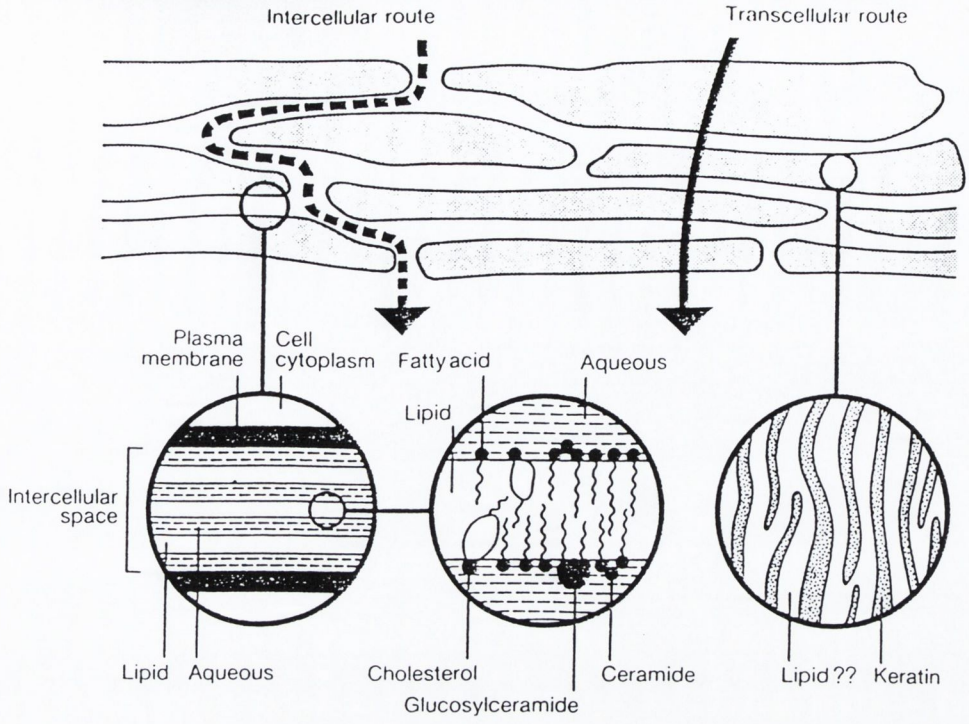


Figure 1:3:4 *Potential routes of penetration: (1) via sweat ducts; (2) across the unbroken SC or (3) through hair follicles or sebaceous glands, (Barry, 1987)*

sweat ducts under a passive potential gradient, (Rothman, 1954, Mackee *et al.*, 1945). Those supporting the latter view stress the fact of the very small surface area of the appendages of the SC, ~ 10^{-3} cm^{-2}. Also cited are the large activation energies required by molecules during steady state diffusion which makes rapid appendageal diffusion relatively unlikely, Sheuplein (1966).

It is widely accepted that the SC is the rate-limiting step in transdermal drug delivery (TDD), (Boddé *et al.*, 1991). However, hair follicles and sebaceous glands constitute areas of lesser resistance to the diffusion of drugs through the epidermis. Direct proof of absorption by the hair follicles was reported by Tregear, (1961), who showed that ^{32}P-labelled tributylphosphate was absorbed through pigskin hair follicles after application. Studies involving guinea pig skin by Scheuplein, (1971) found that cutaneous absorption was less in bald skin than in areas rich in follicles. Scheuplein also postulated on the basis of a mathematical model that shunt diffusion through follicles relative to transepidermal diffusion is of limited importance in steady-state conditions. However, during a lag time transfollicular diffusion can contribute in a significant way in percutaneous absorption and hence remains predominant for very slow-diffusing molecules. This model has been firmly disputed by Illel *et al.*, (1991).



1:3:5 *Diagram of possible permeation routes through the SC, together with idealised representation of intracellular keratin and intercellular bilayer lipids, (Barry, 1987).*

Figure 1:3:2 (A) unwittingly produced the first evidence of an alternative route through the SC. Scheuplein and co-workers whilst studying the effects of partition coefficient on permeability coefficient of a series of alcohols found that the first two molecules in the series (methanol and ethanol), had essentially the same permeability coefficient as water through the epidermis. Little was made of this at the time, but this observation now appears to be related to a different mechanism of permeation for these relatively small and quite polar substances. The three solutes appear to pass through intact skin by a pathway without an oil/water partition dependency. This new route was termed an "aqueous pore pathway".

Wepierre *et al.*, (1989), used specialized techniques developed by Illel and Scheafer, (1988) to grow hairless rat skin without sebaceous glands. The permeability of such skin was compared with that of normal hairless rat skin rich in sebaceous glands. The study found that sebaceous glands contribute significantly in the skin permeation of drugs. After 48 hours, diffusion by the sebaceous route represented 50 to 80% of total absorption.

Illel *et al.*, (1991) continued their studies by producing hairless rat skin with no follicles. Again, comparisons were made with normal skin. Follicles were found to

play a predominant role in the *in vitro* diffusion of a number of tritiated drug molecules, namely, Hydrocortisone, Niflumic Acid, Caffeine and *p*-Aminobenzoic acid. In the absence of hair follicles, the steady-state flux and the amounts diffusing in 24 or 48 hours were 2-4 times lower than in normal skin.

Another frequently posed question regarding the non-appendage transepidermal route is whether diffusion occurs *via* an intercellular space or intracellular route, (see Figure 1:3:5). To answer this question Boddé *et al.*,(1991), investigated the diffusion of mercuric chloride through human SC *in vitro*. Results using electron microscopy indicated that the intercellular route of transport predominated. However, the results also indicated that after longer transport times, corneocytes tend to take up material leading to a bimodal distribution of mercury, i.e., in certain regions of the SC there was mercury both inside and outside of cells. The study observed two types of corneocytes: Apical corneocytes, which tend to take up mercury ions relatively easily, whilst other corneocytes were less capable of doing so.

As Scheuplein, (1967), suggests it may be wrong to try and answer the above scientific questions because they may be bad questions. He further suggests that it is probably wrong to assume that a principal route of penetration exists. In hindsight, from literature published since that time, it appears that Scheuplein was right and that various routes of diffusion are responsible for the overall percutaneous absorption.

1:3:5 Effect of Hydration of Stratum Corneum on Percutaneous Absorption

It has been known for many years that an increase in the permeability of the SC can be achieved by simply increasing the water content of the tissue, (Blank *et al.*, 1969, Idson, 1975). Skin hydration can be readily effected by covering or occluding, e.g., with plastic sheeting, leading to an accumulation of sweat and condensed transpired water vapour, (Hall-Smith, 1962, Sulzberger, 1961). Although the precise mechanism by which penetration is improved is still unclear, hydration by occlusion is known to cause a swelling of the corneocytes, and to increase the amount of water associated with the intercellular lipid domains. *In vivo* there is a continuous diffusion of water from within the body through the SC and into the environment. This is known as transepidermal water loss or TEWL. This water loss is a somewhat unique

example of skin permeability. Blank *et al.*, (1984), found that at any one relative humidity, a water concentration gradient is established such that the concentrations of water in the various layers of the SC differ but each remains relatively constant, and because of the gradient water will still move through the tissue. Figure 1:3:6 illustrates the effect of relative humidity on the concentration of water in the SC. The extent of hydration varies around the surface of the body. The SC is always partially hydrated *in vivo* (5 to 15%), the degree of hydration being dependent on the composition of the lipoprotein environment and on the ambient conditions. Tagami *et al.*, (1980), were able to demonstrate the highly hygroscopic nature of the SC and also the lesser hydration of the extremities than of the trunk of the body.

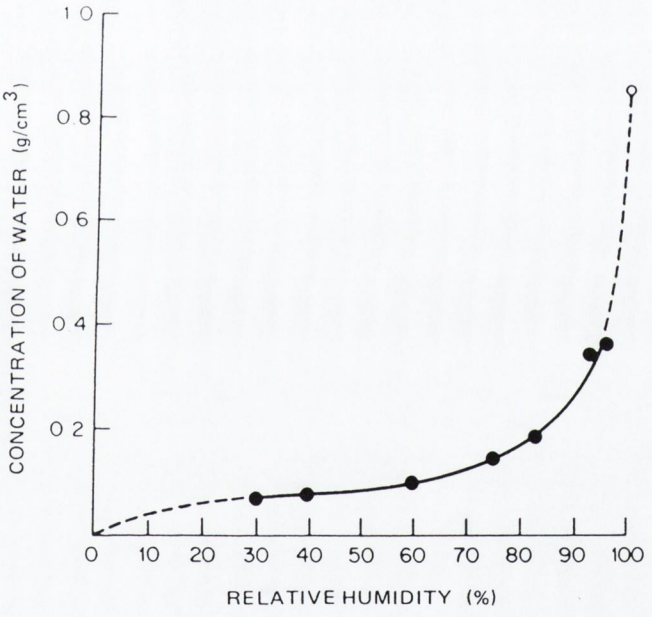


Figure 1:3:6 *Concentration of water in the SC in equilibrium with air at 30°C as a function of relative humidity. (Spencer et al., 1975)*

The evidence for increased penetration of drug molecules due to increased hydration is growing and is now quite extensive. Wurster and Kramer, (1961) showed that hydrating the SC increased the penetration of methyl, ethyl and glycol salicylates. Haigh and Smith, (1995) demonstrated the increased permeation of corticosteroids with occlusion of the SC for 6 hours before application. Interestingly their study also showed that absorption was more significant without an added chemical enhancer

under hydrated conditions. The opposite observation was made when the SC was not occluded. Behl *et al.*, (1980) investigated the influence of hydration on permeation of a series of alcohols. Their results indicated a doubling of the permeability coefficients of butanol and hexanol when the SC is hydrated. However, it was found that the permeability coefficients of methanol, ethanol and water were little affected by such hydration. This reflects the results found by Scheuplein, (1967) i.e., that the permeability coefficients of methanol and ethanol are the same as that of water, indicating that these small polar molecules may follow the same route through the SC regardless of the level of its hydration.

Much of the literature regarding hydration of the SC is concerned with the capability of non-invasively monitoring skin hydration *in vivo*. This is important to accurately determining the effects of hydration on percutaneous absorption. A review by Elsner, (1997) shows that many approaches have been used to assess skin hydration, including electrical measurements, infrared spectroscopy, microwave propagation, heat conductivity, photoacoustic spectroscopy and even indirect indicators such as viscoelastic properties, friction, dye flourescence and topography. Many of the commercially available instruments measure hydration indirectly by electrical means. This means measurement with different devices cannot be compared and that calibration is difficult. This renders these devices unusable in clinical applications. Kohli, (1985), also warned of the wide inter-subject and intra-subject variations in obtaining data, which casts doubt on the validity of some earlier reported results.

In spite of this, researchers have for almost 4 decades used occlusion of the SC to enhance the absorption of drugs such as anti-inflammatory corticosteroids from topical delivery vehicles (Neering *et al.*, 1972). Of all the factors affecting the penetration of drug molecules, hydration of the skin (by preventing TEWL) is by far the least damaging and causes minimal toxicity or irritancy under normal circumstances. Hydration is rapidly and totally reversible and thus has no permanent effects on the barrier condition of the skin.

1:3:6 Vehicle Effects on Percutaneous Penetration

If the range of processes involved in transdermal drug delivery from a typical

TDD system were to be categorised into a few simple steps, the first of these steps would be diffusion of a drug molecule from a vehicle and the effect of the vehicle in this process. One of the first researchers to stress the importance of the vehicle for the permeation of a drug through the SC was Moncorps (1929). He found a large variation in the concentration of Salicylic acid recovered from rat urine depending on the type of vehicle used.

The affinity of the vehicle for the drug molecules will influence the release of the drug molecules from the vehicle, (Higuchi, 1960). Solubility in the vehicle will also determine the release rate of the drug. Generally, the more easily a drug is released from the drug delivery systems, the higher the rate of transdermal permeation. The mechanisms of drug release depends on whether the drug molecules are dissolved or suspended in the drug delivery system and on the interfacial partition coefficient of the drug from the delivery system into the skin tissue.

The pH of the vehicle can influence the rate of release of the drug from the drug delivery system, since the thermodynamic activity of acidic and basic drugs is affected by the pH. Thus, for acidic drugs, the activity changes rapidly when the pH is greater than the pK_a of the drug species. Similarly, the activity for basic drugs is influenced when the pH of the vehicle is lower than the ($pK_w - pK_b$) value of the drug.

The concentration of drug in the vehicle may also have an effect on skin/vehicle partitioning. Raykar *et al.*, (1988) observed increased partitioning of both polar and non-polar steroids into SC as the drug concentration in the vehicle increased. However, in contrast, Surber *et al.*, (1990) recorded a decrease in steroid partitioning as the drug concentration of the aqueous vehicle is increased. The above examples may not be indicative of the effect of concentration profiles as may be appreciated from the following example. Surber *et al.*, (1990), reported considerable partitioning of the lipophilic steroids progesterone and acitretin, into the SC, from aqueous based vehicles. However, when isopropylmyristate is used as a vehicle no appreciable skin partitioning of either steroid is evident. The feasibility of transdermal delivery of a given drug will therefore depend on the selection of the proper vehicle, (Hatanaka *et al.*, 1993).

Another instance is where the composition of the vehicle may affect the transport of the drug e.g., the use of chemical penetration enhancers as the drug

delivery matrix. This type of vehicle can be used to modify the barrier properties of the skin, (Green *at al.*, 1989). Because of the ability of this type of vehicle in this regard, it is best to classify it as a chemical penetration enhancer. Indeed, the option of including another chemical penetration enhancer within a vehicle primarily composed of a penetration enhancer adds to the complexity of the effect a vehicle can have on transdermal drug delivery. A range of these enhancers and their combined effects shall be discussed in depth in a subsequent section.

1:3:7 Reservoir Effect or "Skin Binding"

One of the more interesting factors affecting percutaneous absorption is known as the "Reservoir Effect" or "Skin Binding". These terms generally mean the SC has the ability to prevent molecules from diffusing through it at a faster rate than that at which they partition into the SC and therefore an accumulation of drug is retained within the membrane. The rate of diffusion through the SC is low for most chemicals due to interaction with epidermal tissue in the course of penetration, (Menczel *et al.*, 1985).

The capacity of the SC to retain drug molecules had been speculated upon for a long time. It was not until 1963 that Vickers was able to prove the epidermal reservoir concept. This was exhibited by corticosteroidal reactivation which was achieved two weeks after its topical application by repeated occlusion with Saran wrap. The consequence of this is that the SC can function as a reservoir for delayed action of a drug.

The retention of a chemical in any of the cutaneous tissue is a net outcome of either reversible affinity binding of partitioning of a substance between aqueous vehicle and tissue components, (Artuc *et al.*, 1980). A distinction can be made by analyzing data from equilibration of the chemical dissolved in a suitable buffer with the SC. In the case of partitioning, the partition coefficient (P) can be derived from the concentration of the substance in the SC (D_{SC}) divided by its concentration in an appropriate buffer (D_B)

$$K = D_{SC} / D_B \qquad \textit{Equation 1:8}$$

In its logarithmic form, a straight-line relationship is obtained with a slope equal to unity according to equation 1:9. Reversible binding affinity can be described by

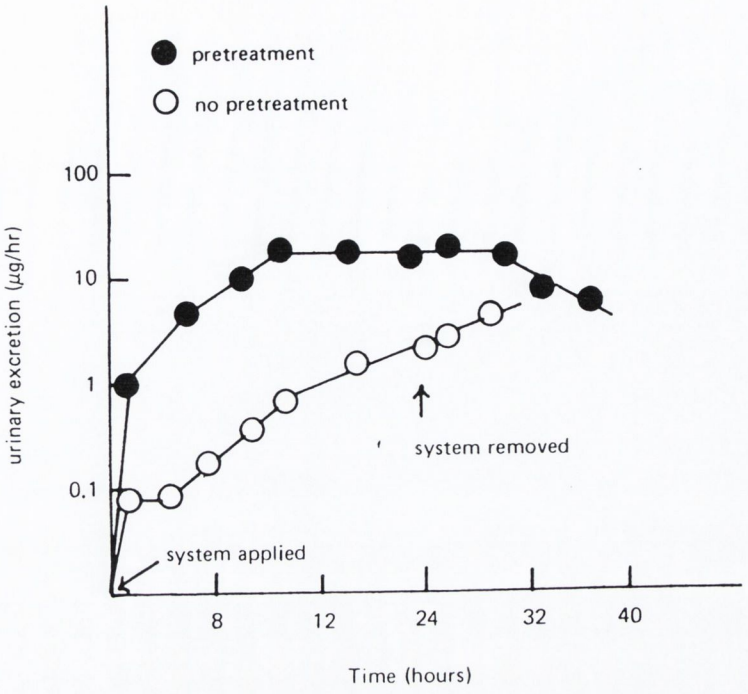


Figure 1:3:7 *Effect of SC pretreatment with Sodium Lauryl Sulphate on the urinary excretion profile of Scolpolamine from a transdermal delivery system, (Shaw et al., 1975).*

introducing the Freundlich adsorption isotherm, where the concentration of the tissue bound substance (D^b_{SC}) is related to that unbound by an exponential term (D_F), as in equation 1:10.

$$\log D_{SC} = \log K + \log D_B$$

Equation 1:9

$$D^b_{SC} = K.D^a_F$$

Equation 1:10

$$\log D^b_{SC} = \log K + a.\log D_F$$

Equation 1:11

Here K and a are computed constants characteristic of the system involved. The logarithmic form of Equation 1:10 is shown by Equation 1:11. This is a linear relationship, where log K is the intercept on the y-axis and a is the slope. The similarities in the relationships in Equation 1:9 and equation 1:11 is evident. When the value of a in Equation 1:11 approximates to 1, it is essentially the same as Equation

1:9. Thus, the determination of slope *a* is indicative of whether the chemical is partitioned or associatively bound, upon equilibration between its solution and the SC, (Artuc *et al,*. 1980; Menczel and Maibach, 1972).

Ideally, for therapeutic purposes, it is desirable to have no reservoir effect in order to deliver a drug into the systemic circulation at a rate determined by the delivery vehicle. It has been reported that the binding of drugs within the SC can be reduced by the pretreatment of the skin with an anionic surfactant. Shaw *et al.*, (1975), demonstrated that the pretreatment of SC with Sodium Lauryl Sulphate (SLS) improved the release of Scopolamine within a set time period compared to a similar experiment without pretreatment, (see Figure 1:3:7). Vickers, (1963, 1961) demonstrated clearly that the main site of the reservoir in the skin is the SC. It was also demonstrated that the reservoir was easily destroyed by stripping the SC with adhesive tape. It has been suggested that using a strong surfactant such as SDS as employed by Shaw *et al.*, (1975) has the same effect on the surface of the SC as has stripping with adhesive tape.

The information gained from these types of experiments helps to formulate new approaches in transdermal drug delivery. Whether there is a need to reduce a reservoir effect or employ an enhancer, or both, is a relevant question in the design of transdermal systems.

1:3:8 Metabolism in the Stratum Corneum

Metabolic processes within the *Stratum Corneum* are often not taken into account in the design of transdermal delivery devices because these processes have been assumed to be unimportant or unlikely. More recently, the SC is regarded less as an inert barrier to the diffusion of drug molecules and this has brought forth many questions as to the extent to which cutaneous metabolism can effect the transdermal delivery of drugs. It has also raised questions as to the relevance of metabolic processes for the selection of candidate molecules and the feasibility of using prodrugs. Metabolism of a drug molecule may reduce its pharmacological activity. However, the reverse can also occur. Skin metabolising enzymes can convert nonactive chemical moieties into active pharmacological or toxicological agents.

The SC is host to a range of metabolic processes similar to those carried out in the liver. These processes in the SC ultimately break foreign substances down into more polar metabolites to aid excretion from the kidneys. These processes are outlined in Table 1:3:5.

Table 1:3:5 *Metabolic reactions known to take place in the Stratum Corneum. Examples of drug molecules which may undergo these reactions are also given, (Tauber, 1989).*

Enzymatic Reaction	*Drug Substance*
Oxidation	
Aliphatic Carbon	*Dimethybenzanthracene*
Alicyclic Carbon	*Dehydroepindrosterone*
Aromatic Rings	*Benzopyrene*
Alcohols	*Cortisol*
Deamination	*Norepinephrine*
Dealkylation	*Ethoxycoumarin*
Reduction	
Carbonyl Groups	*Cortisol, Progesterone, Estrone*
Carbon Double Bonds	*Testosterone, Progesterone*
Hydrolysis	
Ester Bonds	*Gluco-Corticosteroid Esters*
Epoxides	*Styrene Oxide*
Conjugation	
Methylation	*Norepinephrine*
Glutathione conjugation	*Styrene Oxide*
Glucuronide formation	*OH-Benzopyrene*
Sulphate formation	*Aminophenol*

The reactions, as outlined in Table 1:3:5 occur due to specific enzymes within the SC. These reactions include redox, hydrolytic and conjugation reactions, (Noonan and Wester, 1985). When Benzopyrene (BP) is applied to human SC, it is metabolized to several distinct metabolites. These metabolites fall into three classes: phenols,

quinones and dihydrodiols, (Fox *et al,*. 1975). Within these catagories, two phenols, two dihydrodiols and three Quinones are formed. This example illustrates why metabolism within the SC is an important factor when considering drug molecules for transdermal drug delivery. These metabolites may have no therapeutic effect or may even cause adverse side effects.

1:3:8 Other Factors Affecting Percutaneous Absorption

This section will deal with remaining factors, which are considered less significant to percutaneous absorption. The influence on the barrier properties of the skin of its age, anatomic site and race are all factors which require to be understood in order to optimize release from transdermal delivery devices.

Until recently, the influence of the above factors have been assumed rather than quantified. The effect of age on percutaneous absorption was investigated by Wester *et al.*, (1987). The absorption of human newborn foreskin was compared to that of adult abdominal SC. Absorption in the newborn foreskin was found to be 10 times greater than in the abdominal SC. This was attributed to age rather than site as absorption in adult foreskin is comparable to that of adult abdominal SC. Dick and Scott, (1992) studied the effect of age on permeability of rat skin. Results found that dermal thickness, hair follicle depth and to a lesser extent, the surface area occupied by hair follicles all appeared to be influenced by age. These changes were found to have no detectable effect on permeability. The relevance of these findings with regard to human SC are debatable due to the fact that the ageing process of the rat takes 10-120 days.

The rate of transepidermal water loss (TEWL) appears to be unaltered by the age of the skin, (Roskos and Guy, 1989), but SC hydration varies significantly in different age groups, Manuskiatti *et al.*, (1998). Both TEWL and hydration are indicators of permeability. From previous discussion it may be assumed that TEWL is a more important indicator of permeation than is hydration. Regardless of age, it can also be assumed that the same degree of hydration will be reached under the occlusion of a trandermal device when placed on the same anatomical site due to TEWL.

Properties of the skin which frequently vary from site to site are the thickness of the SC, the distribution and number of hair follicles and sweat ducts. Variation of water permeability of bodily regions was investigated by Scheuplein and Bronaugh, (1983). Significant variation was found in the flux and permeability coefficients mainly due to SC thickness. Feldman and Maibach, (1967) found the extent of absorption depends on the anatomical site to which a chemical is applied. Greater absorption occurred at sites at which the distribution of hair follicles was most numerous such as the scalp. Maibach, (1976), carried out a study in which the individual variation in percutaneous absorption of hydrocortisone was characterised. The median absorption of eighteen subjects was 0.9% of the applied dose. However, the absorption of several individuals was 33% higher, whereas one individual absorbed 75% more than the median. The virtue of this experiment is that the median of absorption may be a better representation than the average absorption due to outliers in data caused by individual variations.

Finally, the temperature of the SC has been shown to have a definite effect on the percutaneous absorption of drug molecules, (Stoughton, 1965). A ten-fold increase in the absorption of acetyl salicyclic acid and glucosteroids has been observed when the environmental temperature has been raised from 10 to 30°C. This was rationalised as being due to increased thermodynamic activity within the SC increasing the diffusion coefficient of the drugs. Secondly, it was also be attributed to increased vasodilatation of blood capillaries within the SC leading to an increase in absorption. Thirdly, an increase in temperature is often associated with an increase in humidity. This can lead to an increase in hydration of the SC and a decrease in TEWL. This combination of events can lead to increased permeability.

1.4 Transdermal Penetration Enhancement Techniques

Due to the formidable barrier that the *stratum corneum* represents, very few drug molecules are suitable for transdermal drug delivery. Those which are tend to be of low molecular weight, neutral and lipophilic in nature. This SC barrier must be breached if poorly penetrating drugs are to be administered at an appropriate rate but if enhancers are used then it should be possible to restore the barrier properties of the SC once diffusion of the drug has occurred. The objectives of the design of a TDD are simple to define, but are often very difficult to achieve.

To this end, most recent research has involved the development and optimization of several techniques which enhance the delivery of poorly absorbed drug molecules. It has been found a reduction in the diffusion barrier may conveniently be achieved in a reversible manner via the use of chemical and physical enhancers. The following subsections will outline in detail the potential benefits of these methods.

1:4:1 *Physical Penetration Enhancement*

Iontophoresis

The use of a specific physical technique can be attractive for certain drug classes. Ionized drugs, for example, and complex macromolecules such as proteins and peptides may be induced to permeate the SC at a faster rate than normal by the application of a small electrical current across the membrane, (Singh, 1993). The permeant is repelled form the electrode of similar polarity into the SC, which acts as the electrical conduit to the companion electrode which is placed on the SC. This type of iontophoretically enhanced permeation has one major advantage in that the flux of the permeant can potentially be precisely controlled by varying the assisting current, and thus the delivery can be individually tailored for the patient.

The use of electricity to increase penetration of electrically charged molecules through different membranes, a process known as iontophoresis, has been known for a long time. Biomedical applications of iontophoresis have also been around for several

decades, (Chien and Banga, 1989). The earliest known patent was issued by the American Patent Office on August 19th, 1862, entitled "Electrical Apparatus for Medical Use" which describes a pair of fluid-filled electrodes conducting through the body, with one of the electrodes containing medicament placed on the diseased portion of the body, (Sage, 1995). Whilst iontophoresis for medical use may have first appeared in the U.S. some of the best known early work was published by Stefan Leduc in 1908 in Europe. In a somewhat macabre experiment Leduc using a solution containing strychnine sulphate placed in the anode of an iontophoresis system on one rabbit, and a solution of potassium cyanide was placed in the cathode of the system on a second rabbit. The rabbits were connected to each other, with water filled electrodes serving as a cathode on the first rabbit and as an anode on the second rabbit. When current was applied, both rabbits died. When current was applied with the polarity of the battery reversed, neither of the rabbits died. The only conclusion was that the electric current administered lethal ions in the first setup, but did not when the polarity was reversed.

Since the time of Leduc, resurgence in interest in iontophoresis has occurred in almost every decade. Green *et al.*, (1996) predicted iontophoretic devices would be available by the year 2000. Currently, iontophoresis devices are commercially available on the market. In existence are devices for the delivery of local anesthetics and corticosteroids such as Phoresor®II, Empi®, Life Tech®, and Dynaphor®. Many companies such as Alza (USA), Becton Dickinson (USA), Fournier (France) and Hisamitsu (Japan) are actively trying to commercialize miniature patch systems and are said to be close to the market. Also Dermion, a subsidiary of IOMED, recently bought the iontophoresis technology of Elan Corporation (Ireland) and are working on the development of wearable patches, (Banga, 1999). Iontophoresis has also been used in gene therapy to induce genes into the eye of a rabbit, (Asahara, 1999). The next commercially available product will probably be E-TRANS® which is in phase III clinical trials. The product is an on-demand delivery system of fentanyl intended to allow a patient to manage acute pain by self-titrating the level of fentanyl administered according to his or her needs. There are hundreds of patents on iontophoresis with the majority belonging to Alza and Becton Dickinson. Much of the activity in the area is proprietary and is expected to unfold over the next few years, (Banga, 1998).

Fundamental Theories of Iontophoresis

Many theories have been adapted to try and predict the rate of drug delivery using the applied potential or the delivery current as independent variables. Unfortunately, like many other predictive models, these models do not have closed form equations unless gross simplifications are made. Probably the most accurate model to describe iontophoresis is the basic model known as the Nernst-Planck flux equation. The model asserts that the flux of an ion across a membrane under the influence of an electric field is due to three components: an electroosmotic component, a diffusive component and an iontophoretic component. The relationship is written as:

$$J = Cu - D\left\{\frac{dC}{dx}\right\} + D\frac{zEFC}{kT}$$

Equation 1:12

Where

J = molar Flux

C = molar concentration

u = convective water flow

z = ionic valence

E = electric field

F = Faraday's constant

k = Boltzman's constant

T = temperature (Kelvin)

D = diffusivity coefficient of an ion across a membrane

dC/dx = molar concentration gradient in direction of flux

Most treatments of the problem of drug flux across SC using the Nernst-Planck approach ignore the first or electroosmotic term. This term is also referred to as convective solvent flow. Pikal, (1992), is one of the few researchers to investigate the relative importance of this term. Electroosmosis can be defined as bulk fluid flow which occurs when a voltage difference is imposed across a charged membrane. Electroosmosis is always in the same direction as the flow of counterions and may either assist or hinder drug transport. As human SC is negatively charged above ~ pH 4 and cation permselective, (Burnette, 1987), counterions are positive ions and electroosmotic flow occurs from anode to cathode. Thus anodic delivery is assisted and cathodic delivery retarded by electroosmosis. Osmosis commonly infers the movement of water but in this instance water carried by ions as "hydration water"

does not contribute significantly to the elctroosmotic flow. Rather an electrical volume force acting on the mobile counter ions causes electroosmotic flow.

Both Pikal (1992) and Guy (2000) predict that under certain circumstances electroosmosis may predominate even when the molecule is ionised. Given this assumption Equation 1:12 simplifies to:

$$J = -D\left(\frac{dC}{dx}\right) + D\frac{zEFC}{kT}$$

Equation 1:13

In principle this equation allows the prediction of drug flux through a membrane with the knowledge of only one parameter; the diffusion coefficient D. Unfortunately, Planck assumed the membrane to be electrically neutral and the assumption fails on this account. Assumptions of a constant electric field everywhere in the membrane also fails. This is due to the presence of shunt pathways within the SC in addition to fixed charges. Kasting (1989) found good correlation of the Nernst-Planck equation for voltages up to about 0.5V. For larger voltages, this theory under predicts the drug flux and this becomes progressively worse as the voltage increases.

Guy (2000), theoretically evaluated the effect of ionic mobility on iontophoretic transport, and specifically, on the balance between electrorepulsion and electroosmosis. Guy incorporates Faraday's Law into his model which describes the flux in terms of electric current rather than voltage. Guy assumes that total iontophoretic flux is the sum of the components electrorepulsion and electroosmosis. For simplicity, the contribution of passive flux has been assumed to be negligible;

$$J_{total} = J_{electrorepulsion} + J_{electroosmosis}$$

Equation 1:14

$$J_{total} = \frac{z_p u_p c_p I}{F.\sum z_1 u_1 c_1} + v.c_p$$

Equation 1:15

z_p, u_p, c_p are the permeant charge, mobility and concentration, respectively; I is the current density, F is Faraday's constant and the summation includes all species

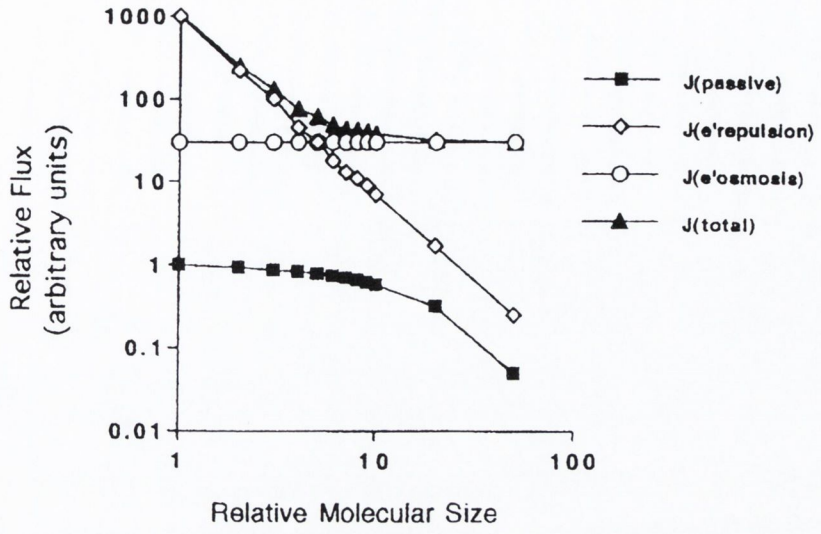


Figure 1:4:1 *The contributions of passive [J(passive)], electrorepulsive [J(e'repulsion)], and electroosmosis [J(e'osmosis)] fluxes to the total iontophoretic transport [J(total)] of cationic drugs as a function of molecular weight, (Guy, 2000).*

present in the solution, v is the electroosmotic solvent velocity, which is positive in the case of cation iontophoresis, but negative for anions.

The average (and upper limit) ionic mobility of both Na^+ and Cl^- in the SC has been estimated to be ~ 1.5×10^{-8} cm^2 V^{-1} s^{-1}, (Kalia, 1998). Using this value, Guy (2000) predicted the contributions of electrorepulsion and electroosmosis to the total flux. For example, for a fast moving cation of mobility 1×10^{-8} cm^2 V^{-1} s^{-1}, at an acceptable current density, electrorepulsion dominates completely. On the other hand for a slow moving molecule (such as a moderately sized cationic peptide having a mobility of ~ 10^{-11} cm^2 V^{-1} s^{-1}), the total flux is almost entirely electroosmotic and is predicted to be about ten-fold less than that of the more mobile species. At intermediate mobilities, the distribution of electrorepulsion and electroosmosis fluctuates between these extremes. Figure 1:4:1 summarizes this discussion schematically.

The equivalent to Figure 1:4:1 for anion iontophoresis has also been predicted by Guy. The electroosmotic flux will be ~30 arbitrary units, suggesting that the delivery of significantly sized, negatively charged species will be extremely difficult.

The idea of delivering high molecular weight anions from the anode, making use exclusively of the electroosmotic effect has also been considered, (Pikal, 1992). However, Guy suggests the chances of success of this strategy are very small, and likely to be of little or no use in "real life" delivery situations.

Electroporation and the Influence of Current Profiles

Does an advantage of pulsed direct current over a continuous direct current for electrically assisted drug delivery exist? This question relates to the rate of drug delivery. It is now known that pulsed current can result in a different mechanistic transport of drug called electroporation. In contrast to iontophoresis, which has been studied for several decades (Chien, 1989), the use of electroporation for TDD was only suggested 7 years ago (Prausnitz *et al.*, 1993) although as a technique has a history of some 25 years of history (Tsong, 1991).

Electroporation involves the application of a high voltage pulse for a very short duration. This technique opens pores in cell membranes allowing DNA or other macromolecules to enter the cell (Weaver, 1995). Electroporation reversibly makes permeable lipid bilayers and possibly involves the creation of aqueous pathways during the application of an electrical pulse. It has been demonstrated that electroporation of SC is feasible (Banga, 1999, Chang, 2000). Hoffman (1995), coined the term electroincorporation which involves the encapsulation of a drug in vesicles or particles and delivery into the skin by applying a pulse of high voltage which causes a breakdown of the SC, (see Figure 1:4:2).

The contribution of electroporation during iontophoresis is also beginning to emerge. Until recently, iontophoresis is believed to primarily transport drugs through pre-existing pathways, (Cullander, 1992). However, it has been shown that changes in the barrier properties of human epidermal membrane during low to moderate voltage iontophoresis are consistent with the induction of new pores or pathways, (Inada *et al.*, 1994). It has also been suggested that even low voltage iontophoresis may electroporate the epithelial layers of the skin appendages which in turn allows transport from the appendages to epidermal cells. At low voltages (<30V), the drop in resistance of skin may be attributed to electroporation of appendageal ducts. At higher voltages (>30V), electroporation of the skin itself leads to a further drop in resistance,

(Chizmadzhev *et al.*, 1998, Hui, 1998). Weaver (1993), considered electroporation to be a non-thermal phenomena since pore formation by membrane rearrangement occurs much before any significant temperature rise takes place in the pulsing medium. Further evidence of the contribution of electroporation to iontophoresis was made by Manabe (2000). Investigations showed that the enhancing effects of iontophoresis on skin permeation by nonionic hydrophilic compounds can be explained by electroporation and increased electroosmotic flow with the electroosmotic flow being monitored using D_2O. Higuchi (2000), also reported that

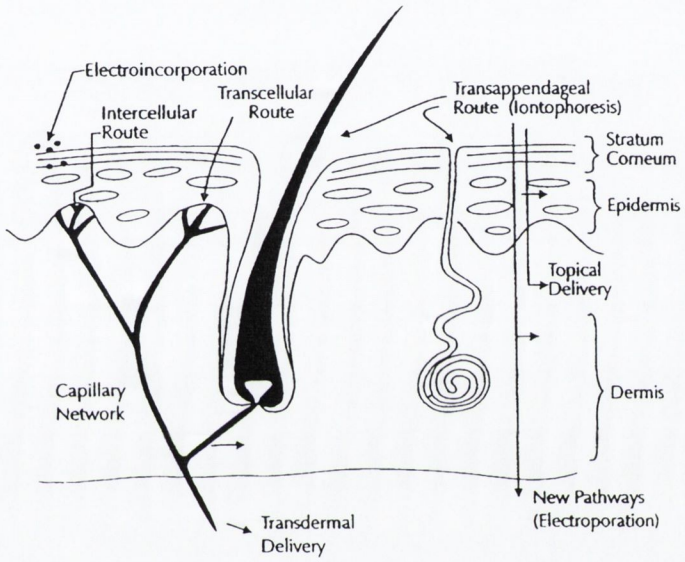


Figure 1:4:2 *Schematic showing the pathways of transdermal delivery, including electrically assisted delivery by iontophoresis, electroporation, and electroincorporation (Banga, 1999)*

the contribution of new pore induction (electroporation) resulted in a 10-100 fold enhancement. This figure is in addition to direct current and electroosmotic effects. Chang *et al.*,(2000), interestingly found that a combination of electroporation (pulsed current) and continuous current iontophoresis resulted in higher transdermal permeation of calcium regulating hormones than either one technique when used separately.

This and other recent evidence is indicative that the knowledge surrounding the mechanisms involved in iontophoresis across human SC are still evolving. In the last five years it has become more apparent that the processes usually called

iontophoresis is the sum of several mechanistic components, namely; electrorepulsion, electroosmosis and electroporation. It is also evident that these components can work with and against each other depending on the nature of the drug. For example, electrorepulsion will work in favour of anionic drugs whereas electroosmosis is biased against ionic drug transport.

Effects of Iontophoresis on the Stratum Corneum

Some of the model equations examined thus far would suggest that any required dose might be achieved simply by adjusting the current. This would be true if the motion of the ions through the skin did not cause secondary adverse effects. Unfortunately, the skin does limit the amount of current that can safely and tolerably pass through it. Exceeding these limits first gives rise to uncomfortable sensations which, if the current is increased, cause a painful, burning sensation, and second, irritates the skin to the point of blister formation. A review by Ledger (1992), reported the average maximum tolerable current density to be 0.5 mA cm^{-2}.

Investigations by Jadoul *et al.*, (1999), into the effects of iontophoresis and electroporation found that for both methods the major effects were: (1) a disorganisation of the lipid bilayers of the SC; (2) an increase in skin hydration; (3) a larger decrease in skin resistance induced by electroporation as compared to iontophoresis. The changes were found to be partly reversible and to depend on the amount of electrical charge transferred. The investigators also issued a cautionary note about the safety of electroporation indicating that its effects on the skin might be irreversible.

In addition to volunteer feedback, methods such as TEWL and skin blood flow (SBF) are used to measure alteration to the SC. Brand *et al.*, (1997) found that current flowing for only 5 minutes was sufficient to cause a significant increase in SBF. Longer periods of current flow induced greater changes in SBF, the elevated level of which persisted for longer times after the termination of iontophoresis. Brand also reported that SBF increased more beneath the anode than the cathode and that a majority of subjects registered greater discomfort at the anode. Apart from occlusion effects there were no apparent changes in TEWL measurements when compared to

no-current controls. The same conclusions for TEWL measurements were also found by Van der Geest *et al.*, (1996).

The perturbation of the SC and quantification of how quickly and to what extent the barrier properties recover from the effects of iontophoresis have not been measured exactly to date due to the large variation in inter and intra-subject samples. Turner *et al.*, (1997) used impedance spectroscopy to assess the resistance of the SC at various intervals after the termination of current. Measurements indicated that the time required for the impedance of hairless mouse skin to return to pre-iontophoresis levels (following 2-hr current passage at 0.5 mA/cm^2) was at least 18 hours. Inada *et al.*, (1994), along with most other researchers concur that the rate of decrease of resistance is strongly dependant on the applied voltage and that reversible recovery times depend on both the magnitude and duration of applied field. Interestingly Inada also found that reversible recovery times were much longer when lower voltages were applied for longer periods to attain the same decrease in electrical resistance than for higher voltages applied for shorter times.

Factors and Variables Effecting Iontophoresis

Many factors influence the efficient delivery of a candidate molecule by iontophoresis. The factors can be generally classified as either physicochemical or electrical. Several physicochemical factors are outlined briefly as follows:

Charge on Drugs

The first and perhaps easiest property of a drug to ascertain is its charge. For iontophoretic delivery to occur, a drug molecule must be in an ionized state with either a positive or a negative charge. Nonionic drugs may on occasion be delivered iontophoretically provided that a charge can be induced on them, or other wise by iontohydrokinesis, (Gangarosa *et al.*, 1980). Multiplicity of charge is also an important factor. Langkjær *et al,*. (1998), found that monomeric insulins with two extra negative charges had fluxes which were 50-100 times those of hexameric insulin. Introducing three additional charges led to a further 2-3 fold increase in flux. The delivery of drugs that are zwitterionic in nature may be iontophoretically enhanced using pH control, giving the opportunity to use either anodal or cathodal

iontophoresis, e.g., low pH will likely neutralise weak acids thus masking a negative charge.

Conductance

Conductivity experiments are useful in ascertaining which drugs are most conductive and therefore the best candidates for iontophoretic delivery. Gangarosa *et al.*, (1978) conducted studies into specific conductivities of various drugs and also into the effect of phosphate buffer being included in the same medium. The study found that the hydrochloride salts of local anesthetics and vasoconstrictors have high conductivities. Subsequent experiments were performed to determine whether drug conductivity can be calculated by determining the conductivities of drug and buffer followed by subtraction of the conductivity of the buffer alone. Results showed that the overall conductivity of the medium was additive, i.e., each ion acts independently in terms of its contribution to conductivity but combined conductivities of drug and buffer add to give the total conductivity, (see Figure 1:4:3).

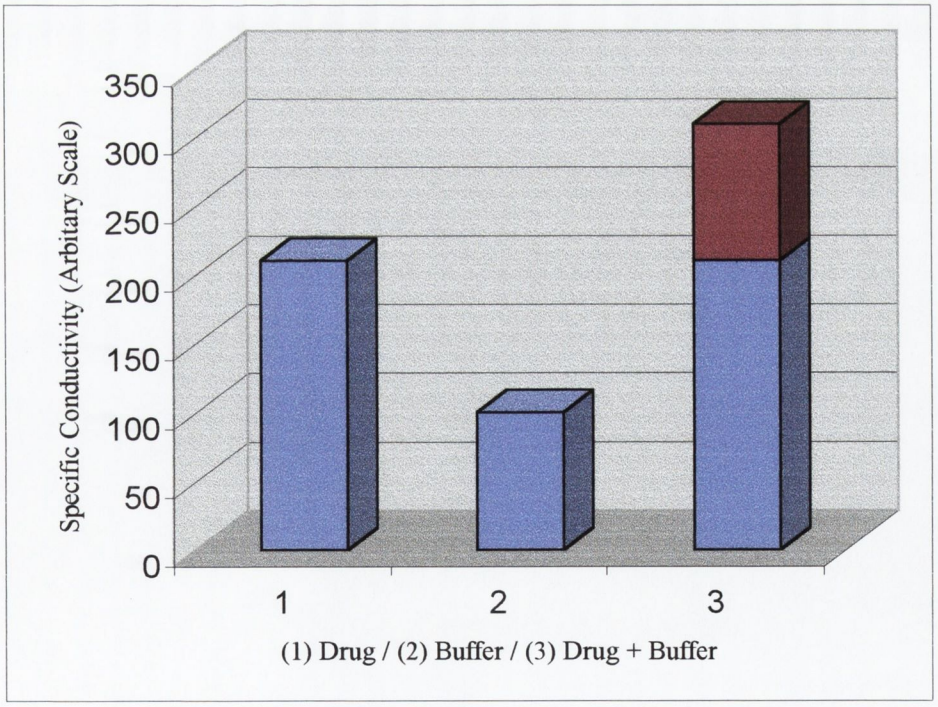


Figure 1:4:3 *Effect of phosphate buffer on the specific conductivity of a drug, (data from Gangarosa et al., 1978)*

Extraneous Ions

The presence of buffer species or salt ions has a negative effect on the iontophoretic transdermal delivery of drug molcules with moderate to strong conductivity, since these extraneous ions compete with charged drug molecules as carriers of the electric current. The presence of counterions in the drug solution does not have any adverse effect on the iontophoretic delivery of charged drug molecules, as these ions carry the opposite charge. However, the delivery of drug molecules will be affected by ions with the same charge in the drug medium as well as by ions with opposite charge when they are transported into the SC. It is desirable to avoid the inclusion of small, mobile ions of the same charges as the drug molecules so as to minimize their effect as competitive current carriers. It is preferable that at least more than one half of the conductivity be attributed to the charged drug molecule, Gangarosa *et al.*,(1978). Thus the use of buffers should be avoided, if possible, or the buffer should be diluted to a concentration with minimum ionic strength, so that the buffering capacity is just sufficient for the particular experiment or vehicle.

Several small ions are typically found in the SC such as Na^+ and Cl^- which are very mobile. These ions move and carry charge from the skin into the drug reservoir, carrying charge and accounting for a portion of the iontophoretic current. This can result in a less than expected efficiency of iontophoretic drug delivery.

pH Range of Ionic Drugs and of the Stratum Corneum

Another important property relates to the pH range in which the drug exists as an ion. True salts dissociate freely and are charged at virtually all values of pH, whereas the salts of weak acids and bases are characterized by their pK_a value. This value indicates the pH at which half of the molecules in solution are dissociated and hence exist in the ionic form. For salts of weak bases, most of the compound is neutral at pH above this critical pH while at pH below this critical pH most of the compound is ionic. The reverse is true for weak acids. Peptides and proteins and other amphoteric molecules are characterized by an isoelectric point (pI). This is at a pH value above which the molecule is anionic, and below which the molecule is cationic.

Nightingale *et al.*, (1990), suggested that the SC can be viewed as an amphoteric ion exchange membrane, the characteristics of which will vary as the pH

changes. At the isoelectric point of the membrane, the sum of the fixed charges is zero and the membrane is ionically non-selective. As the pH increases, fixed negative charges prevail. These restrict the passage of anions and the membrane becomes cation selective. The opposite occurs as the pH is reduced below the pI of the amphoteric molecule. The pI of skin is thought to lie between 3 and 4. Therefore, as the pH increases from 4 to 8 there should be an increase in the selectivity for cations, while the flux of an anionic compound will decrease. Nightingale *et al.*, (1990) also concluded that the pH of the donor medium in iontophoresis is very important. High fluxes are best achieved at low pH values for anionic compounds wheeas the converse is true for cations.

Merino *et al.*, (1999), looked at the effect of pH on the iontophoretic flux of an anionic drug 5-fluorouracil (5-FU). The electrotransport of 5-FU, anode-to-cathode ("anodal") and cathode-to-anode ("cathodal") was determined as a function of the pH of the electrolyte bathing the skin. At pH 8.5, the drug (pK_a~8) is negatively charged and "cathodal", viz. electrorepulsive transport is much greater than that in the opposite direction. At pH 7.4, where ~25% of 5-FU is charged, electrorepulsive and electroosmotic ("anodal") fluxes are balanced. Decreasing the pH to 6, and then 5, reduces the percentage of ionised 5-FU such that "anodal" electroosmosis dominates across the negatively-charged membrane. But at pH 4, "anodal" and "cathodal" fluxes are again equal suggesting neutralisation of the skin (i.e., pI~4). This was confirmed by the researchers at pH 3, where "cathodal" electroosmosis dominated across the now net-positively charged barrier.

The pH range of the skin is highly variable; values range from 4 to 6 on the surface, drop to a minimum value ranging from about 4.0 to 4.5 at a variable depth below the surface of the skin, (Siddiqi *et al.*, 1987), and finally rise to about pH 7.3 in the well hydrated portions of the epidermis. If the candidate molecule becomes uncharged at any of the pH values encountered on its journey into the skin, the major effect of the electric field can be lost. Further, as is the case for certain peptides and proteins which have an isoelectric point at a pH encountered in the skin, further progress into the skin due to diffusion or electroosmosis can result in a charge reversal of the molecule. In this case the force from the electric field is now directs the ion out of and not into the skin. Thus, not only must the molecule be capable of existing in ionic form, it must exist in ionic form over a range of pH of about 4.0 to 7.3, (Sage,

1993). In reality, this means that the pI should be outside the range of about pH 3 to 8.3 to provide most molecules in ionic form.As can be deduced from this short discussion the optimum effect of pH, pK_a and pI in the system can be a difficult and important balance to achieve.

The Electrodes

Recent reviews have explained the importance of electrode choice. An ideal electrode should possess good conductivity, stability and biocompatibility. It should also be flexible in shape and form, so that it can be adapted to the contours of the skin surface and its movement. Also, the size of electrodes and the distance between them plays an important role in skin conductivity. Electrodes with a larger surface area can facilitate the utilisation of lower current densities though the skin, so that iontophoretic assistance can be provided under conditions that can be better tolerated.

A concern in iontophoretic drug delivery is to prevent any pH shifts in the drug solution during delivery. This is especially true for peptide/protein drugs as they carry a positive or negative charge based on the pH of the solution. The pH changes which accompany use of metallic electrodes through the electrolysis of water to provide H^+ ions, may be avoided by employing electrodes such as silver-silver chloride (Ag/AgCl) electrodes (Tapper, 1973).

Another consideration in electrode design which is critically important, is the prevention of any possibility of skin burns. Tapper (1973) designed an electrode with this consideration in mind. The design was based on the view that burns are caused by an uneven distribution of current density in the tissues beneath the electrode.

An example of the interest in the issue of biocompatibility is evident in a letter by Linblad *et al.*, (1987), in response to a review by Tyle, (1986) entitled *"Iontophoretic Devices for Drug Delivery"*. Tyle references Rham *et al.*, (1962), who claimed that tin or steel electrodes are known to be the best, due to the material being harmless to the body. Linblad, who had used brass and steel as anode material in research for the purpose of delivering norepinephrine iontophoretically, disagreed. Using atomic absorption spectroscopy, Linblad found that fluid surrounding the anode contained small amounts of copper, nickel and chromium. This was found with current densities as low as 400 $\mu A/cm^2$ during 2-4 min. As chromium and nickel are

well known allergens to the skin, Linblad suggested that platinum electrodes be used instead and that the use of even medical grade steel should be further investigated.

A new electrode technology reported recently is called a "meander electrode" made by Genetronics (San Diego, CA), (Banga *et al.*, 1999), which consists of an array of interweaving electrode fingers (anode and cathode) coated on a plastic film backing, allowing easy placement of drug medium onto the skin and under the this electrode. This type of electrode has also been used in electroincorporation studies, (Zhang and Hoffman, 1997).

The Vehicle

The composition of the vehicle in drug delivery systems can have a significant influence on percutaneous absorption of drug species. It may affect not only the rate of drug release, but also influence the permeability of the SC by means of hydration, association with skin lipids, or other sorption promoting effects (Ritschel, 1969). This can be illustrated by the example of salicylic acid and its methyl ester, (Reiss, 1966). Methyl salicylate is more lipophilic than its parent acid and, when applied to the skin from fatty vehicles, the methyl salicylate yields a higher percutaneous absorption than does salicylic acid. Also, plasma levels of methyl salicylate obtained from these vehicles are higher than those obtained from propylene glycol vehicles. These differences can largely be attributed to the promoting effects of components in the vehicle composition: these will be dealt with in detail in a subsequent section.

For iontophoretic purposes additional factors must be taken into consideration. The vehicle should not reduce the conductivity of the drug and afford it the highest transport number possible. Nolan (1995) found it impossible to establish a current across a monoglyceride gel unless there was an aqueous component. The vehicles most commonly used for iontophoretic drug release studies are aqueous solutions and hydrogels, (Burnette and Marrero, 1986; Bannon, 1989) but Carr (1992) and Nolan (1995) have shown that the liquid crystalline vehicle used in this study to be suitable for iontophoretically assisted transdermal drug delivery.

Duration and Current Intensity

The quantity of drug electrically transported across the skin during iontophoresis can be predicted using Faraday's law. Here the current density is a

governing factor, i.e., the greater the current passed the greater the quantity of drug which will be transported across the membrane. Both the magnitudes of ionic flux and convective solvent flow increase during iontophoresis. The fluxes of ions, both anions and cations, increase proportionately with current, (Burnette and Marrero, 1986). These conditions allow for a significant degree of control of the flux of drugs both ionised and neutral across the skin.

The current density may also affect the stability of the drug. A high current density may lead to degradation, (D'Emmanuele and Stainforth, 1992). High current density also causes problems for patient tolerance levels. The maximum current density tolerable to humans *in-vivo* is $0.5mA/cm^2$. Densities higher than this tend to cause irritation or burns especially at the anode, (Ledger, 1992).

