INDIGENOUS DEVELOPMENT OF TRANSDERMAL DRUG DELIVERY OF SOME DRUGS

THESIS
Submitted in partial fulfilment
of the requirements for the degree of
DOCTOR OF PHILOSOPHY

By
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CERTIFICATE

This is to certify that the thesis entitled "**Indigenous development of Transdermal Drug Delivery of some drugs**" and submitted by S. K. Gidwani ID No. 93PHXF403 for award of Ph. D. Degree of the Institute, embodies original work done by him under my supervision.

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Dedicated to respected

"DADI SAHEB"

without whose blessings and encouragement it would not have been possible to even begin the work.

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Content

Chapter I.

INTRODUCTION

CHAPTER I. INTRODUCTION:

Intact skin can be employed as a port of drug administration to provide cutaneous transdermal drug delivery into the systemic circulation. While it has been recognised that the benefit of intravenous drug can be closely duplicated without its hazards via transdermal drug delivery, such administrations delivers medication noninvasively. Consequently, most major drug manufacturers are searching for applications of the dermal route of administration for established drugs. Intense research efforts vested in development of transdermal drug delivery technology in the past decade have resulted in development of transdermal drug delivery systems (TDDS) for nine drug entities (clonidine, estradiol, etofenamate, fentanyl, isosorbide dinitrate, nicotine, nitroglycerin, progesterone and scopolamine) that have successfully reached the market place.[1]

1.1. The Skin: Site Of Transdermal Drug Administration

1.1.1. Introduction:

The skin, the heaviest single organ of the body, combines with the mucosal lining of the respiratory, digestive and urogenital tracts to form a capsule which separates the internal body structures from the external environment. The integument not only physically protects the internal organs and limits the passage of substances into and out of the body but also stabilises temperature and blood pressure with its circulation and evaporation system. (Fig. 1.1)

For an average 70 kg. human with a skin surface area of 1.8 m^2, a typical square centimetre covers 10 hair follicles, 12 nerves, 15 sebaceous glands, 100 sweat glands, 3 blood vessels with 92 cm. total length, 360 cm. of nerves and 3×10^6 cells. [1, 2]

1.1.2. Anatomy And Physiology:

The human skin comprises of two distinct but mutually dependent tissues, the stratified, avascular, cellular epidermis and an underlying dermis of connective tissue. At the bottom of the dermis lies the fatty, subcutaneous layer.

Human skin displays two main types. Hairy skin encloses hair follicles and sebaceous glands, but there are no encapsulated sense organs. Glabrous skin of the palms and the soles constructs of a thick epidermis with a compact stratum corneum, but the integuments lack hair follicles and sebaceous glands and the dermis supports encapsulated sense organs.