Drug Concentration

In general, the effect of the concentration of the drug in the vehicle can be predicted by the Nernst–Planck equation, (Equation 1:12). A linear relationship is predicted between the magnitude of the steady-state iontophoretic flux and the concentration of drug present in the vehicle, (Del Terzo *et al.*, 1989). O'Malley *et al.*, (1955), observed increased uptake of radioactive phosphorous by various tissues with an increase in the concentration of ^{32}P after iontophoresis.

In summary, many questions, which have been largely unanswered in the quest for rapid market success in the last decade, still remain to be answered about iontophoresis as applied to electrically assisted drug delivery. Firstly, the profile, duration and intensity of current and interactions with local physiology need to be systematically examined in order to overcome limitations in terms of patient discomfort. Secondly, the formulations to be used in the vehicle must be optimised for drug delivery and stability, electrochemistry and skin compatibility. The balance between the need to reduce competing ions and the requirement for ionic species for the stability of the drug species has to be found, (Merino, 1997). Thirdly, is the mechanism of drug transport primarily electrorepulsive or electroosmotic? How does the mechanism impact upon formulation design and composition? Finally, drug selection and molecular design cannot be overlooked, e.g., modifications made to

peptides to improve pharmacokinetics after injection are not necessarily the same as would be chosen to optimise the molecule for iontophoretic delivery.

Despite these challenges, it is safe to predict that iontophoretic systems will be part of the pharmaceutical tool box of the future in which the delivery of peptides, hormones and more conventional drugs will be available. The prognosis for noninvasive glucose monitoring by reverse iontophoresis is also positive, implying that iontophoresis may play a role in the ultimate development of a smart closed-loop system, the "holy grail" of drug delivery, (Guy, 1995), controlled by a biofeedback mechanism involving the continuous detection of a physiological response, (Merino *et al.*, 1997).

1:4:2 Sonophoresis

Sonophoresis is defined as "the migration of drugs through living tissue, intact skin, under the influence of ultrasonic perturbation". The terms ultrasound and phonophoresis are commonly used do describe the same phenomenon. As early as the 1950's the use of ultrasound was demonstrated in physical medicine to treat localized skin conditions and delivery of drugs into inflamed joints, (Fellinger and Schmidt, 1954).

Ultrasound is usually taken to refer to sound waves of a frequency beyond 20 kHz. The intensity of ultrasound is defined as the rate of energy flux per unit area, watts or milliwatts per square centimeter. The intensity of ultrasound for sonophoresis ranges from 0 to 3 W/cm^2, (Kost and Langer, 1988). Acoustic impedance represents the product of the density of the tissue and the speed of sound in the tissue. This is given as:

$$Z = p.c$$

where Z is the acoustic impedance, p is the density of the tissue, and c is the speed of sound. The speed of sound is expressed by the relationship

$$c = \lambda.f$$ where λ is wavelength and f is frequency.

Because of the total reflection of ultrasound by air, a coupling medium or contact agent is needed to transfer energy between the source of the ultrasound and the skin. Compounds and mixtures such as mineral oil and/or glycerin are typically used.

Successful sonophoretic administration has been reported for a range of drugs including lidocaine, (Novak, 1964), Carbocaine, (Cameroy, 1966), phenylbutazone, benzidamine and other nonsteroidal anti-inflammatory drugs, (Brondolo, 1960, Famaey, 1975, Chatterjee, 1977). Also, antibiotics such as tetracycline, biomycin and penicilin, (Parikov, 1966, Dynnik, 1977, Indkevich, 1971).

The frequency of the ultrasound used has a significant effect on its ability to enhance the drug delivery. An investigation by Bommannan *et al.*, (1992), found that the amount of salicyclic acid absorbed through SC *in vivo* was not significantly increased at 2 MHz for 20 minutes compared to passive absorbtion. However, frequencies of 10 and 16 MHz for 5 minutes increased transdermal delivery by 4 and 2.5 fold, respectively. These results demonstrated that higher frequency ultrasound (10-16 MHz) associated with a relatively low intensity ($0.2W/cm^2$) for 5 minutes was more effective in enhancing the transdermal delivery of salicyclic acid. This supports the Bommannan's theory that higher frequency ultrasound should increase energy deposition within the SC and render the membrane more permeable. As the attenuation of ultrasound is directly related to the frequency, (Bartrum and Crow, 1977), the depth of penetration of ultrasonic energy into the skin is inversely proportional to the frequency. Therefore, the combination of high frequency and low intensity can concentrate the effect of ultrasound on the superficial layers of the skin.

The intensity of the ultrasound is also a significant factor. Mayazaki *et al.*, (1991) demonstrated that a 1-MHz ultrasound for 10 minutes at intensity levels of 0.25, 0.5 and 0.75 W/cm^2 dramatically increased the transdermal indomethacin plasma concentration in rats within 1 hour. The 0.75 W/cm^2 intensity appeared to be the most effective intensity in improving the transdermal absorption of the drug. Simultaneously the skin temperature increased from 30°C to 40°C. Meidan *et al.*, (1998), indicated that the rise in temperature is due to ultrasound standing waves which focus heat generation within the tissue. Mitragotri *et al.*, (1995) has previously shown that the mechanism of sonophoresis under therapeutic conditions is based on ultrasound induced cavitation in the SC. Specifically, cavitation disorders the SC lipid bilayers, thereby enhancing the diffusion of drugs into the SC.

The sonophoretic enhancement of transdermal drug transport has been found to vary significantly from drug to drug. Following a review of the available literature data Mitragotri *et al.*, (1997) fitted the observed variation from drug to drug to an equation. This quantitatively predicts the sonophoretic enhancement of transdermal drug transport, based on the knowledge of two physicochemical properties of a drug: its passive skin permeability and its octanol-water partition coefficient. The equation has the form:

$$e \sim \frac{K_{o/w}^{0.75}}{(4x10^4)P^P}$$

Equation 1:16

Where e = relative sonophoretic transdermal transport enhancement = ([sonophoretic permeability/passive permeability]-1), $K_{o/w}$ = drug octanol-water partition coefficient and P^p is the passive skin permeability of the drug in units of cm/h.

Sonophoresis is a relatively small field of research in comparison to that of iontophoresis, and its full potential has yet to be discovered. The main problem which hinders progress is the lack of consensus on a standardized protocol to study ultrasound efficacy. Questions about the mechanism of action, the influence and optimization of the formulation/coupling medium, the physicochemical properties of the molecule associated with the practical sonophoretic conditions (frquency, power, duration), and the skin model still need to be addressed in order to design an experimental protocol for studying the effect of ultrasound. As with the early days of many fields of research a suitable protocol may emerge, in the not too distant future, in a field that appears to offer an effective technique to enhance drug permeation.

1:4:3 Chemical Enhancement

Substances incorporated into a drug delivery vehicle that help promote drug delivery through the SC and epidermis are referred to as accelerants, penetration enhancers, adjuvants or sorption promoters. Chemical enhancers are believed to operate mainly in the intercellular spaces of the SC, the major diffusion route for lipopholic moieties, although intercellular environments and intracellular protein

environments both form potential routes for drug penetration. The mechanisms by which chemical penetration enhancers function is still not fully understood. They are thought to have multiple effects once absorbed into the SC. Effects to be documented here include an alteration of the solvent potential of the SC biochemical environment and a disordering of the intercellular lipid matrix following the insertion of the enhancer into the bilayer structure. In the former case the stabilized presence of the chemical moiety within the bilayers (consisting mainly of ceramides, sterols, triglycerides, free fatty acids and phospho- and glycosphingolipids) will alter subtly the overall solvent potential of the entire matrix, (Smith and Maibach, 1995). If the matrix exhibits greater affinity for the topically administered drug then the latter has greater potential to partition from the delivery vehicle and dissolve in the SC. Theoretically, a penetration enhancer which has a similar solubility to that of the drug and has a high affinity for the skin would tend to facilitate the dissolution of the co-administered drug. Once in the outermost layer of the SC, the enhanced solubility of the diffusant in the chemically modified strata may also assist with the translocation process through the intercellular environments.

Recent research suggests that the insertion of the enhancer molecule between the parallel carbon chains of the fatty acids enhances the fluidity of the environment, (Bouwstra *et al.*, 1992). A fundamental parameter is suggested to be viscosity; the ordered bilayer structure having a relatively high, gel-like viscosity which is reduced by the incorporation of elongated, alkyl chain enhancers between the lipid molecules, (Francueour *et al.*, 1990). Research has also suggested that in some cases an association of the enhancer molecules may form separate domains within the bilayer structure, which additionally contributes to increasing the fluidity of the matrix, (Ongpipattanakul, *et al.*, 1991). Bommannan *et al.*, (1992) investigated the possibility of both mechanisms operating simultaneously to give an overall additive effect on the rate of drug delivery. The research suggested that if an enhancer or a combination of enhancers, affects both the solubility of the diffusant in the SC and reduces the rigidity of the lipid matrix, then the overall increase in the flux rate should approximate to the product of the increases afforded by either enhancement method alone.

The following section will outline the ideal attribute of a penetration enhancer.

Classification of Chemical Penetration Enhancers

Without a comprehensive understanding of the mechanisms by which chemical penetration enhancers operate within the SC it is difficult to make a simple classification scheme within which to place different compounds. However, several of the more important characteristics of an ideal enhancer can be listed as below (Finnin and Morgan, 1999):

- It should be both pharmacologically and chemically inert and chemically stable.
- A high potency with specific activity and reversible effects on skin properties.
- Show compatibility with formulation and system components.
- Be non-irritant, non-sensitizing, non-allergenic and non-phototoxic.
- Be odourless, tasteless, colourless, and cosmetically acceptable.
- Following removal of the enhancer, the SC should immediately and fully recover its normal barrier property.

Attempts have been made to classify enhancers more rigorously, e.g., Lambert *et al.*, (1993) graded most penetration enhancers into three classes: those that act primarily as solvents and hydrogen bond acceptors (e.g., dimethylsulfoxide (DMSO), dimethylacetamide (DMA) and dimethylformamide (DMF)), simple fatty acids and alcohols, and weak surfactants containing a moderately sized polar head group e.g., Azone, (1- dodecylazocycloheptan-2-one)).

A conceptual diagram was developed by Hori *et al.*, (1990), (see Figure 1:4:4), in which penetration enhancers are assigned organic or inorganic values as proposed by Fujita, (1954). Fujita determined values for compounds depending on their structural components. These values were based on the boiling points. He assumed that the organic properties depend on carbon atoms and inorganic character depends on substituted groups. The diagram was originally developed to predict the properties of organic compounds and has been applied in diverse research activities such as predicting the bio-accumulation of organic compounds in fish, (Kouda, 1984). Figure 1:4:4 depicts the location of penetration enhancers on a diagram in which the organic value is plotted against the inorganic value. As can be seen, enhancers appear to be located in two distinct areas. These different areas suggest that the enhancers

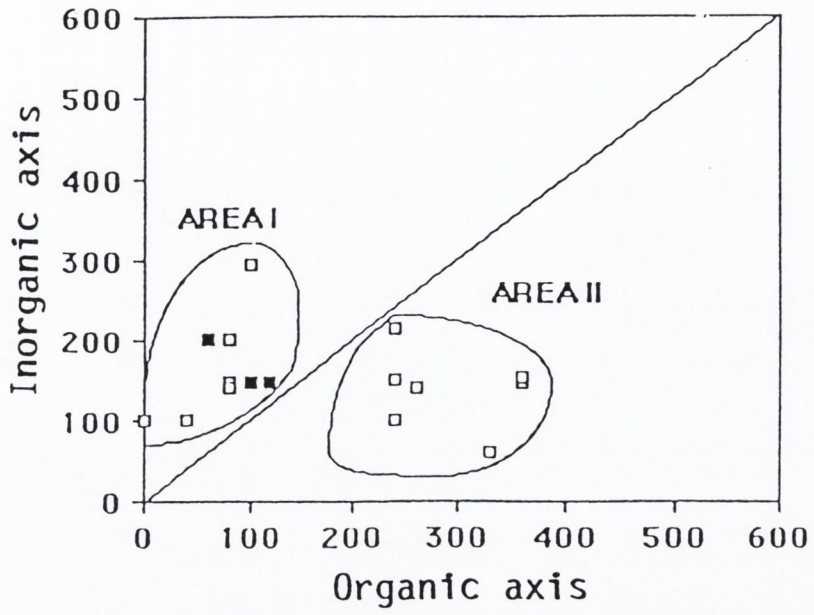


Figure 1:4:4 *Location of percutaneous penetration enhancers on an organic diagram. Closed squares indicate two enhancers. Area I includes ethanol, propylene glycol, N-methyl pyrrolidone and dimethyl sulfoxide. Area II includes oleic acid, lauryl alcohol and Azone, (Hori et al., 1990).*

in the two groups may have different physicochemical properties. This diagram will be discussed further in a subsequent section dealing with co-enhancement.

Pfister *et al.*, (1990), classified chemical penetration enhancers using the Hildebrand solubility parameter (Vaughan, 1985), to describe the compounds as either polar or non-polar. This parameter measures the cohesive forces and sum of all intermolecular attractive forces related to the extent of mutual solubility of many chemical species. Relative hydrophobicity increases with the value of the Hidebrand solubility parameter. Table 1:4:1 shows that enhancers span a range of polarity: hexamethyldisiloxone represents the most nonpolar enhancer of the series and water, the universal solvent and enhancer, is the most polar.

This implies that enhancers with solubility parameters $< 24.5\ J^{1/2}\ cm^{3/2}$ will intersperse with the lipid component of the skin, while those with smaller solubility parameters will selectively partition into the polar components in the skin.

Table 1:4:1 *Solubility parameters of selected permeation enhancers, Vaughan, (1985).*

Classified Enhancer	*Solubility Parameter* $J^{1/2}\ cm^{-3/2}$
Nonpolar	
Hexamethyldisiloxone	13.84
Dimethacone	12.07
Cylclomehticone	12.21
Squalene	12.62
Mineral oil	14.46
Isopropyl palmitate	15.87
Linolenic acid	16.09
Oleic acid	16.13
Isoprpyl myristate	16.04
Macrocycli ketones / acetones	16.91
Lauric acid	17.25
Capric acid	18.11
Oleyl alcohol	18.23
Lauryl alcohol	19.40†
Polar	
Ethanol	25.60
DMSO	27.33
Dimethyl glycol	27.74
Propylene glycol	27.76
Sodium lauryl sulphate	30.12
Glycerol	33.17
Water	47.73

† *Closest solubility parameter to that of the skin, which is ~ 20* $J^{1/2}\ cm^{3/2}$

Using concepts and a model developed by Sloan, (1986), it is possible to calculate the solubility parameters of drugs, vehicles, and enhancers and to use these

parameters to predict relative permeation rates for drugs through the skin, (Sloan, 1986). For drugs that partition mainly into the hydropobic phase, it would be expected that enhanced permeation would result, through the use of an enhancer that is soluble in the nonpolar lipid phase. Such an enhancer would lead to a net increase in the solubility parameter of this lipid phase. In contrast, for drugs that partition into the lipid phase, an enhancer that would decrease the solubility parameter of the hydrophilic phase or polar pathways may increase permeation. Although a significant body of data supports enhancement based on solubility theory, this approach alone cannot be generalised to all drug and enhancer combinations, (Sloan, 1986), primarily because of the heterogenous multiphasic morphology of skin and factors such as hydrogen bonding and dipole moment interactions, (Houk and Guy, 1988). Enhancers can also be classified into a wide variety of chemical classes (Table 1:4:2).

Table 1:4:2 *Chemical classes of enhancers, (Smith and Maibach, 1995)*

Chemical Class	*Example*
Nonionic	*Polyethylene (20) sorbitan monoleate*
Anionic	*Sodium lauryl sulphate*
Cationic	*N, N-bis (2-hydroxyethyl) oleylamine*
Zwitterionic	*Dodecyldimethylammoniopropane sulphate*
Sulfoxide	*Dimethyl sulfoxide, dodecylmethyl sulfoxide*
Ethoxylated	*Dodecanol hexanethoxylated*
Alcohol	*Ethanol*
Fatty acid	*Oleic acid*
Polyols	*Propylene glycol, polyethylene glycol*
Amides	*N, N-dimethyl-m-toluamide (DEET)*
Ureas	*Urea*
Terpenes	*Eucalyptol menthol*
Chelators	*Disodium (EDTA)*
Bile salts	*Glyconolate*
Macrocyclics	*Macrocylic ketones / acetones*
Lactams	*Laurocapram*

A selection of the enhancers in Table 1:4:2 will be discussed here with respect to their development, modes of action and chemical classifications. Firstly, a general understanding of the conformation of the skin lipid bilayers is necessary to interpret fully the different modes of action of the various enhancers.

The lipid fraction of the SC is arranged from several constituents. For whole SC the major fractions are neutral lipids (78%) and sphingolipids (18%) together with a small amount of polar lipid. There is also a considerable amount, totaling ~11%, of non-polar material such as squalene and n-alkanes present. Both saturated and

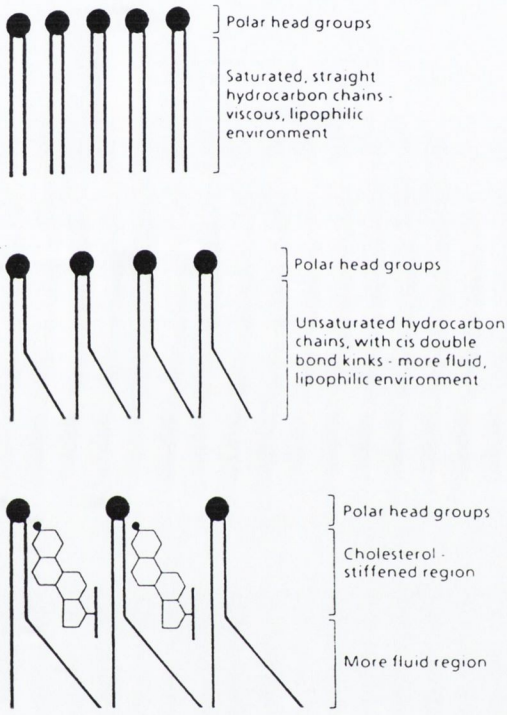


Figure 1:4:5 *The effect of composition on fluidity of the lipid bilayer: (top) saturated fatty acid chains packed tightly together; (middle) kinks arising from cis-double bonds dilate the structure; (bottom) saucer-like steroid molecules of cholesterol interact with and partly immobilise the hydrocarbon chain region closest to the polar head groups, leaving the remainder of the chain flexible, (Barry, 1987).*

unsaturated fatty chains exist in all neutral lipid species, with unsaturated chains predominating except for the free fatty acid fraction. The ceramide (sphingolipid) fraction is comprised of mainly of saturated fatty acid chains. The n-alkanes range in a normal distribution from C_{19} to C_{34}, (Lampe *et al.*, 1983). A combination of the three fractions shown in Figure 1:4:5 forms a lipid bilayer as shown in Figure 1:4:6.

The exact role and location of the non-polar material (i.e. sterol esters, n-alkanes) is at present unclear. Also, no information is available as to whether or not the lipid structure exists in distinct solid and fluid domains or is totally homogenous.

The following penetration enhancers are representative of the various chemical classes, of different modes of action and of the work to be reported in this thesis.

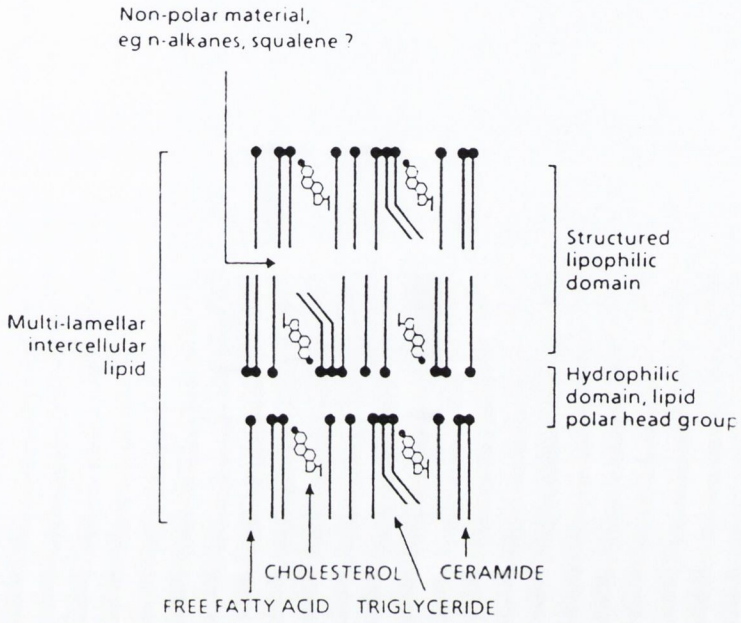


Figure 1:4:6 *Suggested model representing the strucure of lipid bilayers within the intercellular spaces of human stratum corneum, (Barry, 1987).*

Fatty Acids

Fatty acids and fatty acid salts have been known to increase skin permeability since at least 1961, when Bettley (1961) reported increased permeability in epidermis exposed to potassium oleate. Structurally fatty acids consist of an aliphatic hydrocarbon chain and a terminal carboxyl group: oleic acid is an example (Figure 1:4:6).

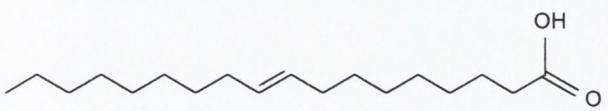


Figure 1:4:6 *Oleic acid (cis-octyldec-9-enoic acid)*

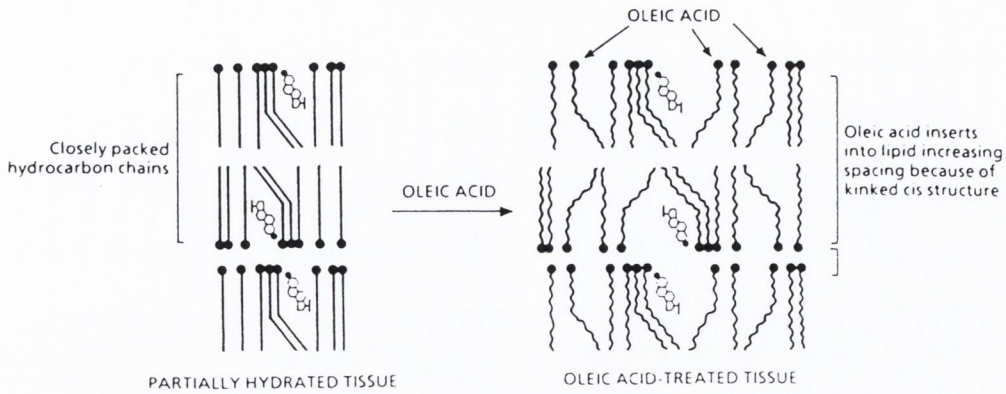


Figure 1:4:7 *Interaction of oleic acid with the intercellular lipids of the SC increasing fluidity, (Barry, 1987)*

The extent of permeation enhancement is greatly dependant upon the vehicle used and the structure of the fatty acid, (Aungst, 1995). Differential scanning calorimerty (DSC) suggests that oleic acid (OA) interacts only with the SC lipids. OA possesses a *cis* double bond halfway along the C_{18} chain, i.e., it is kinked. OA is thought to operate by penetrating into the lipid structure, with its polar end close to the lipid polar heads. Because of its bent structure, it then disrupts and increases fluidity of the lipid region, as illustrated in Figure 1:4:7. Drug mobility in this less tightly packed arrangement will then increase.

The structure of fatty acids can have an influence on skin permeation. Fatty acids can differ in their chain length, in the number, position, and configuration of double bonds, and in having branching or other substituents.

The skin permeation-enhancing effects of saturated fatty acids are consistently greatest for C_{10} and C_{12} fatty acids. Figure 1:4:8 represents a compilation of data from independent studies comparing the effects of various saturated fatty acids on skin permeation. These results cover three compounds using different vehicles, but in each case the maximum enhancement was observed with lauric acid. Another example is the study of the effects of saturated fatty acids on molsidomine percutaneous absortion *in vivo* in rats, where lauric acid (12:0) was more effective than capric acid (10:0) and myristic acid (14:0) and 6:0, 8:0, and 16:0 were relatively ineffective, (Yamada and Uda, 1987). Kandimalla *et al.*, (1999), found a parabolic relationship between the carbon chain length of saturated fatty acids and the enhancement of melatonin permeation across rat and porcine skin.

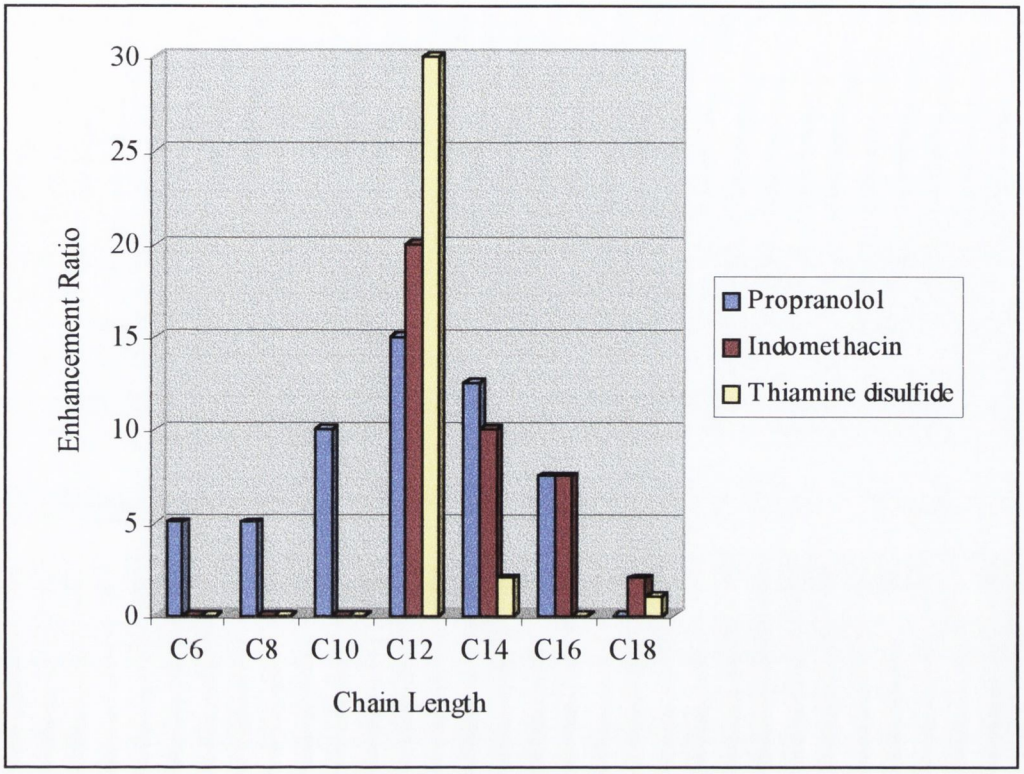


Figure 1:4:8 *The influence of chain length of saturated fatty acids on their effectiveness as skin penetration enhancers for various compounds, (Aungst et al, (1986), Ogiso et al, (1990), Takeuchi et al, (1992)).*

Another relevant parameter of a fatty acid is whether it is saturated or unsaturated. OA (18:1) has been shown in numerous studies to be an effective skin permeation enhancer, whereas stearic acid (18:0) has no enhancing effects on skin permeation. Other long-chain unsaturated fatty acids are also effective enhancers, including myristoleic (14:1), palmitoleic (16:1), vaccenic (18:1), petroselenic (18:1), eicosenoic (20:1), linoleic (18:3) and linoelaidic (18:2), (Cooper, 1984). Kandimalla *et al.*, (1999), also found that an increase in the number of double bonds in cis-9-octadecanoic acid increased the flux of melatoinin across rat skin. Golden *et al.*,(1987), found that whereas *cis* isomers of OA were effective, *trans* isomers had no effect. Although unsaturated long-chain fatty acids ($>C_{18}$) are more effective at increasing skin permeation than the analogous saturated fatty acids, few studies have compared saturated and unsaturated short- and medium-chain fatty acids.

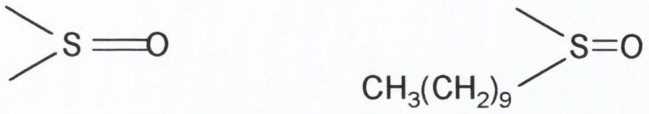
Sulfoxides

Dimethylsulfoxide (DMSO) is probably the earliest used and most extensively studied penetration enhancer. It has a broad spectrum of activity, including the enhancement of penetration through both plant and animal membranes, (Shen, 1976). The reported concentration dependant behavior of DMSO suggests that this permeation enhancer may function either by reducing the resistance of the skin to the drug molecule or by the promotion of drug partitioning from the dosage form, (Barry, 1987). Solutions of DMSO in concentrations exceeding 60% are particularly useful for increasing skin penetration of a wide range of ionic and nonionic compounds of molecular weight < 3000, (Woodford, 1987).

Attempts to elucidate the manner in which DMSO promotes skin permation have resulted in a number of mechanisms of action being postulated, including elution of lipid, lipoprotein, and nucleoprotein structures of the SC, (Embery, 1971, Allenby, 1969), denaturation of the structural protein of the SC, (Montes *et al.*, 1967) and delamination, (Chandrasekharan, 1977). Most current theories on DMSO consider that it displaces bound protein water, thereby substituting a looser structure, (Barry, 1983). The accelerant probably partitions preferentially into the polar cell contents of the tissue until it attains a high concentration after which it also partitions significantly into the intercellular domain. Because of the polar nature of DMSO it is thought not to partition directly into the lipid chains but rather to interact with the lipid polar head groups via hydrogen bonding. DMSO is a powerful aprotic solvent which mixes exothermically with water. The enhancer may displace water from the lipid head groups and create a large solvation shell around these groups. This larger shell can then loosen the lipid packing thereby reducing the barrier function of the skin towards drug molecules.

Another important derivative in the alkylsulfoxide series is decylmethylsulfoxide (DCMS). Unlike DMSO, DCMS is effective at concentrations as low as 0.1% and is reported to enhance polar drug permeation more effectively than that of nonpolar drugs, (Sekura, 1988).

Sekura also reported that in the alkylsulfoxide series, C_{10}MSO was a more effective enhancer of nicotinic acid skin permeation than DMSO, C_6MSO, C_{12}MSO, or C_{12}MSO. More recently, Kim *et al.*, (1999), investigated dimethyliminosulfuranes



DMSO DCMS

Figure 1:4:9 *Structures of DMSO and DCMS permeation enhancers.*

synthesized from activated DMSO by trifluoroacetic anhydride. Of sixteen derivatives synthesized, three showed significant enhancement of hydrocotisone across hairless mouse skin.

Azone

If DMSO is considered the most extensively studied accelerant of the 1970's, Azone® was the most extensively studied of the 1980's, (Woodford, 1987). Azone (Figure 1:4:12), is one of a series of *N*-alkylated cyclic amides and was patented in 1976 as a penetration enhancer by Nelson Research laboratories, California.

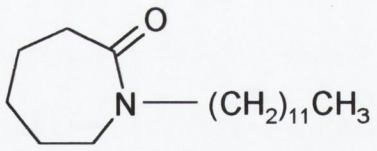


Figure 1:4:10 *Chemical structure of Azone®*

Azone is a chemical combination of pyrrolidone and DCMS. The major advantage of the compound is that it can be incorporated into a variety of topical preparations at low concentrations (in most cases between 1 and 5%) with significant accelerant effects. Pharmacokinetic studies indicate that pure Azone is poorly absorbed into the human body, and although it rapidly penetrates into the upper levels

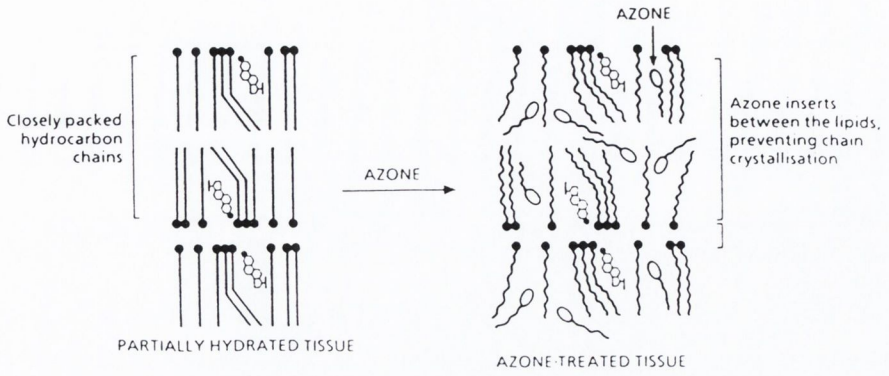


Figure 1:4:11 Proposed interaction of Azone molecules, disrupting the lipid structure of the intercellular region of the SC, (Barry, 1987).

of the skin, it does not accumulate there. The small quantity that is absorbed is readily metabolized and excreted in the urine, (Wiechers, 1987, 1990). Azone has been reported to enhance the permeability of a wide range of drugs both polar and non-polar, e.g., 5-fluorouracil, (Stoughton, 1982), antibiotics, and glucocorticoids and peptides, (Boddé, 1980). Barry and Bennett, (1987) suggested that Azone is likely to be more useful for enhancing the permeability of hydrophilic compounds than for increasing the permeability of lipophilic drugs. However Azone has enhanced skin penetration and retention of a steroid to a significant degree ($p<0.05$), (Michniak, 1993). Barry, (1987) examined in detail the effects of Azone on the SC, in terms of DSC thermograms and permeability studies. In most cases Azone dramatically affected the lipid structure. It was suggested that Azone increases skin permeability by a different mechanism to that proposed for molecules such as DMSO. The DSC data and Azone's non-polar nature suggested that it partitions directly into the lipid bilayer structure, disrupting it as illustrated in Figure1:4:11. Azone also showed no indication of protein interaction, further suggesting
that it does not enter cells in significant amounts.

There is conflicting views whether Azone acts as an irritant of the SC. Chatteraj and Walker, (1995) state that Azone is a colourless and odourless compound that causes minimal irritation to the skin, whereas Finnin, (1999) states that because of its potential to irritate the skin, it has failed to gain general clinical acceptance.

Derivatives of Azone have also been extensively investigated. In brief, it has been reported that the optimal chain length of a saturated tail is C_{12}, (Okomoto *et al.*, 1988). The size of the azacyclo ring has less effect on the enhancing activity than has the chain length. Chains with unsaturated *trans* double bonds were found to have the same enhancing effect as had saturated tails, although Reller, (1982) reported that compounds with double bonds caused more irritation than those with saturated alkyl chains.

Alcohols

The most commonly reported alcohol promoters are ethanol and isopropanol, (Figure 1:4:12). Ethanol is currently found in commercially available TDD systems for estradiol, (Good *et al.*, 1985) and fentanyl, (Gale *et al.*, 1986). The interaction of ethanol with skin in terms of its permeation, primary irritation, and cutaneous metabolism are probably better characterized than any other enhancer, (Berner and Liu, 1995).

Ethanol Isopropanol

Figure 1:4:12 *Chemical structures of ethanol and isopropanol*

These workers proposed that the primary action of ethanol is to dissolve in the fluid lipidic regions of the SC. The three key features of this effect of ethanol on lipophilic drugs are: (a) the flux of the drug across the SC is linear with the flux of ethanol; (b) the enhancement is reversible; and (c) the diffusion constant of drug in the SC is unchanged, and therefore, the enhancement is independent of the of the molecular weight of the drug. For hydrophilic permeants, the major effect of ethanol is the extraction from the SC of lipophilic and peptide solutes to increase the porosity of the SC. Competing with this effect, the solubility of ionized drugs may decrease with increasing alcoholic content of the donor solution, and consequently, the concentration within the pore (the driving force for permeation across the pore) may

decrease. The three key features of these effects of ethanol on hydrophilic drugs are: (a) the flux of drug across the SC is not linear with the flux of ethanol, (b) the enhancement, at best, is partly reversible, and (c) for compounds that are more soluble in water, a substantial concentration of water is required for optimal enhancement.

Magnusson, (1999), reported that the flux of peptides can also be increased, up to three-fold, in the presence of 50% ethanol. Although ethanol can act as a penetration enhancer, and various mechanisms have been postulated, (Manabe *et al.*, 1996), in practice its use as penetration enhancer has relied on the application of a bulk aqueous ethanol vehicle to the skin whereupon the increase in the flux of the drug across the skin is mainly due to a solvent drag effect, (Finnin, 1999). The mechanism of this effect relies on the rapid penetration of the solvent and the subsequent dragging of the penetrant with it. A necessary corollary of this rapid flux is that the concentrations cannot be maintained unless there is a reservoir present, thus limiting the applications of this type of enhancer to reservoir based systems.

Diols

The only reported enhancer in this class is propylene glycol, (Figure 1:4:13). The literature contains conflicting reports as to whether this molecule increases skin permeability, (Barry, 1987, Bendas *et al.*, 1995)

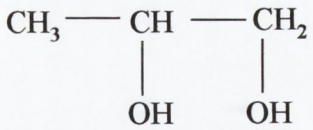


Figure 1:4:13 Chemical structure of propylene glycol

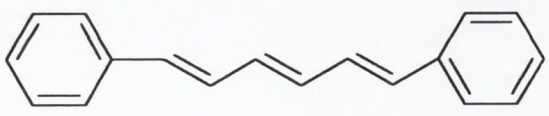
Propylene glycol (PG) is used widely as a constituent in dermatological formulations. Its importance as a cosolvent for lipophilic drugs is widely recognized. Investigations by Bendas *et al.*, concluded that PG is able to enhance the permeation of lipophilic, polar drugs. The penetration of PG into the SC contributes to a favoured partitioning of drug molecules due to changed activity gradients. The solubilizing capacity of the aqueous sites of the SC is increased. The accumulation of PG may cause the

establishment of a drug reservoir, but only drugs that are highly soluble in PG offer enhanced permeation behaviour. Therefore, like ethanol, it seems likely that the sorption promoting effect is related mainly to a solvent drag effect of PG. PG is now known to replace water from its bonding sites in the intercellular spaces. Conformational changes of the keratin structure of corneocytes have been proven using IR techniques and electron microscopy has shown that PG does not infiltrate into cells. The main action of PG is thus fixed on the bilayer structures of the intercellular lipids and it is assumed that hydration spheres of the lipid head groups are disturbed without a measurable influence on the alkyl chain position of the lipids.

Surfactants

Surfactants are classed as either anionic, cationic, or nonioinic. It is 40 years since Bettley and Donoghue, (1960), demonstrated that soaps could enhance the flux of water, glucose, and salicylate across isolated human epidermis. Bettley subsequently investigated the effect of different chain lengths in potassium soaps. Data revealed that 24.4% of dodecanote penetrated the epidermis after 7 days. Shorter chained salts penetrated the membrane in significantly smaller quantities. 48-hour testing in human volunteers revealed a strong correlation between the irritant effects of the soaps and extent to which they penetrated the skin. These studies provided the first evidence that disruption of cutaneous barrier function is directly related to the degree of chemical and physical interaction between the enhancing agent and the skin.

$C_{12}H_{25}SO_4^- Na^+$	Sodium dodecylsulfate	Anionic
$C_{16}H_{33}N(CH_3)_3^+ Br^-$	Hexadecyltrimethylammonium bromide	Cationic



1,6,-Diphenylhexatriene (DPH) Non-ionic

Figure 1:4:14 Examples of various classes of surfactants

Scheuplein and Ross, (1970), proposed that anionic surfactants alter the permeability of the skin by acting on the helical filaments of the SC, thereby resulting in the uncoiling and extension of α-keratin filaments to form β-keratin together with the overall expansion of the membrane. Loden, (1990), discovered the importance of the critical micelle concentration (CMC) when working with surfactants. While studying the effects of sodium dodecyl sulfate (SDS) it was found that permeability coefficients of SDS were almost equivalent at concentrations of 1 and 10%, yet significantly greater than those obtained at 0.1%. This result was unexpected because the highest surfactant concentrations employed in the study resided well above the CMC for SDS in an aqueous vehicle (~ 0.24%), and would presumably contain identical quantities of surfactant monomer. As it is generally accepted that micelles do not penetrate the skin on account of excessive bulkiness and, in the case of SDS, excessive charge density, the results were attributed to micelle-dependant solubilization of skin lipids. Loden concluded that the activity of SDS monomer does not remain constant above the CMC but increases in proportion to the number of micelles present. This causes selective extraction of skin lipids by SDS micelles resulting in the preferential disruption of a pathway through the SC in favour of SDS.

The comparative effects of the different classes of surfactants are significant. In general cationic surfactants cause greater irritation than anionic surfactants which, in turn, are more damaging than non-ionic surfactants. Unfortunately, the ability of surfactants to alter the permeability of the skin follows the same trend, (Ruddy, 1995). Investigations by Ashton *et al.*, (1992) confirmed this trend. Interestingly, Ashton found that non-ionic surfactants penetrate the membrane more rapidly, but interact less extensively than do their ionic counterparts.

Despite the ability of these agents to increase the permeability of the skin to a variety of different compounds, their overall usage as penetration enhancers is limited due to their tendency to cause skin irritation and other irreversible effects, (Zatz and Kushla, 1991).

In this discussion so far, a broad spectrum of different types of enhancer have been considered. The search for new enhancers is continuous. Companies such as Macrochem and Nexmed are seeking to gain regulatory approval for agents such as 2-*n*-nonyl-1,3-dioxolane which have already shown enhancing abilities comparable to

those of Azone, (Finnin, 1999). Enhancers are also being identified from compounds already generally recognised as safe (GRAS). Novel enhancers have been identified in topical sun-screening products. Some of those identified are octyl salicylate, octyl methoxy cinnimate and padimate O, (Murphy *et al.*, 1997).

Before the characteristics of an ideal enhancer can be achieved (as set out on p55) several basic parameters require further investigation such as; structure-activity relationships, possible mechanisms; toxicology evaluations; reversibility of skin alterations; whether deposition of the enhancer in the skin or body takes place and finally compatibility of enhancer with components of transdermal systems, (Pfister and Dean, 1990). These ideals have still yet to be fully described.

1.5 Combined Penetration Enhancement Techniques

1:5:1 Chemical-Chemical Synergistic Penetration Enhancement

The title of this subsection "chemical-chemical synergistic penetration enhancement" has also been referred to as co-solvent effects or vehicle effects in transdermal literature. References to synergistic interactions between vehicle and enhancer have been non-specific and at worst overlooked. More recently, researchers have become aware of possible interactions between vehicle components and to such an extent that it is difficult to reference the singularity of action of an individual enhancer, as was described in the previous section.

In practice, the bulk component of many transdermal vehicles can be classed as penetration enhancers. Although no claims in patents have been made for the use of enhancers in commercial transdermal delivery systems, many are used in estradiol or nitroglycerin-containing patches, e.g., these include co-solvents such as propylene glycol, glycerol, ethanol, silicone fluids and isopropylpalmitate. It is generally believed that these co-solvents act on the skin to enhance permeation of the drug, (Pfister and Hsieh, 1990). Of the 20 transdermal and 23 dermal delivery products on the market in 1990, 12 transdermal and 21 dermal delivery systems are formulated with co-solvents that possess skin penetration-enhancement activity.

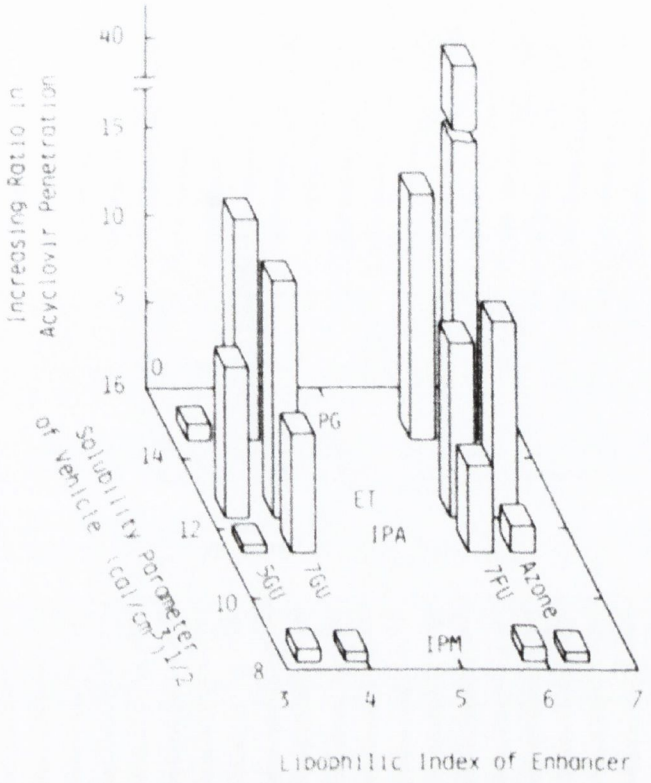


Figure 1:5:1 *Effect of Azone, and Azone derivatives 7FU, 7GU and 5GU on the percutaneous penetration of Acyclovir from various vehicles with different physicochemical properties, ET = ethanol, PG = propylene glycol, IPM = isopropyl myristate, IPA = isopropanol. (Okimoto et al., 1990).*

Okamoto *et al.*, (1990), carried out extensive research into the synergistic effects of "co-solvents" and enhancers on the transdermal delivery through nude mouse skin of acyclovir, an anti-viral drug. All combinations of the four "vehicle" types and the four penetration enhancer derivatives were investigated. They presented their results graphically as reproduced in Figure 1:5:1. No effect of the enhancers was observed from the isopropyl myristate (IPM) vehicle. The estimated solubility parameters of vehicles and enhancers indicated that the polarities of IPM and the enhancers are similar, which prevents effective penetration of the enhancers from IPM. However, the penetration of Acyclovir from the other vehicles was increased by the enhancers. The combination of hydrophilic vehicle and hydrophobic enhancer resulted in a large enhancing effect. The disappearance of enhancers from the vehicle, which was measured experimentally, correlated with their enhancing activity.

Okamoto's results correlate significantly with Hori's conceptual diagram for the classification of penetration enhancers (see Figure 1:4:4, p56). As discussed at that time the enhancers fall into two distinct areas on the diagram. The "vehicle" compounds from Okamoto's study would fall into area I of the diagram and Azone and its derivatives would fall into area II. Wotton *et al.*, (1985) also showed that a combination of PG and Azone synergistically promoted the cutaneous permeation of metronidazole: These would fall into areas I and II, respectively. Cooper (1984), showed that the combination of PG and oleic acid increased the penetration of salicyclic acid compared with delivery from each vehicle on its own. Again, PG and Oleic acid are from the different areas of the diagram. The use of a vehicle comprised of components from these different areas of the diagram apparently promotes absorption better than the use of the same vehicle singly.

Barry (1987), suggested if PG is used in combination with an accelerant which acts on the lipid barrier - such as Azone - then much glycol may enter the tissue and enhance intercellular drug diffusion more than would water. PG promotes Azone penetration of the skin and vice versa. An explanation for this behaviour is that Azone enhances intercellular drug diffusion only. The intercellular protein contents, which offer considerable diffusional resistance, remain unaffected by Azone. However, PG enhances intracellular transport, so the PG + Azone combination is more effective than is the Azone alone.

There are now very many examples in the literature of synergistic co-enhancement. Most recent examples include a significant enhancement of hydrophilic 5-fluorouracil and lipophilic tamoxifen using binary systems of (oleic acid / ethanol) and (oleic acid / PG) (Goa and Singh, 1998). It was also noted that TEWL for the binary systems was significantly less in comparison with ethanol or propylene glycol alone, therefore indicating no correlation between TEWL and mode of action of the binary system. Gabiga *et al.*, (2000) also showed the synergistic enhancing effect of binary systems of (oleic acid / PG) on isosorbide dinitrate (ISDN) through rat skin. Mura *et al.*, (2000) found that the binary system of transcutol (diethyleneglycol monoethyl ether) with PG showed a significant increase in the flux of clonazepam. Chatterjee *et al.*, (1997) reported the synergistic effect of PG /Azone on the permeation of methotrexate. Gorukanti *et al.*, (1999) reported similar effects when an IPM /short chain alcohol system increased the absorption of benztropine. Magnusson

and Runn (1999), used a combination of cinneole and ethanol to increase the permeation of the tripeptide pGlu-3-methyl-His^2-Pro amide (M-TRH) across human SC *in-vitro* in therapeutically relevant amounts. This demonstrates the possibility to deliver classes of compounds that have been viewed as not suitable for transdermal administration. The list could be greatly extended and most of the examples available conform to Hori's conceptual diagram, where the two components of the binary system come from different areas of the graph shown in Figure 1:4:4. These examples confirm Hori's diagram as being useful to predict the synergistic effects of various enhancing compounds.

1:5:2 Chemical-Physical Synergistic Penetration Enhancement

By analogy with the chemical enhancement literature, much of the current literature regarding iontophoresis contains research which shows the combined effect of chemical and physical enhancement. The realisation of this synergy evident in combining physical and chemical enhancement methodologies would seem to have lead to a resurgence of interest in transdermal drug delivery systems.

The precursor to the work in this thesis was carried out by Nolan (1995), which showed a synergistic effect when oleic acid and anodal iontophoresis was used to deliver salbutamol, a cationic drug. Other more recent chronological examples include work by Ganga *et al.*, (1996) who showed that the combination of Azone and an optimum iontophoretic current increased the delivery of metroprolol through human SC 130-fold compared to passive flux. Kalia and Guy (1997), found that the use of Azone, propylene glycol and sodium lauryl sulphate profoundly amplifies the effect of the passage of current. Oh *et al.*, (1998), used a binary system of (oleic acid / PG) plus iontophoresis to show a greater than additive effect on the delivery of AZT, the most widely used anti-AIDS drug. Fang *et al.*, (1998), used the surfactant benzalkonium chloride and iontophoresis at pH 10 to enhance the delivery of enoxacin, an anionic drug. Choi *et al.*, (1999), were also able to show the synergistic effect of chemical ad physical enhancement of a range of drugs. Finally, Kirjavainen *et al.*, (2000) reported that the combination of lipids (egg lecithin) and iontophoresis enhanced the delivery of mannitol. This list could also be extended.

In conclusion to this introductory chapter, it has become apparent that more recent investigations in the field of transdermal drug delivery are reporting continued progress. This is mainly due to a more focussed and logical approach to the problems posed by transdermal drug delivery. The field is maturing and extensive work over several decades has led to a better definition of the factors that are relevant in transdermal delivery. Future investigation may complete the jigsaw. Research such as Kommuru *et al.*, (1999) into the effect of chiral enhancers looks very promising. The SC is stated to be chiral in nature and enantiomers behave differently with respect to their transport across the skin resulting in enantioselective permeation. Their studies showed that flux of the S-form of metroprolol was 35% higher than that of a racemic mixture. The scope for further investigations in this direction are immense.

1.6 Aims and Objectives of this Research

The main aim of the work presented in this thesis was to continue in a logical progression the work previously carried out in our laboratory by Nolan and Bannon. Bannon (1989) had shown that the neutral drug molecule nicotine could be delivered passively across human SC. Nolan (1995) continued research into the passive and enhanced transport of cationic drugs, notably of Salbutamol. Results showed that a combination of chemical (oleic acid) and physical (iontophoresis) enhancement synergistically and significantly increased the delivery of Salbutamol across human SC. Initial experiments by Nolan had failed to reveal any synergism between the use of oleic acid and the iontophoretic delivery of sodium diclofenac, an anionic drug.

The scope of the current investigations is to attempt to find a chemical and physical enhancement system which would significantly increase the transdermal delivery of anionic drugs. As formative investigations by Nolan proved negative, the need for an alternative enhancer seemed obvious.

With this aim in mind, a list of objectives was drawn up which would help in working towards achieving the principal objective. The first was to characterise the release profiles of various anionic drugs from a liquid crystalline gel across limiting (SC) and non-rate limiting (Visking™) membranes and secondly to see the effect of buffering donor and receptor solutions. The next step was to introduce candidate

chemical enhancers into the system and monitor the effects. Before combining enhancement methods, the effect of iontophoresis on the system must also be separately and quantitatively determined. Following this a combination of chemical and physical enhancment can be applied to see if the desired effect of synergistic enhanced delivery is achieved across human SC.

The end goal of this and related research is to broaden the range of drugs suitable for transdermal delivery and to add to the continuously expanding body of knowledge in the field of transdermal drug delivery.

Chapter 2

Experimental and Instrumentation

Chapter 2

Experimental and Instrumentation

2:1 Experimental

This chapter contains details of the materials, preparatory methods and instrumentation used in all experiments reported in subsequent chapters. The bulk of the experiments conducted consisted of diffusion studies in which the molecule being studied diffused from a solid gel vehicle through either a synthetic membrane or through human SC. The synthetic membrane used was Visking ™ which acted as a non-rate limiting barrier and was utilized to ascertain the release characteristics of various drugs from the liquid crystalline or agar gel. The liquid crystalline gel used was formed from an oil / water mixture. The membrane was placed on top of a Franz-like diffusion cell (Franz, T.J., 1978), with the gel placed on top of the membrane. A receptor medium was then poured into the cell which was then stoppered. It was important to make sure to exclude air from under the membrane. A star shaped magnetic stirrer was used in the cell to maintain an even concentration gradient throughout the cell through gentle stirring, whilst at the same time avoiding a vortex which would affect steady state diffusion (Kashery and Chien, 1984). The cell is suspended at neck height in a thermostatic water bath at 310 K. Samples are taken at regular intervals, through the stoppered port, and placed in 1ml HPLC vials. The samples are then injected from an autosampler through a column and tunable detector to give a chromatogram from which concentrations of drug can be determined by comparison with predetermined standards.

The procedure is similar for chemical enhancement trials except the enhancer is incorporated into the gel, along with the drug. For iontophoretic experiments an electrode is placed in the receptor solution directly below the membrane and another placed on the upper surface of the gel. The current is supplied by battery or mains operated adjustable power supplies that were custom built for the work. For

investigations of synergistic methods of enhancement, a combination of the above procedures is adopted.

Any experiments in which the procedure adopted differed from the standard described will be pointed out in the subsequent result sections. In general each experiment takes one half-day preparation, one day to run and the analytical work required at least another day to fully complete.