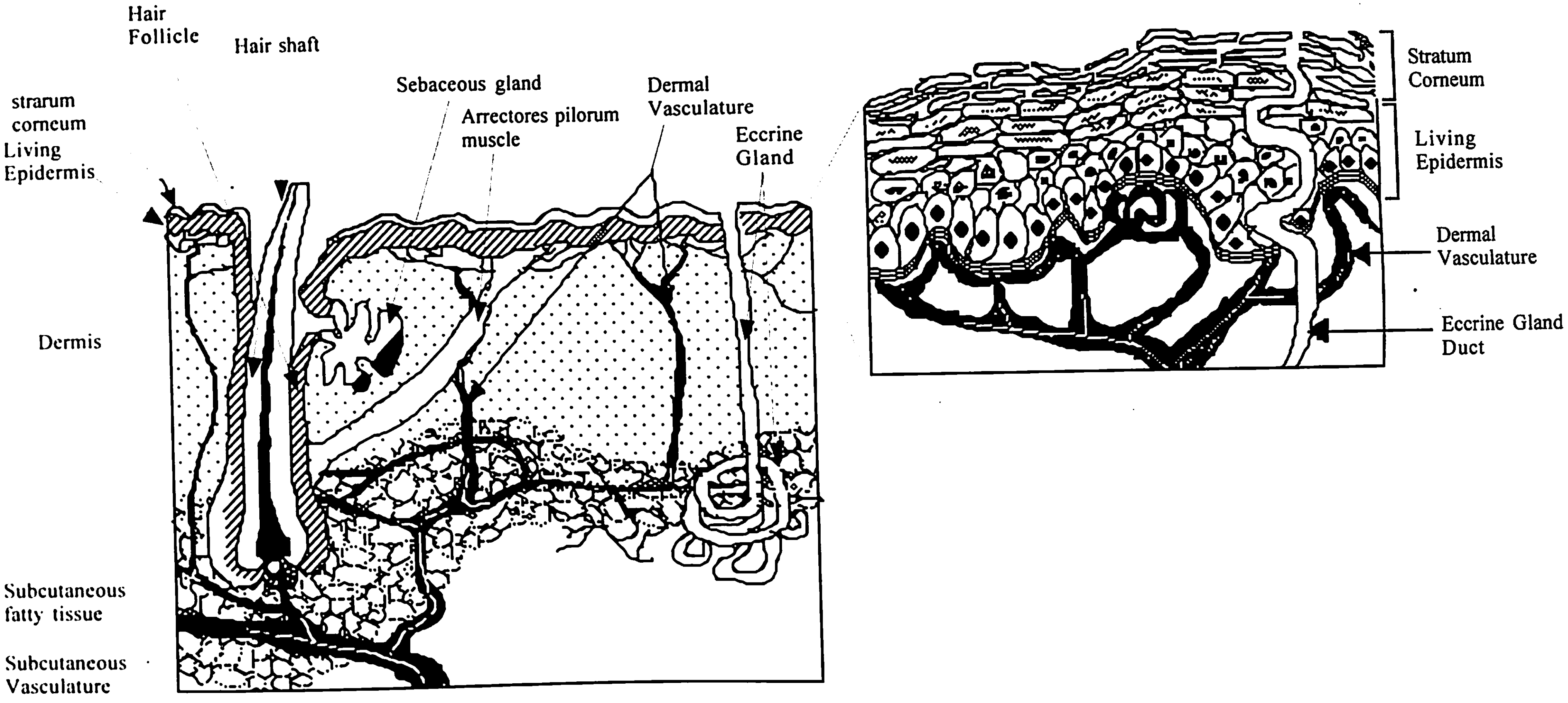


Fig. .1.1 : Schematic cross section of the skin.

A. The Epidermis:

The multilayer envelope of the epidermis varies in thickness, depending on cell size and the number of cell layers, ranging from about 0.8 mm. on the palms and the soles down to 0.06 mm. on the eyelids. Cells which provide epithelial tissue differ from those of all other organs in that as they ascend from the proliferative layer of basal cells they change in an ordered fashion from metabolically active and dividing cells to dense, dead, keratinized protein.

The epidermis comprises of five separate layers:

I. The Basal Layer (Stratum Germinativum) And The Dermoepidermal Junction:

The basal cells are nucleated, columnar and about 6 µm wide, with their long axis at right angles to the dermoepidermal junction, they are connected by cytoplasmic intercellular bridges.

Mitosis of the basal cells constantly renews the epidermis and this proliferation in healthy skin balances the loss of dead horny cells from the skin surface. Thus the thickness of epidermis remains constant.

The basal cells also includes melanocytes which produce and distribute melanin granules to the keratinocytes required for pigmentation, a protective measure against radiation. Below the basal cell layer lies the complex dermoepidermal junction, which constitutes an anatomic functional unit. The junction serves the three functions of dermal-epidermal adherence, mechanical support for the epidermis, the control of the passage of cells and some large molecules across the junction.

We can consider the barrier function of the junction in terms of three species, small molecules, large molecules and cells. There is no evidence that the junction significantly inhibits the passage of water, electrolytes, and other low molecular weight materials.

II. The Prickly Cell Layer: The Keratinocytes (Stratum Germinativum)

As the cells produced by the basal layer move outward, they alter morphologically and histochemically. The cells flatten and their nuclei shrink. It is also called as polygonal cells, prickle cells, because they are interconnected by fine prickles. Each prickle encloses an extension of the cytoplasm, and the opposing tips of the prickles of adjacent cells adhere to form intercellular bridges, the desmosomes. These links maintain the integrity of the epidermis.

III. The Granular Layer (Stratum Granulosum):

As the keratinocytes approach the surface, they manufacture basic staining particles, the keratohyalin granules. This keratogenous or transitional zone is a region of intense biochemical activity and morphological change. The dynamic operation manufactures the keratin to form the horny layer by an active rather by a degenerative process.

IV. The Stratum Lucidum:

In the palm of the hand and the sole of the foot, an anatomically distinct, poorly staining hyalin zone forms a thin, translucent layer immediately above the granular layer. This region is the stratum lucidum.