2:2 Materials

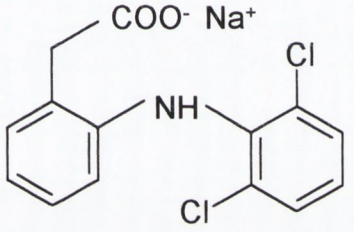
Diclofenac, 2-(2,6-dichloroanilino) phenyl acetic acid is a white crystalline powder with a melting point of approximately 156°C to 158°C and a molecular weight of 296.2 a.m.u. Its sodium salt has a molecular weight of 318.1 a.m.u. and a melting point of 286°C and its molecular structure is shown in Figure 2:2:1.

Diclofenac is classified pharmacologically as a non-steroidal anti-inflammatory drug (NSAID). Introduced in 1974 by Ciba-Geigy, it possesses pronounced anti-inflammatory, analgesic and antipyretic properties in animal models (Krupp *et al.*, 1973) and is a potent competitive and reversible inhibitor of prostaglandin synthesis (Oliw *et al.*, 1978). Sodium diclofenac is well absorbed after oral administration in humans but undergoes significant first-pass metabolism with a bioavailability of about 50-60% (Brogden *et al.*, 1980). Up to 70% of the dose is excreted in the urine in 3 days, including 20 to 40% as conjugates of the major active metabolite 4-hydroxydiclofenac and up to 15% as conjugates of unchanged diclofenac. Other metabolites identified in the urine include 5-hydroxydiclofenac (about 12% of the dose), 3-hydroxy diclofenac, and 4,5-hydroxydiclofenac. Approximately 10 to 20% of the dose is excreted in the bile as 4-hydoxydiclofenac and less than 5% remains unchanged. The mean terminal half-life of the unchanged drug has been determined to be between 1.2 and 1.8 hr (Kendall *et al.*, 1979). A dose of 75 to 100mg of sodium diclofenac daily is reported to be effective (Moffat *et al.*, 1986).

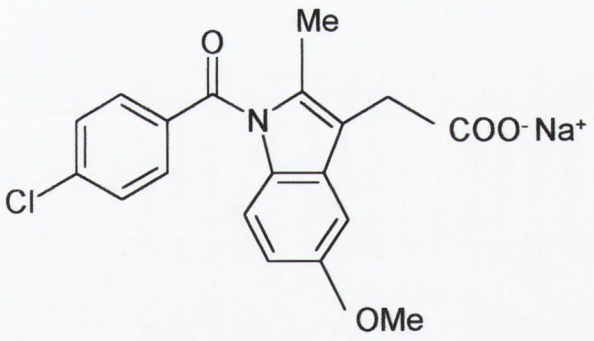
Indomethacin, [1-(4-chlorobenzoyl)-5-methoxy-2-methylindol-3-y] sodium is a pale yellow odourless solid. The compound is decomposed by light. Indomethacin exhibits polymorphism; one form melts at about 155°C and the other at about 162°C.

It may exist as a mixture of both forms, which melts between these two temperatures. Its structure is shown in Figure 2:2:1 (p79).

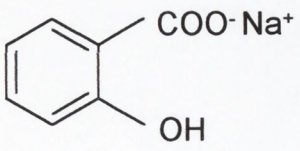
Indomethacin is also pharmacologically classified as an NSAID. Indomethacin became generally available in 1965 and was quickly recognised as an effective anti-inflammatory agent. Indomethacin is readily and almost completely absorbed after oral administration. The major metabolites being mesmethylindomethacin, deschloeobenzoylindomethacin and desmethyldeschlorobenzoylindomethacin. These substances together with indomethacin are excreted in the urine (up to 60% of the dose in 48hr) and in the faeces (up to 30% of the dose in 96hr) in variable amounts. The plasma half-life of indomethacin is between 3 and 15hr.



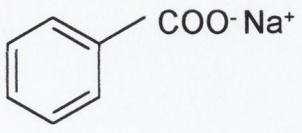
Sodium Diclofenac



Sodium Indomethacin



Sodium Salicylate



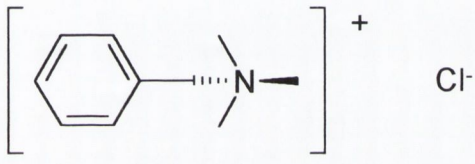
Sodium Benzoate

Figure 2:2:1 *Chemical Structures of Sodium Salts of Drugs Investigated*

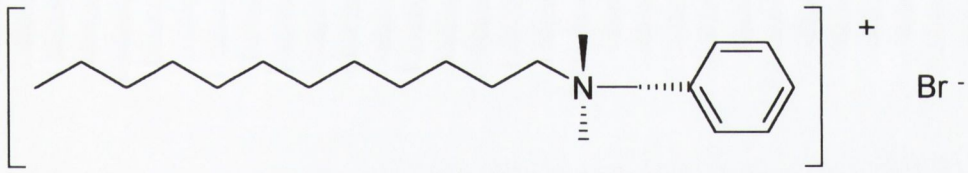
Salicylic acid, (2-hydroxybenzoic acid) and its derivatives is one of the earliest known and most widely used NSAID. The sodium salt is a white odourless compound. In 1928, Lecroux isolated the glycoside salicin from willow bark (*salix alba*), which has been used since antiquity as an antipyretic. The derivative acetylsalicylic known as aspirin is the most common form of the drug (Hamor, 1990).

Finally, sodium benzoate was also chosen as an anionic model compound. Although sodium benzoate is not a therapeutic compound, it was chosen because of its similarities to the other model compounds and because it is readily available.

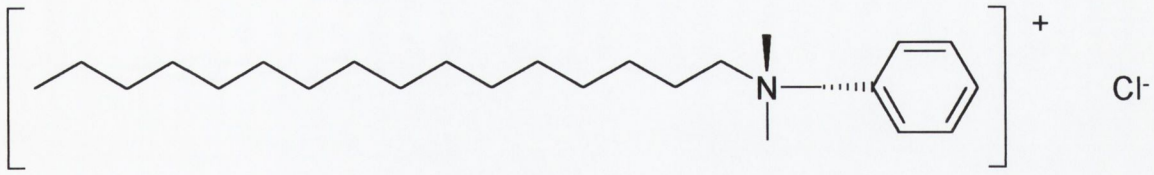
The following analar grade model drugs were obtained from Sigma-Aldrich Chemicals; sodium diclofenac, sodium indomethacin, sodium salicylate and sodium benzoate. Their chemical structures are shown in Figure 2:2:1.



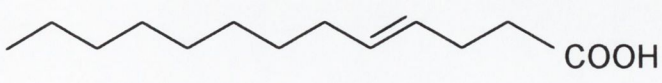
Benzalkonium Chloride



Benzydimethyldodecyl ammonium Bromide (BDDAB)



Benzyldimethylhexadecyl ammonium Chloride (BDHAC)



Oleic Acid

Figure 2:2:2 Chemical Structures of Enhancers and Candidate Enhancers

The following analar grade chemical enhancers and candidate chemical enhancers were obtained from Sigma-Aldrich Chemicals; benzalkonium chloride, and its derivatives benzyldimethyldodecyl ammonium bromide, benzyldimehylhexadecyl ammonium chloride and oleic acid. Chemical structures of these enhancers are shown in Figure 2:2:2. Refer to Table 2:2:1 for physicochemical properties of enhancers.

Potassium bromide and potassium chloride (both 99% purity) were also obtained from Sigma-Aldrich Chemicals. The following chemicals were supplied by Riedal-de-Haan; sodium dihydrogenphosphate, *di*-sodium hydrogenphosphate (both 98% purity). The following analytical grade solvents and acids were also supplied by Riedel-de Haan; acetone, acetonitrile, methanol, sulphuric acid, nitric acid and phosphoric acid.

Table 2:2:1 *Physicochemical properties of model drug and enhancers*

Compound	*Mol. Wt. (a.m.u.)*	*Solubility in Water (g/L)*	*pK_a*	*λ_{max} (nm)*
Diclofenac	296.1	Insoluble	4.0	276
Indomethacin	345	Insoluble	4.5	272
Benzoic Acid	121	4.2	4.19	254
Salicylic Acid	138	2.17	2.97, 13.4	297
Benzalkonium Chloride	172.5	-	-	-
BDDAB	360	-	-	-
BDHAC	416	-	-	-
Oleic Acid	213	-	5.4	-

- *not available*

Myverol 18-92 was kindly donated by Eastman Chemicals (UK) Limited. Myverol is derived from rapeseed oil and consists mainly of monoglycerides. The material contained less than 5% diglycerides. Some food grade antioxidants were also present. Its full composition is given in Table 2:2:2. Myverol was used as the main constituent in the preparation of the liquid crystalline delivery vehicles. Myverol is an off-white, odourless semi-solid and is a dispersing or foaming agent.

Myverol is mainly used as an emulsifier in the food industry in products such as margarine and peanut butter. Its melting point is 313 K and forms a liquid crystalline gel above 303 K on addition of water, it was for this reason and, as will become evident below, work in this (Carr, 1992) and other laboratories had shown it to work well in transdermal systems.

Myverol swells to a water content of approximately 30%. Further addition of water results in the formation of a liquid crystalline system which coexists with water, (Carr, 1992). Engstrom *et al.,* (1992), similarly reported the formation of a monoglyceride liquid crystalline system which can incorporate a water content of approximately 35%.

The spontaneous association of amphiphilic molecules in solution is a well-known phenomenon. Some of the structures formed are known as liquid crystalline phases. These phases can be characterised as either eroding or non-eroding phases in the excess of solvent, (Ericsson *et al.*, 1988). Myverol is used in this study with water to form non-eroding cubic phases. Due to domains of both hydrophilic and of polar character, the liquid crystalline gels can solvate lipophilic and / or hydrophilic substances. Since the monoglycerides are completely physiologically acceptable substances, they have an attractive basis for the formulation of a drug delivery system. Liquid crystalline gels also provide for the need of controlled release, the need of protection of drug degradation and also the need to be able to incorporate absorption enhancing agents, (Engstrom *et al.*, 1988). Engstom also states that many drugs can be incorprated in the cubic phase to the extent of ca. 10-15% above which concentration the drug becomes dispersed. A peculiar characteristic of the myverol / water system is that the more water in a phase the more viscous it becomes forming a paste-like cubic phase. The structure of the phase may be described as a three-dimensional network of bilayers separated by water channels. Typical dimensions between bilayers are 2-20nm. This property is advantageous for the purpose of a drug delivery device as the vehicle is malleable and does not require containment.

Experiments were also carried out using a 4% agar hydrophilic vehicle for the purpose of comparison with liquid crystalline gel vehicles. The purified Agar used was obtained commercially from Oxoid (code L28) as yellowish granules produced by hot water extraction of selected seaweed to yield a polysaccharide mixture of

agarose and agaropectin. It produced a clear viscous gel on addition of water. Agar is one of the strongest gelling agents known (Prendergast, 1975).

Table 2:2:1 *Composition of Myverol used in the preparation of liquid crystalline gels*

% Fatty Acid in Monoglyceride	*Fatty Acid*	*Chemical Formula Degree of Unsaturation*
0.1	Myristic	C14.0
7.0	Palmitic	C10.0
0.1	Palmitoleic	C16.1
0.1	Margaric	C17.0
4.5	Stearic	C18.0
18.7	Oleic	C18.1
67.5	Linoleic	C18.2
0.8	Arachidic	C20.0
0.1	Gadoleic	C20.1
0.7	Behenic	C22.0
Less than 10%	Propylene Glycol; Glycine; Phosphoric Acid; Propyl Gallate; Citric Acid	-

2:3 *Preparatory Methods*

Liquid Crystalline Gels

The following is a typical procedure for the preparation of a liquid crystalline vehicle containing, e.g., Sodium Diclofenac. Each vehicle had an approximate gel volume of 1.8 ml. The gels were prepared according to a method described by Carr (1992). A ratio of 71% myverol to 29% distilled water or buffer solution by weight was used. Carr established experimentally that this ratio was optimum for the release of drugs from the gel, the ratio is also the saturation point of water in Myverol. To prepare a 7 ml gel 4.69g of myverol is required (density = 0.94). The myverol is

carefully weighed and then heated gently over a bunsen burner until it melts. 196mg of Sodium Diclofenac are then dissolved in this liquid myverol. 2.03ml of triply distilled water or buffer are then added to give a solution that is overall 0.1M. The liquid crystalline phase forms immediately and is thoroughly mixed to ensure even distribution of the drug. Vehicles containing enhancers were prepared in the same way, except for the addition of enhancer with the drug into the oil phase prior to the preparation of the liquid crystalline phase.

Agar Gels

The average volume of agar gel used was also 1.8 cm^3. The solution of agar prepared at 60°C was poured into a petri dish and allowed to set then stored in a fridge at 276 K. When set the gel is ready to be cut using a cylindrical cutter to produce disks with a cross-sectional area of 2.54 cm^2. The volume of agar required to fill the petri dish to a depth of 0.9cm, in order to attain this volume was found to be 51.07ml. The quantity of agar required to make a 4% gel is 2.04g. 31.8mg of Sodium Diclofenac are required to make a 0.1M gel. The amount of Sodium Diclofenac required to produce the quantity poured into each petri dish is 1.624g.

Buffer Solution

Isotonic phosphate buffer solution (IPBS) is a physiologically adjusted buffer used in the receptor compartment to closely mimic diffusion into the systemic blood system. The buffer contains 2.2g.dm^{-3} NaH_2PO_4 A.R., 19.1g.dm^{-3} $Na_2HPO_4.12H_2O$ A.R. and 4.4g.dm^{-3} NaCl. The pH of the buffer is 7.4

Mobile Phase (for HPLC analysis)

The mobile phase was prepared using 1.96g H_3PO_4, 600mls of HPLC grade Acetonitrile (ACN) and 400mls triply distilled water. The triply distilled water is filtered through a 0.2 μm filter to remove particulates. The solution is stirred and then sonicated to degas it before use. The mobile phase was degassed on a daily basis and prepared freshly every three days.

Visking Membranes

Visking™ 18/32 cellulose dialysis tubing is a synthetic membrane produced by the Visking Co., Chicago, Ill. It has an average pore size of 2.4nm (Corrigan *et el.* 1980) and an average thickness of 20μm (Bannon, 1989). Any soluble materials, such as sulphur compounds, were removed before use by repeated boiling in triply distilled water, (Molyneux and Frank, 1961). The tubing was cut into lengths of three centimeters and opened flat using a blade for use as a non-rate limiting membrane. This membrane has previously been shown not to significantly affect the diffusion of a variety of drugs from agar or liquid crystalline gels (Bannon 1989, Brady 1991, Foley 1991, Carr 1992, Nolan 1995).

Preparation of Diclofenac Standards

The total amount of Diclofenac in each disk of a gel used is 57.24mg. The receptor cells have an average volume of 59.6ml. It is necessary to quantify percentage release into this volume. Taking a theoretical 50% release to be the stock solution, the following table illustrates the preparation of the remaining standards.

Table 2:3:1 *Example of preparation calculation for sodium diclofenac standards*

Std.	*%*	**mg/cell*	***mg/ml*	*mgs/10ml*	*ml stk.*	*ml (H_2O)*	*Vol. ml*
1	50	28.62	0.47	-	-	-	100
2	40	22.89	0.38	3.8	8	2	10
3	30	17.17	0.28	2.8	6	4	10
4	20	11.44	0.19	1.9	4	6	10
5	10	5.72	0.095	0.9	2	8	10

* Number of mg of Diclofenac corresponding to % release
** Number of mg/ml of Diclofenac corresponding to % release
% Theoretical % release
stk Stock Solution

Preparation of Diffusion Cells

Single compartment Franz-like diffusion cells (Figure 2:3:1) were chosen as the standard apparatus for all experiments. Although more specialized and dedicated diffusion cells are available e.g., the "Enhancer Cell" developed by VanKel Industries, Inc., Edison, NJ (Sanghvi and Collins, 1993), the custom made cells used have been shown to be adequate and also provided continuity from previous diffusion studies in this laboratory (Nolan, 1995). Sanghvi and Collins compared the Enhancer cell to the Franz cell and found a higher cumulative release from the Enhancer cell. The enhancer cell demonstrated more durability and was easier to use during experimentation. In addition it was found after completion of the experiment that there was no apparent change in the condition of the vehicle or the SC when compared to analogous experiments using the Franz cell.

Several other cell types are reported in the literature including the Flow – Through Diffusion Cell (Bronaugh and Stewart, 1985), Rotating Diffusion Cell (Albery *et al.*, 1975), Two-Chamber Diffusion Cell (Patel *et al.*, 1984) and Sweetana-Grass Diffusion Cells (Sutton *et al.*, 1991). Of these only the two-chamber diffusion cell could have provided an alternative to the Franz cell: the other cells would be unsuitable for the work reported here.

The Visking membrane or SC is securely held on the top of the Franz diffusion cell using parafilm wrapped around the neck of the cell. IPBS or triply distilled water is placed in the receptor port of the cell. The viscous gel-containing drug is syringed onto the membrane and covered with Parafilm™ to prevent water loss. A Teflon collar is used to prevent the gel from slipping off the membrane. The receptor port of the cell is then topped up to exclude air bubbles. The cell is now ready to be placed in a heated water bath at 310K with which it is allowed to equilibrate. The first sample is taken after half an hour and subsequently every hour.

The procedure for the iontophoretically assisted experiments was the same except that an electrode (cathode) was placed on top of the gel before the application of parafilm. Another electrode (anode) is permanently fixed in the receptor compartment of the cell just below the barrier. Platinum electrodes (99% purity) were used in all experiments. Figure 2:3:1 shows a typical cell used for both passive and iontophoretic investigations.

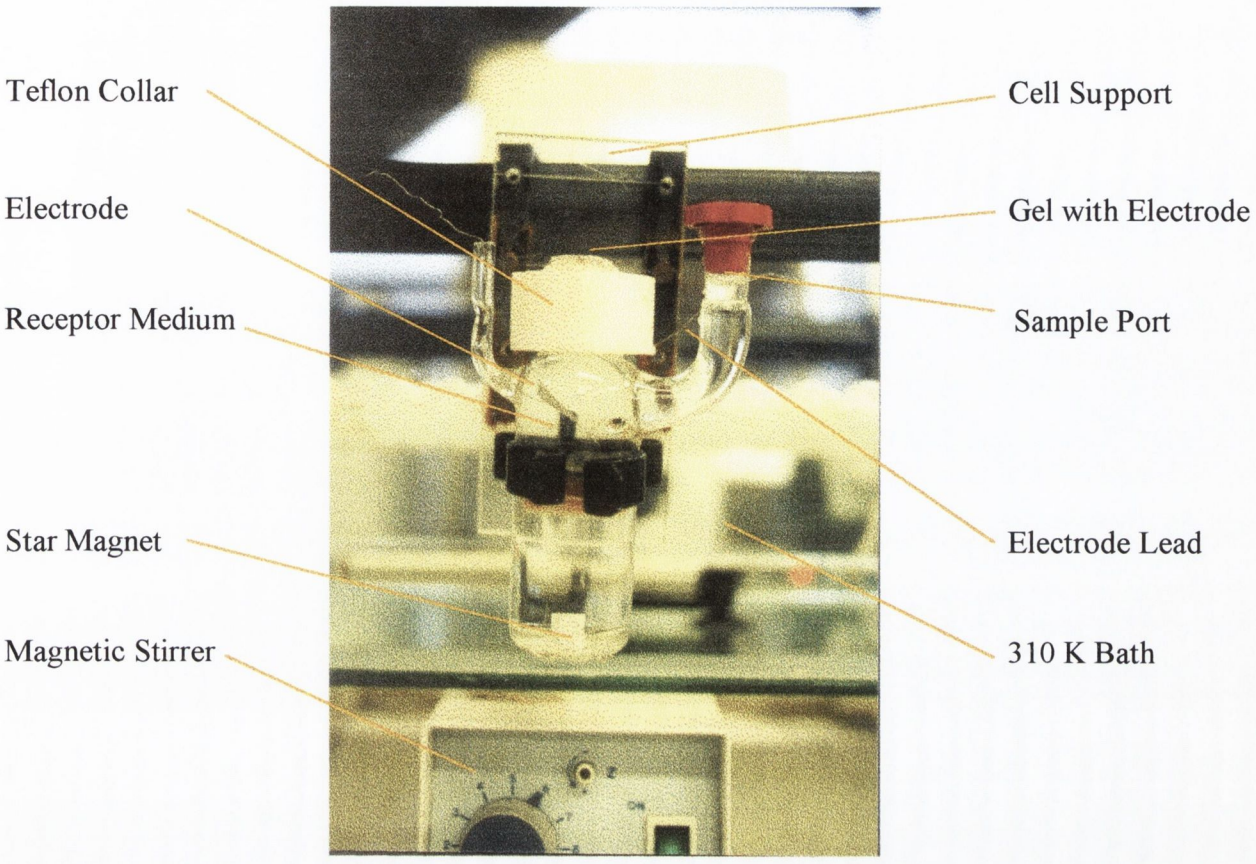


Figure 2:3:1 *Typical Franz cell used in transdermal studies*

In the protocol for the iontophoretic experiments the release of drug was first conducted passively for the first two hours. The experiments were run galvanostatically at currents of 0.25mA and 0.5mA during the following 22 hour period. The voltages and currents involved were monitored using Philips 3456A digital multimeters. A meter to measure either voltage or current can be introduced into the circuit in Figure 2:3:2. Each battery powered power supply was driven by three 9V batteries. Samples were taken at hourly intervals up to 10 hours and at intervals whenever possible there after. However, samples were also always taken towards the end of the experiments, i.e., after the elapse of 23 and 24 hours.

Each sample was of 0.5ml volume taken using a 1ml calibrated Gilson™ pipette. The receptor solution was topped up with a similar volume of the receptor fluid to that withdrawn and the cell was inverted again to exclude air bubbles from under the membrane.

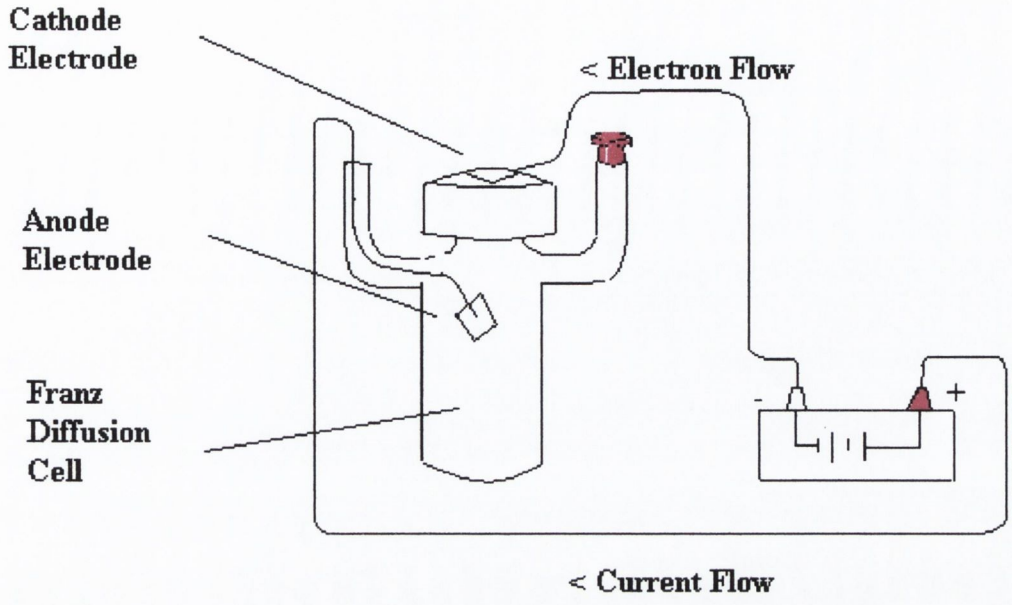


Figure 2:3:2 *Schematic of Iontophoretic Circuit Including Galvanostatic Power Supply*

Partition Co-efficient Studies

These studies were carried out using 60ml vials with self-sealing caps. The drug to be analysed was dissolved in 25ml of octanol or water at a concentration of 0.1M. The final content of the vial was 25ml water and 25ml octanol. The vial was sealed and placed on a shaker stand and shaken for 24 hours. The aqueous solution was then analysed by HPLC to determine the concentration of drug. The concentration of drug in the octanol phase was calculated from these measurements. Figure 2:3:3 exemplifies the partitioning of a drug between two immiscible phases A (aqueous) and O (octanol). P is an equilibrium constant called the partition co-efficient or distribution co-efficient.

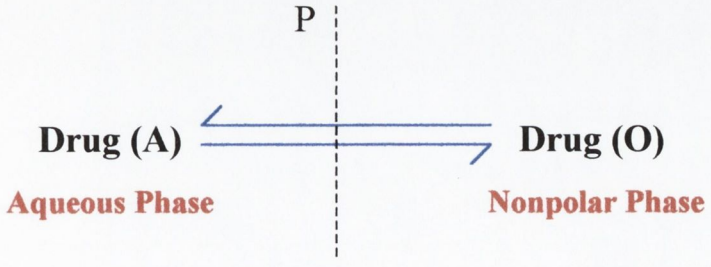


Figure 2:3:3 *Schematic Diagram of the Partitioning of a Drug Between Two Immiscible Phases.*

The distribution co-efficient is represented by the ratio $[Drug]_O$ / $[Drug]_A$ (i.e., the ratio of drug concentration in the two phases). This ratio is expressed most commonly as a log value of P. Polar drugs have small values of P and nonpolar drugs have larger values.

2:4 *Analytical Methods*

High Pressure Liquid Chromatography (HPLC)

The drug content of each sample taken during both the partitioning and drug transport studies described previously was analysed by HPLC. The mobile phases, retention times, flow rates, loop volumes and injection volumes are given in Table 2:4:1.

Table 2:4:1 *Standard parameters of HPLC analysis for the model drugs studied*

Model Drug	*Mobile Phase*	*Retention Time (min)*	*Injection Volume (μl)*	*Flow Rate (ml/min)*
Diclofenac Na	H_2O/ACN 2:3, $1.96gdm^{-3}$ H_3PO_4	6.0	25	1.0
Indomethacin Na	H_2O/ACN 2:3, $1.96gdm^{-3}$ H_3PO_4	5.0	25	1.0
Salicylate Na	H_2O, CH_3OH, CH_3COOH 6 : 4 : 0.1	5.0	25	1.5
Benzoate Na	H_2O/ACN 2:3, $1.96gdm^{-3}$ H_3PO_4	4.0	25	1.0

- *Loop volume for all injections was 250μl*

A Waters 501 reciprocal pump was used to deliver the mobile phase at a constant rate through the system. All drugs were analyzed using a Waters™ C_{18} reverse phase 30cm column. Samples are loaded into a Waters 717 autosampler, which was programmed to sequentially analyse each vial. An average vial takes approximately 10 minutes to run. Each diffusion cell produces 12 vials for analysis. As the experiments are carried out in triplicate, an average of 36 vials / experiment must be processed, resulting in a total run time of 6 hours. Approximately half an hour is also required to analyse standards before injecting unknowns.

Detection of the drug was by a Waters 486 tunable UV absorbance detector. A Waters 746 data module integrator produced chromatograms with arbitrary peak areas: these were then converted to quantitative concentrations form standard values.

Infrared Spectroscopy

This method of analysis was used to relatively quickly and easily ascertain whether the sodium salts of model drugs had changed to the acid form under iontophoretic conditions, as the change is not detectable using HPLC due to insolubility. If diclofenac acid is present a diagnostic sharp peak is evident at 3500 cm^{-1} for the OH bond of the carboxylic acid. The same applies for all the model drugs under investigation.

KBr discs were prepared by estimating the required amount of KBr and drying it in an oven at 373 K. When dry 0.5mg of the solid drug were added and the mixture was thoroughly ground up with a marble mortar and pestle. The mixture was then placed into a stainless steel dye which is then placed in a hydraulic press. Pressure was built up slowly to allow the material to spread evenly in the dye after which the pressure was raised to 10 tonne for 3 mins. A conventional Perkin Elmer 833 was used or alternately a Perkin Elmer FT-IR. Hard copies of spectra were printed for interpretation.

Melting Point Tests

Where relevant the melting points of the various compounds were measured to aid confirmation of IR data. There are very significant differences between the

melting points of the acidic and the ionic forms of the model drugs, e.g., the melting points of diclofenac acid and sodium diclofenac are ~ 157°C and 284°C, respectively. The melting points were measured using a Gallenkamp Variable Heater and glass melting point tube. A brass rod was used to dissipate heat between successive readings

pH Measurements

The pH of the solutions and gels were measured using an Orion model 520A pH meter. Where necessary a combination contact electrode (model 913500) which is also suitable for surface measurements, was used. Readings were necessary to monitor receptor solutions, buffer solutions, mobile phases and electrolysis experiments. The pH meter was calibrated using standard buffers at pH4 and pH 9.

GC Techniques

Gas chromatography was used to analyse precipitates from receptor media. As it was anticipated that the precipitates were the acid from of the model drugs so the corresponding acid forms were used as quantitative and qualitative standards. A typical serial dilution used to obtain a calibration curve is shown in Table 2:4:2

Table 2:4:2 *Typical Serial Dilution for GC standards of Diclofenac Acid*

Standard	*Conc. (M)*	*ml of Stock*	*ml of EtOH*	*Total Vol. (ml)*
1	0.02	2	8	10
2	0.04	4	6	10
3	0.06	6	4	10
4	0.08	8	2	10
5	0.1	-	-	100

The precipitates were weighed and then dissolved in chromatography grade ethanol of known volume. A 1μl GC syringe was firstly cleaned with general purpose acetone

before being washed again with the sample analyte several times prior to injection. Injection volumes ranged from 0.2-2.0μl. The samples were injected into the heating block of a Varian 3300 gas chromatography instrument. Once volatile the sample is transported by nitrogen carrier gas to a varian CP-sil 10m capillary column.

Isothermal analysis was carried out, as the separation of components was expected to take place in less than 5 minutes. Retention times varied with carbon number. A typical chromatogram consisted of three peaks; a small residual acetone peak, a large ethanol solvent peak and model drug peak. The samples were analysed after the column using a flame ionisation detector (FID) fuelled by hydrogen and compressed air. Chromatograms were printed by a Varian 4029 integrator. The analytical parameters used in the GC are contained in Table 2:4:3.

Table 2:4:3 *GC Analytical Parameters for the Detection of Acids of Model Drugs*

Parameter	*Diclofenac*	*Indomethacin*
Injection Volume (μl)	0.2	0.5
Column Temperature (K)	373	120
Injector Temperature (K)	523	250
Detector Temperature (K)	523	250
Carrier Gas Flow Rate (ml/min)	323	60

-FID detection was used for all sample analysis

Condensation checks were carried out regularly with a glass slide to make certain the detector was operational.

UV Spectroscopy

UV spectroscopy was required to obtain the λ_{max} of the model drugs in order to tune the HPLC detector to the relevant wavelength. UV measurements were also used to monitor the complexation reaction between indomethacin and various transition metal complexes. It was expected the tertiary amine lone pair of indomethacin would act as a Lewis acid and form a ligand-metal comlex which would

absorb in the visible region. This would make indomethacin detectable in the visible spectrum.

A Unicam UV/Vis UV4 spectrometer was used for most of the analyses or alternately a Schimadzu UV-2401 PC spectrometer. Quartz glass cells with a path length of 1cm were used for all scans.

Mass Spectrometry

Mass spectrometry was used in conjunction with GC to qualitatively analyse precipitates from receptor media.

Samples were prepared by dissolving 1mg of precipitate in 10 ml of methanol. After complete dissolution, 200μl of the solution were pipetted into another 10 ml of methanol to give a final concentration of 2 ng/ml. The sample was then filtered to prevent undissolved solids from blocking the inlet tube. The sample is then injected onto a Micromass LCT-ToF mass spectrometer. The sample was introduced into the ionizer by electrospray and the analyser uses time-of-flight technology to detect molecular fragments. Computer software generates the required data. A HPLC pump feeds mobile phase to the electrosray unit at a contant rate and pressure.

Nuclear Magnetic Resonance (NMR)

NMR was also used for qualitative analysis of precipitates. The samples were dissolved in either deuterated DMSO or deuterated water. Samples analysed using a Bruker-Spectrospin 400 MHz proton NMR. The same instrument was also capable of 100 MHz ^{13}C NMR spectra. COSY NMR spectra were also available for analysis to match carbons with hydrogens where necessary.

Electrolysis Investigations

Precipitate was observed to form at the bottom of receptor media during iontophretic experiments, especially in the case of diclofenac, and further investigations were required to establish what was happening. It was suspected that the acid form of the drug was forming under iontophoretic conditions but the quantity

of precipitate formed in the experiments was too small to analyze easily. A larger quantity was obtained by placing two electrodes in a 100ml beaker containing a 1M solution of the drug and passing a current density through the solution similar to that used under the normal iontophoretic experimental protocol. In an unstirred beaker, precipitate could be seen to form immediately at the anode. This was then collected for analysis and the results are reported in subsequent chapters.

Cyclic Voltammatry

Voltammograms were obtained by using a Bass CV 50 cyclic voltammetry unit. A 0.1M solution of drug was used in order to run the spectra. No supporting electrolyte was required as the salts of the compounds were used. Parameters were set using a PC with specialized software.

Chapter 3

In Vitro Studies of the Transport of Anionic Drug Molecules Across a Synthetic Membrane

Chapter 3

InVitro Studies of the Transport of Anionic Drug Molecules Across a Synthetic Membrane

3:1 Introduction

The two main investigative aspects of transdermal drug delivery are, firstly, the determination of the release characteristics of the drug from the chosen vehicle and, secondly, the quantitative evaluation of the transport of the drug across the *stratum corneum* and its availability to the systemic system.

This chapter investigates the former aspect of release by carrying out diffusion studies using a non-rate limiting membrane while varying several relevant experimental parameters. These include the nature of the receptor media, the use of buffer solutions in the aqueous phase of the vehicle and the extension of the work to a range of model anionic drug molecules.

Following on from previous studies by Bannon (1989) and Nolan (1995), who investigated the transdermal transport of neutral (nicotine) and cationic (salbutamol) model compounds, respectively, the logical progression was to investigate the release and transdermal transport profiles of anionic drug compounds. Cursory investigations by Nolan of physical and chemical enhancement of sodium diclofenac had given negative results. For the purposes of research outlined here, several anionic drug molecules were chosen to be investigated in varying degrees. The sodium salts of chosen compounds include those of diclofenac, indomethacin, salicylate and benzoate, as shown in Figure 2:2:1. Particular emphasis is given to diclofenac.

It is mainly coincidence that most of the anionic drug compounds under investigation are classified pharmacologically in the same group as NSAIDs. These compounds do not ideally meet all the criteria of suitability for transdermal delivery, (e.g., low molecular weight, lipophilicity, requiring only small quantities to achieve therapeutic affect, and stability). However, equally well they do not match all the criteria for oral administration. Around a quarter of adverse drug reactions reported to the UK committee on the safety of medicines, are attributed to NSAID's and weak

analgesic drugs (Rainsford, 1984). Most frequent side-effects observed with the NSAID's are gastrointestinal. They can be potentially serious, leading in rare cases to life-threatening haemorrage from unsuspected peptic ulcer, but more frequently acute mucosal lesions, dyspepsia, nausea, indigestion, constipation, vomiting and diarrhoea may occur, and while less dangerous, are nonetheless distressing (Rainsford, 1984). Table 3:1:1 contains data on some of the side-effects associated with orally administered NSAID's.

Table 3:1:1 *Side-effects expressed as a percentage associated with the use of NSAID's*

Drug	*Bleeding*	*Peptic Ulcer*	*Nausea dyspepsia pain etc.*	*Central Nervous System*	*Respiratory*
Aspirin	1.0	2.7	2.1, 33.4, 47.9	1.2	0
Diclofenac	0	0.4	12.3	3.7	0
Indomethacin	0.72	2.1	16.5, 25.2, 47	3.5	1.8

- data obtained from Williamson (1987)

As can be seen from the data in Table 3:1:1, there would be considerable benefit if it were possible to deliver these drug molecules via the transdermal route and thus avoiding the gastrointestinal tract and side-effects.

3:2 *The Passive Release of Sodium diclofenac from a Myverol-Based Delivery Device Across Visking Membranes.*

The cumulative amount of sodium diclofenac released passively as a function of time form a monoglyceride liquid crystalline vehicle, incorporating water as solvent, across a Visking membrane, is shown in Figure 3:3:1. This experiment and all subsequent experiments were carried out in triplicate. Averages and error bars were employed throughout. The cell volumes did not vary significantly from each other in volume (standard deviation (δ) = 1.71%). The total amount of sodium diclofenac released over a twenty-four hour period was less than 6% of the initial

loading of 0.1M, increasing to a maximum release of 11% after 72hr. This corresponds to the passage of 6.29mg sodium diclofenac across the membrane. This is significantly lower than the quantities of any other drugs currently or previously investigated in this laboratory that were released under analogous conditions from the liquid crystalline gel. The relevance of this will be discussed later in the chapter when comparisons are introduced with the release of other anionic drug molecules.

The release data in Figure 3:2:1 show a non-linear relationship between the amount of drug released and time. Several mathematical models have

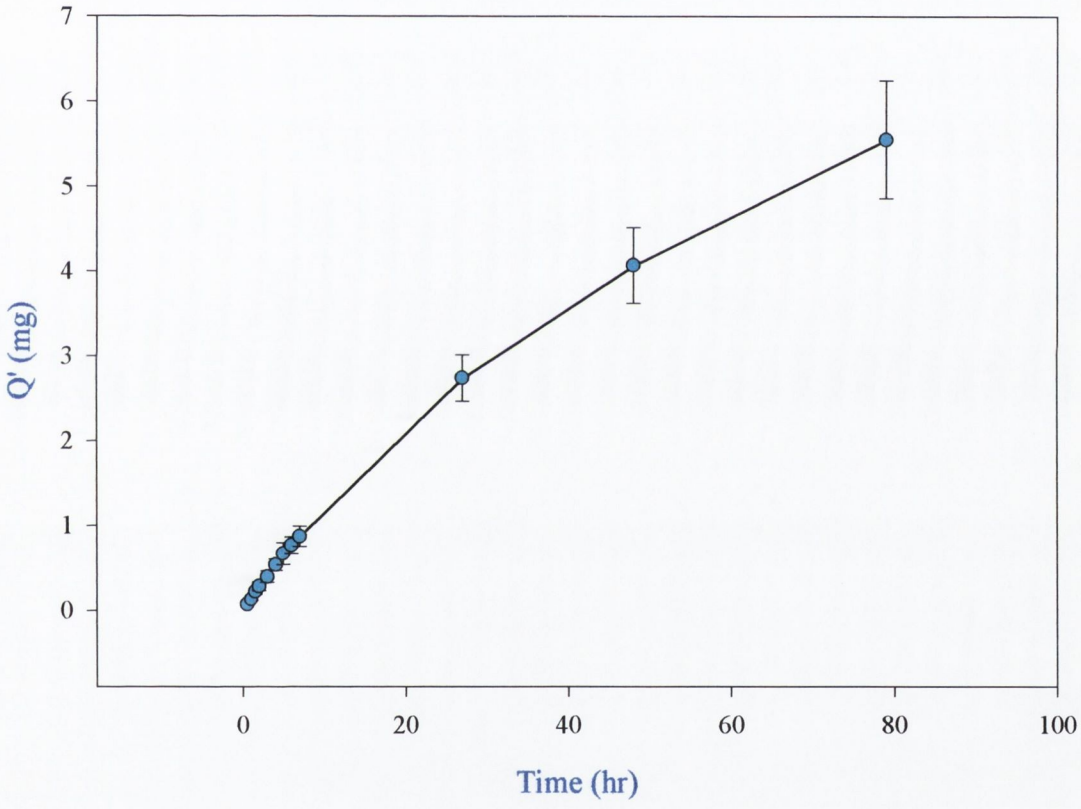


Figure 3:2:1 *The cumulative release of sodium diclofenac transported from a liquid crystalline gel across a visking membrane, into an aqueous receptor medium. All vehicles contained a concentration of 0.1M drug.*

been developed and used to describe the release of drugs from matrix systems (Higuchi, 1960; Paul and McSpadden, 1976; Peppas and Korsmeyer , 1987). The analysis applied to the data in Figure 3:2:1 in this investigation are models proposed by Higuchi (1960, 1961, 1963) and Schwartz *et al.*, (1968). Higuchi's model assumes

that the rate of release of the drug molecules is controlled by their diffusion through the vehicle matrix. If a drug is uniformly dissolved in the gel, drug release from the gel in contact with the membrane can be described by the Higuchi Equation, the most basic form of which is,

$$Q = Kt^{1/2}$$

Equation 3.1

where Q is the amount of drug released per unit surface area of the gel on the membrane ($mgcm^{-2}$), t is the time in seconds, and K is the release constant equal to $2C_o(D/\pi)^{1/2}$, where C_o is the initial concentration ($mgcm^{-3}$) and D is the diffusion coefficient for the drug in the gel ($cm^2\ s^{-1}$). Equation 3.1 is valid for a simple drug species whose diffusion coefficient is constant with respect to time or position within

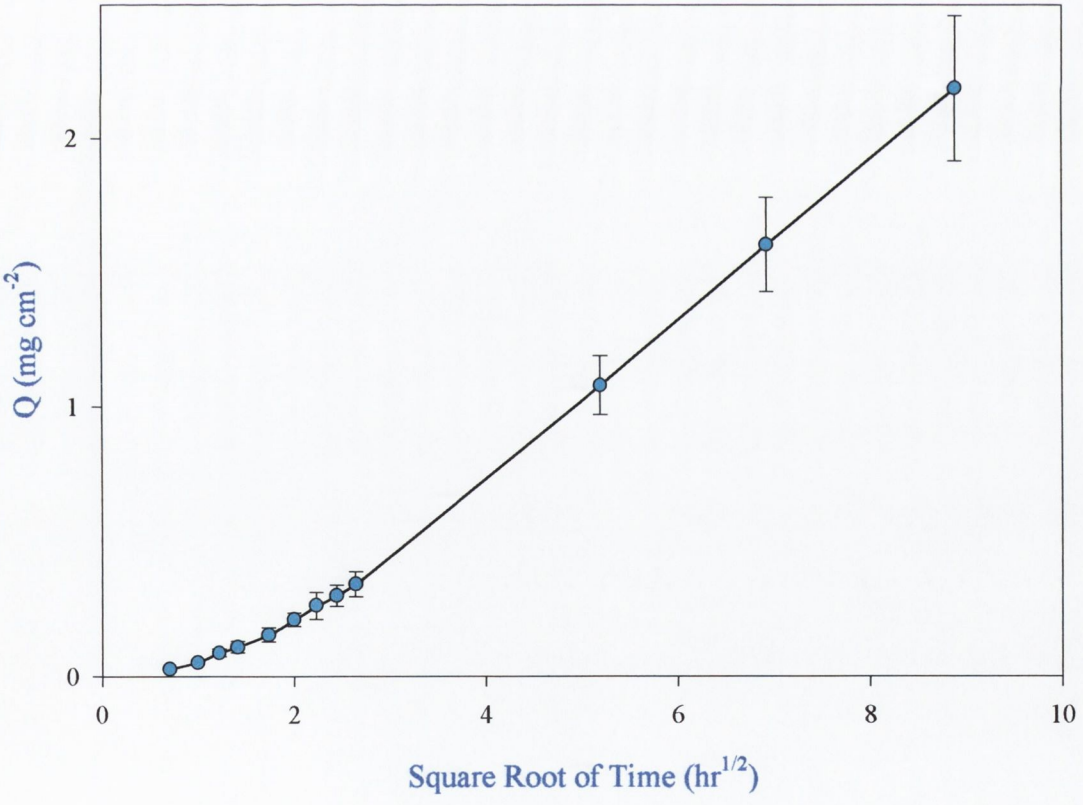


Figure 3:2:2 *Data from Figure 3:2:1 plotted according to the Higuchi model*

the delivery device and which is immediately removed once it reaches the receptor side. The equation also assumes less than 30% of the total drug loading is released although linearity above this percentage release has been found in earlier work in this laboratory to be the norm rather than the exception (Nolan, 1995; Sheerin, 1999; Lynn, 2000). Zarrintan and Groves (1991) also reported that drug release from a matrix delivery system continues to conform to this equation up to as high as 75% release.

Figure 3.2.2 shows release data from figure 3.2.1 plotted according to Equation 3.2, the Higuchi Model. The data conform well to a linear relationship after four hours ($t^{1/2} = 2$). The non-linearity evident in the early stages of release may be due to the system taking time to reach steady state diffusion.

Some previous studies have shown a rapid release of drug present on the surface of the vehicle (Nolan, 1995, Dunbrow and Friedman,1975, Burrows *et al.*,1994). This is known as the burst effect and is due to the vehicle being saturated with drug. It is evident from Figure 3:2:2 this phenomenon was not observed for the release of diclofenac. In contrast at concentrations below saturation level a lag time occurs. This is also due in part to the presence of the Visking membrane. For 0.1M diclofenac the lag time was found to be 0.25hr and for 0.01M it was found to have increased to 0.5hr. The increase in lag time with decrease in concentration is consistent with observations made by Nolan (1995). An initial burst or lag can be expressed by adding the constant C_o to equation 3.1 as:

$$Q = Kt^{1/2} + C_i \qquad \textit{Equation 3.2}$$

If C_i is negative the lag time can be read from the intercept with the x-axis and if positive the burst effect can be quantified with the intercept of the y-axis.

In 1968 Schwartz *et al.*, measured the release of benzoic and salicylic acid from wax matrices into aqueous media.

$$\log W = \frac{kt}{2.303} + \log W_o$$

Equation 3.3

Where W = *quantity of drug left in vehicle* W_o = *initial quantity of drug in the vehicle*
k = *first order rate constant* t = *time in seconds*

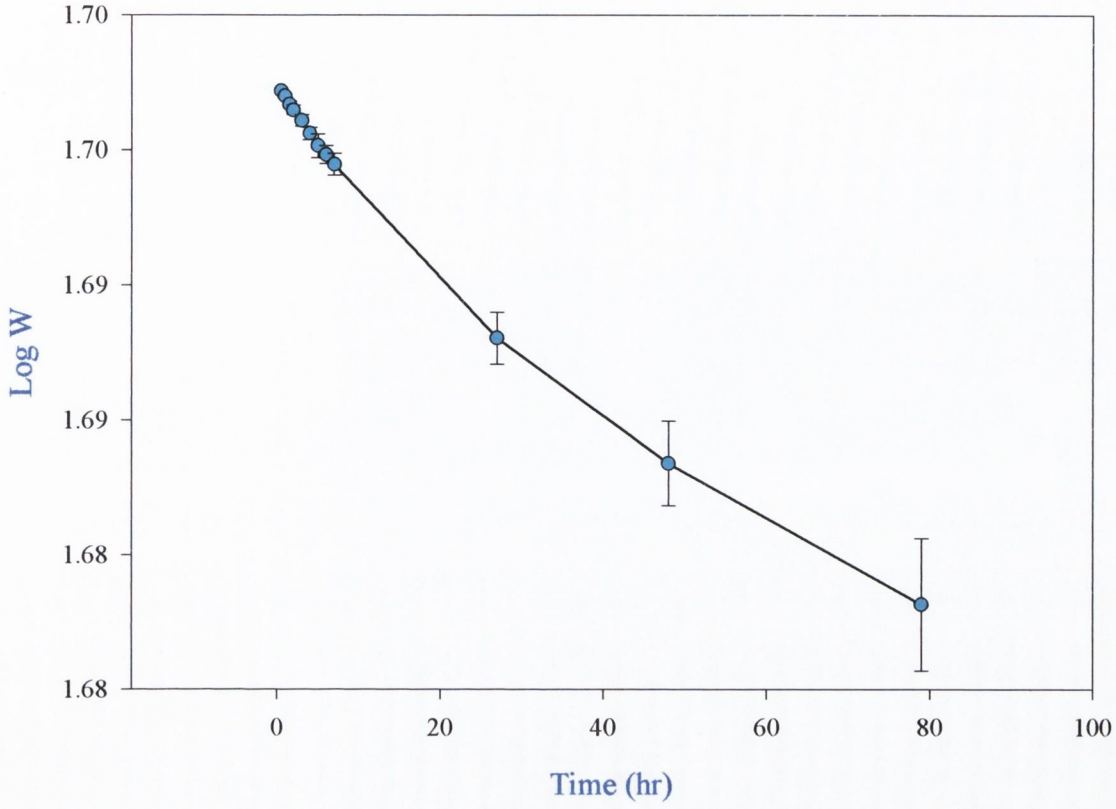


Figure 3:2:3 Sodium diclofenac release data plotted according to first-order release kinetics, (Equation 3.3)

They plotted the data according to the Higuchi equation and obtained a straight line indicating matrix diffusion control. The data were also plotted as predicted by a first-order release in accordance with Equation 3.3. The data were also linear for this model. Schwartz was concerned at the time that in view of the large number of patents issued in this area, it was desirable that the release rates from matrices be unambiguously quantified as the controlling release mechanism had not been firmly established.

The release of sodium diclofenac also adheres equally well to linearity when plotted according to both the Higuchi and Schwartz models (Figures 3:2:2 and 3:2:3). The release data for several other drugs investigated previously in this laboratory also proved linear for both models. Since both first-order and square root of time plots are acceptably linear for diclofenac, a more stringent test was required to distinguish between the mechanisms, as was the case for Schwartz with salicylic and benzoic acid.

Schwartz applied the following treatment to the Higuchi equation (3.1) in an alternative reduced form (Equation 3.3):

$$Q' = Kat^{1/2}$$

Equation 3.4

Where a = surface area of gel in contact with membrane and Q' = amount of drug released (mg). By differentiation of Equation 3.4 and appropriate substitution, Equation 3.5 can be obtained:

$$\frac{dQ'}{dt} = rate = \frac{K^2 a^2}{2Q'}$$

Equation 3.5

Equation 3.5 indicates that for the matrix mechanism, the rate will be inversely proportional to the amount of drug released, Q'.

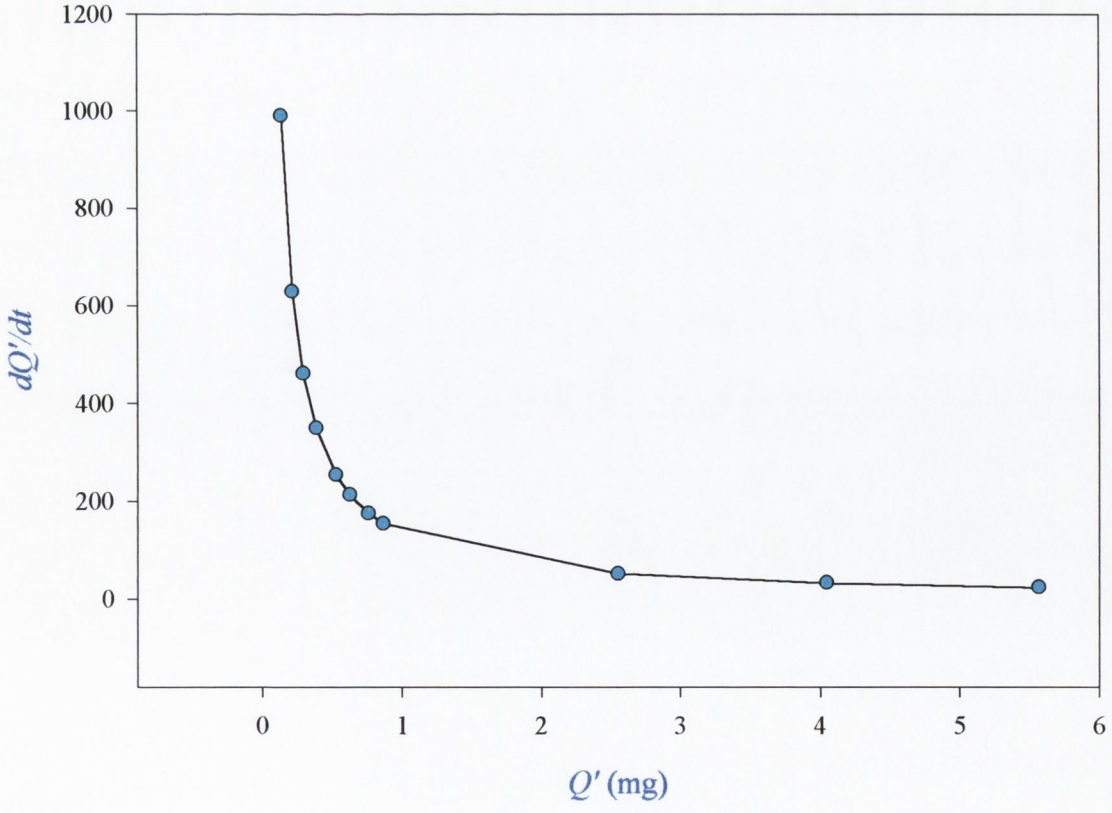


Figure 3:2:4 *The rate at which sodium diclofenac is released from a liquid crystalline gel across visking into water versus the quantity of drug released.*

The rate predicted by first order kinetics, however, is given by the following relationship:

$$\frac{dQ'}{dt} = rate = kW_o - kQ'$$

Equation 3.6

Equation 3.6 indicates that the rate is proportional to Q' for first-order release as opposed to non-linear as indicated by Equation 3.5. Plots of dQ'/dt versus Q' and $1/Q'$ are shown in Figures 3:2:4 and 3:2:5, respectively. The data in Figure 3:2:4 are non-linear and clearly indicate that the release of diclofenac from the vehicle does not

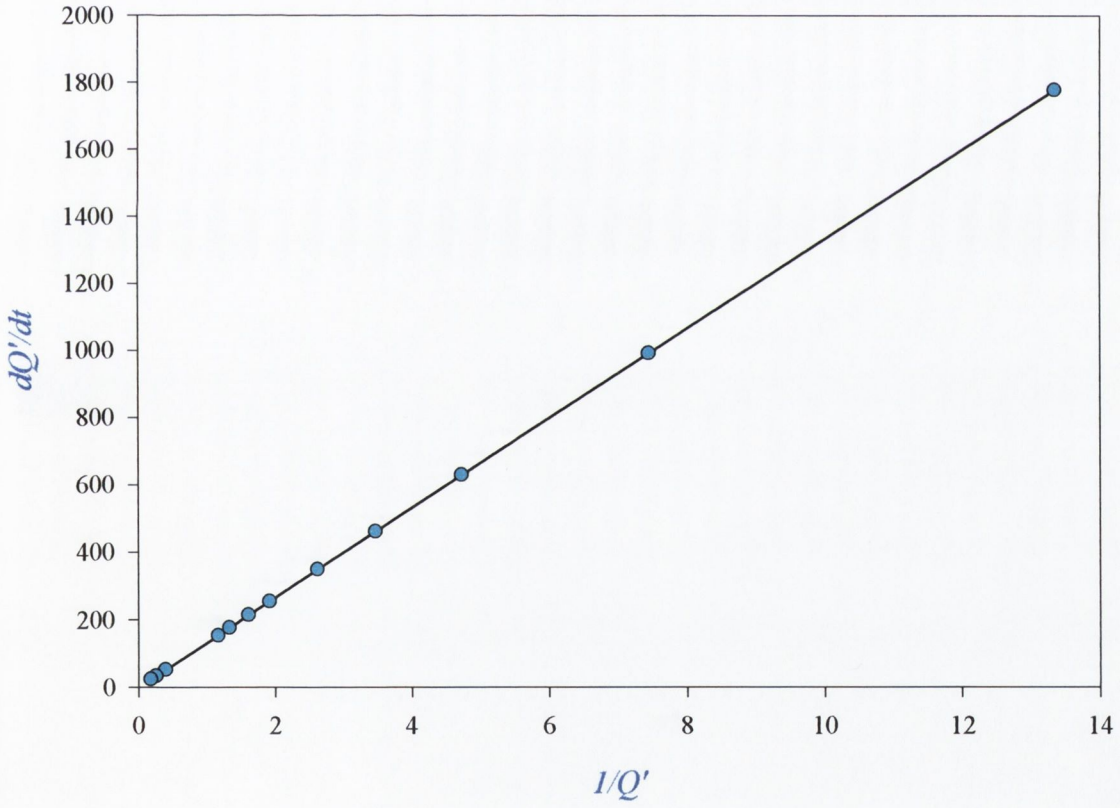


Figure 3:2:5 *Rate as a function of reciprocal drug released of 0.1M sodium diclofenac released from a liquid crystalline gel*

follow first-order kinetics, however the data in Figure 3:2:5 strongly indicate that the release of diclofenac from the vehicle is matrix diffusion controlled. Figures 3:2:4 and 3:2:5 show that the two mechanisms can indeed be differentiated by this treatment.

This conclusion can be further confirmed by the use of equation 3.6 which is obtained by taking logs of equation 3.1. This predicts that a plot of log Q versus log t must not only give a straight line, but should have a slope equal to 0.5.

$$\log Q = \log K + \frac{1}{2}\log t$$

Equation 3.7

The co-efficient of determination (r^2 value) for Figure 3:2:6 data is 0.996 which shows significant correlation between the variables but the value of the slope is 0.82 instead of 0.5 as would be required by first-order release. Zero-order kinetics may prevail above a slope value of 0.66 (Mockel and Lippold, 1993).

The diffusion co-efficient of sodium diclofenac from the liquid crystalline gel was estimated from the slope and found to be 2.2×10^{-8} cm^2 s^{-1}. The diffusion co-efficient of salbutamol base was found by Nolan (1995) to be 1.32×10^{-7} cm^2 s^{-1} and that of salbutamol sulphate was found to be 8.2×10^{-7} cm^{-2} s^{-1} (Carr, 1992).

Table 3:2:1 *Values for the rate, amount released, and the corresponding reciprocal for the release of 0.1M sodium diclofenac from liquid crystalline gels.*

Rate = dQ'/dt (hr^{-1})	*Q' (mg)*	*1/Q' (mg^{-1})*
1778.5	*0.07*	*13.34*
991.7	*0.13*	*7.44*
629.5	*0.21*	*4.72*
462.2	*0.29*	*3.47*
349.5	*0.38*	*2.62*
254.5	*0.52*	*1.91*
213.8	*0.62*	*1.60*
175.9	*0.76*	*1.32*
154.5	*0.86*	*1.16*
52.2	*2.55*	*0.39*
32.9	*4.05*	*0.25*
24.0	*5.56*	*0.18*

Using the Stokes-Einstein Equation (Equation 3.8) it is possible to compare the diffusion co-efficients and offer a possible explanation for the different diffusional behaviour of the drugs concerned.

$$D = \frac{kT}{6\pi r \eta}$$

Equation 3.8

where D = diffusion co-efficient η = dynamic viscosity

r = radius of ion k= *Boltzmann's constant*

The thermal energy driving diffusion is represented above the line by kT, whilst below the line represents the viscous drag force, which is assumed to be proportional to the velocity of the ion. By using the diffusion co-efficients alone it is possible to obtain the ratio of the radii of the solvated to unsolvated ions in the gel by using

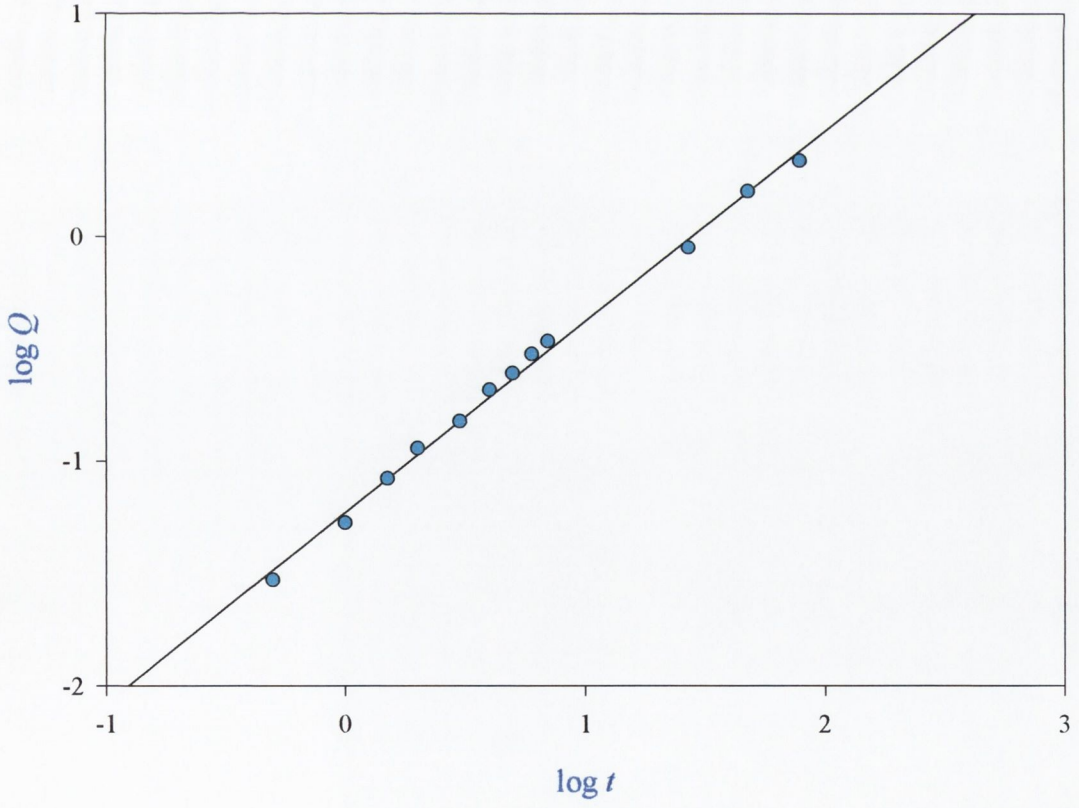


Figure 3:2:6 *Plot of log amount of drug released per unit surface area against the log of time for 0.1M sodium diclofenac from a liquid crystalline gel.*

Equation 3.9, a derivation of the Equation 3.8. Without the requirement to quantify *r* (the radius of the ion) for either molecule, diclofenac is calculated to have a solvated radius 6 times that of salbutamol base and 37 times that of salbutamol sulphate.

$$\frac{D_1}{D_2} = \frac{r_2}{r_1}$$

Equation 3.9

The molecular masses of sodium diclofenac and salbutamol base are 318.1 and 239 a.m.u., respectively. A rough estimate would indicate diclofenac to be 33% larger than salbutamol base in the unsolvated state. Maitini *et al.*, (1994), reported the unsolvated radius of sodium diclofenac to be 0.430 nm. These figures give no indication as to the reason for such a large difference in the solvated state and even less indication as to why the salts of these model drugs have such a large difference in their diffusion coefficients. The Stoke-Einstein equation nevertheless indicates sodium diclofenac to have a substantially larger solvation shell and hence that it will diffuse more slowly. A disadvantage to the application of this equation in the absence of any other effect is that where a diffusion co-efficient is >> than another then very different r values will result.

Another factor that has an influence on the release data is the partitioning of the drug from the oil phase into the aqueous phase of the liquid crystalline gel. The partition co-efficient of sodium diclofenac is 13.4 (octanol/aqueous buffer) and 4.5 (octanol/ water). The partition co-efficient of salbutamol base was found to be 0.46 (octanol/water). The monoglyceride/water partition co-efficient of salbutamol base is 0.8 (Nolan, 1995) and that of salbutamol sulphate zero (Carr, 1992). These values concur with release data which show that the lower the partition co-efficient the greater the release from the liquid crystalline vehicles. The quantity of drug released over a twenty-four hour period follows the trend of salbutamol sulphate > salbutamol base > sodium diclofenac.

The reason for such a significant difference in partition co-efficients between the two salts is unclear. Bhattachar *et al.*, (1992), reported on the ability of sodium diclofenac to form complexes with hydrogenated phospholipids. The oil phase environment of a liquid crystalline gel is not too dissimilar to the one presented by

phospholipids, i.e., negative head group with long alkyl chain being the main composition of molecules in the oil phase.

Further postulations will be discussed towards the end of this chapter.

3.3 The Effect of Concentration of Sodium Diclofenac on its Release from Liquid Crystalline Gels Across Visking Membranes.

Investigations were conducted into the effect of concentration on the release profiles of sodium diclofenac. The studies also provide kinetic data allowing for comparisons of first-order rate constants, and providing further confirmation of the Higuchi matrix diffusion model. Data from initial drug loadings of 0.05M, 0.01M and

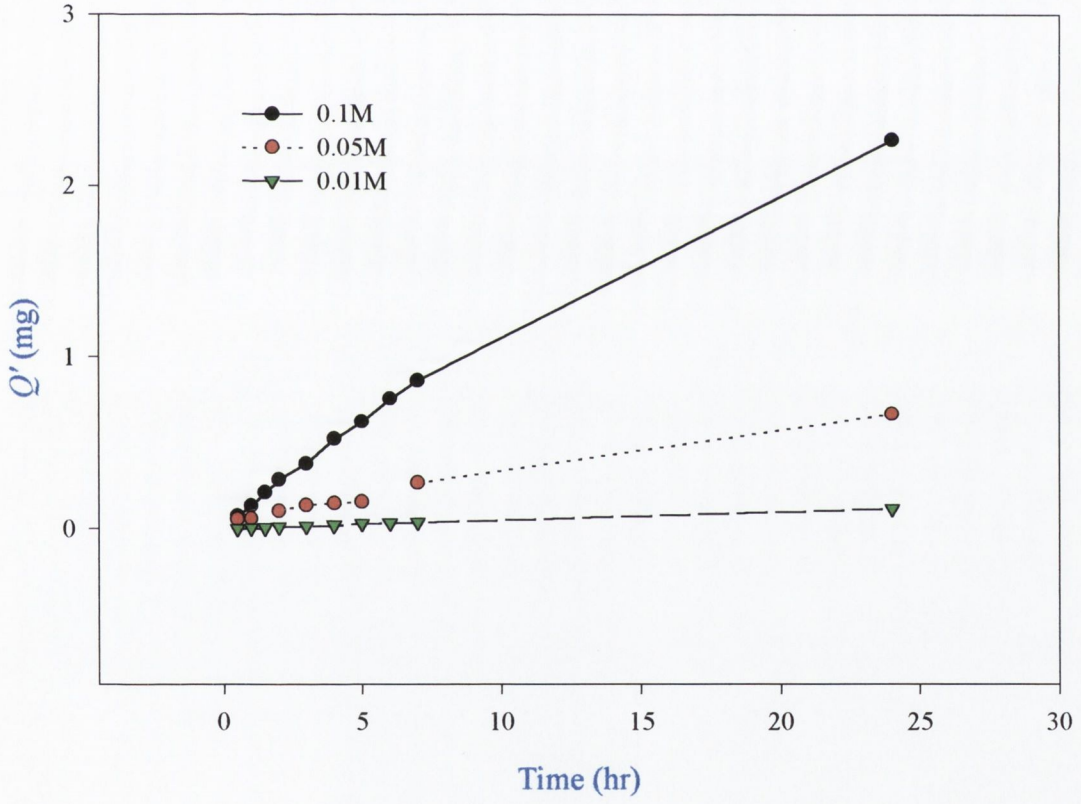


Figure 3:3:1 Cumulative amount of sodium diclofenac released from liquid crystalline gels across visking membrane with initial drug loading of 0.01M, 0.05M and 0.1M.

the previous loading of 0.1M are shown in Figure 3:3:1. Form the profiles it is evident that an increase in the initial drug loading leads to an increase in the quantity of drug

released. Diffusion co-efficients and rate constants of the different loadings are shown in Table 3:3:1.

Table 3:3:1 *Rate constants and diffusion coefficients calculated from the release of sodium diclofenac from liquid crystalline gel across Visking membrane*

Initial Drug Loading (M)	*Rate Constant* (10^{-4} hr^{-1})	*Diffusion Co-efficient* (10^{-7} cm^2 s^{-1})
0.1	9.20	1.60 ± 0.15
0.05	3.69	0.44 ± 0.03
0.01	5.54	0.90 ± 0.08

It is evident from the rate constant values that the release is not first-order as there is considerable variation in the data. This further confirms the matrix diffusion control hypothesis for the release of sodium diclofenac. The diffusion co-efficients are within acceptable limits.

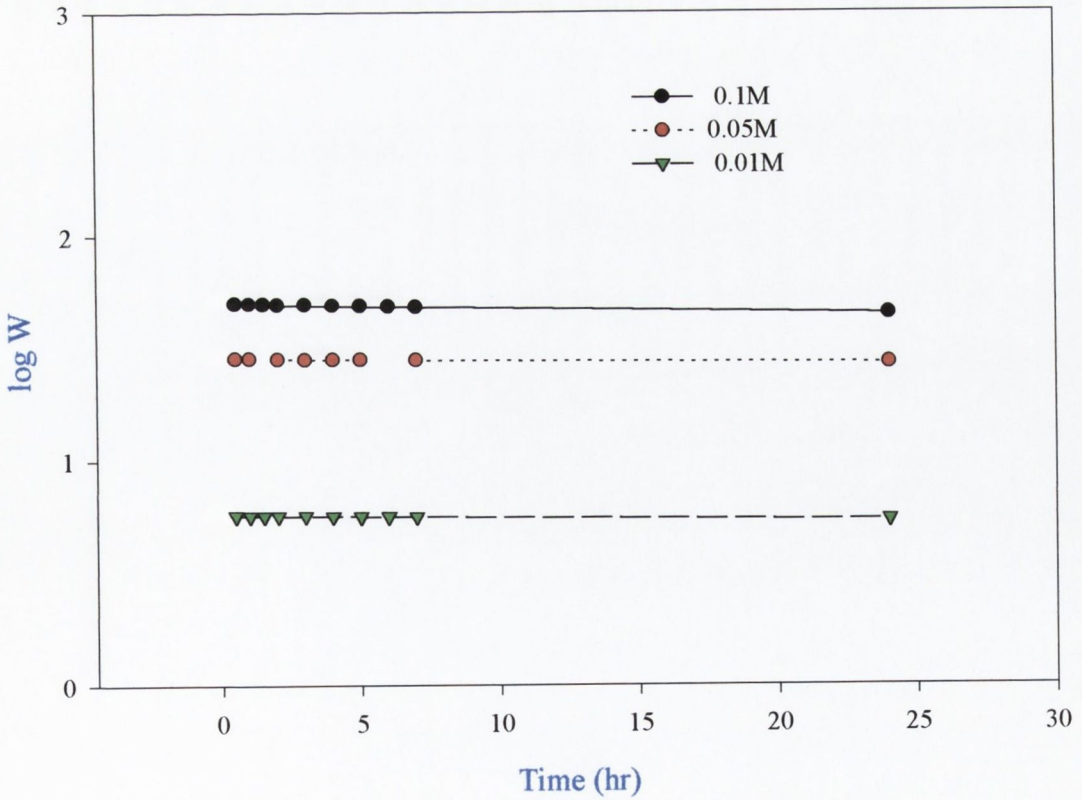


Figure 3:3:2 *Log W (quantity of drug remaining in the vehicle) versus time for the release of sodium diclofenac from a liquid crystalline vehicle, across a Visking membrane*

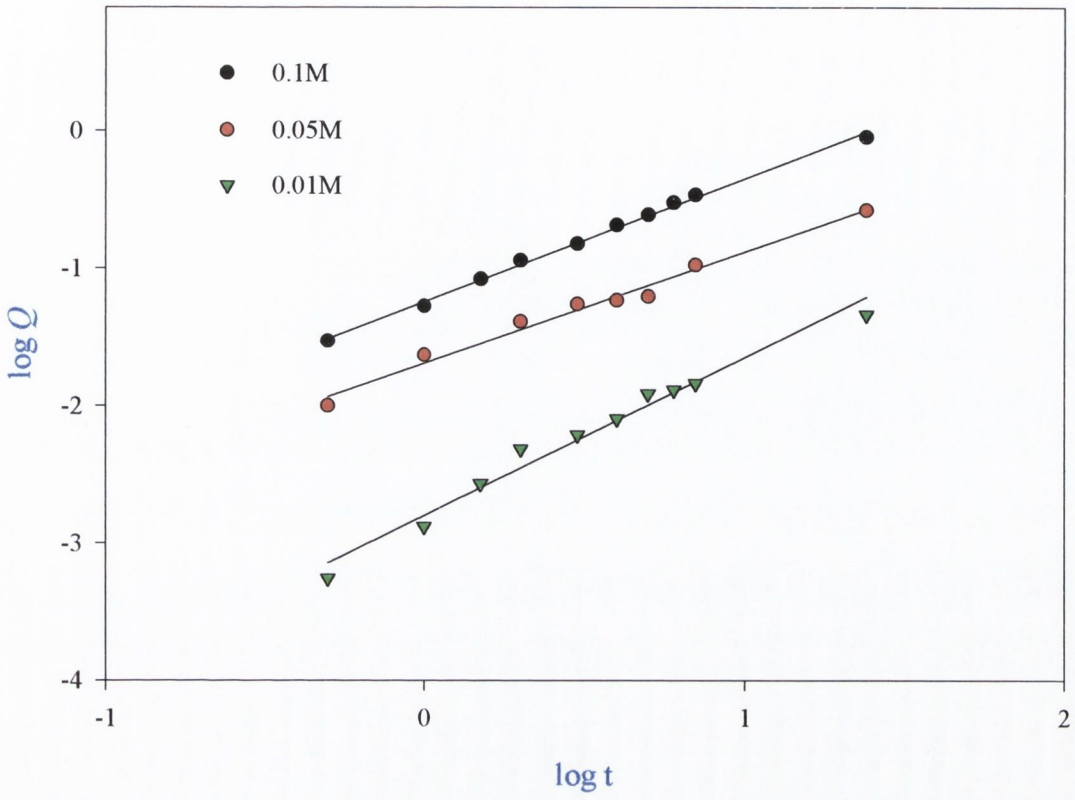


Figure 3:3:3 Log Q (quantity of drug released) versus log time for the release of sodium diclofenac from a liquid crystalline vehicle

Analysis of the relevant data reveal that the release kinetics conform to matrix control for all initial drug loadings of sodium diclofenac. Although the data is linear for first-order kinetics (Figure 3:3:2), analysis of the data indicates the release conforms to matrix controlled diffusion tending towards zero-order.

Arellano *et al.*, (1998) reported that the diffusion of sodium diclofenac across a synthetic membrane (0.2μm cellulose nitrate) from a binary system of carboxypolymethylene (Carbopol 940) / distilled water was best described by the Higuchi diffusion model. They reported a maximum release rate of 5.8×10^{-3} mg cm^{-2} min^{-1} compared to a release rate of 3.27×10^{-4} mg cm^{-2} min^{-1} from the myverol / water binary system used in this study. The reason for a ten-fold difference in release rates could be attributed to a larger ratio of aqueous phase in the carbopol system. Their systems contained 98% water w/w compared to 29% w/w for myverol systems. This increased ratio insures a greater proportion of sodium diclofenac is dissolved in the aqueous phase giving a more rapid and significant release.

Ho *et al.*, (1994), also reported the diffusion of sodium diclofenac across a synthetic membrane (Durapore) from a binary system of carbomer 940 and water with a 1:90 ratio, respectively. They used 10% triethanolamine as a gelling agent and also found that the release was best described by the Higuchi model. A release rate of 14.66 mg cm^{-2} min^{-1} was reported. Fang *et al.*, (1999) also make mention of sodium diclofenac adhering to matrix controlled release in the absence of rate-limiting membranes from aqueous / polymer formulations.

Another factor which may influence the release profile of a drug is the viscosity of the matrix. Mockel and Lippold (1993), investigated the release kinetics of proxyphilline from hydrocolloid matrices. The mechanism of release from the matrices depended on their viscosities. Gels of low viscosity exhibited zero-order release kinetics whilst those of higher viscosity adhered to matrix diffusion control.

By substituting the calculated rate constants of sodium diclofenac into the matrix diffusion equation (Equation 3.2), theoretical release profiles may be calculated.

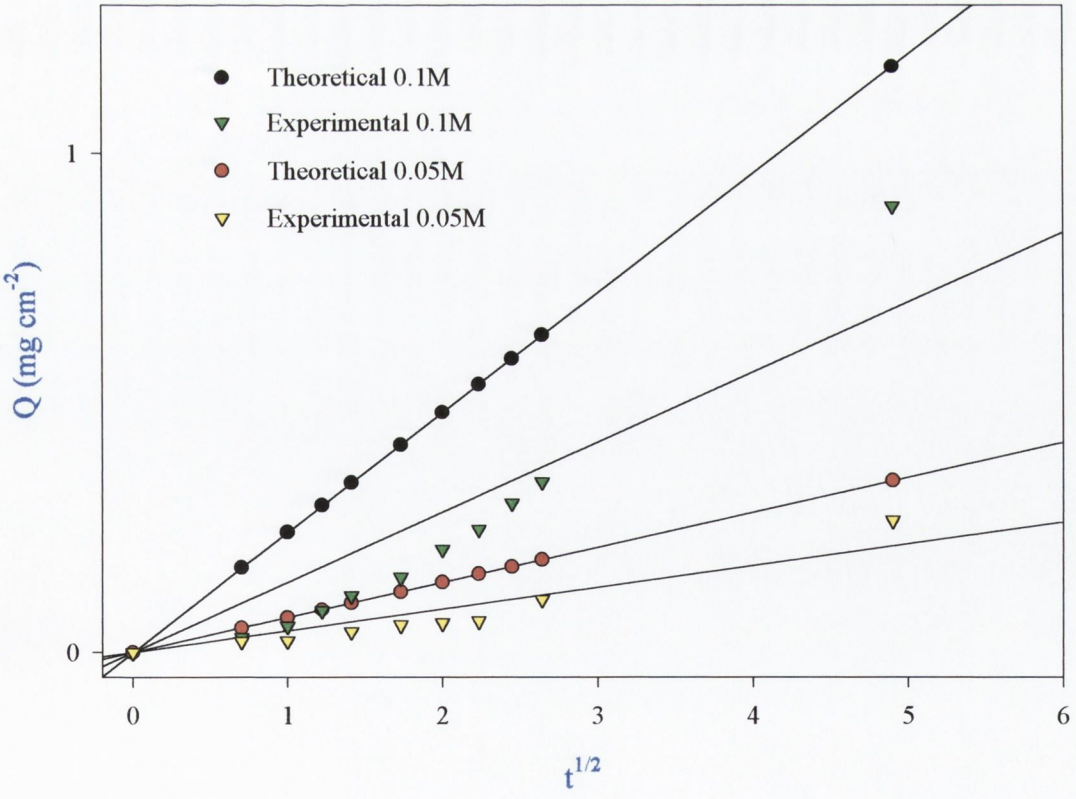


Figure 3:3:4 *Comparison of theoretically calculated profiles and experimental profiles for the release of sodium diclofenac from liquid crystalline gels.*

Theoretical profiles generated in this manner are compared to experimental profiles in Figure 3:3:4. The graph shows that experimental release falls below that calculated theoretically for both concentrations. This is the opposite to that observed by Nolan (1995) for the salbutamol base comparisons where the experimental release was ~10% greater than that calculated theoretically in the same manner.

The initial drug concentration can have an effect on the release kinetics exhibited by a drug. Burrows *et al.*, (1994), found that the release of atenolol from systems of concentrations up to 10% w/w could be fitted to both diffusion controlled or first-order kinetics. The release of atenolol at initial drug loading of concentration of 15 to 20% w/w could be fitted to zero-order release model.

3:4 Effect of Buffering Vehicle and/or Receptor Medium

As subsequent *in-vitro* investigations will involve the use of excised SC, it was necessary to assess the release characteristics of the model drug under physiological conditions. These were mimicked by buffering the delivery vehicle and/or the receptor medium. Isotonic phosphate buffer (pH=7.2) was used throughout the investigation. The respective data are tabulated in Table 3:4:1 and reveal that the release of sodium diclofenac was greatest when the vehicle and receptor medium were not buffered. There was a minimal decrease in the release when the aqueous phase of the vehicle was buffered and an almost threefold decrease when the receptor medium was buffered.

Table 3:4:1 Effect of buffering vehicle and/or receptor medium

Aq. Gel/Receptor Medium	*% Release after 7 hr*	*% Release 24 hr.*
H_2O/ H_2O	1.70	4.50
Buffer/H_2O	1.44	3.27
H_2O/Buffer	0.63	1.80
Buffer/Buffer	0.57	1.40

- Triply distilled water was used in all experiments and for the preparation of buffer solutions.

In contrast, the above trends are exactly opposite to those found for cationic drug (Salbutamol) release (Nolan, 1995). In that case buffering the aqueous phase of the vehicle resulted in a minimal increase in release and when the receptor medium was also buffered there was a dramatic increase in the release. The trend continued for up to 72 hours.

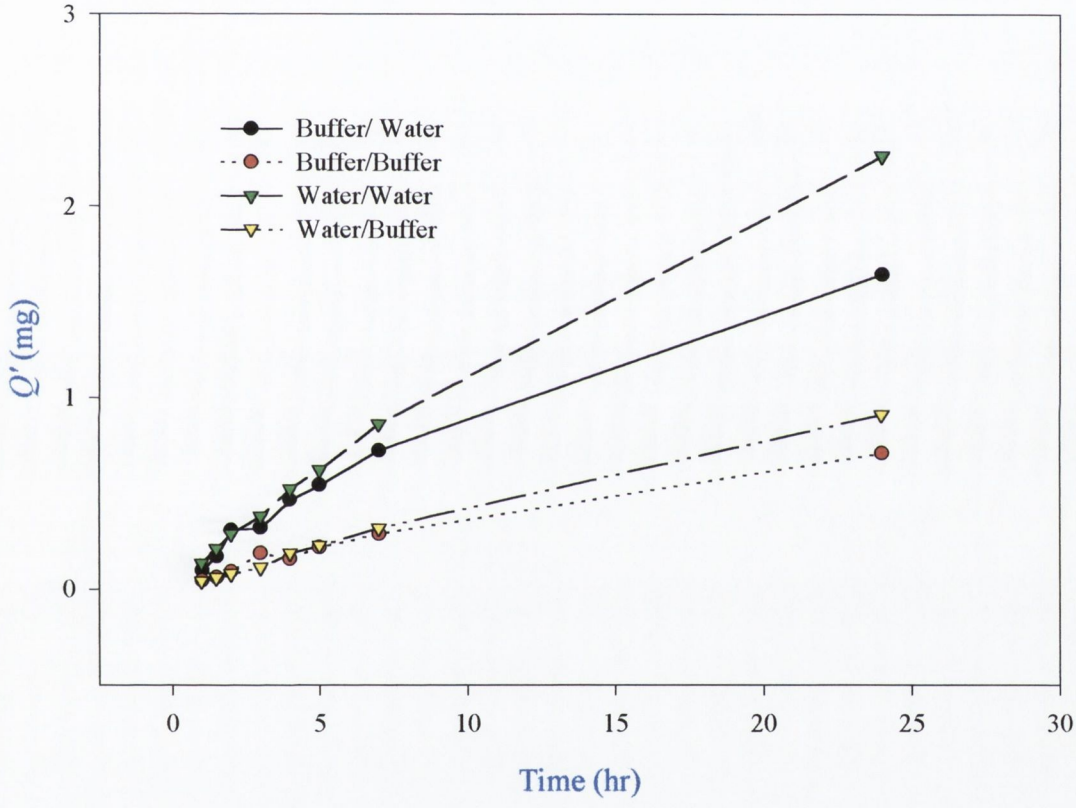


Figure 3:4:1 *The effect of buffering aqueous phase of vehicle and/or receptor medium. The insert shows the presence of buffer in the aqueous phase of the vehicle and/or in the receptor medium. All concentrations 0.1M sodium diclofenac.*

The effect of buffering the system on the release rates is illustrated in Figure 3:4:1. The release characteristics for diclofenac and salbutamol base, from the same vehicle have been shown to be distinctly different. After a period of 24hrs the release of sodium diclofenac at 4.50 % is approximately one sixth of that recorded for salbutamol base at 26.5%. The reasons for such a difference in passive release profiles

may be explained by factors such as lipophilicity, charge on drug ions, solubility, pK_a values and partition coefficients. For example, the lower partitioning of diclofenec into buffer than water may be due to several factors. When the aqueous phase of the gel is buffered there is an ion gradient between the two phases against which the drug must work. The concentration gradient favours transport of the drug in the required direction, as it is dissolved in the oil phase and must diffuse into the aqueous phase before being released. The presence of sodium ions in the buffered aqueous phase may inhibit the free dissociation of sodium diclofenac from the oil phase due to sodium being the main counter ion to diclofenac.

In the case of salbutamol base its octanol/water partition coefficient was determined to be 0.43, i.e., it is almost 2.5 times more soluble in water. Salbutamol in its basic form is cationic which will give it hydrophilic properties. Its pK_a is 9.5 which indicates it is not appreciably dissociated at pH = 7.0. Salbutamol sulphate partitions into water more strongly than does its basic form. Carr (1992), observed a greater release of salbutamol sulphate passively than was observed for salbutamol base by Nolan (1995).

Despite its ionic nature sodium diclofenac is more soluble in octanol than water. This may be due to the difficulty of the salt to dissociate in a lipophillic environment and also because the undissociated salt is neutral and well solvated in the neutral myverol phase. Fini *et al.* (1996), found that by changing the counter ion of diclofenac the partition coefficient changes in favour of water. Using a diethyl amine cation they found a coefficient of 1:1. It may be that the larger the counter ion the easier it is for the salt to dissociate. If this is the case it may explain why the sulphate salt of salbutamol dissociates so well in comparison with the sodium salt of diclofenac.

3:5 Effect of Gel Preparation and Vehicle Composition

The preparation of the liquid crystalline gel has been previously described in the experimental section (Chapter 2). This section details how the sodium diclofenac was dissolved in the myverol phase before the addition of water. To gain more information of the release characteristics of the gel the reverse procedure was also

carried out, i.e., the sodium diclofenac was dissolved in the aqueous phase before it was added to the myverol.

The results of this investigation are shown in Figure 3:5:1. The main conclusion of this experiment is that there is no significant difference in the cumulative release from the vehicle regardless of the phase in which the drug is initially dissolved. This was a surprising result as it was thought the lack of dissociation of the salt within the vehicle was a factor in the comparatively low percentage of release from the vehicle.

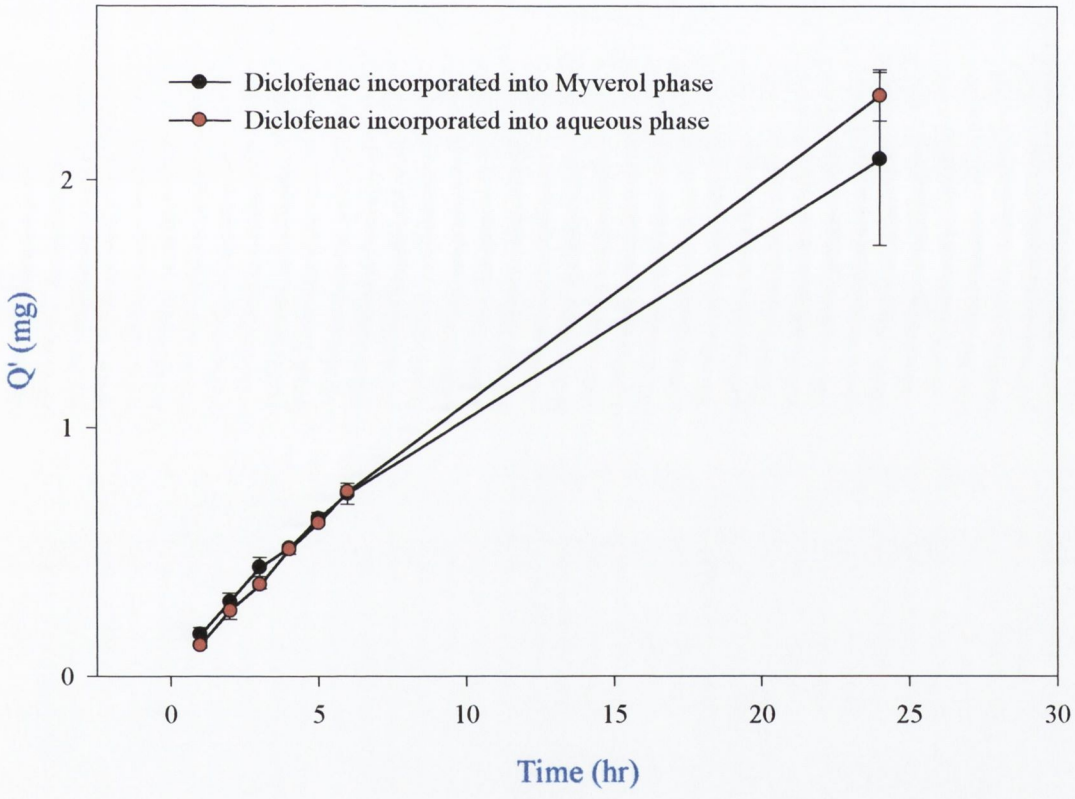


Figure 3:5:1 *Effect of initially incorporating the sodium diclofenac into the different phases of the liquid crystalline gel on its release characteristics when preparing the vehicle. For clarity just two of each experimental profiles are shown.*

The deviation at the lowest time interval (24hr) in Figure 3:5:1 is most likely due to experimental error such as an accumulation of air under the gel during the overnight duration of the experiment. It would seem that regardless of whether the drug salt is dissociated or not that the drug encounters difficulty in diffusing from the vehicle.

The addition of a third component (drug) to these liquid crystalline systems often results in a modification of the phase properties of the system, with possible consequences for solute release. Collete *et al.*, (1990), identified the release from liquid crystalline gels as a two stage process, and a three stage process if the gel was saturated. Saturation need not be considered in respect of the sodium diclofenac investigations as 0.1M concentration is equivalent to 0.05% w/w and the drug was still found to be soluble at concentrations more than twice this level. Stage one is the diffusion of the drug through the liquid crystalline sample and the second is the diffusion of the drug away from the sample surface into the receptor medium. As the diffusion of sodium diclofenac follows the same release profile as other model drugs it can be assumed that it follows the two-stage process of release in a similar manner. This investigation adds further evidence to the postulate that the immobilisation of sodium diclofenac within the liquid crystalline gel maybe due to the comparatively large solvation radius of the drug as calculated from the Stoke-Einstein equation (Equation 3.8).

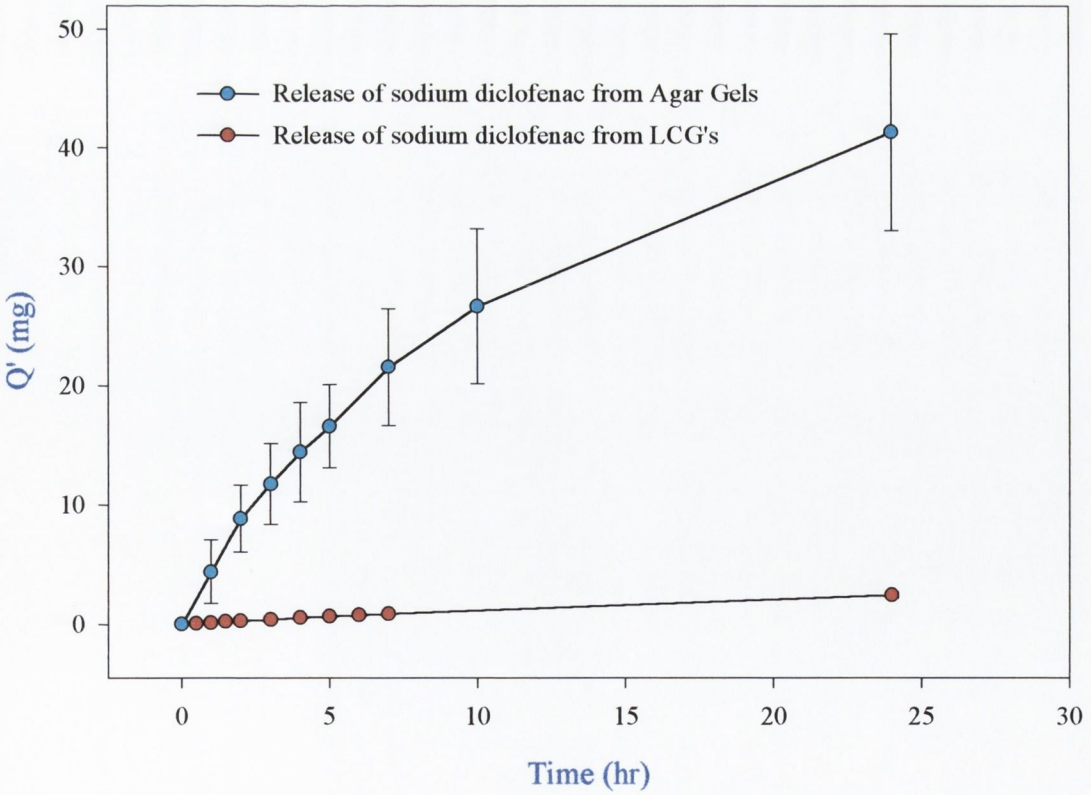


Figure 3:5:2 *Comparative release of sodium diclofenac from Agar and liquid crystalline gels over a 24hr period.*

In comparison, the release of sodium diclofenac from another vehicle type was investigated. Purified agar gel was selected due to the considerable experience with its use in the laboratory by Bannon *et al.*, (1989), Foley (1991) and Carr (1992) to investigate other model drugs. The release profile of sodium diclofenac from Agar gels is shown in Figure 3:5:2. where it is compared with its analogous release from the liquid crystalline gel.

As can be seen from Figure 3:5:2 the release of sodium diclofenac from agar gels is very significantly greater than from the liquid crystalline gels. The quantitative release from Agar is between 70.5-88.0% compared to 4.5% from a liquid crystalline vehicle over the same time period of 24 hrs. The significant standard deviation of 7.5% of sodium diclofenac release may be as a result of varying degrees of evaporation from the largely aqueous Agar gels.

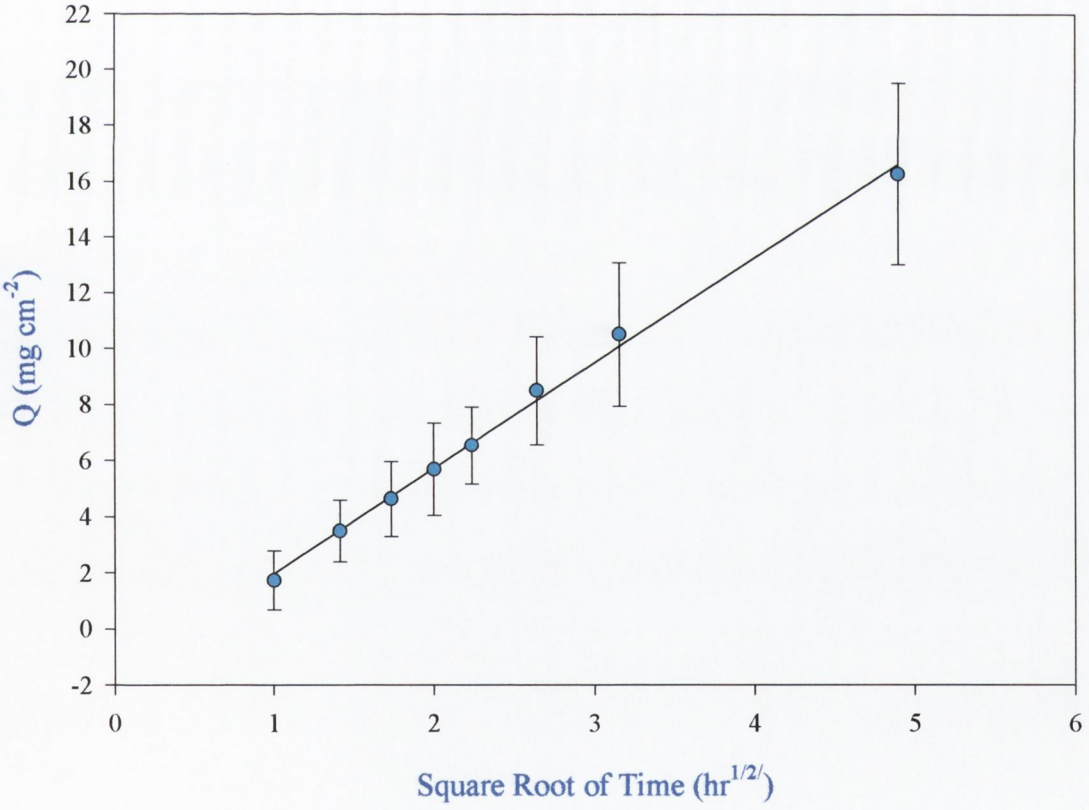


Figure 3:5:3 *The release data of sodium diclofenac from Agar gel plotted in accordance to the Higuchi diffusion model. All concentrations 0.1M.*

The aqueous nature of the Agar gel may account for the significant release of sodium diclofenac. Agar is distinguished physically by its gelling temperature and chemically by its methoxy content. Agar is a complex mixture of polysaccharides of which the gelation involves a shift from a random coil to a double helix to bundles of helices in the final stage forming a network through which the diffusion of a solute is facilitated.

The release data of sodium diclofenac from Agar gel also conform to the Higuchi matrix diffusion model as shown in Figure 3:5:3. An estimated lag time of 15 minutes was obtained from the intercepts on the y-axis. The data yielded a diffusion co-efficient of 3.1×10^{-6} cm^2 s^{-1}, which is one of the largest figures reported from this laboratory for any drug released from an Agar gel.

A comparison of diffusion co-efficients obtained for anionic, cationic and neutral drug molecules from liquid crystalline gels and Agar gels are is given in Table 3:5:1.

Table 3:5:1 *Diffusion Co-efficients of Cationic, Anionic and a Neutral Model Drug from Liquid Crystalline Gel and Agar Gel. All initial drug loadings are 0.1M.*

Model Drug	*Diffusion Co-efficient from Liquid Crystalline Gel* (10^{-7} cm^2 s^{-1})	*Diffusion Co-efficient from Agar Gel* (10^{-7} cm^2 s^{-1})
Sodium diclofenac (anionic)	1.87	31.0
Salbutamol Sulphate (cationic)	9.70[a]	9.33[b]
Nicotine (neutral)	3.04[a]	26.0[b]

- *a = data obtained from Carr et al., (1997)*
- *b = data obtained from Bannon (1989)*

No statistical conclusions can be drawn from the data in Table 3:5:1. More data would be required to carry out a one-way ANOVA (analysis of variance) hypothesis test. It is interesting to note that diclofenac and nicotine have rather similar diffusion co-

efficients from the same vehicles whereas salbutamol sulphate has almost identical diffusion co-efficients for both vehicle types.

It can be expected that both salbutamol sulphate and sodium diclofenac will both remain in the ionic state as they have pK_a values of 9.5 and 4.0 respectively. As these differ from the vehicle pH (~7.0) by more than 1.3 units both species would be expected to be ionised, (Padmanabhan and Surnam, 1991).

The data in Table 3:5:1 show that both sodium diclofenac and nicotine have low diffusion co-efficients from the binary myverol/water systems but would exhibit significant release from an aqueous environment. The diffusion co-efficient of salbutamol sulphate from Agar is marginally less than that from the liquid crystalline vehicle. Further investigations are required to identify the main parameters governing the release of model drugs from aqueous and binary systems.

The following section will further characterise the release of various anionic drugs from liquid crystalline gels. The objective is to further delineate the roles of solubility, charge, pK_a and molecular size on release profiles.

3:6 Comparison of Release Profiles of a Range of Anionic Drugs.

Due to the low diffusion co-efficient of sodium diclofenac and nicotine (Carr 1997) from myverol/water liquid crystalline vehicles, it is worthwhile investigating the release of similar anionic model compounds in order to ascertain whether this type of vehicle is generally suitable for use as a delivery vehicle.

The model drugs chosen for comparison were the sodium salts of Indomethacin, salicylate and benzoate. These molecules differ in physicochemical characteristics such as molecular weight, partition co-efficient, pK_a and solubility but all are related in having negatively charged anions when dissociated. The release profiles of these compounds in comparison to that of sodium diclofenac are shown in Figure 3:6:1. The data in Table 3:6:1 indicates, from a quantitative viewpoint, that sodium indomethacin has a very significantly greater release than that of the other model compounds. From the viewpoint of percentage release, sodium diclofenac would appear to be anomolous. The question is which of these results is indicative of the behaviour of the release of an anionic drug from a liquid crystalline vehicle? The three compounds in Table 3:6:1 other than sodium indomethacin could be viewed as

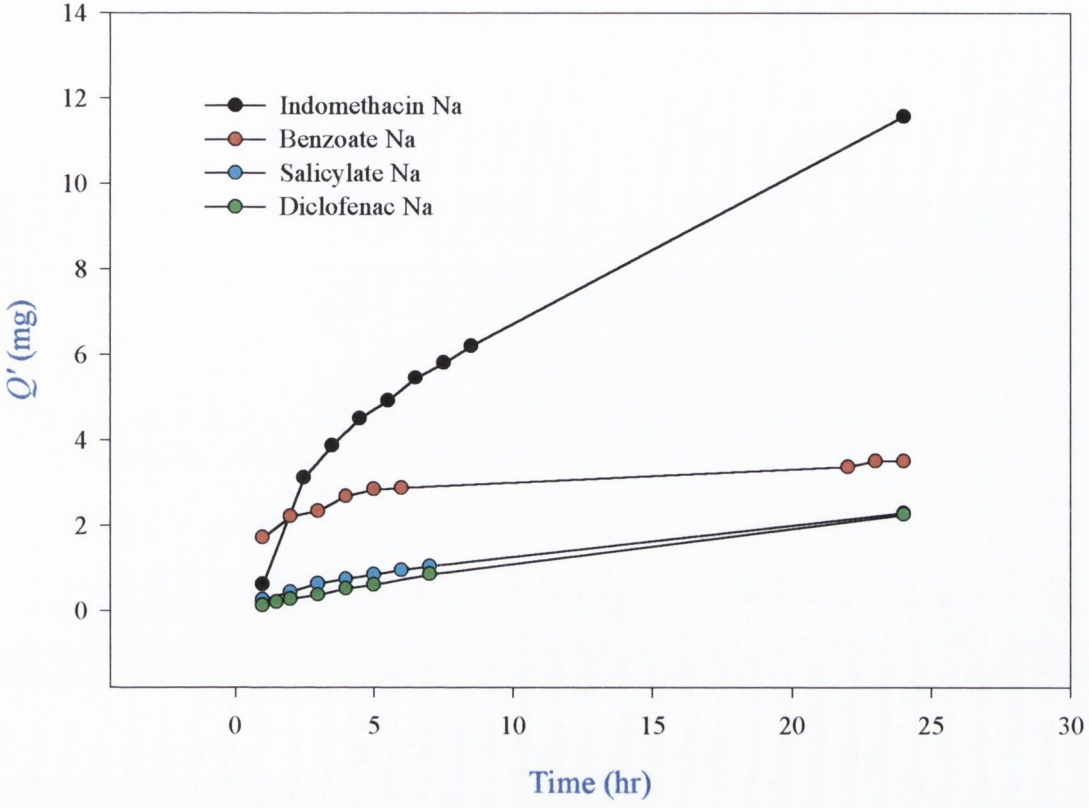


Figure 3:6:1 *Comparison of the Release of Anionic Drugs from the Liquid Crystalline Vehicle. Data for Sodium Benzoate and Sodium Salicylate were obtained from Lynn (2000) and Cadre (1999), respectively.*

being related in a physicochemical manner by one or more properties yet to be defined. The result of the similarities lead to a fixed quantitative release over a set time period regardless of concentration below saturation state, as shown in column three. On the other hand the percentage release data clearly indicate that the three compounds, other than sodium diclofenac in Table 3:6:1 are related and in this latter scenario the similarities are concentration dependent. The former suggestion is less likely to be representative of actual processes taking place, particularly in light of previous experiments which have shown the effect of initial drug loading on cumulative release. In brief, the evidence would suggest concentration dependent release profiles and the fact that three of the drugs have similar quantitative release maybe coincidental taking into account molecular weight differences.

No direct correlation could be made between partition co-efficient and percentage release, e.g., sodium indomethacin has the lowest log P value and the highest diffusion co-efficient yet is third on the percentage release scale.

Table 3:6:1 *Quantitative Release Data and Diffusion Co-efficients of Anionic Drugs from Liquid Crystalline Vehicles with an initial drug loading of 0.1M.*

Model Drug	*Diffusion Co-efficient* (10^{-7} cm^2 s^{-1})	*% Release of Initial Loading after 24hr*	*Quantitative Release after 24hr (mg)*
Na Benzoate	0.12	25.2	3.51
Na Salicylate	0.37	26.0	2.29
Na Indomethacin	1.50	19.1	11.6
Na Diclofenac	1.02	4.0	2.27

It is evident that sodium diclofenac has the smallest percentage release from myverol/water liquid crystalline vehicles. The reasons for this are probably many. The results of this investigation can only serve to eliminate some of the more obvious parameters such as pK_a, partition co-efficient, concentration, molecular weight and solubility from the list of potential factors which may inhibit the release of diclofenac. Factors, which have not been eliminated, are complexation of some form or ion–paring which would change the properties of the drug making it more stable within the cubic phase of the matrix.

Chapter 4

In–vitro Studies of the Transport of an Anionic Drug from a Liquid Crystalline Gel Incorporating Chemical Enhancers Across a Synthetic Membrane

Chapter 4

In–vitro Studies of the Transport of an Anionic Drug from a Liquid Crystalline Gel Incorporating Chemical Enhancers Across a Synthetic Membrane

4:1 Introduction

The objective of the investigations described in this chapter is to evaluate the release characteristics of anionic drug molecules from a liquid crystalline gel incorporating a chemical enhancer across a Visking membrane. There is special reference to sodium diclofenac as in previous experiments.

Earlier preliminary experiments by Nolan (1995) found that the combined enhancement of sodium diclofenac by iontophoresis and by the addition of oleic acid to the gel were unsuccessful. Because of these negative results it was decided that an alternative enhancer would be selected in the place of oleic acid for the purpose of current investigations. It was assumed that the enhancement properties of oleic acid in relation to salbutamol were due to the opposite charge exhibited by the drug and the enhancer when ionised under vehicle conditions (pH = 7.2). This assumption was the main criterion for the selection of the new model enhancer. A molecule with similar properties to oleic acetate was sought with the exception that it should have a positive head group instead of a negative one. As oleic acetate possessed a long alkyl chain so too should the selected molecule for investigations involving anionic diclofenac. It was decided to proceed with derivatives of benzalkonium chloride which best fit the selection criteria of the desired molecule. The derivatives chosen are shown in Figure 2:2:2 (p80). The molecules are tertiary ammonium salts which vary in counter ion type and in chain length. The main focus in the investigation was on benzyl dimethyldodecylammonium bromide (BDDAB) which has a chain length of twelve carbons. The other molecule used was benzyldimethylhexadecylammonium chloride (BDHAC) which has four additional carbons in its chain length and also differs in its counter ion.

It was likely that the molecules chosen using the selection criteria, would be classified as surfactants which are known to have permeation enhancement characteristics, (Ruddy, 1995). Benzalkonium chloride and its derivatives are also considered clinically safe and are use as anti-spermicides in commercially available preparations and birth control products.

4:2 *The Passive Release of Anionic Drug Molecules from a Liquid Crystalline Gel Incorporating Benzalkonium Chloride Derivatives*

The model enhancers at a concentration of 0.1M were incorporated with the drug into the hydrophobic region of the vehicle during its preparation. The investigation first involved the use of the simple experimental protocol used in previous passive studies with water as the gel solvent and receptor medium. The study was then extended to assess the effect of including buffer ions in both these parts of the system. Physiologically adjusted buffer (pH = 7.4) was used throughout the study. The experimental conditions are summarised in Table 4:2:1.