V. The Horny Layer (Stratum Corneum):

As the final stage of differentiation, epidermal cells construct the most superficial layer of the epidermis, the stratum corneum. Human beings owes their ability to survive in a nonaqueous environment to the almost impermeable nature of this refractory horny layer. On the general body areas the membrane provides 10-15 layers of much flattened, keratinized dead cells, stacking them in highly organised units of vertical columns. The horny layer may be only 10 μm thick when dry, but swells in water to several times this thickness. When dry it is a very dense tissue, about 1.5 gm. cm^{-3}. Each thin polygonal cell measures approximately 0.5 to

1.5 μm thick, with the diameter ranging from 34 μm on the forehead to 46 μm on the thigh axilla.

We can distinguish two types of horny layers by anatomic site, function and structure. The horny pads of the palms and soles adapt for weight bearing and friction and the membranous stratum corneum over the remainder of the body is flexible but impermeable. The horny pads are at least 40 times thicker than the membranous horny layer. Holbrook and Odland [2] carried out an ultrastructural analysis of the stratum corneum to find out the regional differences in the thickness (cell layers) in humans (Table 1.1). The methods that were utilised for measurement of the layers were :

1. Scotch tape stripping to remove cell layers, the number of strippings were correlated with the numbers of cell layers.
2. Treatment of paraffin embedded and frozen biopsies with alkali to cause swelling, hence better visualisation and more accurate counting.
3. Application of standard chemical fixation, paraffin embedment and staining procedures for histological sections. Thickness was measured with a micrometer eyepiece.

Table 1.1: Regional Differences in the thickness and cell layers of the Stratum Corneum.

Body Region	Thickness of S.C.(μm)		No. of cells layers	
	Mean	Range	Mean	Range
Abdomen	8.2	6.9-9.8	18.0	15.0-20.9
Flexorforearm	12.9	8.1-16.2	21.6	16.7-30.0
Thigh	10.9	7.7-15.3	19.3	14.3-22.7
Back	9.4	8.2-11.3	15.8	14.0-21.1

Human beings constantly shed the outermost layers of the stratum corneum as lipid-soaked horny flakes with an average daily loss from the whole body surface of 0.5 to 1 kg.

The stratum corneum plays a crucial role in controlling the percutaneous absorption of drug molecules. The selective permeability of its elegant structure provides a central theme in many aspects of the study of the biopharmaceutics of transdermal delivery systems [3,4].

1.1.3. Barrier Functions Of The Stratum Corneum:

1. Location Of The Barrier:

Until 1944 it was not established that the water barrier, which prevents desiccation of all terrestial vertebrates is localised in the stratum corneum. This basic fact was first demonstrated by Winsor and Burch [5], who monitored the flux of water while they sand papered through human epigastric skin and observed a sharp rise in water flux just as they breached the stratum corneum. The rates of water vapour transpiration was further studied by Oken et al [6]. These observations that the stratum corneum represents the barrier to water loss through the skin was soon verified by Blank [7], who observed that water loss did not increase much until the lowest layers of the stratum corneum were reached, and accordingly concluded that the barrier is located at the bottom of the horny layer.

Early solvent extraction experiments indicated that lipids, especially polar lipids, played a critical role in the barrier [8,9], but it was not until Breathnach et al [10] applied the freeze-fracture technique that it was clear that these lipids forms multiple broad bilayers, filling the intercellular spaces. It was further showed that the bilayers are present throughout the horny layer. These intercellular membranes provide the barrier to water loss through the skin as well as limit the penetration of water-soluble agents from the environment.

2. Two Compartment Model Of The Stratum Corneum:

The picture of the stratum corneum which emerges from the above considerations is one of the individual corneocytes with Keratin rich interiors surrounded by a continuous lamellar lipid phase. This view of the stratum corneum was first formally presented by Shaw and co-workers [11], who developed it as a quantitative model for predicting the permeability of the stratum corneum to specific penetrants.

Elias [12] referred to the two compartment model as the bricks and mortar model and used it as a qualitative picture of the horny layer. Smith et al. [13] were able to physically disaggregate corneocytes after delipidization and could reaggregate sheets of stratum corneum by recombining the extracted lipids (mortar) with the delipidized corneocytes (bricks).