Table 4:2:1 *Possible choices with respect to the use of the buffer solution*

Protocol	*Aqueous Phase of Gel*	*Receptor Medium*
1	H_2O	H_2O
2	H_2O	Buffer
3	Buffer	H_2O
4	Buffer	Buffer

Figure 4:2:1 shows the cumulative amount of sodium diclofenac released from the liquid crystalline vehicle containing 31.8 mg cm^{-3} of drug and 0.1M BDDAB. For comparative purposes the drug release rate from the standard vehicle, without any BDDAB is also shown. The stoichiometric ratio of drug to enhancer is 1:1. It is evident that the presence of BDDAB in the vehicle has a substantial effect on the

release of the drug. As both the drug and enhancer are incorporated in their respective salt forms it is expected that both will be fully ionised when diffusing through the aqueous phase of the gel. This introduces the possibility of ion-pairing between the oppositely charged monovalent ions present in the vehicle. Both the drug and trial enhancer each bear one charge centre and consequently the association should result from a 1:1 interaction. An ion-pair association of this type in effect increases the lipophilic properties of the drug, which is of benefit with regard to transdermal drug delivery. However, it would appear that the apparent ion association between anionic

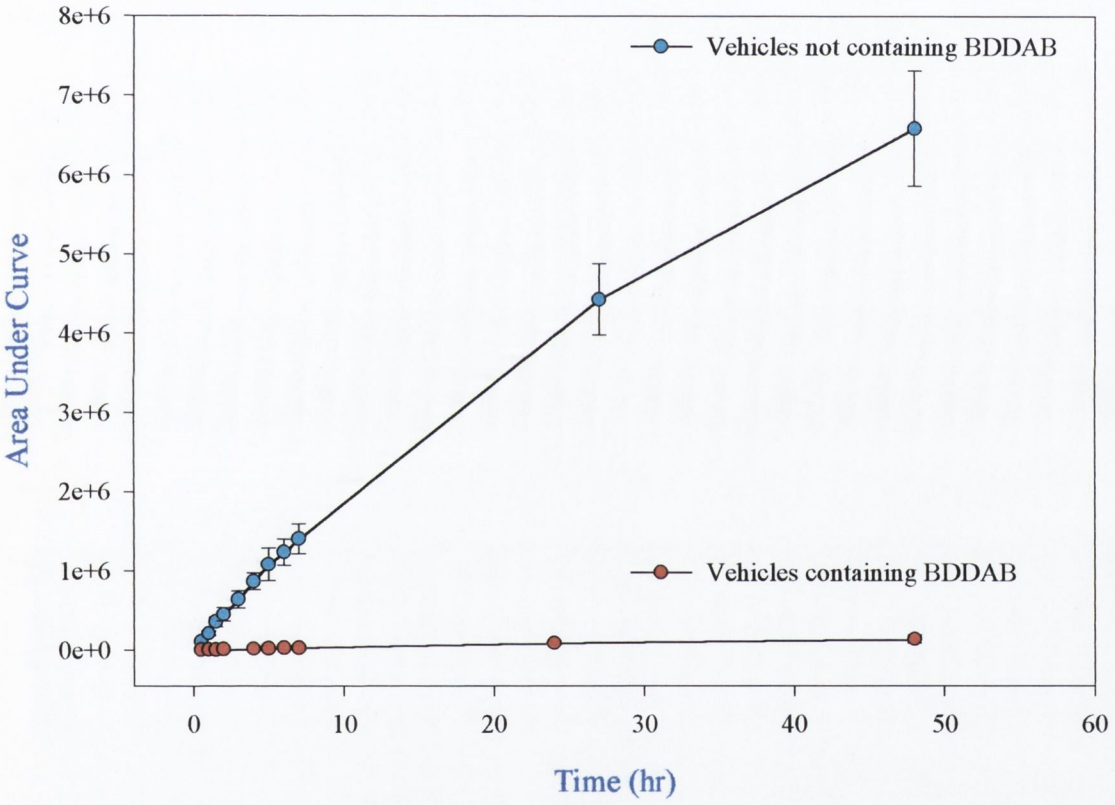


Figure 4:2:1 *The Comparative Release of Diclofenac from a Liquid Crystalline Gel with and without BDDAB. (Water/water system) (1mg = 1619629.4 AUC)*

sodium diclofenac and cationic $BDDA^+$ also reduces to almost zero the concentration of drug which can diffuse across the Visking membrane and into the aqueous receptor medium.

Nolan (1995) also encountered a similar result during studies of the passive release of salbutamol base (drug) and oleic acid (enhancer) from the same liquid

crystalline gel. Because an acid and a base were used in the investigation, total dissociation of either the drug or the enhancer were not expected at vehicle pH. It was observed that the quantity of salbutamol base that associated with oleic acid was approximately 66% and that the rate of release had decreased by a factor of three. The release of sodium diclofenac maybe affected dramatically due to a greater association between ions of opposite charge and increase number of ions from the dissociation of salts rather than incomplete dissociation of a weak acid and base. The results reflect this expectation as shown in Figure 4:2:1. The release of sodium diclofenac was estimated to be 0.1% of initial drug loading after 24 hours and this can be regarded as negligible in terms of passive release.

Despite the previous conclusive results of negligible release for the trial enhancer it was decided to investigate whether incorporating buffer ions into the vehicle could interrupt the association of drug ions and BDDAB. The experimental

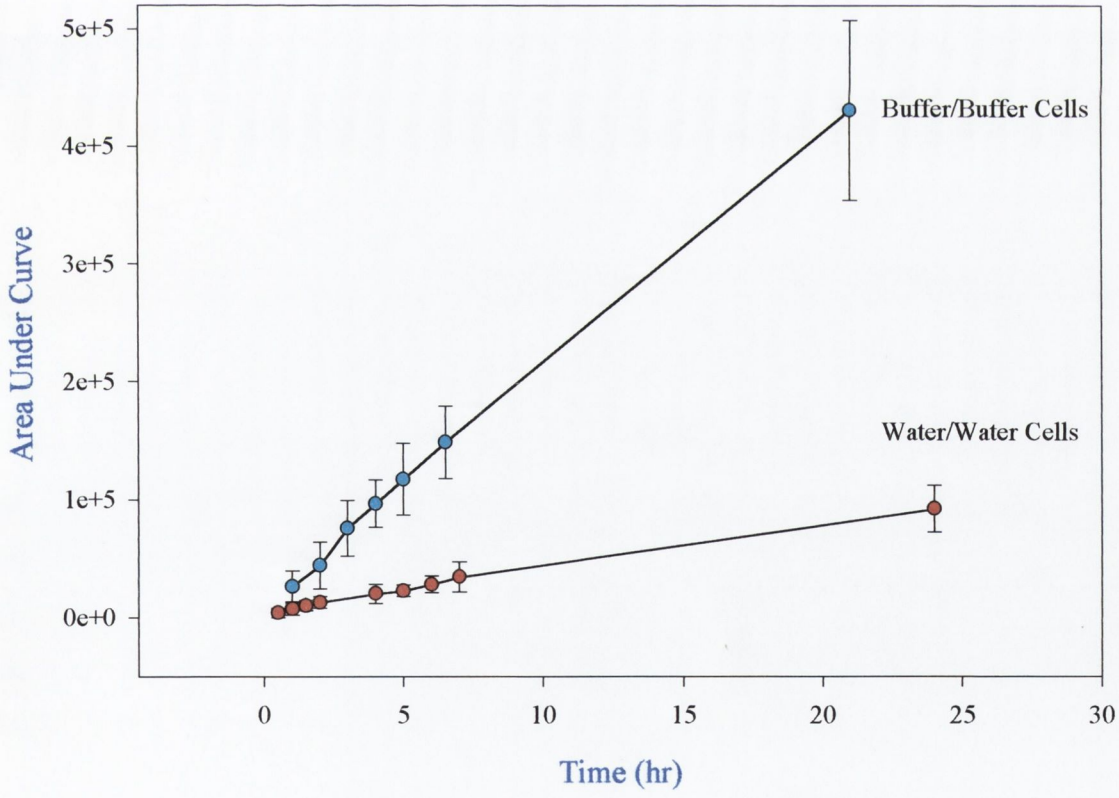


Figure 4:2:2 *The Effect of Buffering both the Aqueous Gel and Receptor Medium on the Release of Sodium diclofenac from a Liquid Crystalline Gel Incorporating BDDAB compared to the release rate using only triply distilled water. (1mg = 1619629.4 AUC)*

protocol is number 4 of Table 4:2:1. The most significant difference in quantitative release is evident in the comparison of water/water system compared to buffer/buffer system and is shown in Figure 4:2:2.

Although the release from the buffer/buffer system in Figure 4:2:2 appears significant in comparison to the release from the water/water system, the area under the curve for maximum release still only represents half of one percent release of the initial drug loading.

Introducing buffer ions into the aqueous phase of the gel and/or receptor medium increases the number of extraneous ions that can associate with the drug or enhancer. Table 4:2:2 contains the data from the experimental protocol of Table 4:2:1.

Table 4:2:2 *Effect of Buffering Aqueous Gel and/or Receptor Medium on the % Release of Sodium diclofenac From a liquid Crystalline Gel Incorporating BDDAB.*

Protocol	*% Release after 7 hr*	*% Release after 24 hr*
1	0.04	0.11
2	0.19	0.60
3	0.14	0.47
4	0.24	0.60

In general the more buffer that was added to the system the greater the release of drug. Overall the buffer/buffer system containing the model enhancer BDDAB showed a 78% decrease in the amount of sodium diclofenac detected in comparison to the same system without BDDAB, whereas the water/water system showed a 98% decrease in comparison to the same system without BDDAB. The effect of including buffer ions in the system is thus to increase the release of diclofenac by a ten-fold margin. However, passive investigations in section 3:4 have already shown that the inclusion of buffer in the system inhibits the release of sodium diclofenac. Data in Table 3:4:1 showed that buffer decreased the release of diclofenac by a factor of three. Buffer has thus been shown to have two effects in the system so far, one effect is to inhibit the release of the drug by reducing the ion gradient between the phases of

the vehicle which is non-beneficial and the other effect is to reduce ion-pairing in the presence of the enhancer which is beneficial.

In order to further explore ion-pair formation a different model enhancer was used in place of BDDAB. It was expected that the incorporation of BDHAC as a model enhancer would have the same effect on the system. The dissociation of the chloride salt produces cations with which diclofenac anions may be expected to pair. The release profiles of sodium diclofenac with and without BDHAC in the vehicle is shown in Figure 4:2:3. The graph shows that the inclusion of BDHAC in the vehicle significantly inhibits the release of sodium diclofenac. This reduction in the cumulative release of sodium diclofenac is also thought to be due to ion-pair formation within the vehicle. Due to the 1:1 ratio of drug to model enhancer the ion-pair association is almost total. The inclusion of BDHAC in the vehicle accounts for a 93.2% decrease in quantitative release in comparison to a 98% reduction by BDDAB in a similar system. A comparison of release profiles for both model enhancers with sodium diclofenac are shown in Figure 4:2:4

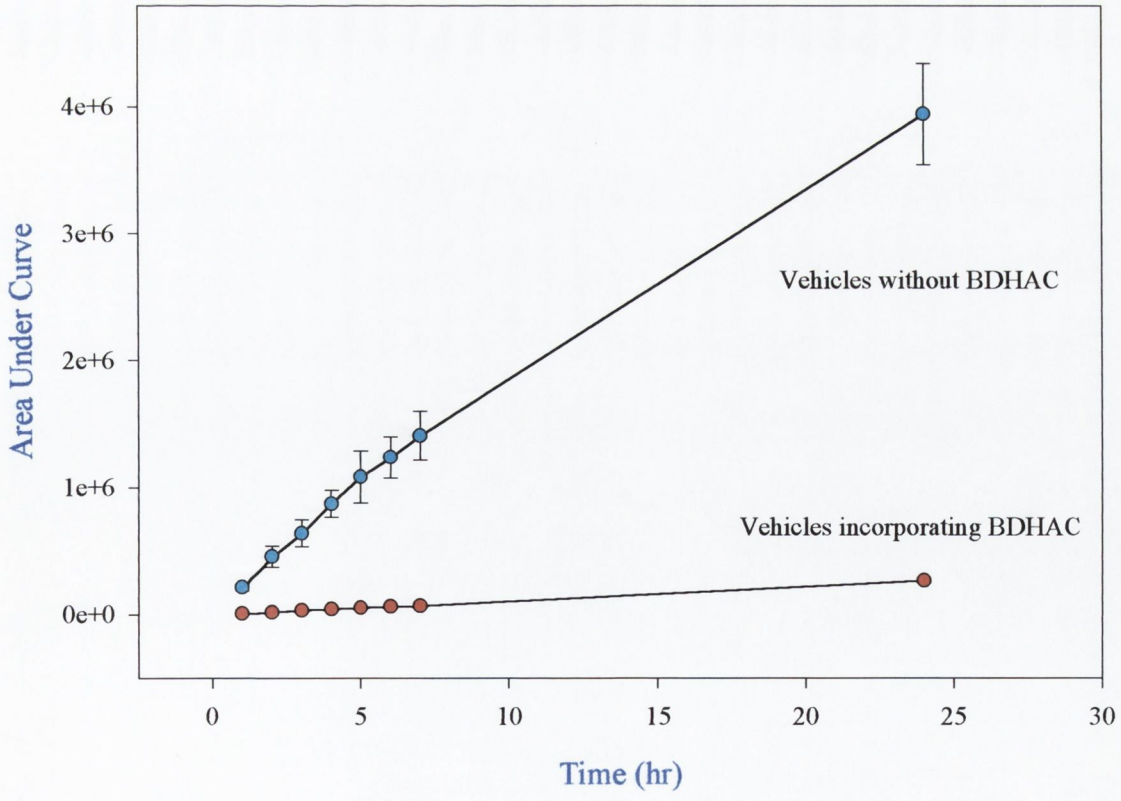


Figure 4:2:3 *Comparison of the Passive Release of Sodium diclofenac from the Liquid Crystalline Gel with and without BDHAC. (1mg = 1619629.4)*

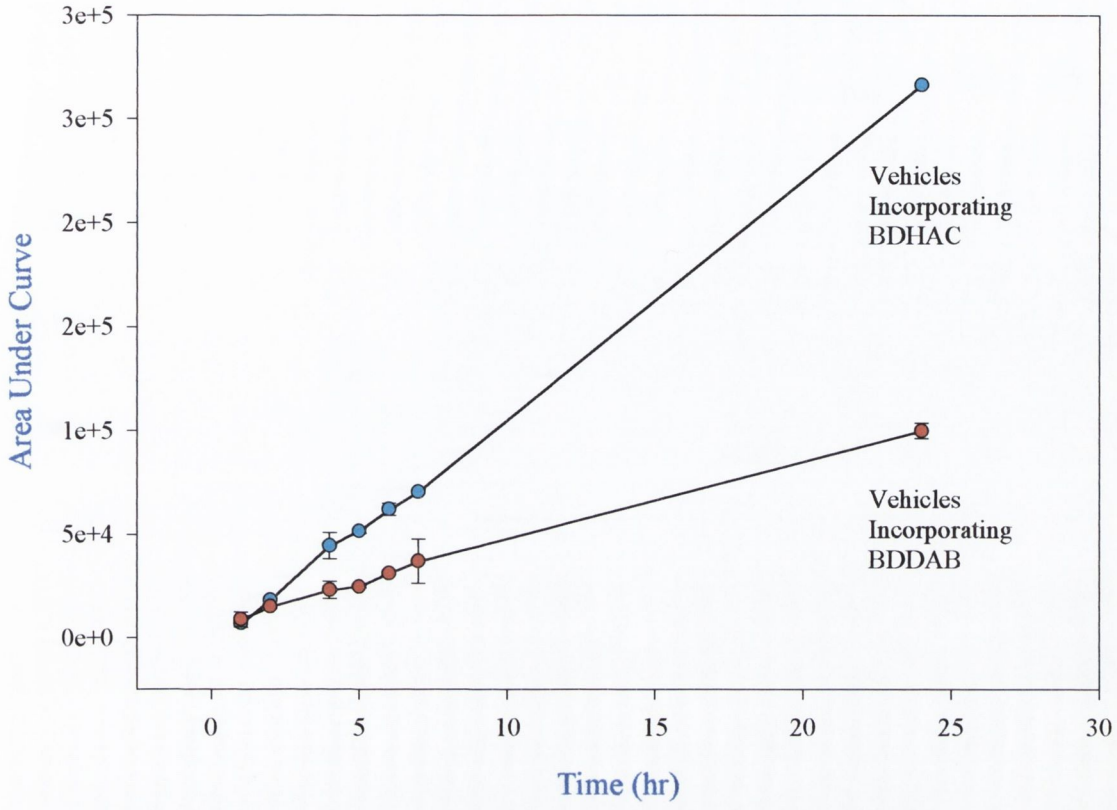


Figure 4:2:4 *Comparison of the Passive Release of Sodium Diclofenac from a Liquid Crystalline Gel Incorporating BDDAB or BDHAC. (1mg = 1619629.4 AUC)*

4:3 Effect of Vehicle Preparation and Ratio of Enhancer to Drug

The results of incorporating the long-chain benzalkonium chloride derivatives into the liquid crystalline vehicles are conclusive from the point of view that the release of sodium diclofenac is reduced to being almost negligible because of ion-pair formation.

If both drug and enhancer could diffuse from the vehicle there may still be an enhancing effect, if the enhancer reduces the barrier properties of the skin or ion-pairs with the drug, making it more lipophilic and increasing its skin permeability. Further investigations were undertaken to illustrate the effect of incorporating the drug in the aqueous phase and the enhancer into the myverol phase of the vehicle in an attempt to delay ion-pair association before diffusion of the drug or enhancer from the vehicle. The results of this investigation are shown in Figure 4:3:1.

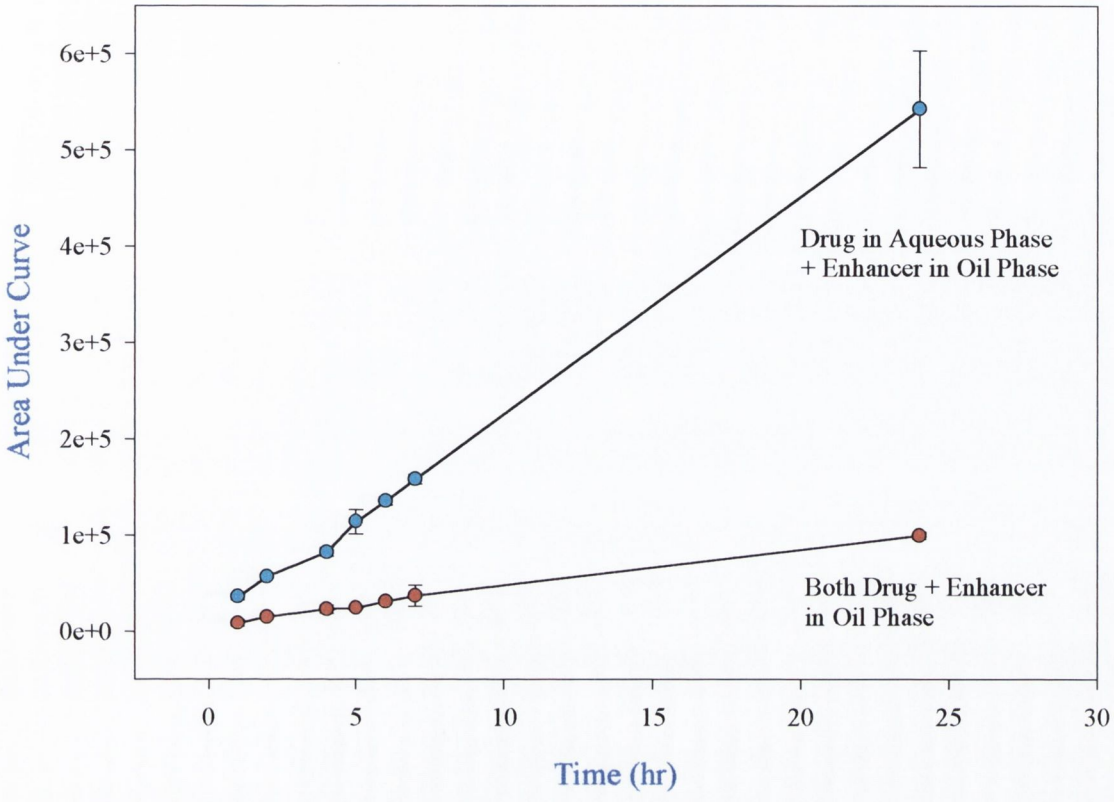


Figure 4:3:1 *Effect of Incorporating the Sodium Diclofenac into the Aqueous Phase and Enhancer (BDDAB) into the Myverol Phase compared to putting both in the Myverol Phase in the preparation of the Gel. (1mg = 1619629.4 AUC)*

It is evident from the profiles on Figure 4:3:1 that by separating the drug and enhancer (BDDAB) into different phases of the vehicle during preparation increases the amount of drug which diffuses from the system. Although the effect of incorporating drug and enhancer into different phases results in a six-fold increase in release, the quantity released is still minimal in comparison to passive release in the absence of enhancer.

To determine definitively that the inhibited release of sodium diclofenac was due to ion-paring with the model enhancer, investigations were undertaken in association with Blanc (1998) into the effect of concentration. Several ratios of drug/enhancer were investigated to establish if there was a relationship between the amount of enhancer present in the vehicle and the cumulative release of drug.

It was first necessary to establish whether ion-pair formation would mask chromophores or in some way change the absorption characteristics of the drug in the concentration range in which investigations were to be carried out.

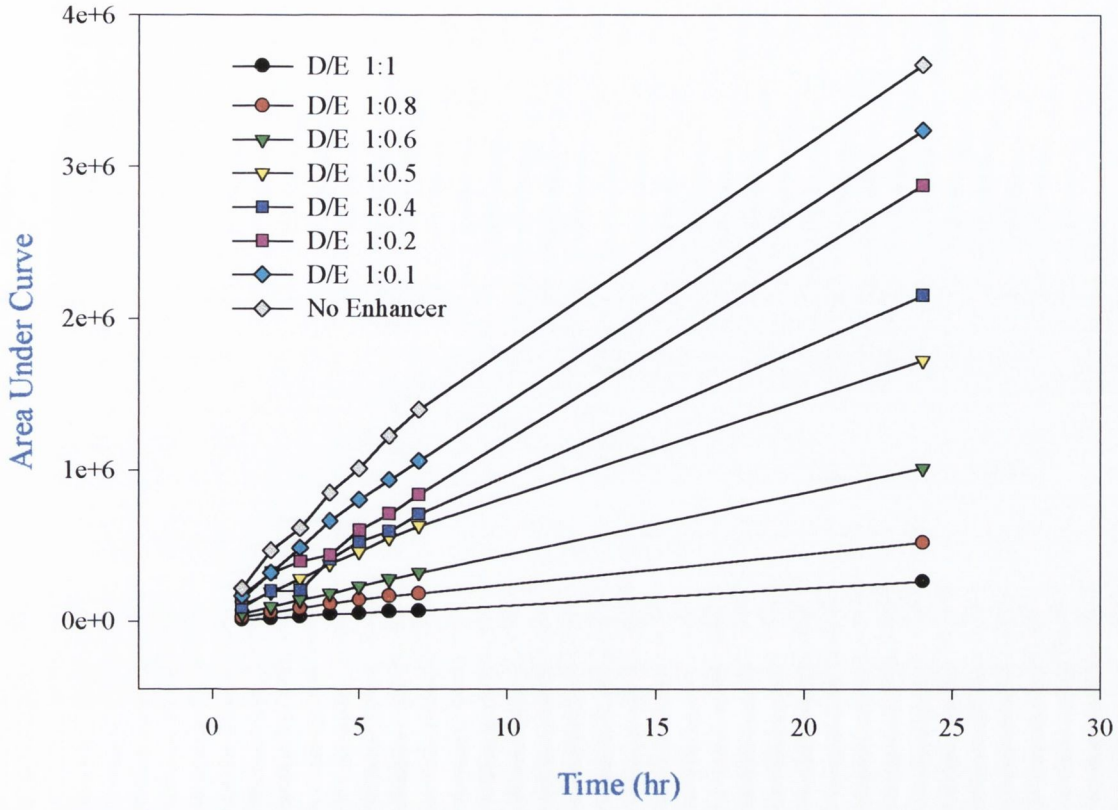


Figure 4:3:2 *Passive Release of Sodium Diclofenac from the Liquid Crystalline Gel Incorporating Incremental Concentrations of BDDAB. The insert shows the ratio of Drug (D) to Enhancer (E). (1mg = 1619629.4 AUC)*

UV detection studies showed that from 0% concentration of BDDAB to a 1:1 ratio of drug/BDDAB there was no decrease in the absorbance of sodium diclofenac in aqueous solution. As the ratio of enhancer increased above that of sodium diclofenac, a dramatic fall off in absorbance was noticeable. This was mainly due to an increase in the cloudiness of solutions and the subsequent dispersal of the light caused by micelle formation.

Figure 4:3:2 shows the effect of varying the ratio of enhancer concentration from zero to 1:1, whilst keeping the drug concentration at 0.1M. The graph indicates an obvious relationship exists between the ratio of drug/enhancer. A plot of percentage release after 24 hours versus the ratio of drug/enhancer shows a linear relationship as shown in Figure 4:3:3.

The linear relationship exhibited in Figure 4:3:3 gives conclusive evidence of a link between the concentration of enhancer in the vehicle and the percentage release of sodium diclofenac from the system. An r^2 value of 0.98 was obtained for the linear

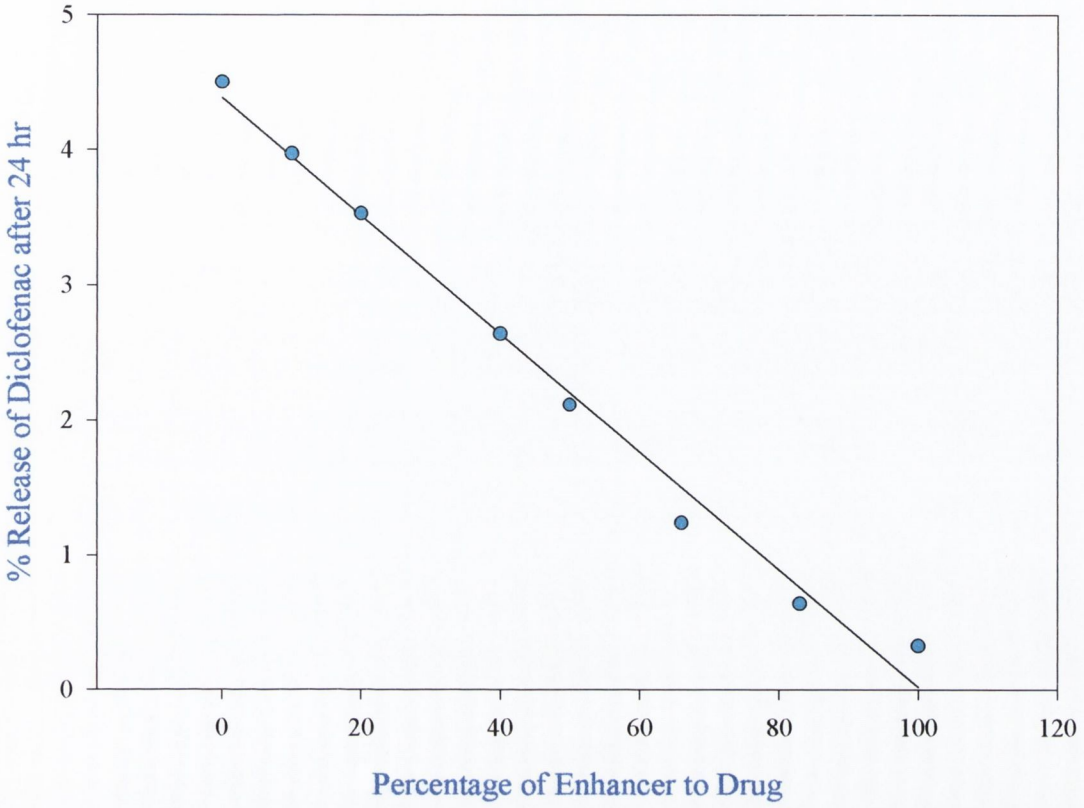


Figure 4:3:3 *Relationship of Drug/Enhancer Ratio versus % Release After 24 Hours.*

regression of the two variables. Fini *et al.*, (1996) have reported that the diclofenac anion has the ability to form complexes and also possess colloidal properties due to its structure which somewhat resembles that of a common anionic surfactant. These properties maybe responsible for the unexpectedly low % release in the absence of enhancer but are unlikely to be the reason for the decrease of release in the presence of the model enhancer.

4:4 *The Passive Release of the Sodium Salt and Acid Form of an Anionic Drug from a Liquid Crystalline Gel with and without the Incorporation of BDDAB into the Vehicle*

Variables, which have been investigated so far include; effect of buffering the system, changing counter ion and chain length of the enhancer, and method of preparation of the vehicle. In an attempt to more fully understand the effect of

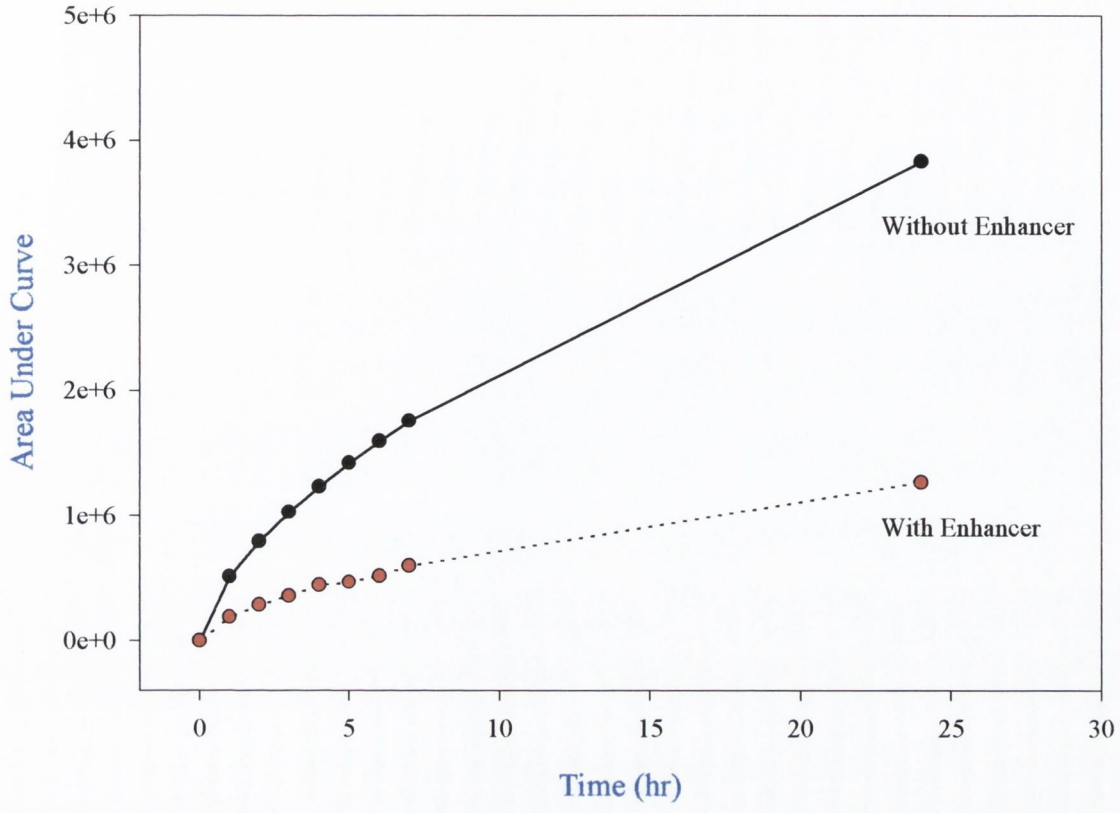


Figure 4:4:1 *Comparison of the effect of incorporating BDDAB on the passive release of Sodium Salicylate from the Liquid Crystalline Gel to that without enhancer (1mg = 78905 AUC)*

incorporating BDDAB into the vehicle, its was decided to exchange sodium diclofenac with the sodium salt of another drug. Sodium salicylate was chosen for these investigations. Measurements also included the use of the acid form of the drug (salicylic acid) to establish whether an increase in release is observable due to decreased ion-pairing, which is due to incomplete dissociation of the weak acid. The passive release of sodium salicylate with and without the incorporation of BDDAB into the vehicle is shown in Figure 4:4:1. A similar graph for the release of salicylic acid is shown in Figure 4:4:2

As expected the release of salicylic acid was marginally less inhibited than that of its sodium salt. The decrease in the amount of sodium salicylate observed was 67% compared to cumulative release in the absence of BDDAB. The decrease in the release of salicylic acid in comparison was 60%. The difference of 7%, although not significant, maybe anticipated due to the incomplete dissociation of the acid, thus all of the acid is not available to form ion pairs.

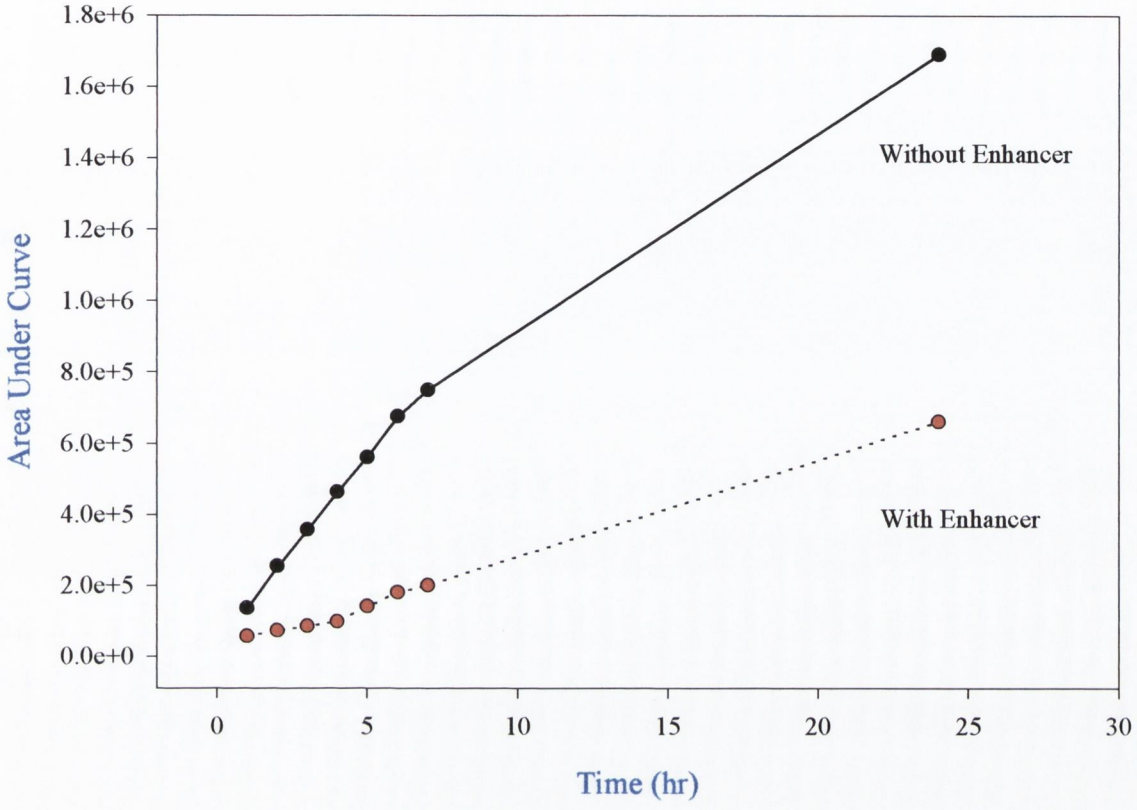


Figure 4:4:2 *The Effect of Incorporating BDDAB on the Passive Release of Salicylic Acid from a Liquid Crystalline Gel. (1mg = 78905 AUC)*

Compared to the >90% association of diclofenac anion and BDDAB, the reason is unclear as to why the association of the salicylate anion with BDDAB is ~67% when there are equal concentrations of both compounds in the vehicle. However, Nolan (1995) measured the decrease in the cumulative release of salbutamol base in the presence of oleic acid to be ~66%. This represents a similar degree of ion pair association in both studies. In the latter system the drug (salbutamol) is cationic and the enhancer (oleic acid) is anionic.

What is clear is that BDDAB and other benzalkonium salt derivatives associate strongly with anionic drugs. In the case of the acid form of the drug the ion-pair association may be expected to be pH dependent. Association will not occur where the pK_a of the drug is within 1.8 pH units of the vehicle pH (6.8 or 7.2 buffered). For most weak acids this exception will not occur.

4:5 *The Passive Release of Sodium diclofenac from the Liquid Crystalline Gel Incorporating Oleic Acid.*

Due to the difficulties encountered in the study so far because of the ionic association of sodium diclofenac and the model enhancers (benzalkonium salt derivatives) under various conditions, it was decided to now test the use of oleic acid as an enhancer.

As mentioned previously, preliminary investigations by Nolan (1995) found that oleic acid and iontophoretic assistance did not enhance the transdermal delivery of sodium diclofenac. However, for reasons which will be fully reported on in the next chapter the lack of a synergistic enhancement of transport of sodium diclofenac can not in fact be attributed to the use of oleic acid.

Passive release of sodium diclofenac from a liquid crystalline gel incorporating oleic acid is shown in Figure 4:5:1. The graph indicates that incorporating oleic acid into the vehicle in a 1:1 ratio with diclofenac has an

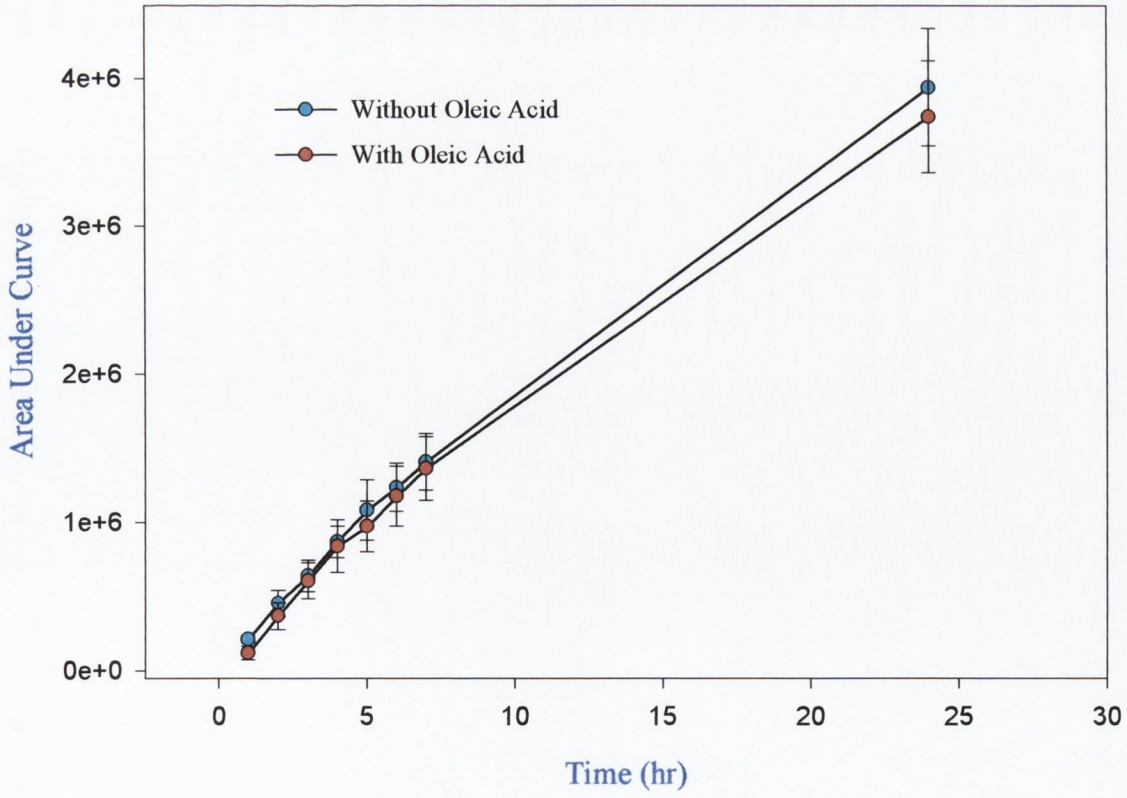


Figure 4:5:1 *The passive release of Sodium diclofenac from the liquid crystalline gel with and without Oleic Acid. Each set of measurements was made in triplicate. (1mg = 1619629.4 AUC)*

insignificant effect on the passive release of the drug from the gel. Oleic acid would not be expected to form ion pairs as was observed in the presence of a cationic drug (Nolan, 1995) because both sodium diclofenac and oleic acid both produce anions. The lack of association between the drug and enhancer leaves the drug free to diffuse across a membrane and the enhancer free to disrupt the barrier properties of the skin. This result provides a firmer basis on which to continue investigations with this combination of enhancer and drug. Investigations by Maitini *et al.,* (1996) used ethanol treated silicone membrane in studies involving the passive diffusion of sodium diclofenac in the presence of oleic acid. Results indicated oleic acid enhanced the diffusion of the sodium diclofenac through non-pore lipophilic routes. These results are not directly comparable due to the use of a non-rate limiting membrane in this investigation. Also, the use of ethanol could be viewed as a co-enhancement technique given the evidence of Hori's work, (Hori *et al.*,1990). Further comparisons will be given in subsequent chapters.

4.6 Discussion

Studies involving sodium diclofeanc and the incorporation of benzalkonium salt derivatives proved negative from the point of view that the diffusion of the drug from the vehicle was not found to be enhanced across the non-rate limiting membrane. Further studies using sodium salicylate and benzalkonium derivatives as enhancers will be described in subsequent chapters. Although sodium salicylate release was inhibited by ~67% by BDDAB so too was salbutamol base by oleic acid in studies by Nolan (1995) and this system was later shown to exhibit a significant synergistic increase in the transdermal transport of salbutamol when iontophoretically assisted.

There is still merit in using surfactants as chemical enhancers but the idea that a cationic enhancer is required for an anionic drug and vice-versa has been shown to be ill-founded and this is further exemplified in the next chapter. Studies involving BDDAB will still remain central in the remaining investigations but also in tandem with oleic acid. Oleic acid is considered preferable as an enhancer because BDDAB, as a cationic surfactant, is more damaging to the skin. Despite their potential side effects, surfactants have been widely investigated for use as enhancers for

transderamal delivery but no reference has been found with to their use specifically with sodium diclofenac: references to other anionic drugs do occur. An investigation by Aoyagi *et al.*, (1990) into the transdermal transport of 5-fluorouracil (5-FU)(an anionic drug) found that by polymerizing benzalkonium chloride derivatives they could effectively reduce irritation to the skin whilst enhancing the permeation of the drug. The derivative possessing a hexadecyl chain showed the most effective enhancement of permeation of drug.

At this point it is necessary to try and illuminate the nature of the vehicle and additives. Incorporating a surfactant into a binary system effectively creates a tertiary system. The further addition of a drug could also be classed as producing a ternary system. It is known that the addition of surfactant to an oil/water mixture leads to the formation of a variety of phase structures (Attwood *et al.*, 1983) and a surfactant or micelle cannot be considered as inert. The drug and other additives may (depending on the amount present) dramatically alter the type and range of aggregates formed (Lawrence, 1994). Little work has been performed in this area and it is difficult to predict the effect of a drug or other additive on a phase structure as it is expected to vary according to whether the additive (a) is water soluble, (b) adsorbs at the aggregate surface, (c) co-aggregates with the surfactant, or (d) resides in the interior of the aggregate. Evidence suggests, however, that the phase structure experience the most disruption when the additive is itself surface active. For example, the presence of the drug lignocaine hydrochloride at concentrations >5 wt % converts the cubic structure formed from 10 wt % monoolein in water into a lamellar phase, (Engstrom and Engstrom, 1992). The influence of the presence of a drug is further complicated because most drugs are administered as salts, hence the amount of amphiphilic salt to lipophilic free drug varies according to the pH. Consequently the effect of the drug on the phase structure may vary with the pH of the surrounding environment. This effect is more likely to be significant if ionic surfactants are used. Yet another complication is that if the drug promotes a phase transition, this transition may conceivably be reversed as the release of a surface-active drug from the aggregate proceeds (Engstrom and Engstrom, 1992). This phase reversal may lead to two different patterns in the release of the drug.

Much of the work that has been reported in the literature examining the use of liquid crystalline phases in drug delivery has not characterised the nature of the phase structure, that is, whether the liquid crystalline phase is hexagonal or lamellar in

nature, yet it is known that the release pattern differs depending on the phase structure present (Ibrahim, 1989).

It was noted during the experiments with the salts of drugs that the vehicles in which BBDAB was incorporated had a cloudy appearance rather than being clear. This may be indicative of a phase change in the gel from cubic to one of a number of other phase types including hexagonal, lamellar or vesicles, all of which are cloudy birefringent phases (Lawrence, 1994). A wide range of surfactant concentration is possible in the formation of these phases (Lawrence, 1994). The gels continued to be observed over a period of days. The cloudiness eventually cleared from all gels and the formation of solid crystals was observed. The geometry and appearance of the crystals depended on the drug type. Sodium salicylate formed large snowflake like crystals whereas sodium diclofenac formed smaller more spherical crystals. Gels in which BDDAB and salicylic acid were present remained clear initially and became cloudy over a period of days. All gels were prevented from dehydrating during this period by encapsulation in parafilm, they were also stored at room temperature.

From the available information it appears most likely that the cloudiness of the vehicle was more likely due to the presence of both enhancer and drug. The cubic phase of the binary myverol/water system has the ability to solvate a wide range of drugs due to its very large interfacial area – in the order of 400 m^2/g (Ericsson *et al.*, 1991).

Evidence firmly suggests that an ion pair association exists between the drug and model enhancer. The association takes place with the carboxylate group of the drug and the quaternary nitrogen head group of the surfactant. This association may have a beneficial effect on the transdermal permeation of the drug should the ion-pair form after both drug and enhancer have diffused from the vehicle. However in this instance the ion-pair association occurs within the vehicle and results in the decrease in the transport of the drug out of the gel. Ion pair association has been employed by many researchers to facilitate the percutaneous absorption of drugs. The lipophilicity of hydrophilic ionized drugs can be increased by ion pair formation with lipophilic counter ions. The concept was first introduced by Bjerrum in 1926 to explain the decrease in electrical conductance of sodium chloride in liquid ammonia, (Smith and Maibach, 1995).

Studies by Hadgraft and Wooton (1984) of Ethomeens, a group of ethoxylated tertiary amines derived from natural fats and oils, to assess their ability to facilitate

percutaneous absorption of sodium salicylate used a pH gradient of 6 to 7.4 to provide a driving force. Green *et al.*, (1989), investigated the effect of long chain fatty acids on the transdermal absorption of cationic drugs. The driving force in this investigation was a pH gradient in a direction opposite to that described above. The pH of the donor was >7.4 so that the ion pair could dissociate into single ions. Using the same mechanism, Azone was found to be capable of enhancing the transport of salicylate anions, (Hadgraft *et al.*,1985). There are numerous further examples.

The careful control of pH within the vehicle could be considered as a mechanism to prevent ion-pairing of the drug and enhancer. To achieve this the pH must be at a value where either drug or enhancer is non-ionic in form. This may be possible by shifting the pH downward to make the drug a neutral acid or by shifting the pH upwards to make a tertiary ammonium head group neutral. The only option in the diclofenac/BDDAB system or salicylate/BDDAB system is to shift the pH downward. The problem in doing so is that the pH required to obtain a neutral drug would be unsuitable for transdermal applications. With a pK_a=3 for salicylate the pH would have to be below 4 in order to have an appreciable quantity of drug as required. In the case of BDDAB, a pH shift will not affect the ionic nature of the head group due to the difficulty in eliminating methyl groups or a benzyl group.

The following chapters will investigate the behaviour of these systems under iontophoretic conditions.

Chapter 5

In-vitro Studies of the Iontophoretically Assisted Transport of Anionic Drug Molecules Across Visking Membranes from a Liquid Crystalline Gel

Chapter 5

In-vitro Studies of the Iontophoretically Assisted Transport of Anionic Drug Molecules Across Visking Membranes from a Liquid Crystalline Gel

5:1 Introduction

The work reported in this chapter investigates the effect of a constant electrophoretic current on the delivery of anionic drugs across a non-rate limiting membrane from liquid crystalline gels. The use of iontophoresis as a physical enhancement technique was described in Chapter 1. The suitability of sodium diclofenac for enhanced drug delivery by this method is due to the negative charge of the carboxylate group of the molecule in the ionised state. Such charged moieties will be expected to carry a proportion of the established current and to move towards the electrode of opposite charge, in this case the anode. All electrodes used were made of platinum (99% purity) and the anode was placed in the receptor medium just below the membrane barrier. The cathode was placed in contact with and on top of the vehicle. The ionised drug, having a negative charge, will be repelled away from the negatively charged electrode by electrorepulsion, which is one of the main driving forces of electrically assisted transport. In the preceding section the Visking membrane was shown not to significantly affect the diffusion of the drug from the vehicle. Consequently it was assumed that neither would the membrane influence the iontophoretically assisted release of the drug from the delivery device. The parameters which will be investigated include: the effect of buffering; the current density; and the nature of the drug molecule with specific emphasis on sodium diclofenac

5:2 The Iontophoretically Assisted Release of Sodium diclofenac from the Liquid Crystalline Gel Across a Visking Membrane.

The initial investigations of the release of sodium diclofenac had water as the gel solvent and also in the receptor medium. Triply distilled water was used to avoid the

possibility that extraneous ions could participate in the conduction process across the system. The water/water protocol had also been found to give the most significant release in passive studies with sodium diclofenac. Nevertheless, all protocols listed in Table 4:2:1 were investigated in order to obtain a full picture of the behaviour of the system.

The system was allowed to equilibrate in Stage I (passive) for two hours before current was established between the electrodes (Stage II). Potential and current were both monitored during the course of experiments using a multimeter and all experiments were carried out in triplicate. The standard current used throughout the investigation was 0.5 mA (0.2mA/cm^2), which is within generally accepted levels of tolerance for iontophoretically assisted transdermal transport.

The iontophoretic release of sodium diclofenac from liquid crystalline vehicles in comparison to passive release is shown in Figure 5:2:1

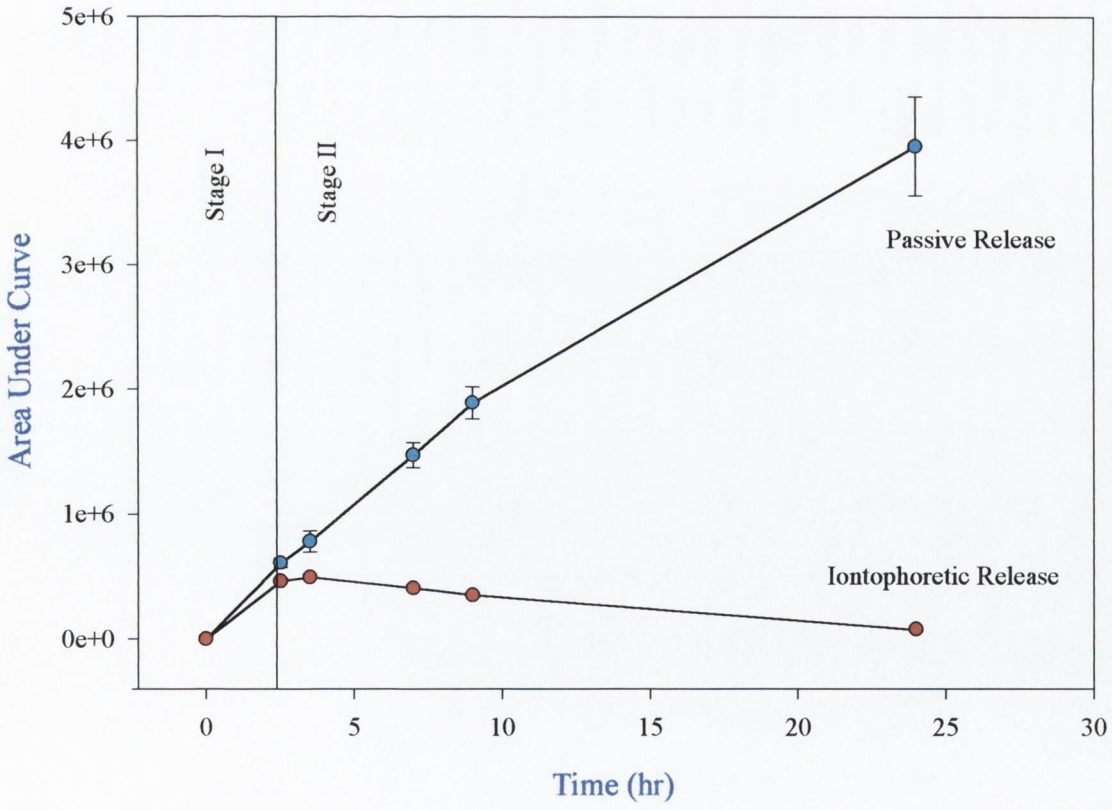


Figure 5:2:1 *Comparison of the passive and iontophoretically assisted (0.5mA) release of Sodium diclofenac from the Liquid Crystalline Gel Across Visking Membrane. Both the gel and receptor were based on triply distilled water. (1mg = 1619629.4 AUC)*

Surprisingly, the release of sodium diclofenac was not found to be enhanced by the assistance of current. In fact the complete opposite effect to that expected was observed. The initial release of the drug, during the passive stage I, was as expected and corresponds to the release rates in earlier similar experiments. When the potential was applied in stage II, further release of the drug is inhibited and, even more surprisingly, the concentration of the drug which has diffused into the receptor cell gradually becomes lower or perhaps it is somehow removed from the receptor medium.

5:3 *The Effect of Current Reversal and Intensity of Current.*

Due to the unexpected release profile observed using cathodal iontophoresis it was decided to compare the profiles with those of anodal iontophoresis by reversing

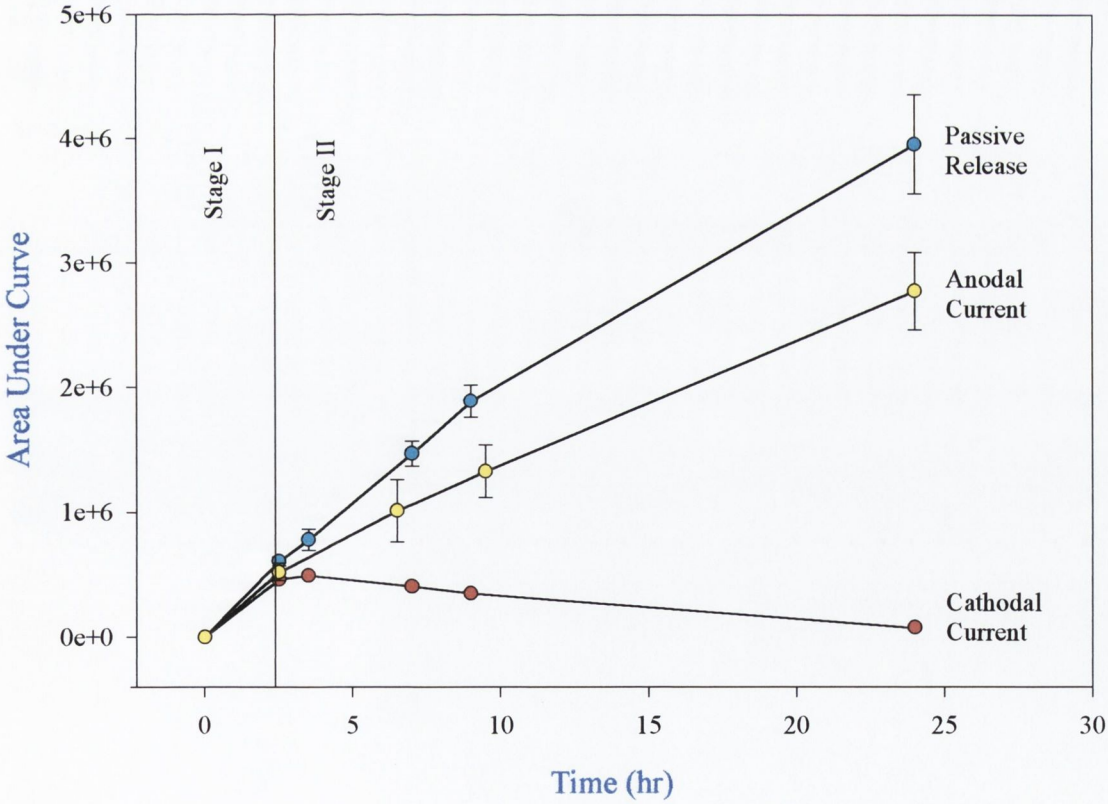


Figure 5:3:1 *Comparison of the rates of the release of Sodium diclofenac from the Liquid Crystalline Gel across Visking Membrane passively and with cathodal and anodal iontophoretic assistance. (All concentrations 0.1M) (1mg = 1619629 AUC)*

the polarity of the electrodes. If the polarity had been incorrect, the electrode (anode) on top of the gel would be expected to attract the drug, thus preventing it from leaving the vehicle. Experiments were carried out in which all other conditions remained the same as those used in Figure 5:2:1 except the polarity of the electrodes was reversed. The results of the experiment are graphically shown in Figure 5:3:1.

A thorough systematic examination of the apparatus and power supplies suggested there was no error involved in the initial set up of the experiments, i.e., the voltage has been applied with the correct polarity. From the profile of anodic iontophoretic data in Figure 5:3:1, there is evidence that the application of a constant current significantly decreases the release of drug compared to passive release, although this decrease is not as substantial as that for cathodal iontophoresis. A decrease in release was anticipated because the drug would be expected to be attracted towards the anode, which was now on top of the vehicle.

The total drug delivery rate from an iontophoretic system, R, can be divided into two components: R_p due to the chemical potential gradient and R_i due to the electric potential gradient. The driving forces resulting from the chemical and electrical potentials act simultaneously. Consequently the following expression can be written for the total delivery rate, R.

$$R = R_P + R_i$$

Equation 5:1

The iontophoretic release rate, R_i, may be expressed to be the product of the current, i, and the iontophoretic flux, F_i, defined as the amount of drug (on a weight basis) delivered per unit time, per unit current.

$$R_i = iF_i$$

Equation 5:2

Consequently, if R_p is constant, R should vary linearly with respect to the current, as illustrated in the combination of Equations 5:1 and 5:2.

$$R = R_p + iF_i$$

Equation 5:3

Linear relationships between the release rate of the drug and the iontophoretic current flowing have often been reported for iontophoretic drug delivery (Nolan, 1995). The release profile of sodium diclofenac under anodal iontophoresis, in Figure 5:3:1, shows more linearity compared to that of passive release. The surpressed release of sodium diclofenac under anodal iontophorsis is ~ 30% less than that of passive release after 24 hours. It may be expected that under cathodal iontophoretic assistance that the assisted release of the drug would also be in the order of 30% or greater than passive release. This increase would be due to molecules, under passive and iontophoretic delivery, moving in the same direction rather than opposing directions to each other, as is the case under anodal conditions.

Returning to the very reduced release profile of sodium diclofenac after 24 hours of cathodal current in Figure 5:3:1, it was decided having confirmed that the correct polarity was used in the course of experiments to closely examine the iontophoretic experimental conditions used. It is commonly known that the electrolysis of water occurs at an electrical potential of ≥2V at a platinum electrode. With cell potentials significantly in excess of this value (~24V), the production of hydronium and hydroxide ions in the system is almost certain. Although the pH of the system will not change due to the formation of equal amounts of positive and negative ions, it is possible that in the presence of a large surplus of hydronium ions that the acid form of the drug may occur.

Another possibility under iontophoretic conditions is the oxidation of the diclofenac anion as shown by the cyclic voltammogram shown in Figure 5:3:2. This voltammogram was taken using the drug itself as the supporting electrolyte. Platinum electrodes (99.99% purity) were used for the experiment.

It is evident from the voltammogram in Figure 5:3:2 that oxidation of sodium diclofenac occurs at a potential of 0.7V. This figure concurs with standard literature values, (Merck Index). The reduction of the drug is shown to take place at a potential of –0.4V. The over-potential is shown to be ~1.0V indicating that the reaction is non-reversible under cathodal iontophoretic conditions. The other peak and trough at lower potentails is due to hydrogen evolution due to the aqueous environment.

Further investigations were undertaken to establish the cause of the unexpected release profile of sodium diclofenac under iontophoretic conditions (Figure 5:3:1).

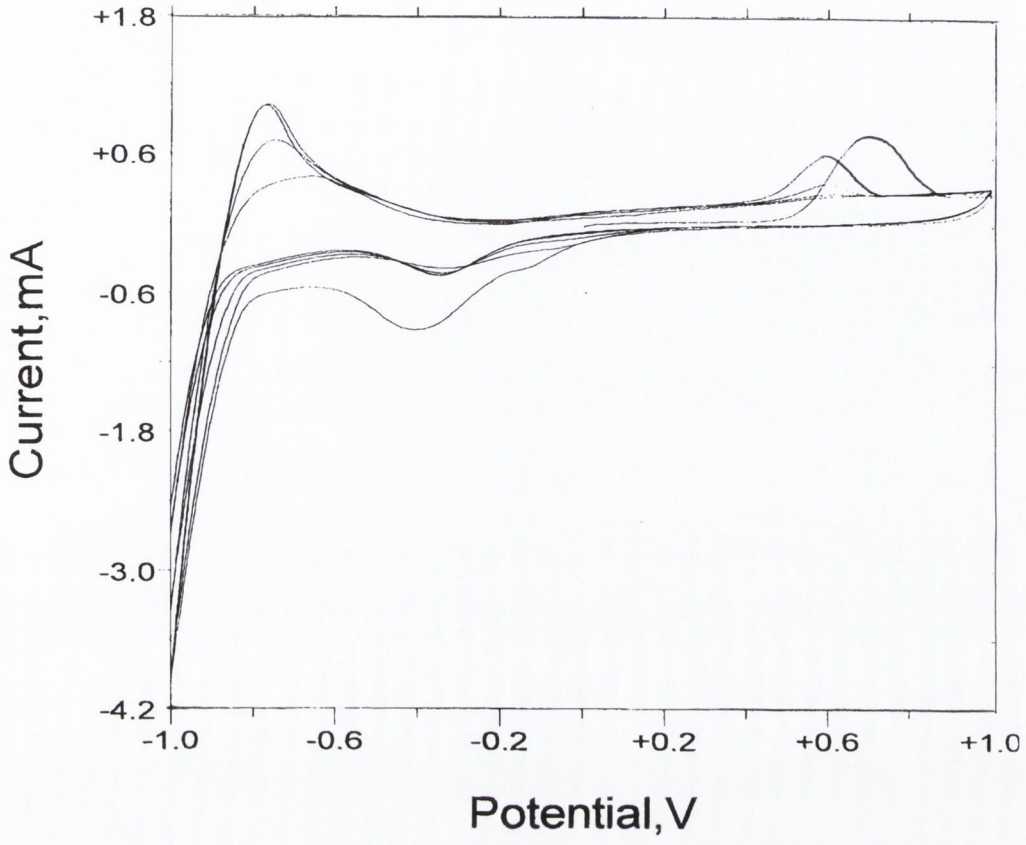


Figure 5:3:2 *Cyclic Voltammogram of Sodium diclofenac showing Oxidation at 0.7V.*

Endeavours in this respect involved the simplification of the iontophoretic process to its most basic components. A 0.1M solution of sodium diclofenac in triply distilled water was prepared into which both electrodes were placed. The solution was not stirred after the dissolution of the drug. It was noticed that as soon as the current was established a precipitate formed on the anode. The precipitate continued to form over a period and eventually caked over the whole electrode. Under cathodal iontophoretic protocol, the anode is placed in the receptor medium. Because the product was insoluble in solution it was assumed that the acid form of diclofenac was being produced at the anode or perhaps a complex of diclofenac due to oxidation.

The product from the simplified experiment was collected, dried, and prepared for NMR analysis by dissolving the compound in deuterated DMSO. Several other methods were also used to identify the compound including Mass Spectrometry, I.R

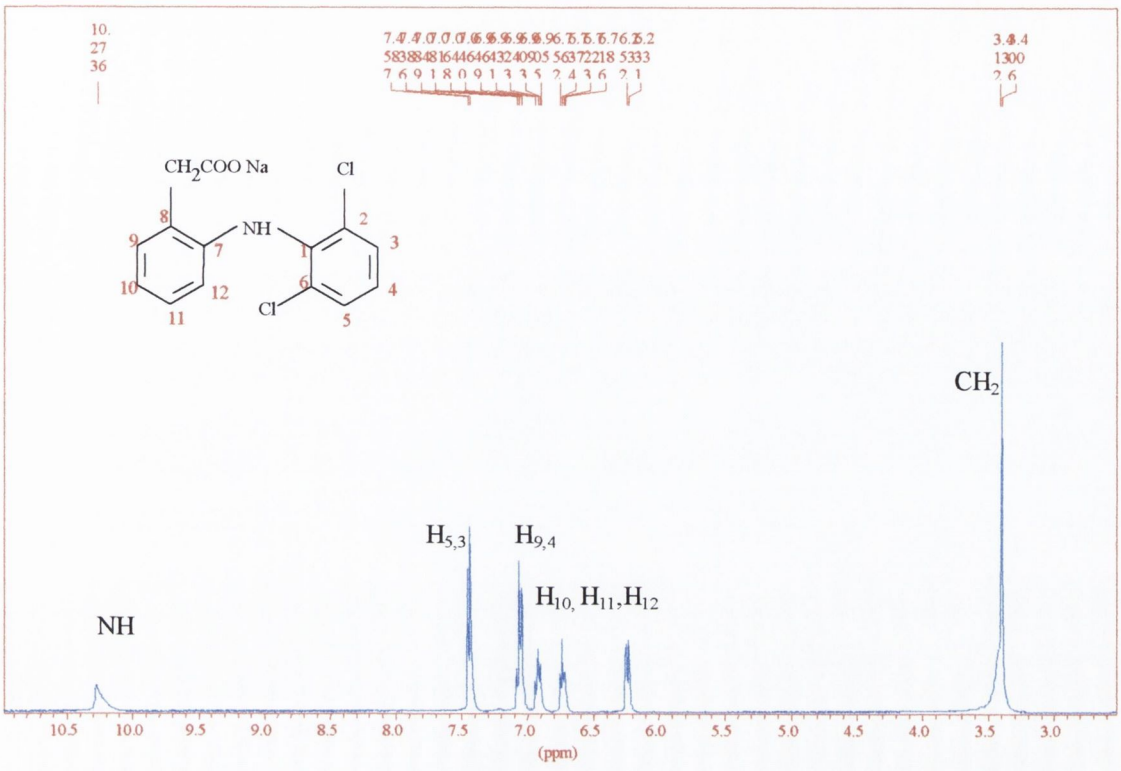


Figure 5:3:3 *^1H NMR Spectra of Sodium diclofenac in DMSO solvent.*

and melting point tests. A comparison of ^1H NMR (400mHz, DMSO) spectra of sodium diclofenac and the collected product are shown in Figures 5:3:3 and 5:3:4.

A cursory inspection of the two spectra shows that they are very similar. The spectrum in Figure 5:3:4 indicates that the product which was collected is diclofenac acid. The key diagnostic feature of the spectrum is the presence of a peak down field at δ = 2.5ppm. This peak represents the OH group of the carboxylic acid which is absent in the spectrum of sodium diclofenac in Figure 5:3:3. All other down field shifts correspond exactly with the presence of the acid form of the drug.

Melting point tests also gave confirmatory evidence that the product was the acid form of the drug. The electrolysis product melted at a temperature of 431K, which is the literature value of the melting point of the acid, (Merck Index). By comparison the melting point of the sodium salt is 559K.

Other confirmatory tests carried out also indicated the presence of the acid form of the drug with IR data showing the presence of a sharp carboxylic OH peak at 3500 cm^{-1}, which is not present in the spectrum of the sodium salt.

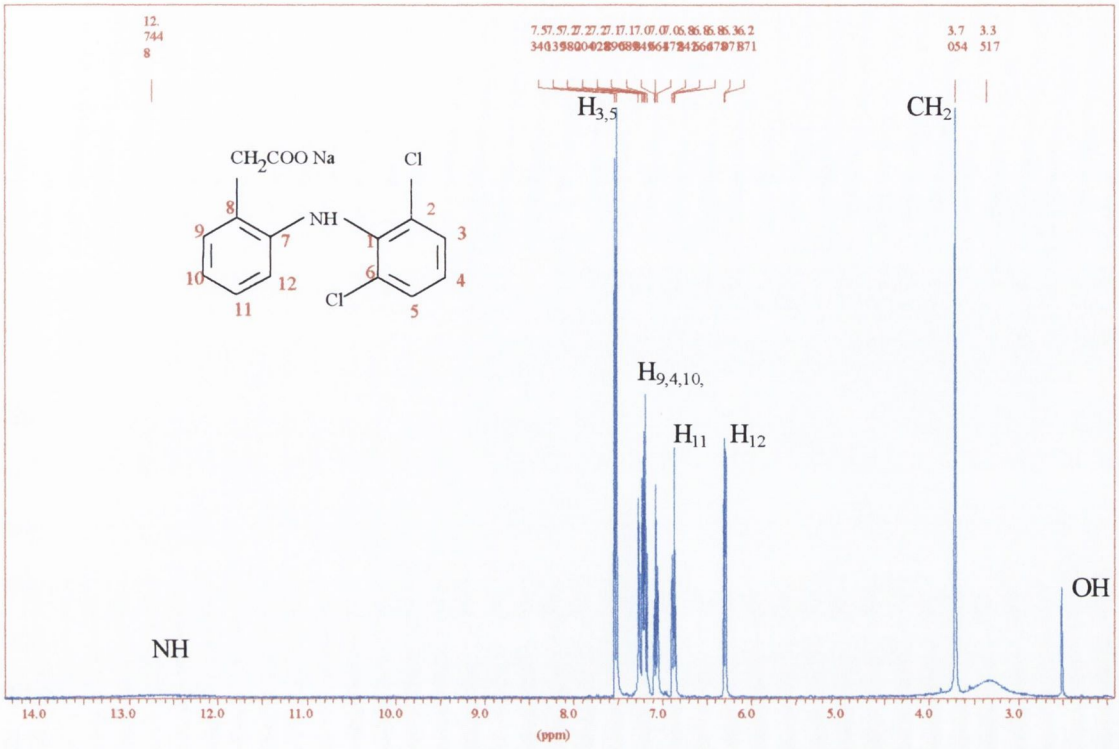
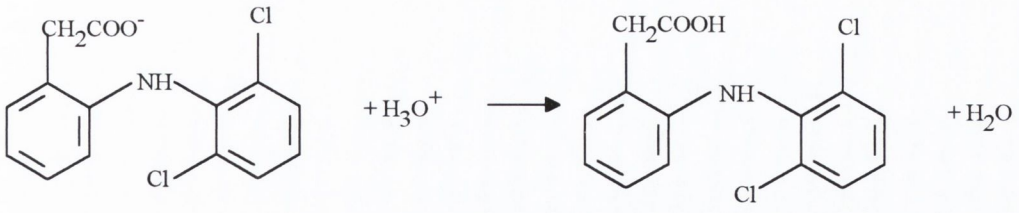


Figure 5:3:4 *^1H NMR of product collected from Iontophoretic Experiment. This NMR spectrum identifies the product as Diclofenac Acid.*

Finally, Time of Flight (ToF) mass spectrometry was used and the spectrum indicated the presence of the acid by presenting a peak at 296 m/z (see Appendix A) as opposed to 318 m/z for the sodium salt. The acid also had a distinctly different appearance. Sodium diclofenac is white in colour whereas its acid is off-white to beige in colour. The formation of the acid follows the reaction shown by Equation 5:4. This reaction mechanism is driven to the right under the conditions used. If the formation of the diclofenac acid is taking place under iontophoretic conditions it would explain the reason for the release profile in Figure 5:3:1. As the diclofenac anion diffuses into the receptor medium it is protonated making the molecule insoluble.

One doubt remained as to the explanation of the observed iontophoretic experiment. During the basic electrolysis experiment the acid collected on the anode whereas in standard iontophoretic experiments there was no apparent formation of



Equation 5:4

this product. Even though the expected quantity would be far below that of a 0.1M solution the product might still be expected to be visible. The simplified experiment was carried out again but on this occasion the solution was continuously stirred. This stirring of the solution had a significant effect. Instead of the acid forming at the anode, the anode remained clear of acid formation and it was noted that solution was slightly cloudy with a very fine particulate suspension. At the end of the experiment the solution was allowed to stand so that the particulate matter collected at the bottom of the beaker. This precipitate was then collected and analysed using the techniques previously described. The precipitate was also found to be diclofenac acid.

In light of these results, the previous iontophoretic experiments were repeated and when they had been completed the receptor cells were inspected for precipitate. After standing overnight a fine precipitate was indeed evident at the bottom of all three cells. To further confirm this finding, the experiments were carried out using 4% agar gels as the vehicle since this vehicle had been shown to release diclofenac in greater quantity. In these experiments a substantial amount of precipitate was found at the bottom of the cells and again identified through analysis as diclofenac acid.

Reversing the polarity in the cell turned out to be a useful indicator that the correct polarity had indeed been used in the initial experiments. It has been proven beyond doubt that sodium diclofenac diffuses from vehicles in substantial quantities under iontophoretic conditions but undergoes an electrochemical change to diclofenac acid during the experiment. As diclofenac acid is insoluble in aqueous media it forms very fine particulate, which eventually precipitate. This would explain why the drug would not be detected using standard sampling techniques and HPLC. This is also therefore the reason why the tentative experiments reported by Nolan (1995) showed negative results for iontophoretic delivery of diclofenac in the presence of oleic acid.

5:4 *The Effect of Buffering on the Iontophoretic Delivery of Sodium diclofenac.*

As buffering the receptor medium was shown to have significant effect on the passive release of sodium diclofenac it was decided to investigate the effect buffering may have under iontophoretic conditions. The effect is shown graphically in Figure 5:4:1. Experiments were carried out using both cathodal and anodal current. It is evident from the graph that the detection of diclofenac improves due to the presence of the buffer ions in the receptor solution. The dashed box in the graph encloses the data points for release under both polarities after 24 hours. All the data points in the box are seen to lie between those for iontophoretic delivery with both polarities but without any buffer in the receptor medium.

The buffer has a dual effect in these experiments. Firstly, the graph shows that for buffered cells with the cathodal assistance that detection of diclofenac is significantly greater than it is the case without buffer where it is seen to be negligible.

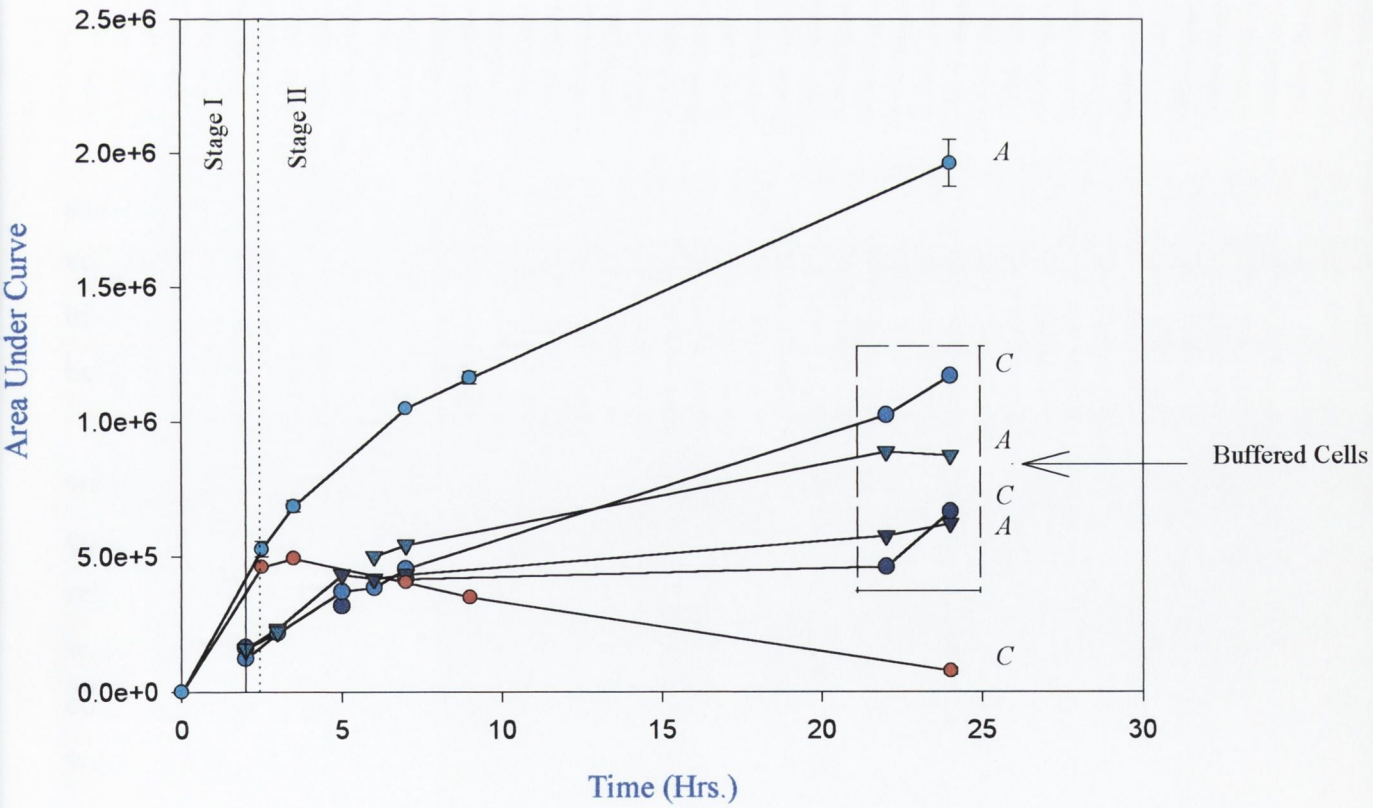


Figure 5:4:1 *The Effect of Buffering the Receptor Medium and Reverse Polarities on the Iontophoretic Release (0.5mA) of Sodium diclofenac.(A = Anodal current, C = Cathodal current, dashed line=delayed current flow of light blue profile). (1mg = 1619629 AUC)*

This increase is due to the buffer action, which reduces the amount of hydronium ions in solution so that less of the drug anions are removed as precipitate in the acid form. Secondly, the graph shows that for buffered cells with anodal assistance the detection of diclofenac is significantly reduced from that in the analogous case without the buffer present. This effect has been noted already in chapter 3 where the passive release of sodium diclofenac was reduced due to a reduced ion gradient within the vehicle between the two phases of the gel.

It is most likely therefore that it is coincidental that the data for cathodal and anodal assisted delivery in cells in which the receptor solution is buffered, fall and overlap in the same portion of the graph. These results are again in accordance with the suggestion that diclofenac acid is formed in the receptor solution under iontophoretic conditions.

5:5 *The Effect of Current Level, Applied Potential and Vehicle Type on the Iontophoretic Release of Sodium Diclofenac.*

Another variable which may have an effect on the iontophoretic release of sodium diclofenac is the current level. The level of current depends on the applied voltage which may also have an effect on the release profiles. This section investigates the effects of altering these parameters and discusses any changes in behaviour of the system.

The iontophoretic release profiles of diclofenac from liquid crystalline gels with current levels of 0.25mA and 0.5mA are shown in Figure 5:5:1 where they are compared to the analogous passive release. The graph indicates a five-fold increase in release when the current is halved from 0.5 to 0.25 mA. Although the current level was decreased from 0.5 to 0.25mA, the electrical potential required to maintain a constant current was still significantly above the potential at which electrolysis of water occurs, 2V. The reduction of the formation of diclofenac acid would not be expected to occur until the electrical potential used to give iontophoretic assistance was reduced to less than 2.0V. This could not be tested in practice because at such low potentials it was not possible to establish a current through the system.

Similar studies were carried out using 4% agar as the delivery vehicle. It was expected because of the significant release of diclofenac from agar (>70%) the

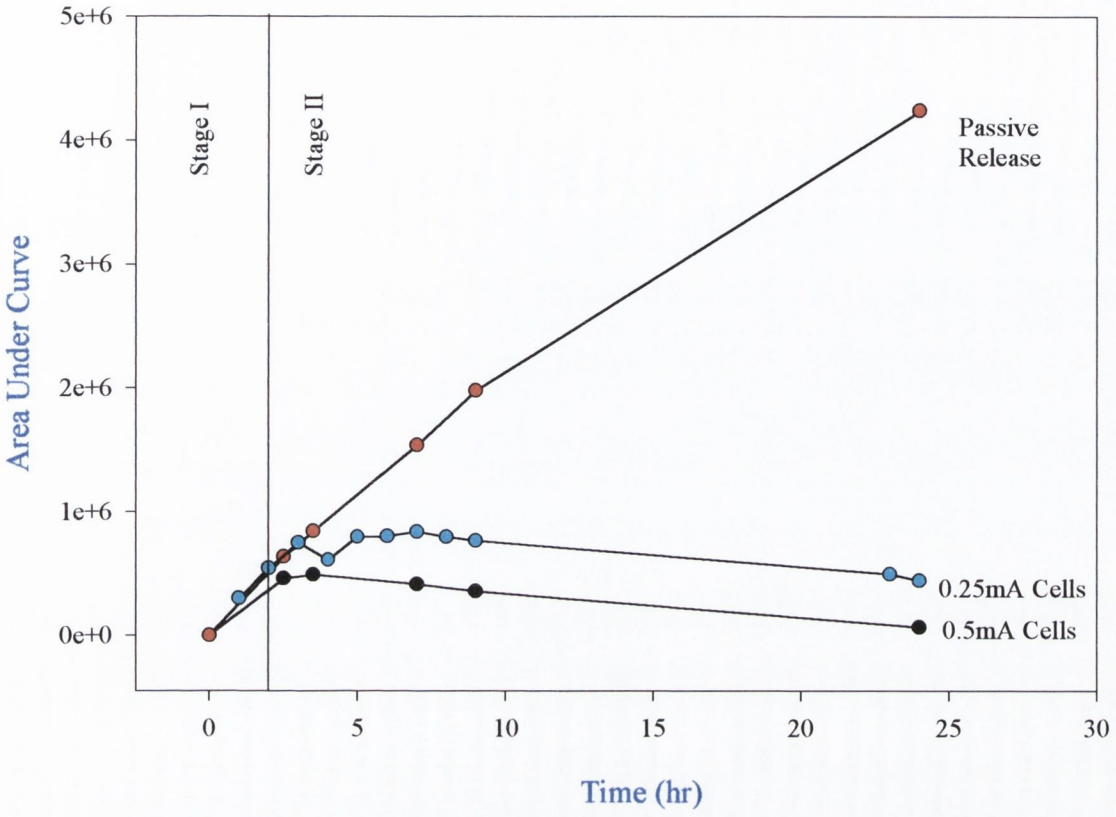


Figure 5:5:1 *The Iontophoretic Release of Sodium diclofenac from Liquid Crystalline Gels at Current Levels of 0.25 and 0.5 mA , in Comparison to Passive Release. (1mg = 1619629 AUC)*

release of the drug might proceed faster than its conversion to the acid form. The iontophoretic (0.5mA) release profile is shown in Figure 5:5:2 in comparison to passive release. The profiles are similar in character to the corresponding data shown in Figure 5:2:1 for the release of sodium diclofenac from liquid crystalline gels. The only difference is that the scale of release from the agar is very significantly greater. The graph would suggest that the rate of conversion of the drug to the acid form will occur as rapidly or more rapidly than the diffusion of the drug from the vehicle.

During the period between 4 and 10 hours after the experiment is started the level of diclofenac measured in the receptor department is seen to remain more or less constant. This would suggest that the rate of diffusion from the gel is equal to the rate of conversion of the drug during this time period. The total conversion to the acid form occurs at some point between 10 and 24hours (overnight). The total conversion is probably gradual as indicated by the graph or may fall on a hysteresis loop around the profile which would indicate either the equilibrium being maintained for several

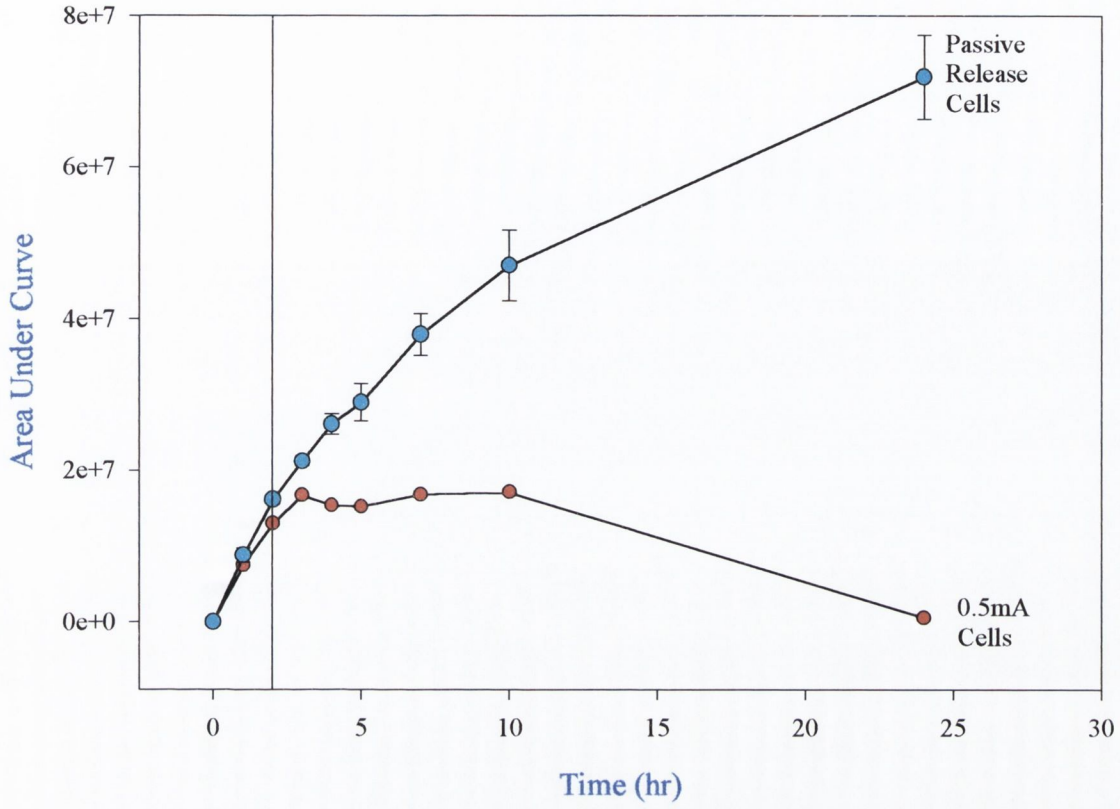


Figure 5:5:2 *The Iontophoretic Release of Sodium diclofenac from 4% Agar at a Current Intensity of 0.5mA in Comparison to Passive Release. (1mg = 1619629 AUC)*

hours longer and then a decrease in detection or a decrease in detection shortly after 10 hours to zero and a flat baseline to the 24 hour value.

An experiment was carried out at a potential between the oxidation potential of sodium diclofenac and that of the electrolysis of water, i.e., between 0.7 and 2.0V. In this case it was possible to establish a minimal current due to a lower resistance provided by the agar gel in comparison to the myverol vehicles. The release profile matched that of passive release in Figure 5:5:2. There was no enhanced delivery from the vehicle as the current level (0.005mA) was insufficient to transport significant quantities of the drug.

An attempt was made to collect product from iontophoretic cells by filtration. 11.7mg were collected from one cell, which amounts to 25% of initial drug loading of 50.4mg. As the acid form has a lower molecular weight than the sodium salt the appropriate calculation was carried out to obtain this percentage value. The quantitative retrieval of product was approximate but was later improved upon as reported in the next chapter.

5:6 *Comparison of Iontophoretic Release of Similar Anionic Drug Molecules.*

Investigations so far have had special reference to sodium diclofenac. Experiments in this section are broadened to investigate the effect of iontophoresis on a range of anionic drug molecules. The molecules under investigation are those for which their passive release rates from liquid crystalline gels, have been already measured, namely; sodium indomethacin, sodium slicylate and sodium benzoate. As all the model drugs are salts of carboxylic acids, it is anticipated that they will behave similarly to sodium diclofenac under analogous ionotphoretic conditions.

The iontophoretic release of sodium indomethacin from the liquid crystalline gel is shown in Figure 5:6:1. The initial 2 hour passive period (Stage I) shows a similar profile for all cells. The profiles remain the same for a further 2 hour period during stage II. After this time period the iontophoretically assisted cells show a

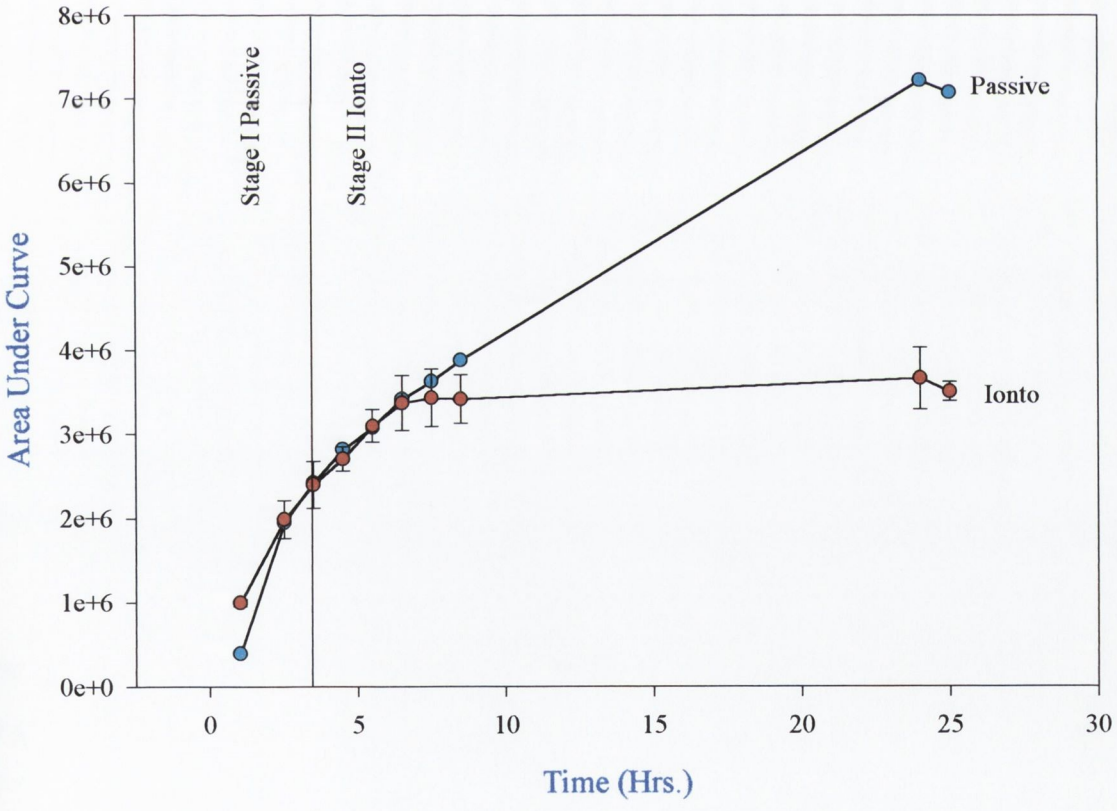


Figure 5:6:1 *The iontoporetic (0.5mA) release of sodium indomethacin (0.1M) from liquid crystalline gels in comparison to that of passive release. (1mg = 321338.9 AUC)*

levelling off of the detection of the drug. This also is an unexpected result under cathodal iontophoretic assistance. This deviation from the expected profile was also attributed to an electrocemical change of the drug to its acid form. Similar profiles were obtained for sodium salicylate during experiments carried out in association with Cadre (1998). The iontophoretic release of sodium salicylate in comparison to its passive release is shown in Figure 5:6:2.

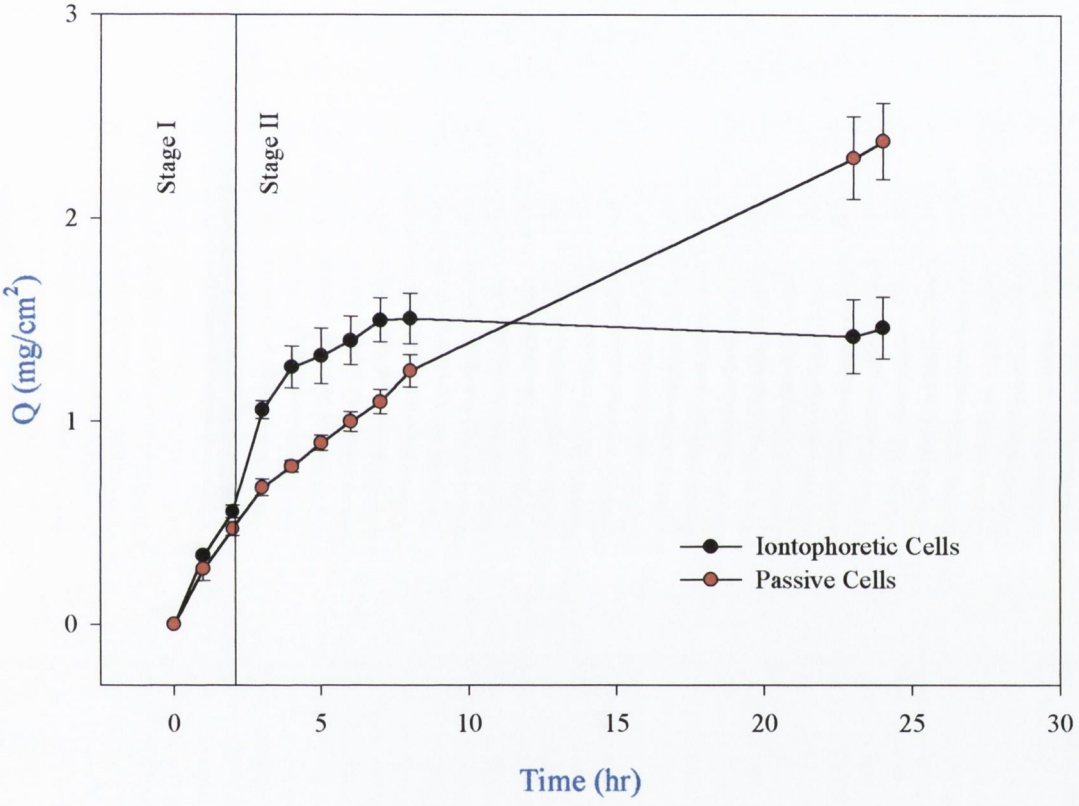


Figure 5:6:2 *The iontophoretic (0.5mA) release profiles of sodium salicylate (0.1M) from liquid crystalline gels in comparison to passive release. (1mg = 78905 AUC)*

The graph shows that the release is similar for both experiments in the initial passive stage. During the following 5 hours of stage II the drug showed enhanced release from the vehicle compared to passive release. Thereafter the detection of the drug begins to level off and, at 11 hours the release profiles fall below that of passive release.

In order to obtain a clear picture of the relative behaviour of these molecules an arbitrary passive release profile is used in Figure 5:6:3 to compare percentage release of each drug under iontophoretic conditions.

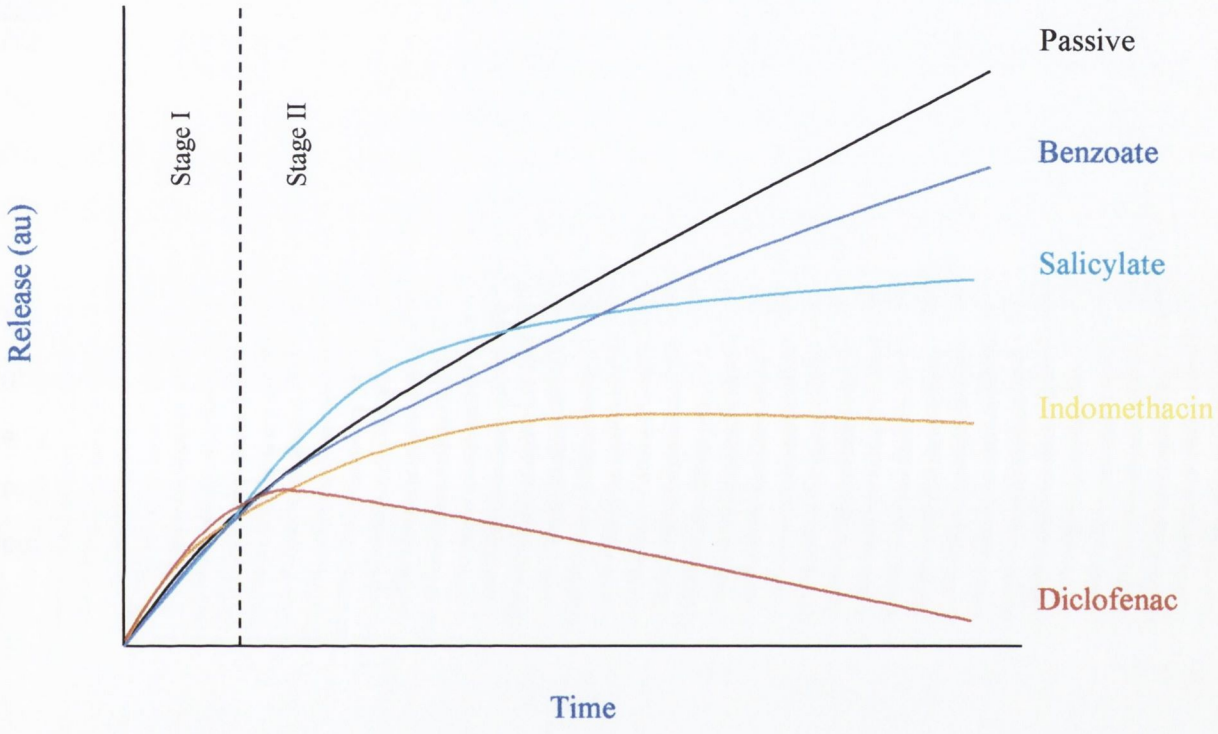


Figure 5:6:3 *The Comparative Iontophoretic Release of Several Anionic Drugs from Liquid Crystalline Gels during a 24hr Time Period. (All vehicle concentrations and current assistance were 0.1M and 0.5mA, respectively).*

Figure 5:6:3 shows that none of the iontophoretic release profiles of the drugs are as large as their passive release profiles, i.e., there is no enhancement of release apparent. A decrease occurs shortly after the current is established in all cases except for sodium salicylate which shows enhancement over a short period of time. The percentage reduction in the release compared to passive for each drug due to iontophoretic current is outlined in Table 5:6:1. These data reveals an emerging trend for the drugs under investigation.

Table 5:6:1 *The percentage reduction in the release of anionic drugs from liquid crystalline gels under iontophoretic conditions compared with passive release.*

Model Drug	*Initial Drug Loading (M)*	*Iontophoretic Current (mA)*	*% Release Reduction*
Na Benzoate	0.1	0.5	15
Na Salicylate	0.1	0.5	30
Na Indomethacin	0.1	0.5	65
Na Diclofenac	0.1	0.5	99

It has been noticed that the trend for the model drugs in Figure 5:6:3 and Table 5:6:1 observe a similar trend to that for the solubility of the acid form of each drug in aqueous solution, i.e., the greater the solubility of the acid the less the percentage reduction observed. The pK_a's and aqueous solubility of the model drugs are contained in Table 5:6:2.

Table 5:6:2 *pK_a and Solubility Data of the Acid Form of Model Drug Molecules Investigated.*

Model Drug	*pK_a*	*Solubility (g/L)*
Benzoic Acid	*4.2*	3.33
Salicylic Acid	*3.0*	9.13
Indomethacin	*4.5*	Practically Insoluble
Diclofenac	*4.0*	Insoluble

Evidence has shown that when a potential is applied and a current is established across the system, the anionic drugs will move out of the vehicle, into the receptor medium and undergo an electrochemical change. It is a reasonable assumption that the acid form of salicylate, indomethacin and benzoate are also forming, given the in-depth analysis provided earlier of the analogous process which occurs with sodium diclofenac.

It was necessary to determine in each case which form of the drug is detected by HPLC with UV detection. Investigations were undertaken to establish if a

distinction could be made between the detection of sodium benzoate and benzoic acid. This drug was chosen because both acid and salt forms of the drug are soluble in aqueous solution and have a comparable solubilities. Standard solutions of both compounds were prepared for analysis. Results showed that both compounds had the same λ_{max} (254nm), they both also had the same retention time through a reverse phase column and finally and most importantly, both have the same peak response ratio, i.e., a 0.1M solution of benzoic acid will give exactly the same integration area as a 0.1M solution of sodium salicylate. These results show that a differentiation cannot be made between the two compounds on the basis of HPLC analysis alone.

Because the peak area response ratios are the same for both salt and acid forms of benzoic acid, it cannot be said that its profiles in Figure 5:6:3 represent either the acid or salt form of the drug alone. It is most likely that the profile represents the cumulative amount of both acid and salt which is soluble in the receptor medium under the prevalent conditions. However, for the other drugs investigated it is more likely that the profiles represent the salt form of the drugs as the acid forms are increasingly insoluble in the aqueous receptor media.

5:7 *Discussion*

The results in this chapter were expected to be straightforward with a significant enhancement of drug evident upon application of the correct potential. Instead the results have proven at once disappointing, surprising and in the end revelatory as it was confirmed that indeed the release of anionic drugs is enhanced iontophoretically.

Further evidence towards understanding the electrochemical change of the drugs came about by monitoring the pH and current profiles during the experiments. It was measured that over the course of the experiments that the pH of the receptor medium changed from an initial pH of 6.8 during Stage I to a final pH of ~9.0 at the end of Stage II. This would indicate that hydronium ions produced by electrolysis were not in solution by the end of the experiment. The formation of the acid form of the drug at the anode requires hydronium ions. Once the acid forms the product is

insoluble thus leaving an excess of hydroxide ions in solution from the electrolysis of water.

Secondly, it was noted that as the experiments progressed the current would gradually decrease. A greater and greater potential was required to maintain the current at the stated values, from 7.0V initially to > 20.0V by the end of the experiment. The explanation for this is the decreased number of drug and hydronium ions available to carry the current as the experiments proceeded.

In experiments to date, the effects of buffering are notable. Buffering the system is now known to have three effects depending on the type of experiment. In passive studies it was recognised that buffering the system reduced the ion gradient within the vehicle thus reducing the release of sodium diclofenac. During enhancer studies the buffer was found to reduce the degree of ion-paring in the vehicle, thus increasing the amount of drug released and finally in iontophoretic studies buffer has been shown to reduce the amount of acid formation therefore increasing the amount of drug detected by standard methods.

The results of this chapter lay down a firm basis on which to proceed with investigation into the enhancement of the release and delivery of anionic drugs using combined methods of physical and chemical enhancement across Visking membrane and human *stratum corneum*. Although detection methods may be inadequate to obtain a continuous release profile it is expected that an enhanced quantitative delivery of drug should be obtainable after a 24 hour period whereby any precipitates may be collected and quantified.

Chapter 6

Simultaneous Physical and Chemical Enhancement of the Transport of Anionic Drugs from Liquid Crystalline Gels Across Visking and Human Stratum Corneum Membanes.

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Simultaneous Physical and Chemical Enhancement of the Transport of Anionic Drugs from Liquid Crystalline Gels Across Visking and Human Stratum Corneum Membanes.

6:1 Introduction

Investigations reported so far have focussed exclusively on the release characteristics of anionic drugs from different vehicles across non-rate limiting Visking membranes. This chapter describes primarily investigations into the transport of anionic drugs across full thickness human *stratum corneum* (SC). It was first necessary to comprehensively characterise the release of drugs from the liquid crystalline vehicles in order to determine subsequently their release rates across a rate limiting membrane. Investigations involving the passive, chemically enhanced and physically enhanced anionic drug transport across Visking have been presented in earlier separate chapters whereas for SC investigations, all results will be presented in this section for convenience.

Introductory results will firstly complete the investigations across Visking membranes with the characterisation of the system under combined methods of enhancement. Nolan (1995) found that the combined use of oleic acid and iontophoretic assistance significantly increased the delivery of salbutamol base across the SC. Because it has now been established by the work reported here that anionic drugs are released at an enhanced rate from the vehicle by iontophoresis (albeit electrochemically modified), enhanced transport across the SC should also be observed for anionic drugs. Some synergy should also be expected so that the enhancement obtained when the chemical and physical techniques are applied simultaneously may even be greater than the sum of that provided by either method alone. Oleic acid has been shown not to ion-pair with anionic moieties under cathodal iontophoresis. The enhancer remains in its neutral acid form in which it is most

effective for the disruption of the barrier properties of the SC. Due to a lower interaction between drug and enhancer, there may be a greater enhancement than was observed in the case of oleic acid and salbutamol base by Nolan, (1995).

6:2 *The simultaneous physical and chemical enhancement of the transport of sodium diclofenac from a liquid crystalline gel across Visking membranes.*

As outlined in section 1:5:2, examples of simultaneous physical and chemical enhancement are now numerous in the transdermal literature. Among the examples more relevant to this study are the investigations by Oh *et al.*, (1998) which showed a synergistic enhancement of AZT in the presence of oleic acid and iontophoretic assistance. Also relevant are investigations by Fang *et al.*, (1998), which provided evidence for the enhanced transdermal delivery of enoxacin (an anionic drug) by the combined use of benzalkonium chloride and iontophoresis. These experiments were carried out at a vehicle pH = 10.