3. Relationship Between Lipid Structure And Barrier Function:

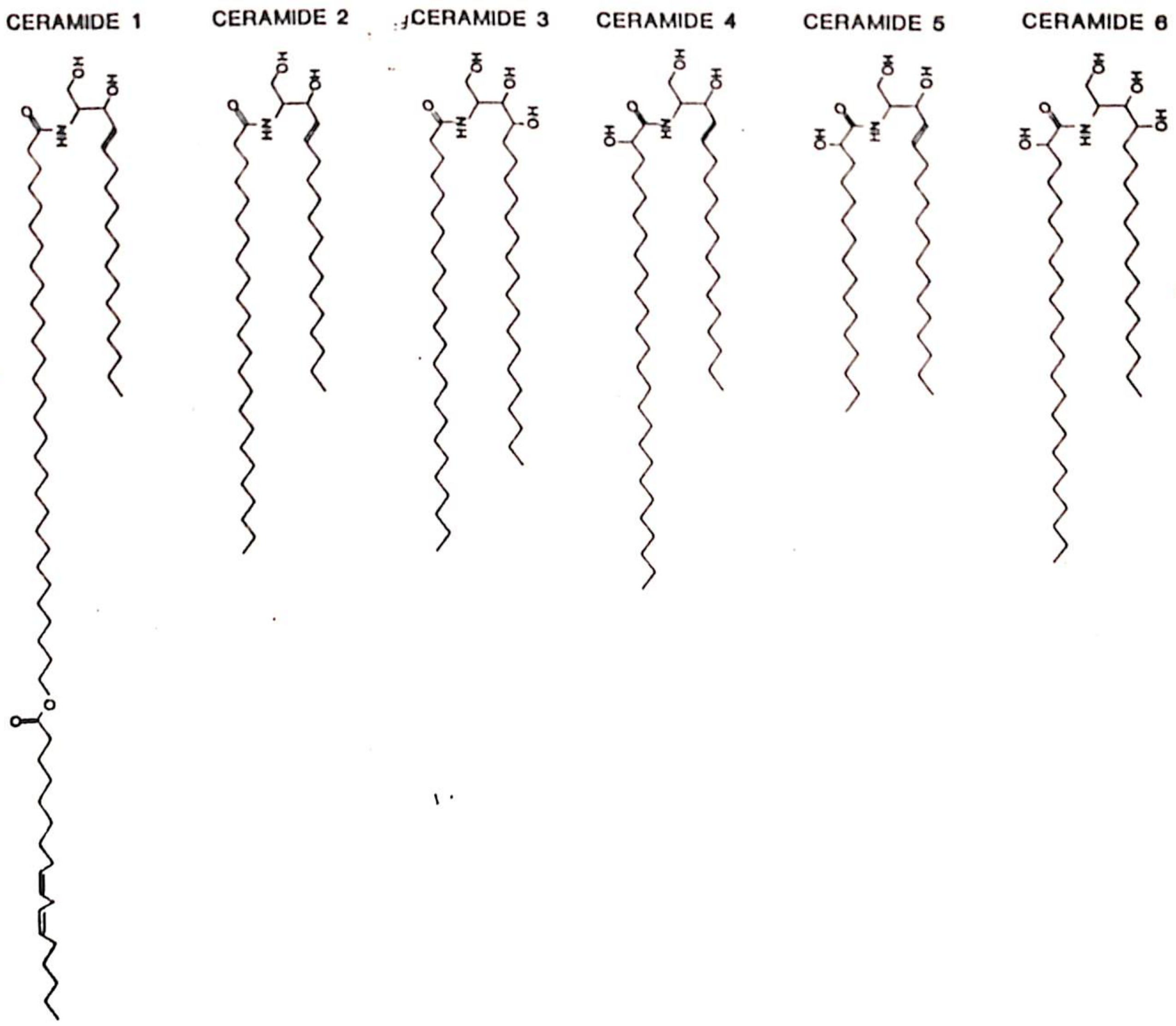
Mammalian cell membranes, composed of a variety of phosphoglycerides, sphingomyelin and cholesterol do not constitute an appreciable barrier to water or small water molecules [14]. The phospholipids most typically contain a range of saturated and unsaturated 16-through 20- carbon fatty acyl chains. Occasionally a methyl branched fatty acyl chains are also noted. The methyl branches and double bonds within the interior of these chains inhibit the formation of a highly ordered hydrophobic membrane interior. The result of this disorder among the fatty acyl chains is a fluid-permeable bilayer.

The membranes found within the stratum corneum, however, do not contain phospholipids or the usual assortment of fatty acyl chain structures. These bilayers are composed mainly of cholesterol, fatty acids and ceramides (Table 1.2) [15].