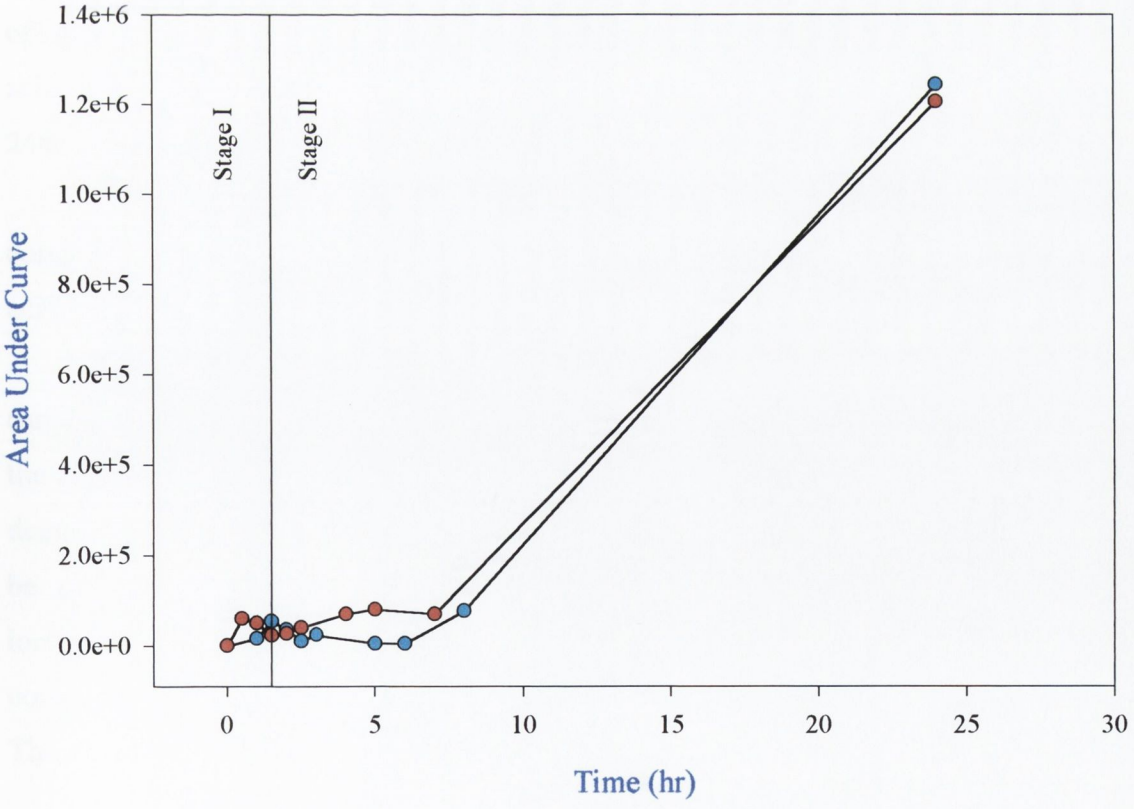


Figure 6:2:1 *Iontophoretic Release Profiles of 0.1M Sodium Diclofenac across Visking Membranes from Liquid Crystalline Gels Incorporating 0.1M BDDAB.(Current = 0.5mA, the experiment carried out in duplicate)(1mg = 1619629 AUC)*

It was anticipated that ion pairing of BDDAB and sodium diclofenac within the vehicle leading to non-release of the drug would produce the same difficulty under iontophoretic assistance as has been encountered in passive experiments. The iontophoretic release profiles of sodium diclofenac from a liquid crystalline gel containing BDDAB is shown in Figure 6:2:1. The profile shows a negligible release during stage I and the following six hours of stage II of the experiment. The increase evident in the release rate after an eight our period of the experiment was not expected. There are several possible reasons for this delayed increase in the amount of drug detected in the receptor medium and it may not be a direct result of iontophoretic assistance.

The most plausible explanation for the delayed increase observed in the release is that the production of additional ions from the electrolysis of water, reduces the ion pairing between drug and enhancer. This in turn increases the release of drug from the vehicle in the same manner in which buffer ions have been shown to act. After the hydronium and hydroxide ions are produced at the anode they must diffuse upwards into the vehicle to have an effect, hence the delayed increase. The presence of BDDAB has in some way reduced the amount of diclofenac being converted to the acid form. In earlier experiments there was no diclofenac evident in solution after 24hr under iontophoretic conditions alone.

Although the release after 24hr is only 27% of that released under passive conditions, the results are encouraging. It can be assumed that additional diclofenac in acid form is also present in solution but not enough to form a quantifiable precipitate.

Experiments using combined physical and chemical enhancement were also carried out using oleic acid as the model enhancer. As oleic acid has been ruled out as the cause of the inhibited release of sodium diclofenac in previous experiments, it was deemed suitable again for current investigations. Also oleic acid has been proven to be an effective enhancer by many researchers, (Nolan, 1995, Oh *et al.*, 1998). The iontophoretic release profile of sodium diclofenac in the presence of oleic acid in comparison to that when BDDAB was used as an enhancer is shown in Figure 6:2:2. The concentration of both drug and enhancer is 0.1M. The current level was 0.5mA.

The graph shows the model enhancer to have significantly different effects on the release of the drug under iontophoretic conditions. The profile of sodium diclofenac and oleic acid is similar to that observed in the absence of oleic acid except that the level of detection of diclofenac remains the same after the first hour of Stage

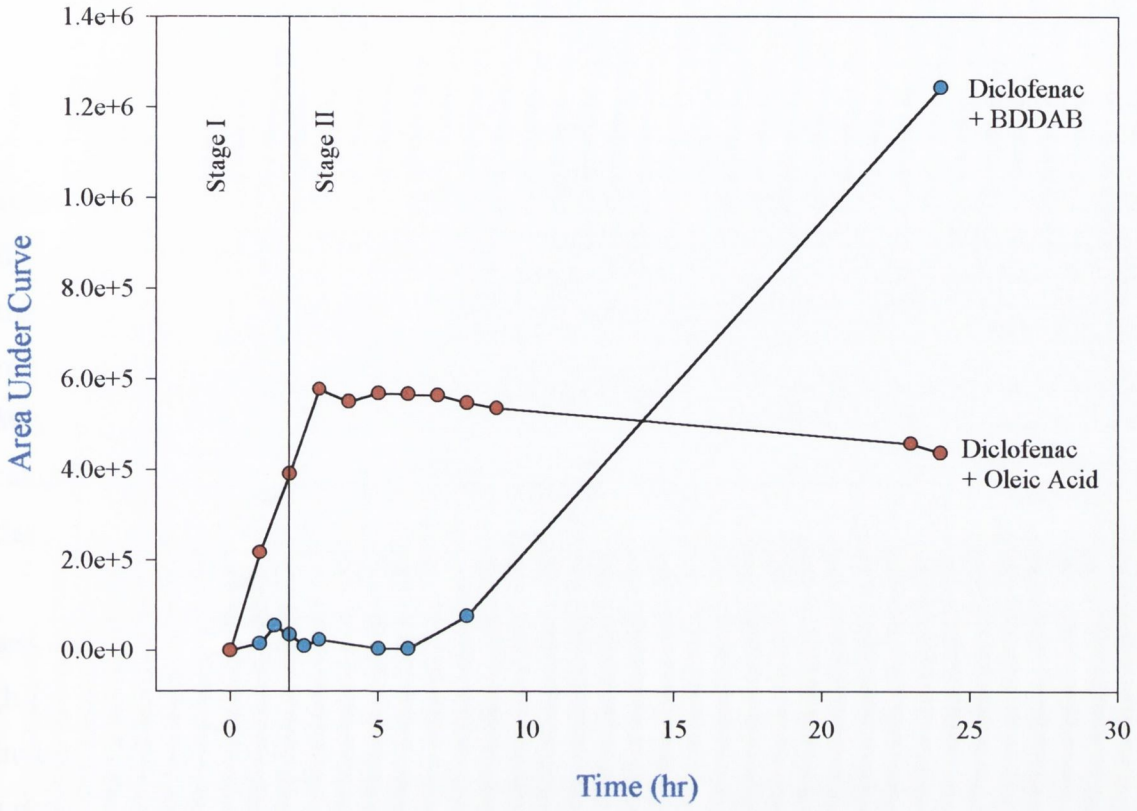


Figure 6:2:2 *A comparison of the iontophoretic release of Sodium diclofenac from liquid crystalline gel incorporating oleic and BDDAB. (All concentration were 0.1M with 0.5 mA current)(1mg = 1619629 AUC)*

II and with only a slight decline by the end of the experiment. The levelling off of the quantity detected compared to that observed without the acid is probably due to the buffering action of the oleic acid within the vehicle. Oleic acid will thus reduce the number of hydronium ions which may otherwise associate with sodium diclofenac resulting in a greater quantitative detection of the drug. The total quantity of sodium diclofenac detected after 24hr is 13.6% of that detected after the same period for passive release but also represents a 13-fold increase in detection compared to the same experiment in the absence of oleic acid. The effect of iontophoresis can be said to be dominant for both enhancer systems and detection of the true cumulative amount of drug delivered, in both salt and acid forms, remains as an analytical problem.

6:3 *Transdermal Transport of Anionic Drug Molecules Across Human Stratum Corneum.*

The final stage of the work is to analyse the transdermal transport of the selected anionic drug molecules across human SC in the light of information obtained from the work thus far and from experiments carried out by Nolan (1995) into the transdermal transport of cationic salbutamol base. The experimental protocol remained the same except that human SC was used instead of Visking membrane. With the behaviour of the drugs under different conditions already established from release from liquid crystalline and agar vehicles, it is now possible to characterise the delivery of the drugs through a rate limiting membrane.

Human *stratum corneum* was obtained within 24hr *post-mortem*: the age and sex of each tissue sample was recorded. Subcutaneous fat was removed and the full thickness SC was dissected into the appropriate size. The majority of experiments involved the use of skin as received. However, due to the irregularity of donors, some samples were frozen and stored below 273K until required and thawed prior to use. Kastings and Bowman have verified that human skin stored in this manner is entirely suitable for iontophoretic transport studies.

6:4 *Passive Release of Anionic Drugs Across Human Stratum Corneum*

Due to their charged state, anionic drugs do not fall into the category of drugs which are ideal for passive transdermal drug delivery. However, studies were carried out to establish the amount of drug which may diffuse passively in order to compare and quantify the contribution of passive diffusion to any enhanced delivery reported later.

The result of passive experiments under all conditions revealed no detection of diclofenac over the initial 10.5hr. The experiment was allowed to run for a period of 48hr, after which a release maximum of 0.62% of diclofenac was detected, which is equivalent to 0.35mg. The release from 4% agar across SC was also investigated and also found to be negligible. These results indicate that regardless of the type of vehicle used or the release rate from a vehicle the SC membrane will determine the rate of drug transport. The references to passive transdermal diffusion of sodium

diclofenac are rare. Most references are in respect of animal models such as hairless mouse skin (Ho *et al.*, 1993), abdominal rat skin (Arellano *et al.*, 1998, Calpena *et al.*, 1999), snake skin (Bhattachar *et al.*, 1992), and rabbit abdominal skin (Wong *et al.*, 1999, Huang *et al.*, 1995). Others have sought to predict the passive flux of diclofenac using synthetic membranes to mimic the barrier properties of the skin. These include the use of silicone membranes (Maitani *et al.*, 1994, 1996).

Of the few reports of passive diffusion of sodium diclofenac across human SC, Nishihata *et al.*, (1988) found the relative bioavialability of the drug from a topical gel of one volunteer to be ~7% of that after rectal administration of a commercial suppository. Kurowski and Dunky, (1987) carried out a double-blind crossover test on twelve patients with degenerative joint disease and knee joint effusions. Ointments were applied once a day over three days radially 10cm up and down from the knee joint. After the last application of ointment, samples of synovial fluid were taken from the joint at regular intervals by punctuation of the joint. After 1.5hr the peak plasma concentration reached was 45.0 ± 29.0 ng/ml of drug and after 3hr the figure obtained was 37.9 ± 20.2 ng/ml. These concentrations are in the range of data published by Reiss *et al.*, (1986) and prove the penetration of the compound through human SC into synovial fluid. Due to the nature of the experiments carried out by Nishihata it is difficult to compare quantitative release *in-vivo* with *in-vitro*. If the data obtained for passive investigations is extrapolated linearly back to zero hours, the value for 3hr is ~366ng/ml which is 10 times greater than the range of those discussed. However, the human body can be regarded as an infinite sink in comparison to the volume of the receptor medium of the Franz-like diffusion cell used for the work reported here.

Assandri *et al.*, (1993) calculated the plasma levels of diclofenac acid after three hours in human volunteers to be ~17.4 ng/ml from a gel formulation with repeated dosing. Devi and Paranjothy, (1999) reported a plasma level of the diethyl ammonium salt of diclofenac in human plasma to be ~34ng/ml after 8 hours. Again interpolating back to three hours for Devi and Paranjothy's data will give a value in the same range as the other cited literature.

Most researchers concluded that the levels obtained were, if sustained, adequate for therapeutic use, even though plasma levels were up to 100 times lower than those achievable via oral administration. A study by Brown *et al.*, (1995) of the

in-vitro diffusion of ^{14}C-labelled diclofenac across human SC found that a depot or reservoir of the drug was formed in the epidermis and that it was probably this layer that determined the rate of release of diclofenac within the SC. Nishihata *et al.*, (1988) also reported a large accumulation of diclofenac in dorsal subcutaneous tissue of rats.

From *in-vitro* experiments carried out and from literature reports, it is evident that diclofenac diffuses across human SC and more so across animal models. However, quantitatively amounts are very small and very close to the limits of detection using UV detection. More sensitive methods such as luminescence may be required if passive studies alone were of interest.

The concentration of sodium indomethacin detected in the receptor medium is very similar to the passive release of sodium diclofenac across human SC.

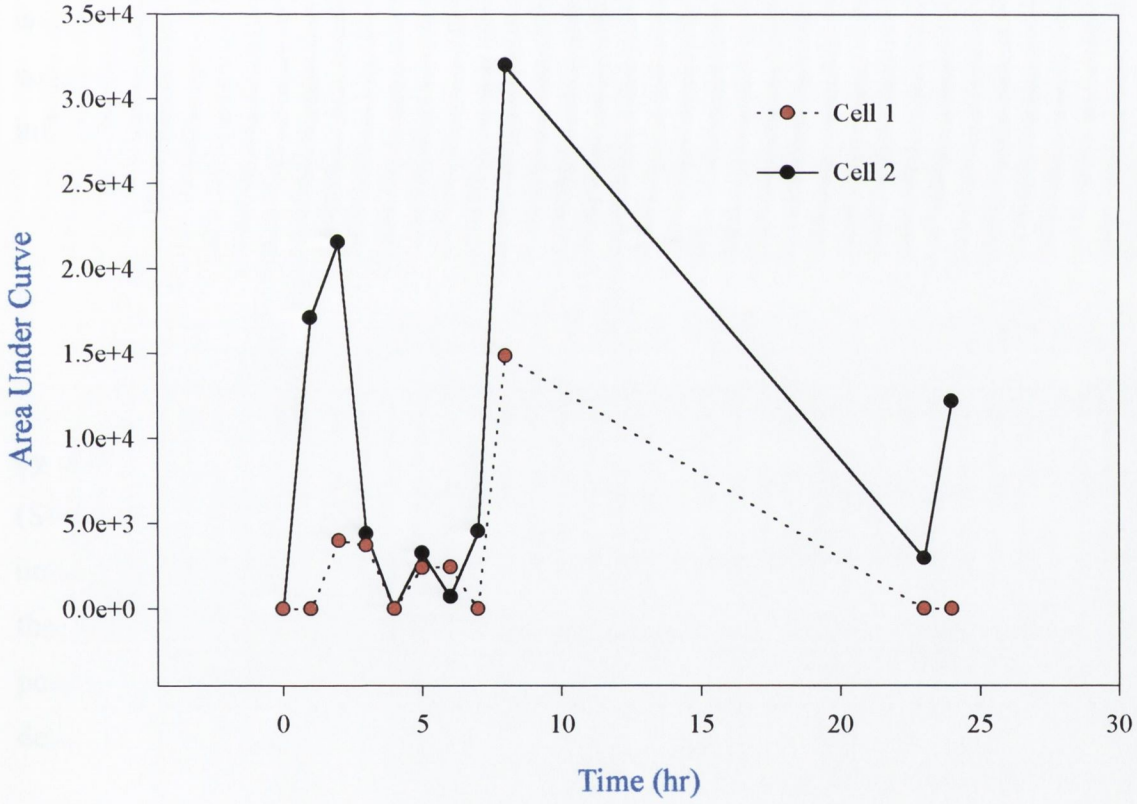


Figure 6:4:1 *The baseline passive release of Sodium Indomethacin across human Stratum Corneum from liquid crystalline gels in large scale. (All concentrations 0.1M). (1mg = 321338.9 AUC)*

The passive release profile of sodium indomethacin across SC is shown in Figure 6:4:1. If plotted in the same range as passive release across Visking membranes, the profiles in Figure 6:4:1 would appear as a flat line. In a sense the profile is an

amplified baseline with an erratic profile close to the limits of detection. The average maximum concentration at three and eight hours corresponds to a percentage release of 0.056%, which is equivalent to ~353 ng/ml. This compares significantly with the interpolated value for sodium diclofenac after three hours of ~366ng/ml.

The three to eight hour value for sodium indomethacin is in good agreement with values obtained by Jona *et al.*, (1995) for a prodrug derivative of indomethacin. They obtained a maximum concentration after 24hr of ~150μg/ml, which was not obtained here during investigations as shown in Figure 6:3:1. However, the derivative was designed and synthesised specifically to improve upon the transdermal penetration of indomethacin itself.

From this investigation and limited literature reports it is evident that sodium indomethacin also passively diffuses through human SC but in very small amounts. It is also a possibility that the drug would have a therapeutic affect at such low concentration levels, as in the case of sodium diclofenac, due to its potent anti-inflammatory capability, e.g., five times that of cortisone (Hamor, 1990).

6.5 *The Iontophoretic Transport of Anionic Drug Molecules Across Excised SC*

Ionised drugs may be caused to permeate the SC at a rate faster than normal by establishing a current following the application of a potential across the membrane, (Singh, 1993). Drugs such as sodium indomethacin and sodium diclofenac are ionised under the standard vehicle conditions used here but there are very few references to the transport of these or other anionic drugs in the iontophoretic literature. It is possible that attempted investigations have never been completed due to unresolved detection problems or electrochemical changes not being recognised.

The theory of iontophoresis has been introduced in detail in section 1:4:1 where concepts such as electrorepulsion, electroosmosis and electroporation were discussed. With the release behaviour of a range of anionic drugs now established from liquid crystalline gels it is possible to undertake investigations to establish the true behaviour of this class of compound when transported across SC with iontophoretic assistance. Earlier work reported here (Chapter 5) has established that sodium diclofenac is caused to diffuse in a greater quantity from a liquid crystalline

gel using cathodal iontophoresis than by passive diffusion alone. Here it will be shown that the drug is also caused to diffuse in increased quantities across excised SC under the same conditions.

The main difficulty is to find the correct analytical technique or model to accurately estimate the transport of the drug. The nature of the experimental procedures restricts the ability to change parameters such as receptor medium without compromising the integrity of the investigation itself. As the transported drug may be electrochemically altered to an insoluble material it may only be possible at best to obtain an estimate of the final quantity of drug which has diffused rather than a time dependant profile. The polarity of the receptor medium may not be changed to suit the solubility of the acid form of the drug. However, if similar profiles are obtained to those described in the previous chapter, e.g., in Figure 5:6:3 it provides convincing if indirect evidence of enhanced transdermal delivery.

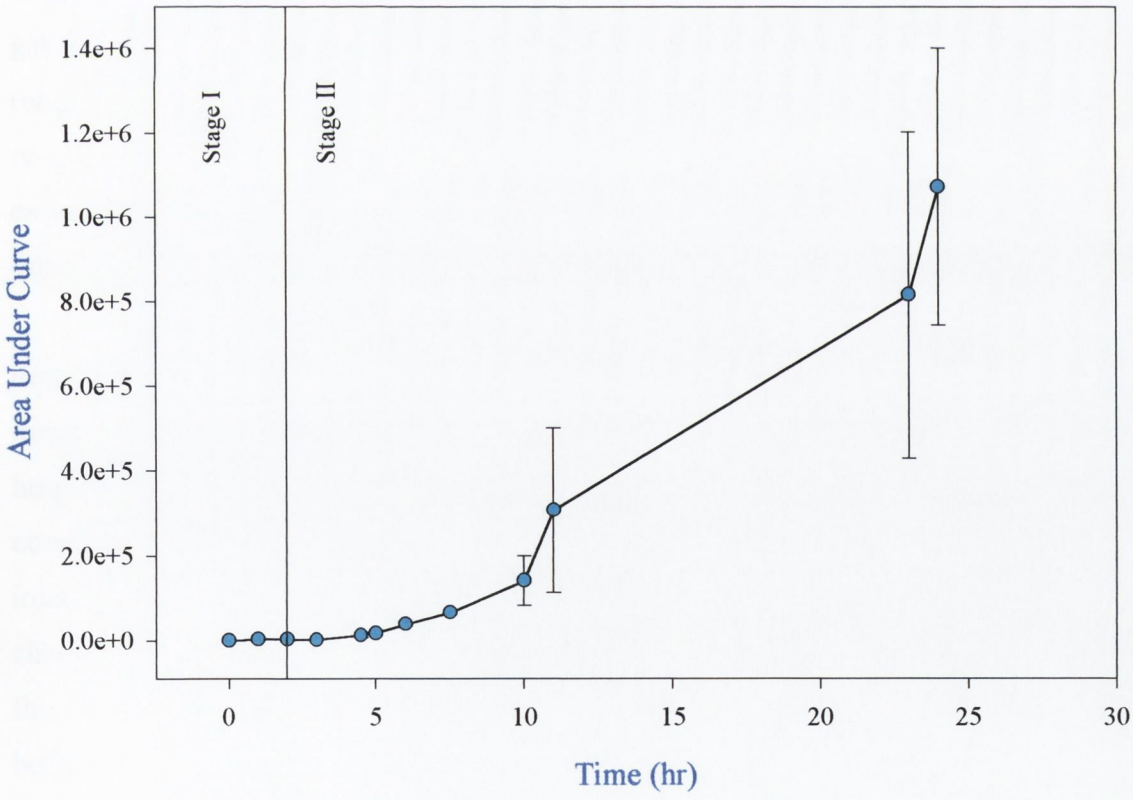


Figure 6:5:1 *Iontophoretic release of Sodium Diclofenac from Liquid Crystalline Gels across Human Stratum Corneum. (Concentration = 0.1M, Current = 0.5mA). (1mg = 1619629 AUC)*

The iontophoretic release profile of sodium diclofenac from a liquid crystalline gel across SC is shown in Figure 6:5:1. The receptor medium used throughout investigations was buffer solution (IPBS) in order to optimise detection of the drug as shown in Figure 5:4:1.

The profiles in Figure 6:5:1 are remarkably similar to those in Figure 6:2:1 which represent the release of diclofenac under similar conditions across Visking membranes except for the inclusion BDDAB in the latter experiment. The most obvious feature of the profiles in Figure 6:5:1 is the extended lag time of 2 hours, before detection of the drug becomes apparent, compared to Figure 6:2:1. This may be due to the reservoir effect of the skin reported by Nishihata *et al.*,(1988) and Brown *et al.*, (1995). However as BDDAB was included in one of the experiments it may not be a comparison of like with like.

The steep increase in detection after 23hr is due to the expulsion of bubbles of gas, which form overnight, from under the gels. The volume of gas is sufficiently large enough to prevent contact of the membrane with the receptor medium. Once the gas has been expelled any drug which may have diffused through the skin and reaching the underside will then move into the receptor medium as soon as contact is re-established. The formation of gas is more prevalent during iontophoretic experiments than during passive delivery. It is possible that more gas was formed in one cell than the other hence the reduced value of the red profile after 10hr.

The other significant deduction from the data is that the 24hr value for the detection of sodium diclofenac is not zero as was the case with the Visking membrane. Comparisons of results from investigations using Visking and SC may help to explain the difference in the release profiles of diclofenac under iontophoretic conditions. For example, it is now possible to envisage the formation of hydronium ions at the anode by electrolysis which then diffuse towards the electrode of opposite charge, i.e., the cathode above the vehicle. On route the hydronium ions encounters the SC membrane, which may in turn absorb or neutralise the ions. Alternately the buffering capacity of the receptor medium (IPBS) may also neutralise their effect thus increasing the detection of diclofenac anions in the receptor medium.

A quantitative comparison of the amount of drug transported after 24hr, with water as the aqueous phase of the gel and buffer as the receptor medium, shows the iontophoretic delivery of sodium diclofenac across human SC to be ~ 732.6μg

compared to ~ 183μg for the analogous passive delivery. This amounts to a four-fold increase in the quantity of sodium diclofenac transported iontophoretically compared to passively.

The experiment was repeated with SC from a different donor to estimate the variation arising from different donor sources. A comparison of the same experiment with the two different donor sources is shown in Figure 6:5:2.

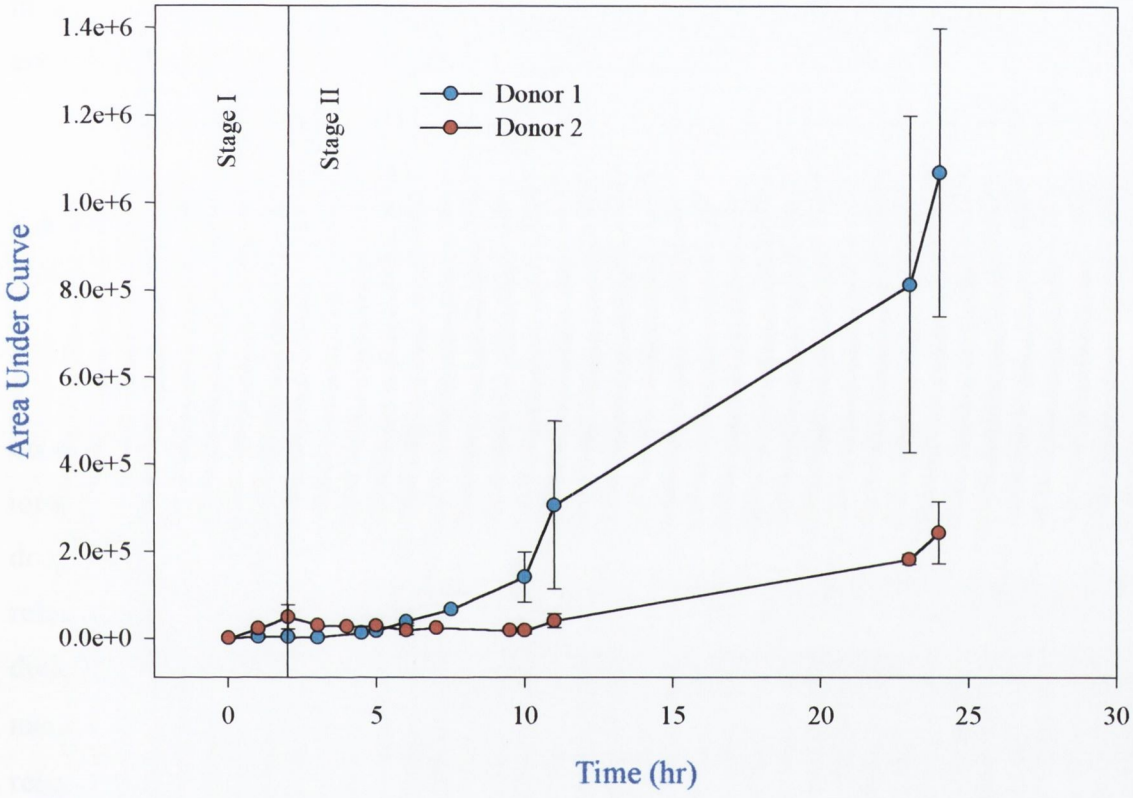


Figure 6:5:2 *The iontophoretic release of Sodium Diclofenac from Liquid Crystalline Gels across SC of two different donor sources, (Concentration 0.1M, Current = 0.5mA). (1mg = 1619629 AUC)*

The graph indicates there is a significant difference between the mean values of drug transport across the membranes after 24hr. The experiment was carried out in triplicate.

Human skin is known to display considerable inter sample variation in both its physicochemical and transport properties (Behl *et al.*, 1989, De Nuzzio and Berner, 1990, Sims *et al.*,1992). In terms of drug permeability, the co-efficients of variation indicate that the inter-sample variation to be ~89% whereas the intra-sample variation was found to be 30%. Investigations by Nolan (1995) found that the inter sample

variation of the transdermal transport of salbutamol base to be ~ 35%. This value reduced to ~20% for intra-sample variation.

With these margins of error it is difficult to make predictions as to the merits of the enhancement techniques being employed. However, it is possible to reduce the standard deviation by making comparisons only with intra-sample investigations, i.e., samples from the same donor source. Under these conditions the transdermal delivery of sodium diclofenac is significantly enhanced by iontophoretic assistance as shown in Figure 6:5:1, when compared with passive diffusion. The passive and physical enhancement comparisons are made using intra-sample specimen membranes only.

6.6 *Effect of Initial Drug Loading and Buffering on the Transdermal Transport of Sodium diclofenac.*

The initial drug loading was doubled from 0.1M to 0.2M to estimate the effect on the quantitative transdermal delivery of drug across SC. In the absence of buffer ions in the receptor solution the release of diclofenac is negligible regardless of initial drug loading. However, when buffer is used as the receptor medium the increase in release is significant with increase in loading. A comparison of the release of sodium diclofenac with initial drug loadings of 0.1M and 0.2M is shown in Figure 6:6:1. The mean cumulative 24hr transdermal releases are 166μg and 759μg for 0.1M and 0.2M, respectively.

There is very little error in the data between the four to eleven hour period. This time period is also interesting in that the 0.2 M profile shows distinct undulations once the current has been established. This may be indicative of the system going through several equilibria as the system goes through changes induced by the current. It is conceivable that once the potential is applied, the instantaneous electrolysis of water, producing hydronium and hydroxide ions, also reduces the detection of the drug. As the current becomes established and diclofenac is drawn across the SC the detection of the drug increases. These two opposing processes may be responsible for the nature of the profile until the quantity of drug coming across is sufficient to increase its detection towards the end of the experiment. Buffer ions must also be playing a part in this process by reducing the amount of hydronium ions available in

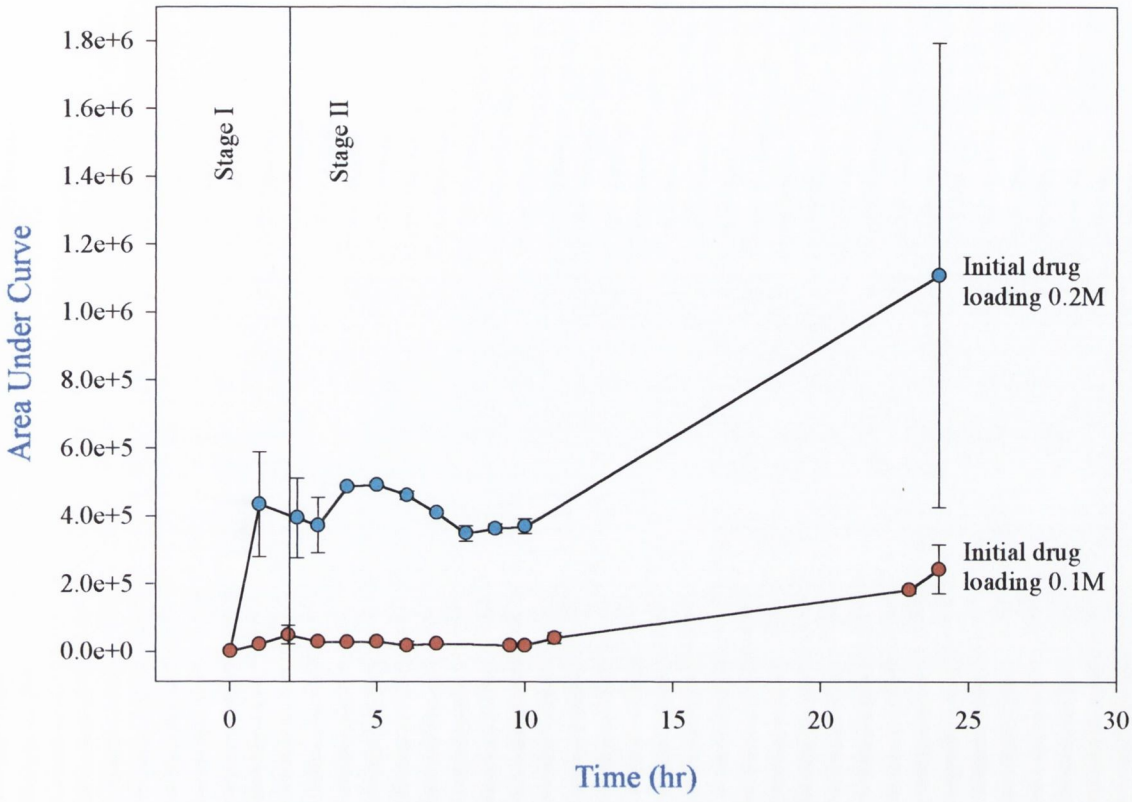


Figure 6:6:1 *The iontophoretic release of Sodium diclofenac across SC with initial drug loadings of 0.1M and 0.2M. (Aq. Phase of Gel=water/receptor medium buffered, current=0.5mA, the experiment was carried out in triplicate). (1mg = 1619629 AUC)*

solution to convert the anion of the drug to its acid form. As with non-rate limiting Visking, the true quantitative transport of sodium diclofenac may be considerably higher than is apparent from the analysis. Efforts to ascertain the values will be described later in this chapter.

6.7 *The effect of buffering on the iontophoretic transport of Sodium Diclofenac from a Liquid Crystalline Gel across Stratum Corneum*

Investigations were also carried out into the effect of buffering the receptor. Experiments consisted of an initial drug loading of 0.2M and the receptor medium was either aqueous or buffer solution. The effect of buffering the receptor medium is apparent in Figure 6:7:1.

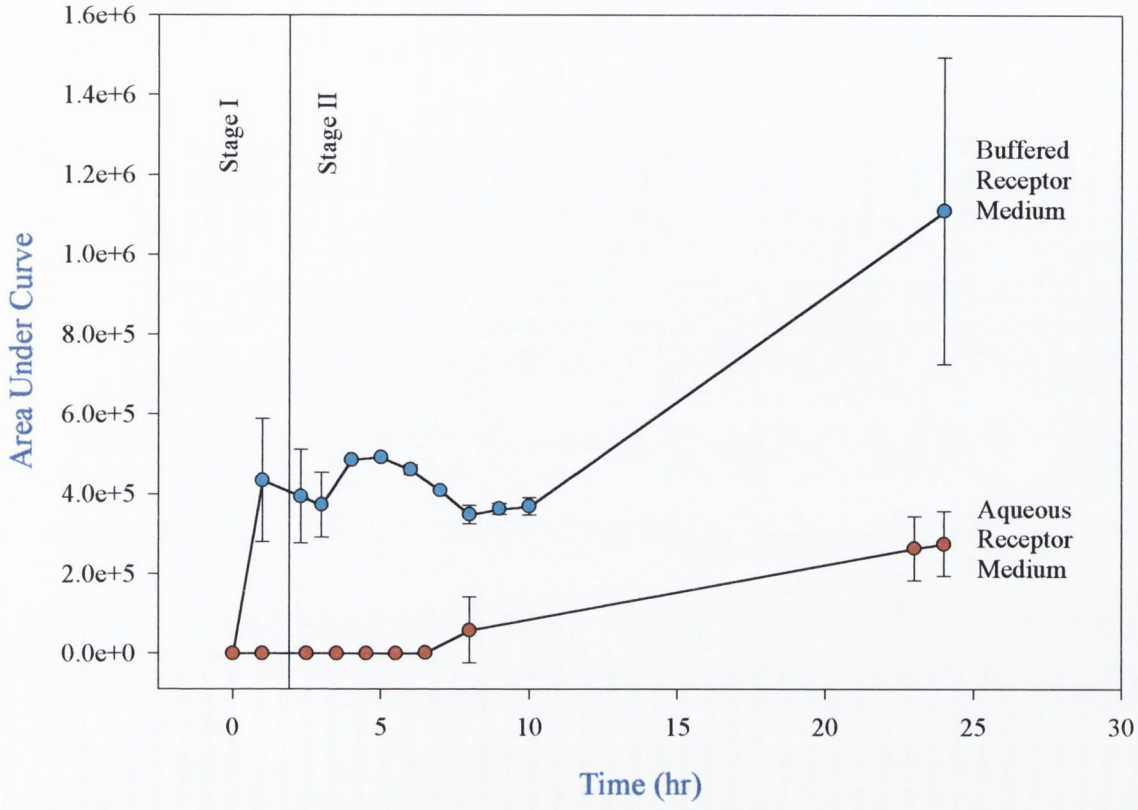


Figure 6:7:1 *The effect of buffering the receptor medium on the iontophoretic transport of Sodium Diclofenac across SC. (Drug concentrations 0.2M, 0.5mA, the error bars represent the standard deviation of the experiments carried out in triplicate) (1mg = 1619629 AUC)*

The effect of buffering the receptor medium is significant. The apparent increase in release may be due to an increase in drug detection. A similar result was observed during investigations across Visking membranes as shown in Figure 5:4:1. An approximately four-fold increase in diclofenac detection is evident in Figure 6:7:1.

The increase in the quantity of drug detected may be as a result of several processes in addition to the buffer action of the receptor medium. The negatively charged phosphate ions of the buffer solution will also migrate towards the anode. This in turn may reduce the rate at which diclofenac anions will reach the electrode. This process may also inhibit the electrolysis of water in the same way and in one sense "quench" hydronium ions at source by association with phosphate anions. The use of buffer as the receptor medium has been shown to reduce the optimum release of diclofenac under passive conditions, as shown in Figure 3:4:1. However, in Figure 6:7:1 the release of diclofenac is shown to increase dramatically in the presence of

buffer, during the initial passive period of stage I. This may be due to an exchange of drug and buffer ions within the SC. It must be noted that the drug loading used in the investigation is twice that reported in the investigations reported in Chapter 3.

These and other hypotheses will be further explored in the final discussion.

6.8 *The Iontophoretic Transdermal Transport of Sodium Indomethacin*

The iontophoretic transport of sodium indomethacin across Visking membrane was shown in Figure 5:6:1. The graph indicated that the quantity of indomethacin detected under iontophoretic conditions was less than that detected in the absence of the current. This result was attributed to the protonation of the indomethacin anion. This form of the drug is slightly more soluble than diclofenac acid hence a greater quantity of the drug was detected in comparison to diclofenac. It is expected this will be the same for transdermal iontophoretic experiments. The iontophoretic transdermal delivery profiles of sodium indomethacin are shown in Figure 6:8:1.

The graph indicates a significantly enhanced delivery of the drug across the SC under iontophoretic assistance. The mean 24hr iontophoretic value represents the transport of 7.8% of the initial drug loading, which corresponds to a quantitative cumulative release of 5331μg. A comparison of the passive and iontophoretic transdermal deliveries of sodium indomethacin and sodium diclofenac are shown in Table 6:8:1.

Table 6:8:1 *Mean 24hr cumulative passive and iontophoretic transdermal drug release data. (All comparisons are intra-sample and 0.1M drug concentration)*

Drug	*24hr Passive Release (μg)*	*24hr Iontophoretic Release (μg)*
Sodium diclofenac	183.0	732.6
Sodium Indomethacin	34.2	5331.0

The data in Table 6:8:1 indicate that there is a 4 fold increase in the transdermal transport of sodium diclofenac and a 156 fold increase in the release of sodium

indomethacin. The data relate to experiments where water was used as the aqueous phase of the gels and buffer was used as the receptor media. Comparison will be made with available literature values in the next chapter.

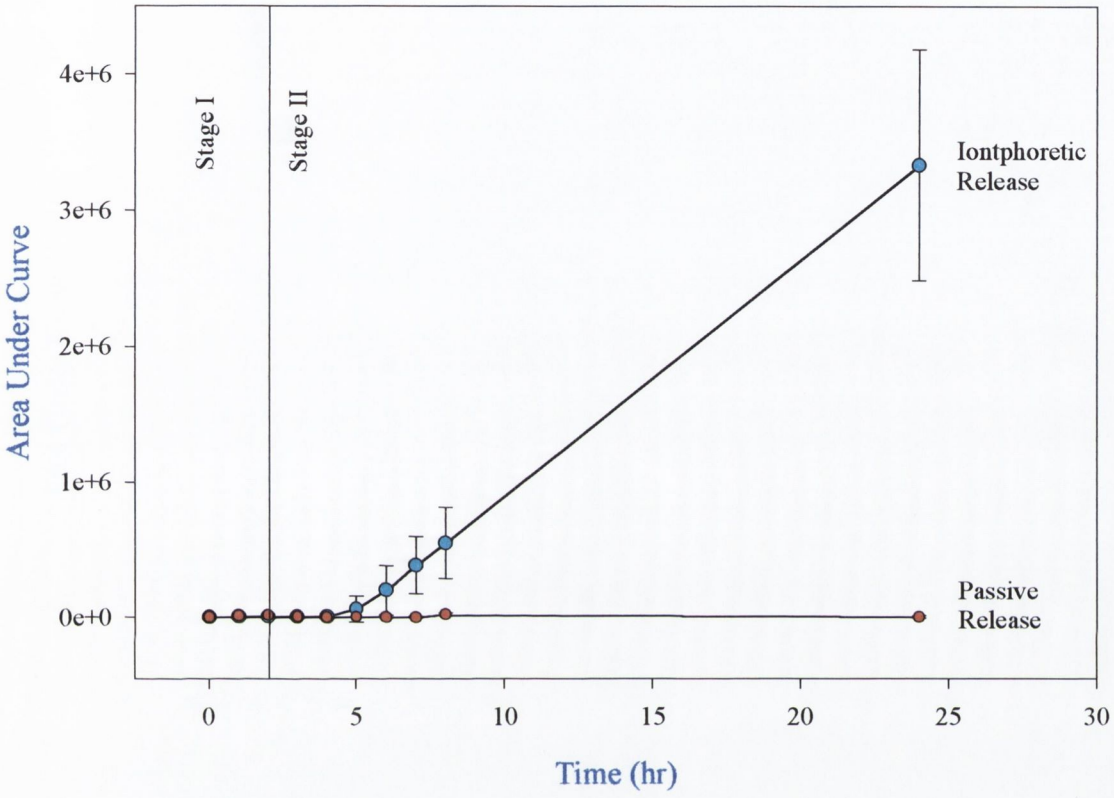


Figure 6:8:1 *A comparison of iontophoretic and passive transdermal release profiles of Sodium Indomethacin. (1mg = 321338.9 AUC)*

6.9 *Simultaneous Physical and Chemical Enhancement of Sodium Diclofenac Across Stratum Corneum*

Many examples of the synergistic enhancement of drug delivery of combined iontophoretic and permeation chemical enhancers were given in Section 1:5:2. In particular, earlier work in this laboratory (Nolan, 1995) has shown synergistic enhancement of *in-vitro* transdermal delivery of cationic salbulatmol base across SC from the liquid crystalline gel.

It has now been established that anionic drugs are enhanced by iontophoretic assistance across human SC. Analogous investigations to Nolan's were performed

incorporating oleic acid or BDDAB in addition to the drug compound to further examine if evidence of synergy between the two enhancement techniques exists. Due to the limited availability of human SC, experiments were curtailed to a comparison of data obtained from liquid crystalline vehicles only.

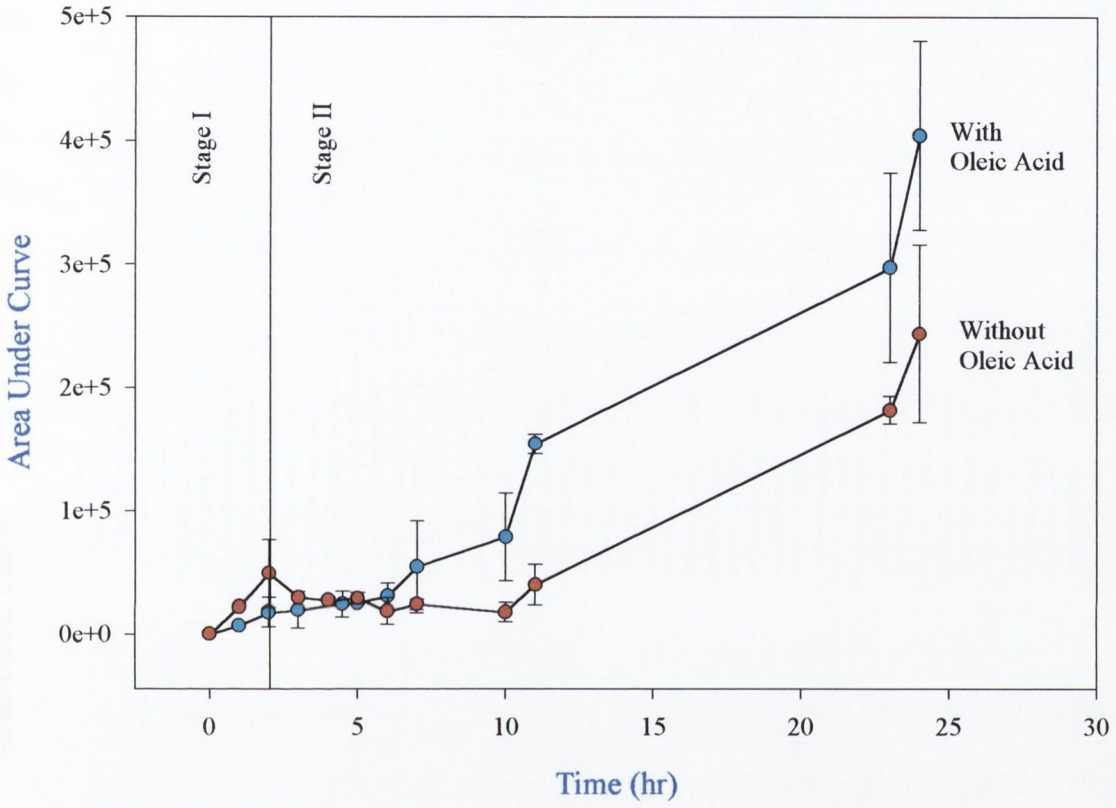


Figure 6:9:1 *The iontophoretic release of Sodium Diclofenac from Liquid Crystalline Gels with and without Oleic Acid. (All concentrations 0.1M, 0.5mA, water/buffer system). (1 mg = 1619629 AUC)*

Comparisons of the iontophoretically assisted release of sodium diclofenac from a liquid crystalline gel with and without oleic acid across SC is shown in Figure 6:9:1. The aqueous phase of the gel was water and the receptor medium was buffered.

The mean cumulative 24hr value in Figure 6:9:1 (blue profile) indicates that the release of sodium diclofenac increases by a significant margin of 66% in the presence of oleic acid. The experiments were carried out in tandem using intra-sample SC membranes. The presence of oleic acid would appear to reduce the lag time by several hours with the profiles diverging at the six hour time interval. This is indicative of a disruption of the barrier properties of the skin. The increase in diclofenac detection when oleic acid is incorporated into the vehicle may not be

attributed to any process involving oleic acid in the receptor medium when compared to the release of the drug in the absence of the enhancer or across Visking membranes. It is not expected that oleic acid will diffuse into the aqueous receptor medium due to its high log *P* value. Oleic acid having the same carboxylic chromophore as diclofenac acid would also be expected to show up in HPLC chromatograms at the same wavelength but with a different retention time. No evidence of this was found. The effects of oleic acid are therefore attributable to its actions within the SC only. The mean quantitative release of diclofenac in the absence of oleic acid after 24hr is 150μg compared to 250μg in the presence of oleic acid.

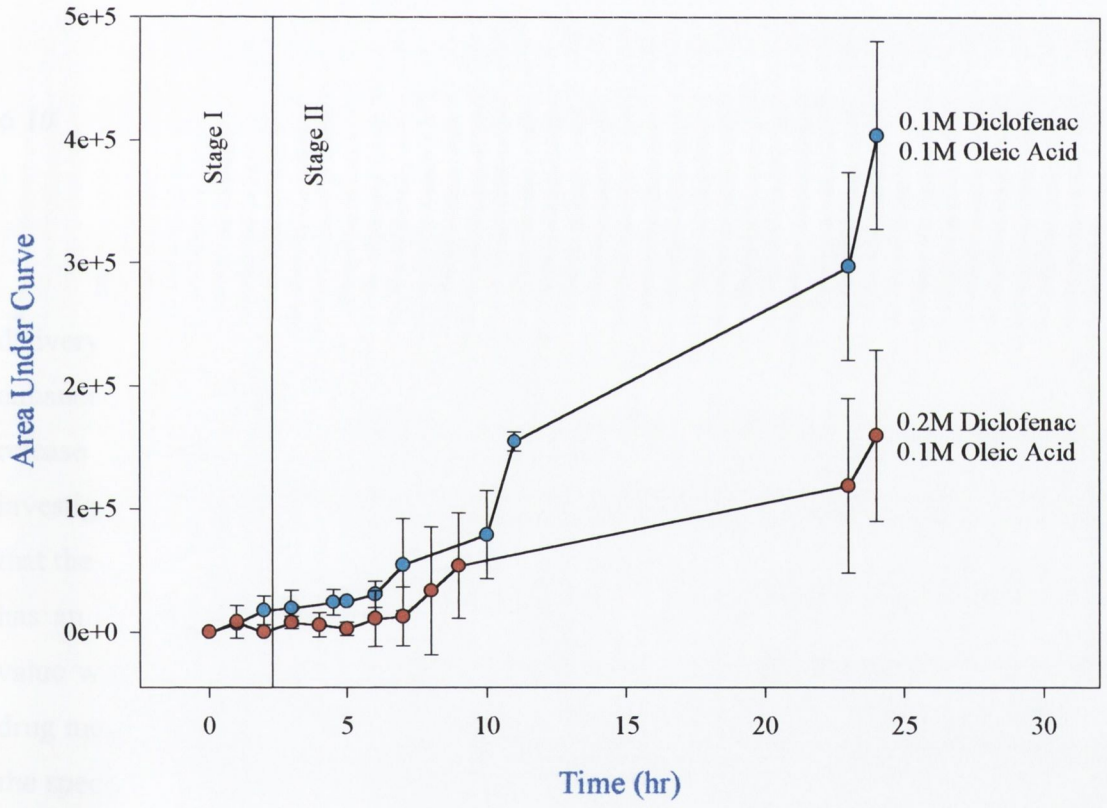


Figure 6:9:2 *The effect of the concentration ratio of drug to enhancer on the iontophoretic release of Sodium Diclofenac form Liquid Crystalline Gels. (0.5mA, water/buffer system, the experiments were carried out in triplicate) (1mg = 1619629 AUC)*

The effect of altering the ratio of the concentrations of drug to enhancer was also investigated during iontophoretic transdermal experiments. The release profiles of 1:1 and 2:1 ratio of drug / enhancer are shown in Figure 6:9:2. Surprisingly, the

detection of diclofenac decreased when the initial drug loading was doubled from 0.1M to 0.2M, in the presence of oleic acid. It is difficult to reasons as to why increasing the initial drug concentration from 0.1M to 0.2M should result in a decrease in the amount of drug detected. This decrease may be a reduction in the apparent detection of the drug. It data shown in Figure 4:5:1 demonstrated that the inclusion of oleic acid in the vehicle did not affect the passive release of the drug. A 1:1 ratio may be the optimum ratio at which the enhancer performs. It may also be possible that the 2:1 ratio of drug to enhancer affects the cubic phase formation of the vehicle which may consequently affect the release characteristics of the drug from the vehicle.

6.10 *The effect of BDDAB on the Iontophoretic Release of Sodium diclofenac from a Liquid Crystalline Gel across SC*

Results reported in Chapter 4 have shown the passive and iontophoretic delivery of sodium diclofenac across Visking depends on the ratio of drug to model enhancer (BDDAB). Figure 4:3:2 indicates that a 1:1 ratio almost totally inhibits the release of the drug. This result does not necessarily make further transdermal investigations futile using this combination of drug and enhancer. It may be possible that the ion-pair of sodium diclofenac-BDDAB (or more correctly diclofenac-BDDA) has an increased thermodynamic potential when in contact with SC relative to its value when it is in contact with the receptor medium. The driving force behind the drug movement may be the difference in the value of the thermodynamic potential of the species in the vehicle and that of the drug in the deeper tissues. In order to obtain the maximum rate of penetration, the highest thermodynamic potential must be used (Higuchi, 1960, Kydonieus, 1987). The estimated log *P* value of sodium diclofenac between octanol/buffer is 2.2. If the log *P* value between the drug and the ion-pair is lower than this value diffusion into the SC is more likely to occur, but the drug or ion-pair must still be transported into the receptor medium to be detected.

The iontophoretic release of sodium diclofenac across human SC with a 1:1 loading of BDDAB was found to be insignificant. All the main parameters such as buffering effects and current level were also investigated but to no avail. It may be possible that the drug partitions from the vehicle into the SC but does not

subsequently partition from the SC into the receptor medium. However, ion-pairing between the drug and model enhancer seems a more plausible explanation for the lack of detection even if transdermal delivery takes place. Ion-pairing was shown to decrease the release of the drug from the vehicle by the data presented in Chapter 4 and it is evident that the partitioning of the drug is not affected when in contact with SC or under iontophoretic conditions. Ion-pairing between the drug and enhancer would negate the negative charge of the drug and this will reduce its electrorepulsion from the cathode above the vehicle. The release of drug was found to be negligible during stage I and stage II of the experiments which further conforms to the hypothesis of ion-pair formation.

It must be noted at this point that throughout all transdermal investigations involving human SC that HPLC analysis provided 1-2 peaks other than those of

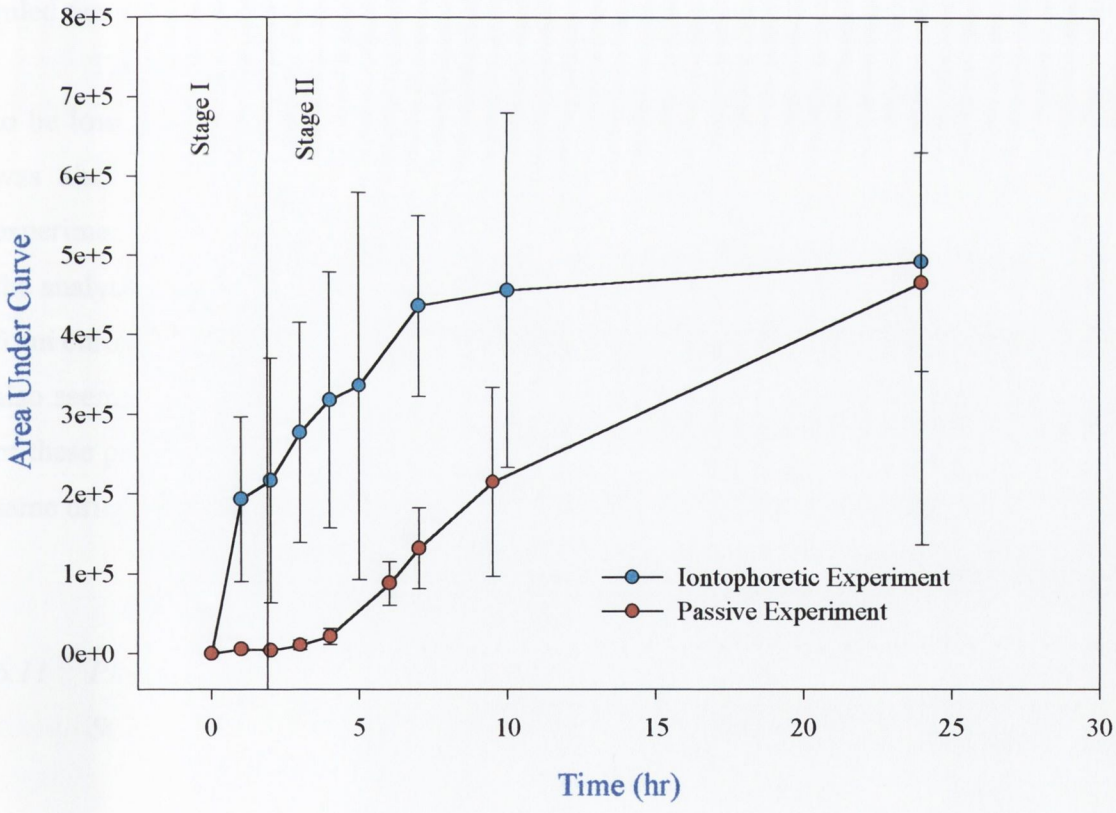


Figure 6:10:1 Release Profile of Unknown Component into Receptor Medium of SC Experiments

the drug molecules under analysis which were not found in the Visking experiments. Figure 6:10:1 shows the profile of an analyte which consistently appeared at a retention time of 2.2 minutes. The data, in this instance, are taken from the

experiments discussed in the previous paragraph, i.e., under the following conditions: 0.1M BDDAB, 0.1M sodium diclofenac, from liquid crystalline gels, 0.5mA current and water/buffer system.

The profiles in Figure 6:10:1 represent the maximum variation that was observed for this analyte throughout all transdermal experiments, both passive and iontophoretic. In general, the analyte is present in the receptor medium from the outset during stage I, which suggests the compound may be present on the underside of the SC or that it may diffuse quickly out of the SC. Stage I showed a large variation in passive flux of the analyte. By examining the blue iontophoretic profile it would appear that the analyte is perhaps negatively charged. The compound has a profile similar to that of indomethacin under iontophoretic conditions. It would be expected that the profile would be more linear if the compound was not charged. Note that the mean cumulative 24 hour value of both profiles is roughly the same. Buffer ions were ruled out as a source of the absorbance peak as they do not absorb at 276nm.

The short retention time and the wavelength of detection indicate the analyte to be low in carbon number, polar and a small molecule. Another unidentified peak was also present at a retention time of 3.15 minutes, but was only present in experiments where liquid crystalline gels were used as the vehicle. This indicates that the analyte peak may be that of one of the vehicle components. The peak was absent from chromatograms where agar was used as the vehicle. The transport of the analyte also seems to be unaffected by current density. Despite diligent searches no evidence of these peaks was found in any literature describing analogous experiments using the same drugs and detection technique.

6.11 *Physical and Chemical Enhancement of Sodium Indomethacin Across Porcine SC*

Due to the lack of availability of human SC, the remainder of experiments had to be carried out using porcine SC. Previous studies have demonstrated that porcine skin may be a useful *in-vitro* alternative animal model for percutaneous absorption and transdermal drug delivery studies (Carver *et al.*, 1989; Williams *et al.*, 1990).

In order to make comparisons, the results of the simultaneous enhancement experiments need to be measured against those of passive and iontophoretic

experiments. Therefore, these experiments were initially carried out. The passive release profile of sodium indomethacin from liquid crystalline gels are shown in Figure 6:11:1.

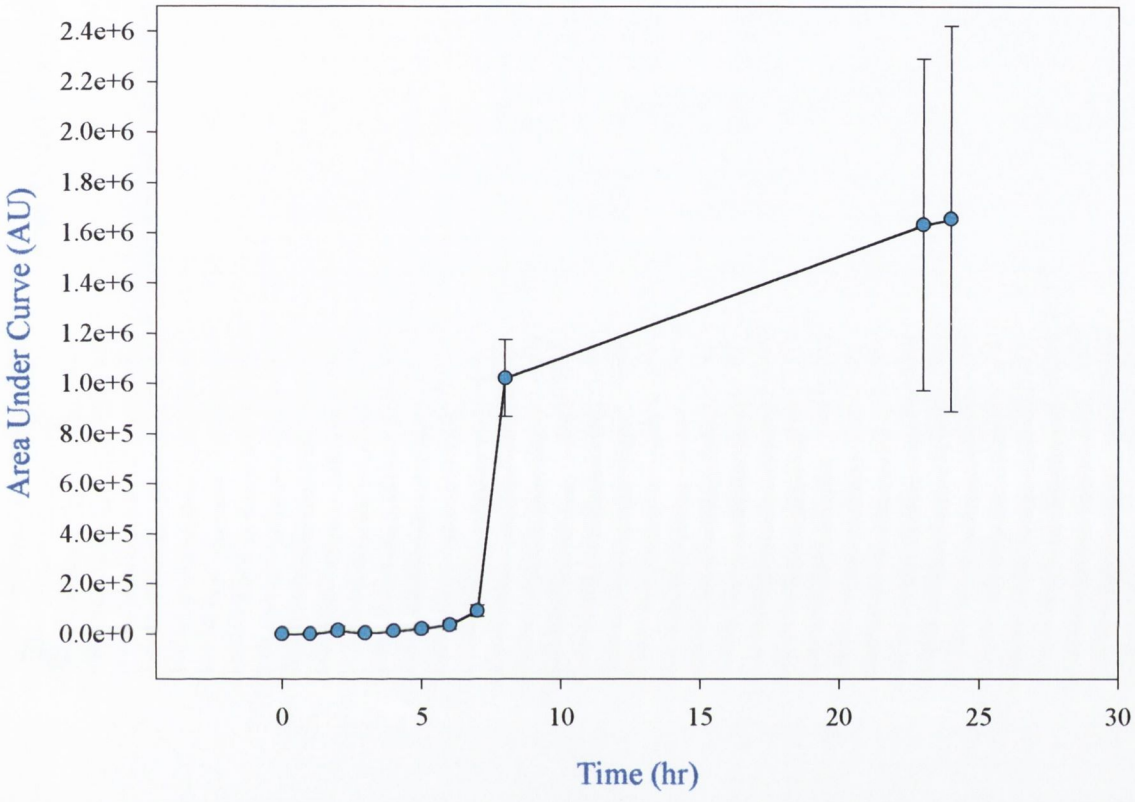


Figure 6:11:1 *Passive release of Sodium Indomethacin across porcine SC from Liquid Crystalline Gels. (0.1M drug concentration, water/buffer system, experiments were carried out in triplicate) (1mg = 321338.9 AUC)*

The release profile in Figure 6:11:1 shows a seven hour lag time before a dramatic increase in release between the seven and eight hour period. The cumulative mean 24 hour transdermal transport of sodium indomethacin was calculated to be 5270 μg/ml. This represents approximately 7.7% transdermal release of the initial drug loading of the vehicle. Sodium indomethacin has been shown to exhibit a lag time under the same conditions across Visking membranes (Figure 3:6:1). Therefore, the lag time in Figure 6:11:1 may be attributed in total to the time it takes for the drug to diffuse through the SC membranes. The next stage of the investigation was to carry out the same experiment under iontophoretic conditions. The iontophoretic release of sodium indomethacin across porcine SC is shown in Figure 6:11:2

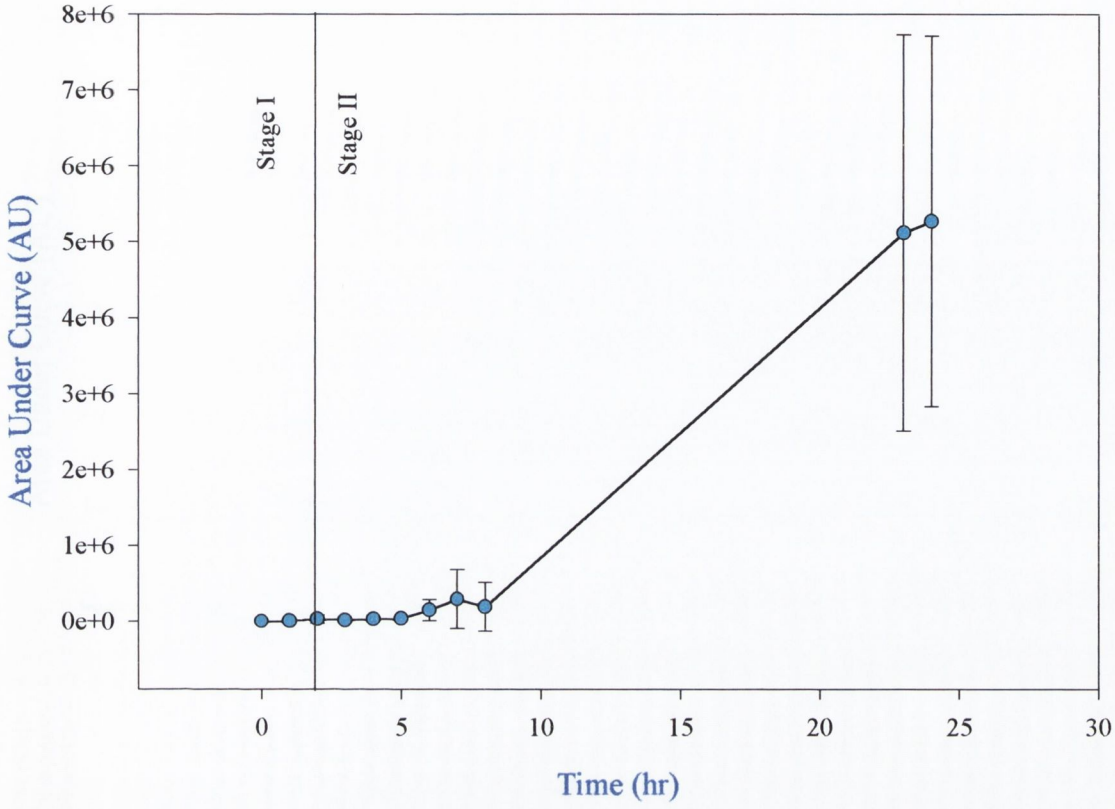


Figure 6:11:2 The iontophoretic release of Sodium Indomethacin across porcine SC from Liquid Crystalline Gels.(0.1M drug concentration,0.5mA current, water/buffer system, the experiment was carried out in triplicate) (1mg = 321338.9 AUC)

The iontophoretic profile for the release of sodium indomethacin across porcine SC shows a dramatic increase in the cumulative quantity of drug transported after a 24 hour time period. The cumulative release was estimated to be 16810 μg of drug. This represents more than a three-fold increase in the transdermal transport of the drug or 200% enhancement over passive delivery. The lag time was observed to be eight hours which is one hour longer than that observed for passive experiments.

The final stage of the investigation was to repeat the iontophoretic experiment but also to incorporate oleic acid as an enhancer at a 0.1M concentration which gives a 1:1 ratio of drug to enhancer. The results of this experiment are shown in Figure 6:11:3. The release profile in Figure 6:11:3 is similar in character to that for iontophoretic transport alone, i.e., the lag time is also eight hours and the standard deviation of data between the three experiments is not significant.

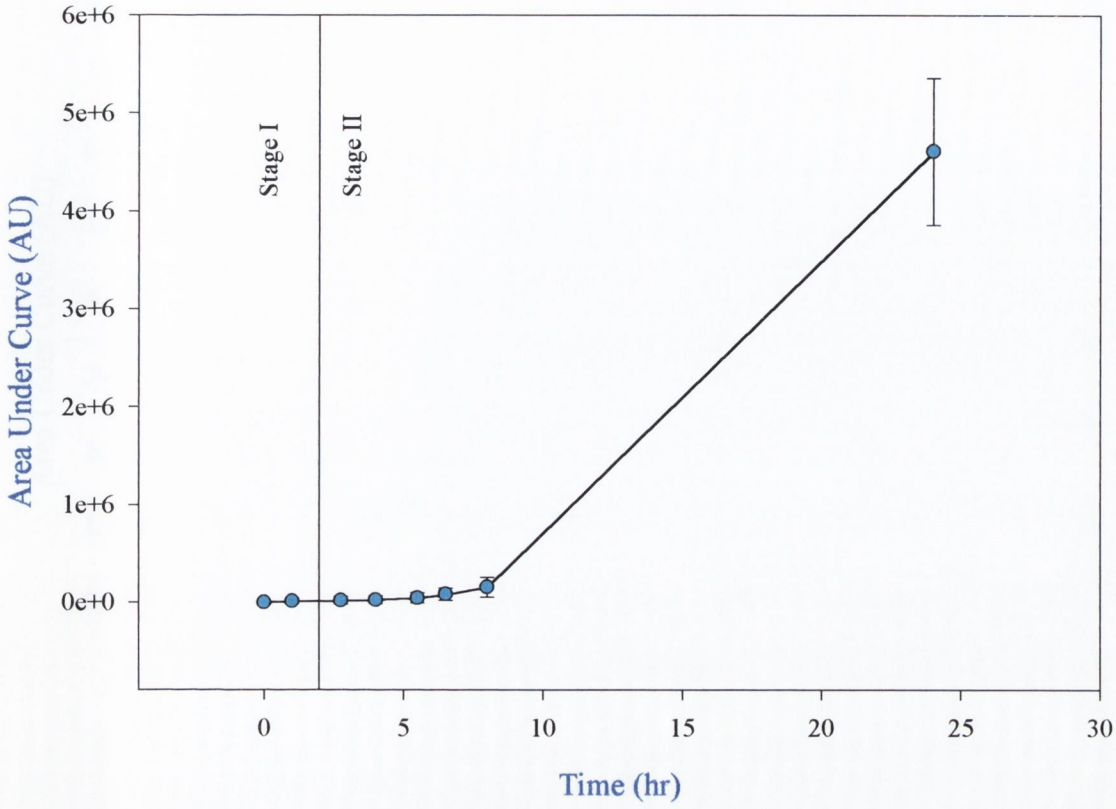


Figure 6:11:3 *The iontophoretic transdermal release profile of Sodium Indomethacin from Liquid Crystalline Gels incorpoating Oleic Acid.(Drug and enhancer concentration 0.1M, 0.5mA current, water/buffer system) (1mg = 321338.9 AUC)*

However the cumulative release after 24 hours is slightly below that for iontophoretic transport alone, (see Figure 6:11:4). The cumulative release was estimated to be 14.69 mg after 24 hours had elapsed or 21.5% transdermal transport of the initial drug loading.

Table 6:11:1 *The percentage and cumulative release of Sodium Indomethacin from liquid crystalline gels across porcine SC under various experimental conditions.*

Experiment	*Percentage Release*	*Cumulative Release (mg)*
Passive	7.77	5.27
Iontophoretic	24.6	16.8
Iontophoretic +Oleic Acid	21.5	14.7

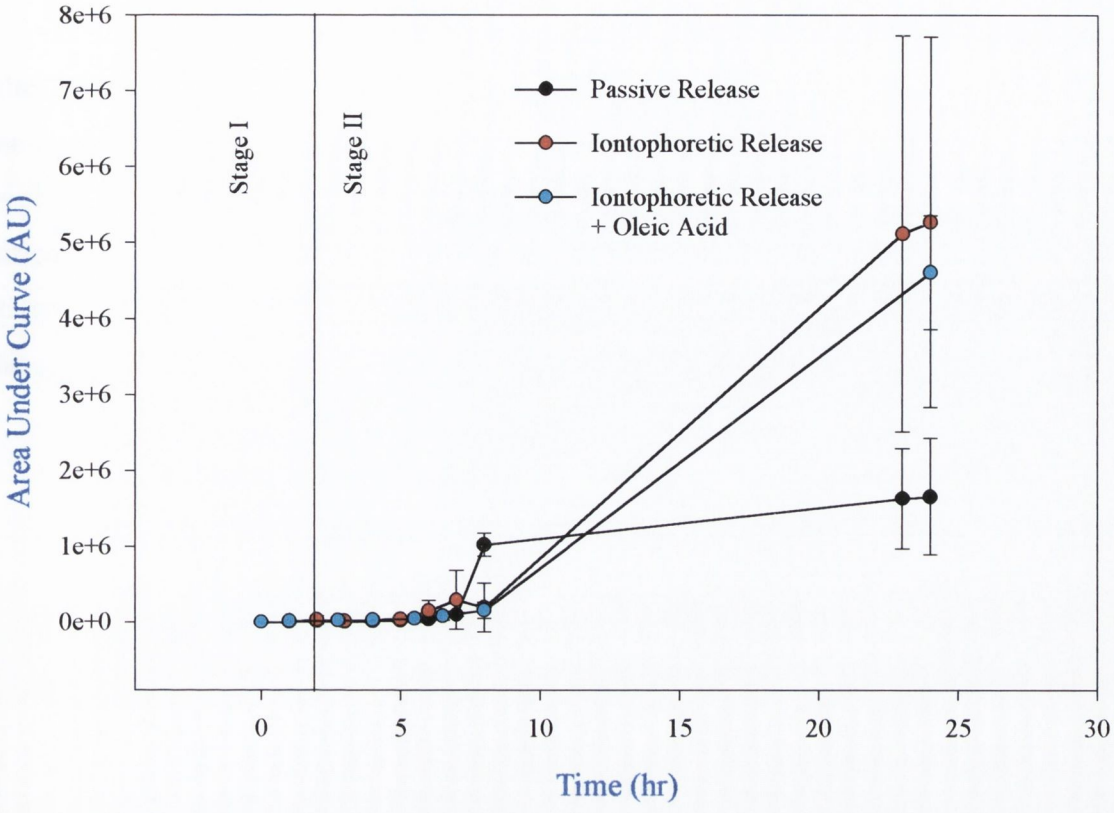


Figure 6:11:4 Comparison of the transdermal release profiles of Sodium Indomethacin from Liquid Crystalline Gels across porcine SC. (all drug and enhancer concentrations 0.1M, 0.5mA current and water/buffer systems) (1mg = 321338.9 AUC)

A summary of the percentage and cumulative release data is presented in Table 6:11:1. A comparison of the release profiles of porcine SC experiments is shown in Figure 6:11:4. The graph shows a distinct difference in the release profiles of passive and iontophoretic delivery. The sharp increase in detection between the seven and eight hour interval for passive release is notable. It can be said with a high level of confidence that this sharp increase will not occur under iontophoretic conditions. This may be due to an electrochemical change of the drug molecule as observed in Figure 5:6:1 which leads to a decrease in detection. However, the 24 hour iontophoretic release of sodium indomethacin across porcine SC was measured to be 33% greater than that measured across Visking. This may be as a result of increased partitioning of the drug from the vehicle into the porcine SC. It may also be due to components of the skin acting to reduce the amount of drug which is electrochemically altered. This was also noted in relation to diclofenac for human SC.

The results of the experiments do not provide conclusive evidence in respect of the objective of the experiments which was to observe whether synergistic enhancement of the transdermal transport of sodium indomethacin using iontophoresis and oleic acid occurred. The cumulative 24 hour release was not significantly affected with only a slight decrease in the mean value in the presence of oleic acid. Conclusions from these results will be assessed in the context of the results of the other experiments in the next Chapter.

Chapter 7

General Discussion

Chapter 7

General Discussion

7.1 Introduction

This thesis has investigated the passive, chemically enhanced and iontophoretically assisted transport of a range of anionic drug molecules across an artificial membrane and also through human *stratum corneum* from liquid crystalline gels. It has also sought to quantify any synergy that might exist when both chemical and physical enhancement methods are used simultaneously.

The following discussion will deal with the results reported, expand on some of the issues raised and discuss them in the context of current literature.

7.2 Discussion

The two main categories of the study reported here were firstly, to characterise the release of the model drugs from liquid crystalline gels across non-rate limiting membranes under the conditions described above and in some instances to compare this with the release from water-based agar vehicles (Chapters 2,3,4and 5).

Secondly, with the knowledge acquired from the former investigations, it was then possible to characterise the transdermal transport of the drugs from the liquid crystalline vehicle. Deductions have been made as to the true nature of the transdermal transport by taking into account the behaviour and contributions of the release characteristic of the drug from the vehicle across Visking and applying this knowledge to results obtained during investigation utilising human SC and porcine SC. These results and simultaneous enhancement investigations were reported in Chapter 6.

The passive release profiles of the sodium salts of diclofenac, indomethacin, salicylate and benzoate were measured across Visking membranes. All were found to conform to the Higuchi model of matrix diffusion controlled release from the liquid

crystalline gels. Sodium Diclofenac was shown to have a significantly lower release from liquid crystalline gels compared to the other drugs investigated (Figure 3:6:1). The poor release from liquid crystalline gels was in sharp contrast to that observed from water-based agar gels. It is possible the drug may be complexing with an unknown component of the vehicle. Bhattacar *et al.*, (1992) have reported on the complexation of sodium diclofenac and other non-steroidal anti-inflammatory drugs with hydrogenated phospholipids. Any complexation of the drug may result in a decrease in its diffusion co-efficient. Desai *et al.*, (1965) stated that very often it has been found that a matrix which provided a desired release profile for one drug was unsuitable when used to incorporate another drug.

The composition of myverol, the main constituent of the liquid crystalline gels was outlined in Table 2:2:1. The table lists more than 16 components. This may be considered as too complex for the studies undertaken. Future investigation may benefit from a gel which has fewer constituents and thus reducing the number of possible interactions between the drug and the vehicle. This ought to also reduce the variation observed in release characteristics between the drug molecules. In the current work the effect of the vehicle components on the release data can not be fully quantified due to the complexity of the myverol vehicle.

The effect of buffering the vehicle and/or the receptor fluid on the passive release of sodium diclofenac across Visking membrane was thoroughly investigated. It was demonstrated that the best release was obtained when water was used as the receptor media and for the aqueous phase of the gel. There was almost a four-fold decrease in the release observed when buffer solution was used instead. This was attributed to a reduced ion gradient between the oil and water phases of the vehicle. No literature could be found to which these results could be directly compared in respect of the transport of these drugs across non-rate-limiting membranes. However, limited information exists on the influence of the receptor medium on the transport of drugs across human SC which will be introduced later in the discussion. Previous work by Nolan, (1995) in this laboratory reported the opposite result for the release of the cationic drug salbutamol sulphate. There was a three fold decrease in the diffusion co-efficient of the drug when water was used as the aqueous phase of the gel and the receptor medium. Nolan offered by way of explanation of these observations that the buffer ions may alter the "tortuosity" of "porosity" of the vehicle thus increasing its rate of release. However, taking the results of diclofenac into account it is not likely

that this is the case. It is more probable that a preferential cation-exchange process may be taking place between the buffered aqueous phase of the gel and the myverol.

The effect of the initial drug loading was also investigated. Nolan observed a burst effect for salbutamol sulphate with an initial drug loading of 0.1M from liquid crystalline gels. For a similar drug loading sodium diclofenac was observed to have lag time of 0.25hr which increased to 0.5hr when the initial drug loading was decreased to 0.01M. The gradients of the release profiles were shown to increase in proportion with each indicated increase in the initial drug loading concentration. These observations are consistent with observations made by other researchers in this laboratory including Nolan, (1995) and Carr (1991). Falson-Reig *et al.*, (1990) have also reported similar concentration effects from liquid crystalline gels.

The effect of initially incorporating the sodium diclofenac into either the aqueous phase or the oil phase of the gel was investigated. There was no apparent effect on the release profiles for either method. However, Carr *et al.*, (1997) reported a 15% decrease in the release of salbutamol sulphate when the drug was initially incorporated into the myverol phase of the gel. It was thought that the drug incorporated in this way may be entrapped between the surfactant bilayers and hence tend to diffuse more slowly through the more lipophilic region of the myverol to the aqueous channels from whence it is released across the membrane.

The next phase of the investigations was to characterise the effects of incorporating a model enhancer into the gel on the release profiles of the drug. The surfactant molecules chosen were shown to have a dramatic effect on the release of the drugs from the liquid crystalline gels. A negligible amount of drug was detected when the enhancer was incorporated at a 1:1 ratio with the drug in the vehicle. Changing the counter ion or chain length of the vehicle had no beneficial effect on the release of the drugs. This phenomenon was attributed to ion-pairing between the drug and enhancer.

Incorporating the drug and enhancer into separate phases of the vehicle, instead of the placing both in the same phase, resulted in a six-fold increase in the release of the drug but this was still minimal in comparison to release in the absence of enhancer. The increase was due to a reduction of the ion-pair formation of drug with enhancer. More drug was able to diffuse from the vehicle before ion-pairing could take place.

Buffering the aqueous phase of the gel and receptor medium also resulted in a six-fold increase in the release of the drug. The presence of buffer ions reduced the extent of ion paring between the drug and the enhancer. Using the acid form of the drug was also found to reduce ion pair formation and increase the release of the drug. The increase expected may be dependent on the pK_a value of the drug, i.e., the less ionised the drug the less ion pair formation takes place. Further research is required on this matter.

Much is known about the effects of surfactants on the barrier function of the skin. However, most literature refers to the use of small radiolabelled molecules such as water or butanol as the permeant and not drug molecules, (e.g., Loden, 1990). Of the few reports referring to enhanced permeation due to surfactants, most cite the use of non-ionic surfactants, e.g., Hans *et al.*, (1994) and Kadir *et al.*, (1989). This class of surfactant is known to be less effective but also less irritating to the skin. Aoyagi *et al.*, (1990), have reported the enhanced permeation of 5-fluouracil across rabbit abdominal skin *in-vitro* using a polymerised form of benzalkonium chloride. Both monomer and polymer forms enhanced the permeation of the drug but the polymer form caused less irritation to the skin.

Although ion pair formation takes place between anionic drugs and benzalkonium derivatives its use may still be conducive to enhanced percutaneous penetration of the drugs if a suitable vehicle were available. Fini *et al.*, (1999), reported that the formation of diclofenac ion pairs can play a positive role in the absorption of the drug deposited on the skin, which then extracts the active agent in the form of an ion pair and transports it through the hydrophobic domains of the horny layer. Tan *et al.*, (1989), have shown that benzalkonium chloride significantly enhances the permeation rate of indomethacin. Young *et al.*, (1988), found that *in-vitro* percutaneous absorption of isopropamide iodide through hairless mouse skin was increased by sodium salicylate. Devi and Paranjothy, (1999), used the diethyl ammonium salt of diclofeanc to enhance the delivery of the drug from pressure sensitive adhesive systems. These examples are but a few. Ion pair formation may also reduce the irritancy of the surfactant molecule, which is also a desirable characteristic in a transdermal system.

Ultimately, enhanced transdermal transport due to ion pairing may be a delicate balance, between the increased partition co-efficient of the ion pair and the resultant changes in physicochemical properties of the drug once bound as an ion pair.

For example, an ion pair may be twice the molecular weight and size of the drug initially intended for delivery. Increased size may result in a reduced diffusion co-efficient. However, this may be outweighed by the fact that the drug and enhancer are no longer ionised and are hence more suitable for transdermal transport.

Oleic acid, a known penetration enhancer, did not effect the release rate of sodium diclofenac from liquid crystalline gels under any protocol. As esters of oleic acid are a component of Myverol it was not expected the enhancer would effect the vehicle in any significant way. Maitini *et al.*, (1996), have reported that the enhanced transport of sodium diclofenac across ethanol treated silicone membranes in the presence of oleic acid. However, ethanol itself can be considered as an enhancer, therefore, this example may be viewed as co-enhancement. Other compounds have been reported to enhance the transdermal delivery of sodium diclofenac and sodium indomethacin. Huang *et al.*, (1995), have reported that cardamom oil increases by 25 fold the cumulative amount of diclofenac detected across abdominal rabbit skin and also reports a 36 fold increase in the cumulative amount of indomethacin detected in the same time period. It must be noted that the salts of these drugs were not used.

Chapter 5 contains the most significant results of the work reported here. Indeed, the results may have repercussions for all transdermal researchers. The main finding of the investigations was that anionic drugs undergo electrochemical change under cathodal iontophoretic conditions *in-vitro*. This electrochemical change leads to a decrease in the concentration of the drug that can be detected due to changes in its solubility. Although the cumulative release of the drug is enhanced, the apparent release is reduced in comparison to release in the absence of current assistance.

Because *in-vitro* investigations are usually the precursor to *in-vivo* studies, the possibility of using iontophoresis to deliver anionic drugs may have been overlooked by many researchers as investigations may appear to give negative results at an early stage. This was the case when investigations by Nolan (1995), reported that no enhancement of sodium diclofenac took place under iontophoretic conditions. The results reported here are also convincing evidence for the relative under-reporting of the iontophoretic transdermal transport of anionic drugs in general. In one of the few reports in which iontophoretic delivery of sodium diclofenac is cited there is evidence of the same electrochemical change occurring. Fang *et al.*, (1999), reported the synergistic enhancement of sodium diclofenac across rat skin using 0.5mA

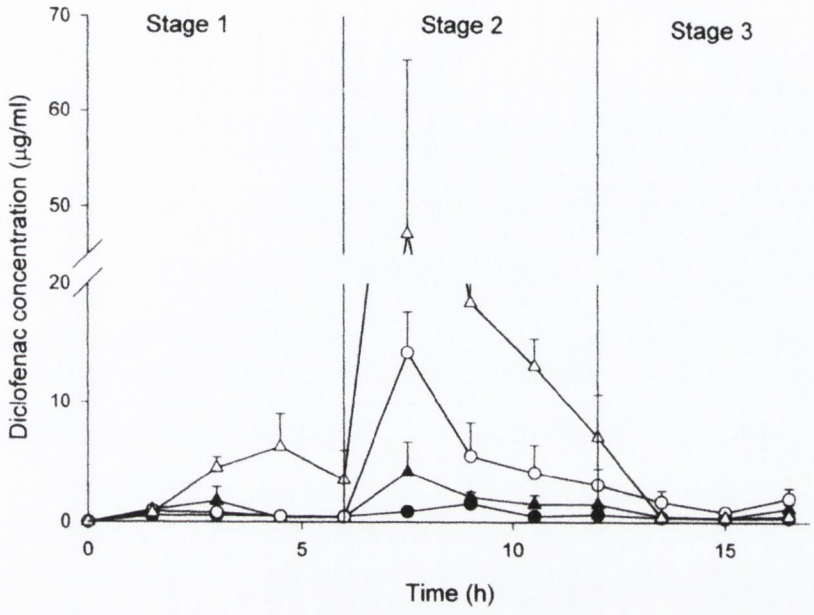


Figure 7:2:1 *Sodium Diclofenac concentration in dialysate collected from the dermis with hydrogels:* (●) *Poly-vinylpyrrolidone* (PVP); (▲) *Hydroxypropyl methycellulose* (HPMC); (○) PVP-HPMC; (Δ) PVP-HPMC *pretreated with cardamom oil on skin. (Current Density 0.5mA/cm^2) Reproduced from Fang et al., (1999).*

iontophoretic current and cardamom oil. The profile of release is represented by the open triangular data points in Figure 7:2:1 which is taken from their work. It is evident that all profiles show an initial increase in the release of the drug after the current is established which is then followed by a decrease in the amount of drug detected. In this respect the profile is very similar to that represented in Figure 5:2:1. Although this experiment was carried out *in-vivo* the microdialysis methodology described is very similar to *in-vitro* experiments in that the receptor medium consisted of Ringer's solution contained in a probe under the skin, both electrodes were made of platinum, a microfraction collector was used to obtain samples from the probe and HPLC analysis was used to analyse the concentration of drug collected. A glass cylinder with a diffusion area of 3.8 cm^2 was placed on the skin in which 8 grams of polymer formulation containing drug was placed. The researchers attributed the increase in drug detection to a higher quantity of drug diffusing through shunt pathways and especially because of the increased number of hair follicles in furry rat skin than human skin.

Varghese and Khar, (1996) have also reported the enhanced iontophoretic delivery of sodium diclofenac across rat skin. In this case however the transdermal delivery is measured indirectly by the percentage inhibition of induced swelling in a rats paw. Their *in-vitro* experiments showed a reduced permeability flux profile for continuous direct current iontophoresis in comparison to passive flux.

Ideally, *in-vivo* measurements of iontophoretic transdermal diffusion of sodium diclofenac should be taken systemically, i.e., from blood samples or by analysing metabolites in the urine. It is not expected that anionic drugs will undergo electrochemical change in controlled iontophoretic experiments. For example, the drug, although attracted towards the anode, may never actually reach it. Once the drug enters the systemic circulation it is taken away. The abundance of water available to undergo electrolysis is also significantly lower than that available during *in-vitro* studies. Hydronium ions produced by electrolysis may be taken away by the systemic circulation or neutralised by components of the skin itself, or by the blood which is a buffer. Should the drug convert to the acid form, against the odds, it still possesses its therapeutic properties.

The *in-vitro* results of transdermal investigations of anionic drugs that were reported in chapter 6 do not exhibit the same level of electrochemical change of the drug as are reported in chapter 5. The results even show evidence of enhanced delivery, however, the amount of drug detected may only be apparent and it may be difficult to fully quantify the cumulative amount of drug which has been transported transdermally. Nevertheless, the results are encouraging and show evidence of enhanced transdermal transport of both sodium diclofenac and indomethacin.

The investigations in chapter 6 showed synergistic enhancement of the transdermal transport of sodium diclofenac across human SC in the presence of oleic acid and current assistance. However, the same enhancement was not evident for the transdemal transport of sodium indomethacin across porcine SC. Pershing *et al.*, (1993), have reported disparities of *in-vitro* and *in-vivo* results of investigations into the percutaneous penetration enhancement of estradiol by oleic acid. *In-vitro* experiments showed a four-fold increase in drug transport in comparison to *in-vivo*.

Evidence such as that reported by Pershing *et al.*, (1993), opens the debate as to how accurate *in-vitro* investigations are in mimicking what actually happens *in-vivo*. Critical analysis of *in-vitro* apparatus leaves much room for improvement but this may not be possible when taking all factors into account. For example, a drug

may partition into the SC but may not then partition into the receptor medium. This may be a fundamental problem of *in-vitro* investigations. Jones *et al.*, (1993), reported that receptor fluid also influenced the extent of penetration of drugs such as hydrocortisone through full thickness skin. Their results suggest that the osmotic strength of the receptor medium and its ability to exert delipidising/back-flushing effects will influence the amount of penetrant able to traverse the skin barrier. Factors such as this and partitioning phenomena may need to be corrected for when assessing the permeability of drug compounds. It is difficult to foresee how a standardised protocol for *in-vitro* transdermal investigations could be formalised.

7.3 Conclusions

The main findings of the work reported here are the effect of the receptor medium, the electrochemical change of anionic drugs under iontophoretic conditions, ion pair formation between cationic model enhancers and anionic drug molecules and the synergistic enhancement of the transdermal transport of sodium diclofenac by chemical and physical enhancement.

Buffering the aqueous phase of the liquid crystalline gel and the receptor medium results in a reduction of the cumulative release of sodium diclofenac across Visking membranes under passive conditions. Buffering the receptor medium results in an increase in diclofenac detection under iontophoretic conditions. This is a result of buffer ions reducing the hydronium ion concentration in the receptor medium which in turn reduces the amount of diclofenac anions converted to the acid form. Buffering the receptor medium and aqueous phase of the vehicle also reduces the ion pair formation between anionic drugs and cationic model enhancers, resulting in an increase in diclofenac detection.

Cathodal iontophoresis (>2V) with platinum electrodes results in the hydrolysis of aqueous receptor media. The production of hydronium ions at the anode causes drug anions to be converted to their acid form. This conversion of the drug molecule induces a change in the solubility of the drug in the receptor medium which results in a decrease in drug detection. Therefore, the apparent release of the drug is not representative of the actual amount of drug which is transported with iontophoretic assistance. Iontophoretic assistance did not reduce the ion pair formation between drug and enhancer within the vehicle.

Sodium diclofenac and sodium indomethacin were shown to diffuse passively across human SC but probably not in sufficient quantities to attain steady state therapeutic levels. Iontophoretic assistance improved the transport of sodium diclofenac significantly across human SC and similarly enhanced the transport of sodium indomethacin across porcine SC in quantities which would be deemed therapeutic. The SC membranes were also thought to play a role in the reduction of hydronium ions in the receptor media. Synergistic enhancement of the transport of sodium diclofenac across human SC was observed when oleic acid was incorporated into the liquid crystalline gel in addition to iontophoretic assistance .

7.4 *Future Investigations*

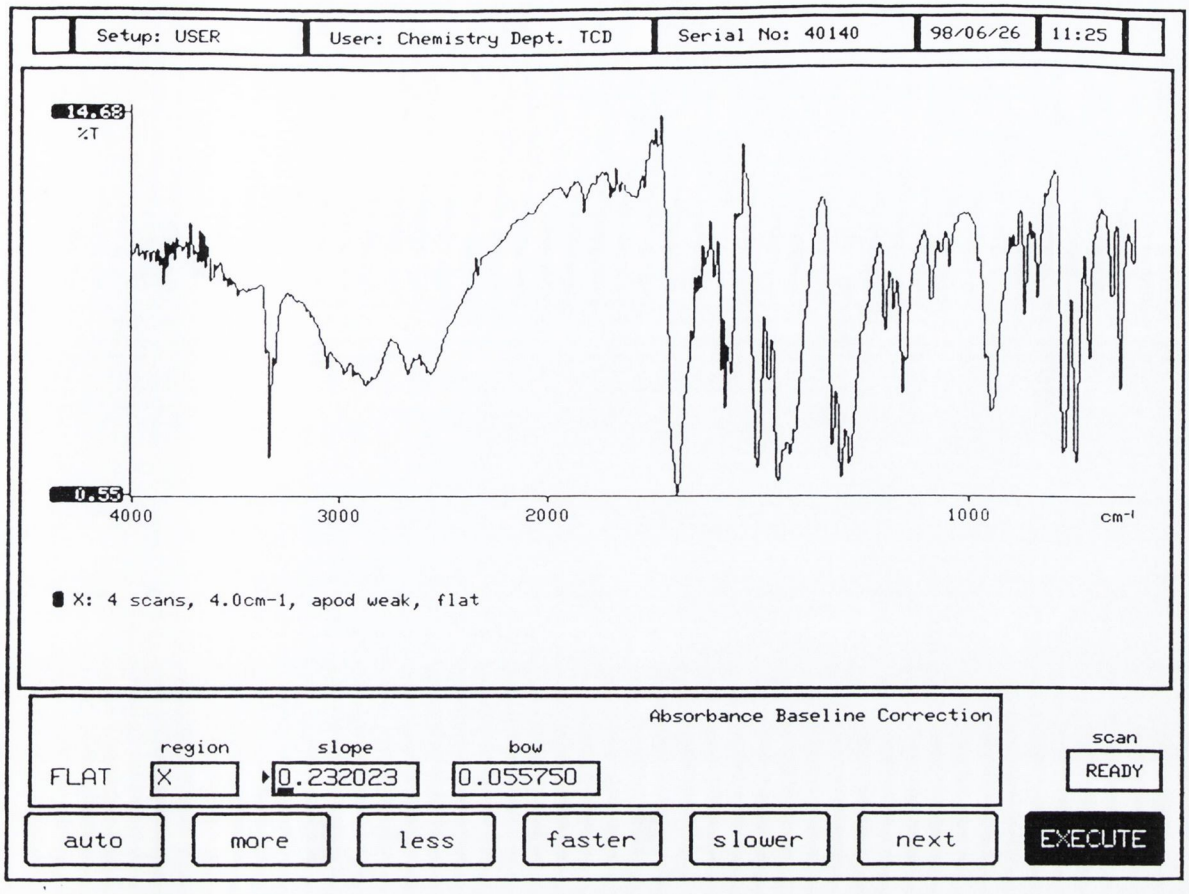
The results presented in this thesis taken in context of those available through literature reviews, make it possible to focus on the important aspects of the work carried out and to narrow the scope of possible future investigation to obtain the optimum results in achieving the objectives set out.

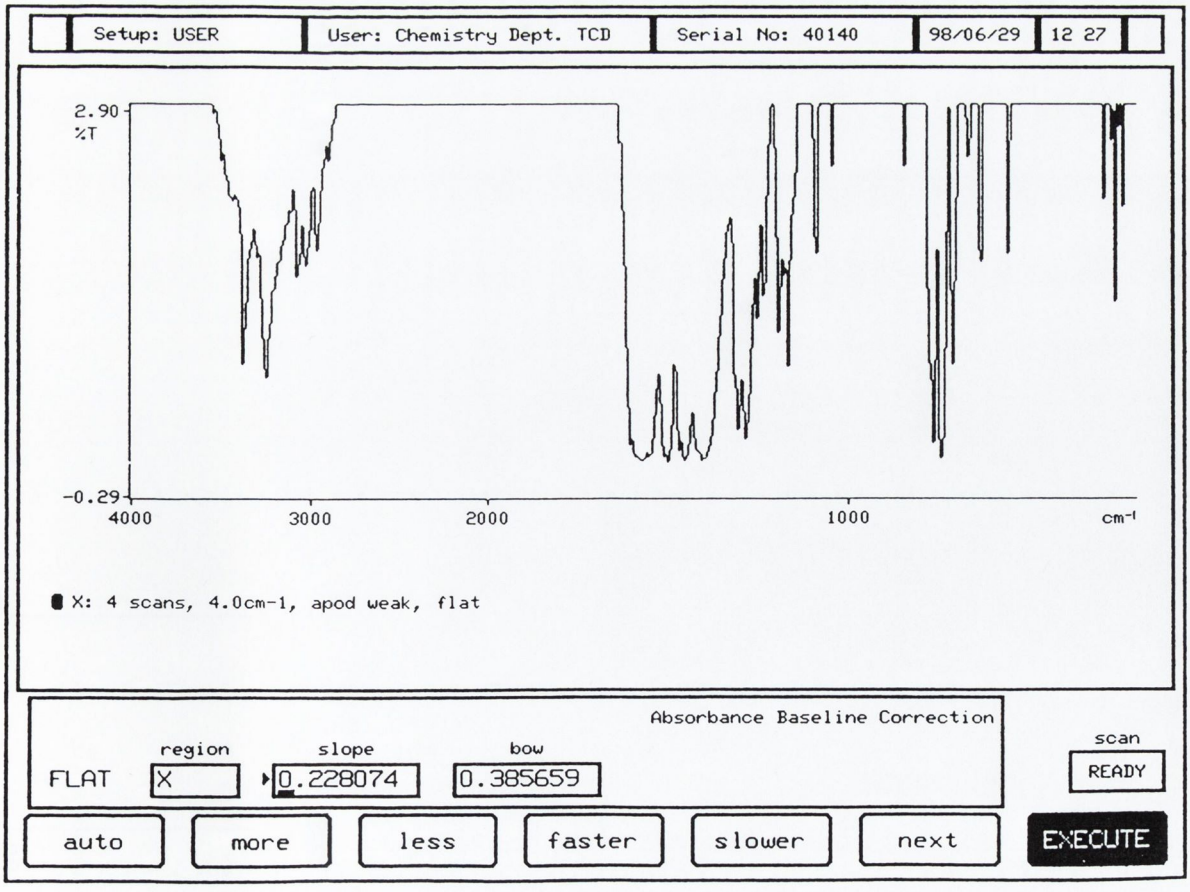
For any researcher who has carried out *in-vitro* investigations it would seem a logical and desirable step to be able to extend their investigation into the realm of *in-vivo* studies. This is not always possible but is merited for a number of carefully chosen systems in the light of results presented here.

If asked to administer an anionic drug by the transdermal route in a hypothetical situation, an educated approach would be to strip the skin four or five times with selotape then treat the skin with two enhancers from different areas of Hori's diagram (Hori *et al.*, 1990), e.g., ethanol and oleic acid and finally apply the drug containing vehicle to the skin and administer cathodal iontophoretic assistance over several hours. Although a patient would find the use of selotape uncomfortable, much of the logic to this approach is contained in the introductory chapter. To apply lessons learned from the work reported here, it would be necessary to avoid a cationic enhancer which is extensively ionised at neutral pH. Sensitive sampling and detection techniques would be required to measure the concentration of drug in the systemic system. If the investigations were carried out *in-vitro,* the electrochemical change of the anionic drug would be required to be quantified or avoided if possible.

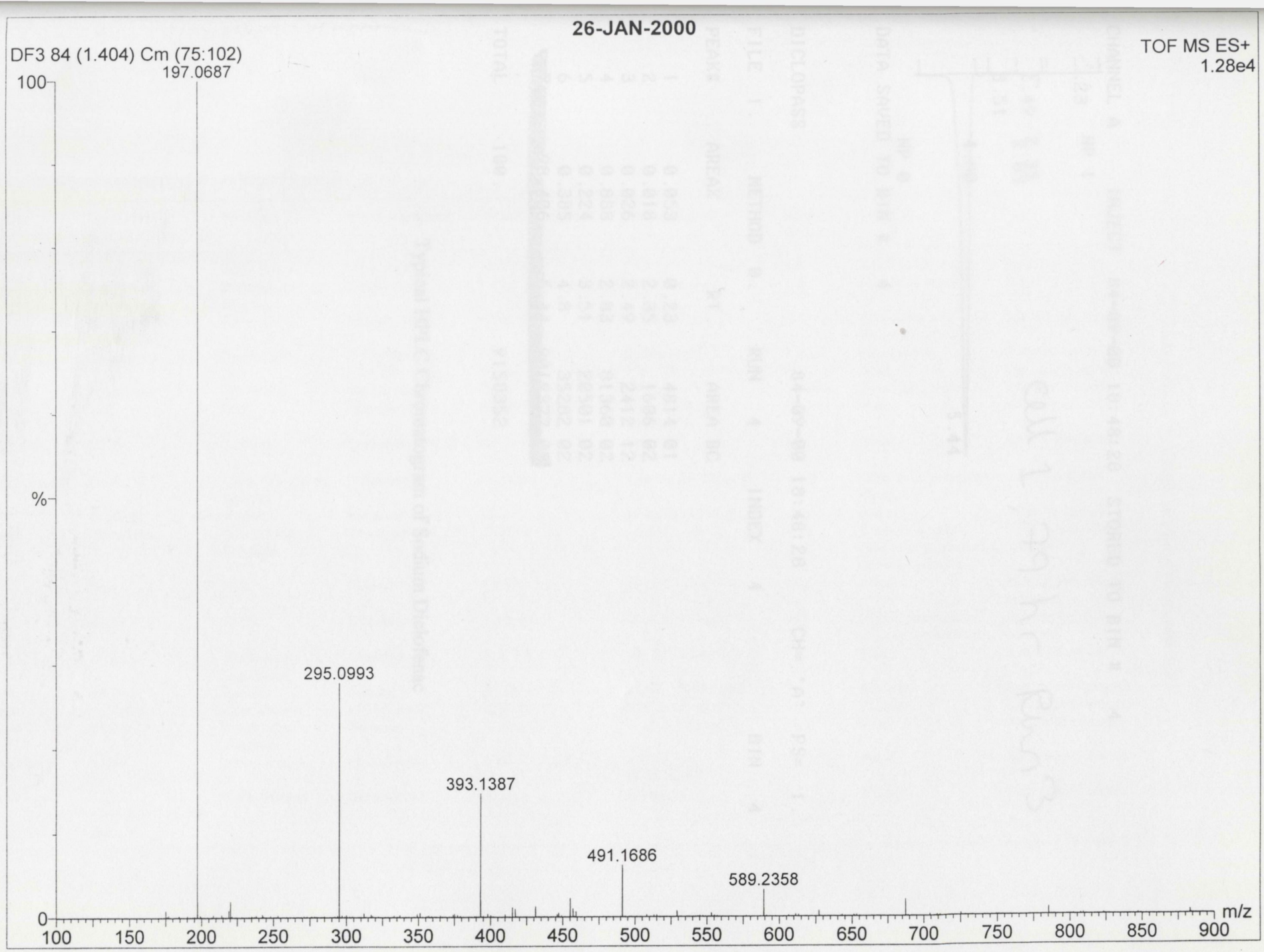
In the long run it will take a co-ordinated approach involving many laboratories to try and systematically overcome the challenges and obstacles of the barrier properties of the skin and transdermal drug delivery.

Appendix



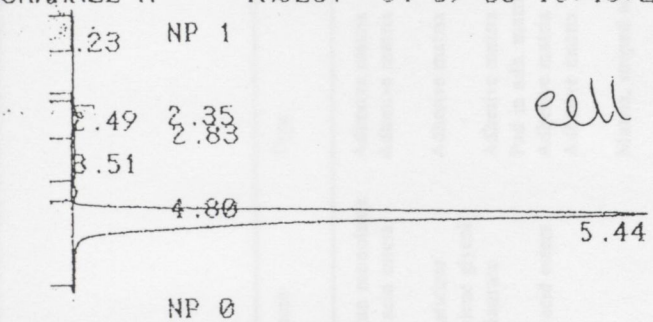


Infrared Spectrum of Diclofenac Acid (Top) and Sodium Diclofenac (bottom)



(Time of Flight) Mass Spectrum of Electrolysis Product indicating Diclofenac Acid

CHANNEL A INJECT 84-09-00 18:48:28 STORED TO BIN # 4



cell 1, 79 hr Run 3

DATA SAVED TO BIN # 4

DICLOPASS 84-09-00 18:48:28 CH= "A" PS= 1.

FILE 1. METHOD 0. RUN 4 INDEX 4 BIN 4

PEAK#	AREA%	RT	AREA	BC
1	0.053	0.23	4814	01
2	0.018	2.35	1606	02
3	0.026	2.49	2412	12
4	0.888	2.83	81360	02
5	0.224	3.51	20501	02
6	0.385	4.8	35282	02
7	98.406	5.44	9012377	03

TOTAL 100. 9158352

Typical HPLC Chromatogram of Sodium Diclofenac

Appendix. Family of commercial transdermal systems

Product name	Innovator	Marketer	Active ingredient	Systems area(s) (cm^2)	Delivery rate(s)	Rated duration	Enhancer	Type
Alora®	TheraTech	P&G Pharm.*	17β-estradiol	18,27,36	0.05, 0.075, 0.1 $mg\,d^{-1}$	4 d	Sorbitan monoleate	Adhesive matrix
Climara®	3M Pharm. Lohman	BerlexLabs Pharma	17β-estradiol	12.5 & 25	0.05 & 0.1 $mg\,d^{-1}$	7 d	Fatty acid esters	Adhesive matrix
Deponit®	Neuwied FRG	Schwarz	Nitroglycerin	16 & 32	0.2 & 0.4 $mg\,h^{-1}$	12–14 h	'A plasticizer' Propylene glycol monolaurate	Adhesive matrix
FemPatch®	Cygnus	ParkeDavis	17β-estradiol	30	0.025 $mg\,d^{-1}$	7 d	None	Adhesive matrix
Habitrol®	Ciba	Ciba	Nicotine	10, 20, 30	7, 14, 21 $mg\,d^{-1}$	24 h	Fatty acid esters	Pad in adh. matrix
Minitran®	3M Pharm.	3M Pharm.	Nitroglycerin	20	0.6 $mg\,h^{-1}$	12–14 h	None	Adhesive matrix
Nitrodur®	Key	Key	Nitroglyerin	10, 20, 30, 40	0.2, 0.3, 0.4, 0.6, 0.8 $mg\,h^{-1}$	12–14 h	Oleic acid, propylene glycol,	Adhesive matrix
Testoderm® with adhesive	ALZA	ALZA	Testosterone, USP	60	6 $mg\,d^{-1}$	24 h	None	Matrix, striped adh
Menorest®, Vivelle®	Noven	Ciba, RPR-Novo Nordisk	71β-estradiol	11 & 29	4 Rates, 25–100 $\mu g\,d^{-1}$	3–4 d	Ethanol, glyceryl mono oleate, methyl laureate, glycerin	FFS**, peripheral adhesive
Nicotrol®	Cygnus	McNeil	Nicotine	30	15 mg 16 h^{-1} (1 d)	16 h	None	Adhesive matrix
Androderm®	TheraTech	Smith Kline Beecham	Testosterone, USP	37 & 44	2.5 & 5 $mg\,d^{-1}$	24 h	Ethanol, glyceryl mono oleate, methyl laureate, glycerin	FFS**, peripheral adhesive
Prostep®	Elan	Elan	Nicotine	3.5 & 7	11 or 22 $mg\,d^{-1}$	24 h	None	Matrix, peripheral adhesive
Nitrodisc®	Searle	Searle	Nitroglycerin	8, 12, 16	0.2, 0.3, 0.4 $mg\,h^{-1}$	24 h	Polyethylene glycol, Isopropyl palmitate	Matrix, peripheral adhesive
Catapres TTS®	ALZA	Boehringer Ingelheim	Clonidine	3, 5, 7 & 10.5	0.1, 0.2, 0.3 $mg\,d^{-1}$	7 d	None	Rate-control memb.
Durageslc®	ALZA	Janssen	Fentanyl	10, 20, 30, 40	25, 50, 75, 100 $mg\,d^{-1}$	3 d	Ethanol	Rate-control memb.
Estraderm®	ALZA	Ciba Geneva	17β-estradiol	10 & 20	0.05 & 0.1 $mg\,d^{-1}$	3 d	Ethanol	Rate-control memb.
Nicoderm®CQ	ALZA	Smith Kline Beecham	Nicotine	7, 15 & 22	7, 14 & 21 $mg\,d^{-1}$	24 h	None	Rate-control memb.
Transderm-Nitro®	ALZA	Ciba Geneva	Nitroglycerin	5, 10, 20, & 30	0.0, 0.2, 0.4 & 0.6 $mg\,h^{-1}$	12–14 h	None	Rate-control memb.
Transderm Scop®	ALZA	Ciba SelfMed	Scopolamine	2.5	0.5 $mg/3d^{-1}$	3 d	None	Rate-control memb.

*Proctor & Gamble
**FFS = Form-fill-seal

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Above: Myself hard at work in the Physical Chemistry Research Laboratory (Arch 2)

Below: Apparatus used for Transdermal Investigations