Table No. 1.2 : Composition of Stratum corneum lipids.

Sr. No.	Components	Pig	Human
1.	Cholesteryl ester	1.7	10.0
2.	Triglycerides	2.8	0.0
3.	Fatty acid	13.1	9.1
4.	Cholesterol	26.0	26.9
5.	Ceramide 1	4.1	3.2
6.	Ceramide 2	16.7	8.9
7.	Ceramide 3	6.9	4.9
8.	Ceramide 4	4.4	6.1
9.	Ceramide 5	4.5	5.7
10.	Ceramide 6	7.6	12.3
11.	Glucosylceramides	1.0	0.0
12.	Cholesteryl sulphate	3.9	1.9
13.	Others	5.7	11.1

Fig. 1.2: Structures of Ceramides.



CERAMIDE 1 CERAMIDE 2 CERAMIDE 3 CERAMIDE 4 CERAMIDE 5 CERAMIDE 6

The free fatty acids include only 7 % of unsaturated species and no methyl-branched components. With the exception of ceramide, the hydrophobic chains in the ceramides (Fig. 1.2) are almost entirely straight and saturated. These lipids with their straight, saturated aliphatic chains would seem to be ideally suited to form highly ordered, impermeable membranes and to resist oxidative damage on exposure to air at the skin surface. Of special importance to the barrier function is ceramide 1. This acylceramide is one of the principal carriers of linoleic acid in the epidermis. This molecule is thought to function in the fusion of the discs extruded from the lamellar granules and in the stabilisation of the resulting multilamellar sheets.

B. The Dermis:

The dermis, as indicated in the Fig. 1.1, is the non descriptive region lying in between the epidermis and the subcutaneous fatty region. It consist mainly of the dense network of structural protein fibres i.e. collagen, reticulum and elastin, embedded in the semigel matrix of mucopolysaccharidic, "ground substances". It is about 0.2 to 0.3 cm thick. It also consist of the epidermis and reticular layer, which is the main structural body of the skin.

Of particular relevance to biopharmaceutical studies is the fact that this generous blood volume usually functions as a "sink" with respect to the diffusing molecules which reach it during the process of percutaneous absorption. The sink condition ensures that the penetrant in the dermis remains near zero and therefore the driving force for diffusion, as an abundant supply assists percutaneous absorption.

C. Skin Appendages:

The skin as interspersed with hair follicle and associated sebaceous glands, so called pilosebaceous glands and in specific regions two types of sweat glands, eccrine and apocrine glands. Collectively, these are referred to as the skin appendages.

Hair follicles are distributed over the entire skin surface except sole of the feet, the palm of the hand, the red portion of the lips, and selected portion of the sex organs. It consist of concentric layers of cellular and noncellular components and is placed at an angle. Smooth muscle fibres, arrectores pilorum, attaches the hair to the dermal connective tissues. The hair shaft is formed by a process of cellular division and migration of the cells similar to that which forms the stratum corneum. Hairs are thus formed of kertinized cells compacted together into plates and scales.

Each hair follicle is associated with one or more sebaceous gland which are referred as the acid mantle of the skin. In some selected region of the skin these exist in the absence of the hair follicle. Their size varies from region to region (200 to 2000 μm) and are highest in nose. It secretes oily material, sebum, which lubricates the skin and stratum corneum and also maintains the pH of the skin at 5 [16].

D. Eccrine Gland And Apocrine Gland:

Eccrine glands or salty sweat glands are distribution over the surface of body. They consist of simple, coiled tube as shown in Fig. 1.1 and have a density from 100 to 200 glands per cm^2 of the body surface depending on the body region. The secretion is dilute aqueous secretion of the salt and some other minor components and it has a pH of about 5. The principal function of the gland is heat control. It secretes dilute aqueous solution of salt and due to this it regulates the body temperature.

Apocrine glands are present only in the selected region of the body viz. axillae (armpits), in anogenital region and around the nipples. They are ten times larger than eccrine glands and secretes a milky substance like protein, lipoprotein, lipids and diverse proteins. The secretion is mainly stimulated due to emotional stress and sexual stimulation.

1.1.4. Skin Permeability Of The Drugs:

When the skin serves as the port of administration of drugs for systemically active drugs, the drug applied topically is distributed, following absorption, first to the systemic circulation and then transported to the targeted tissues which can be relatively remote from the site of drug application to achieve the intended therapeutic action. Following is the diagrammatic representation of the steps involved in drug transport (Fig. 1.3).

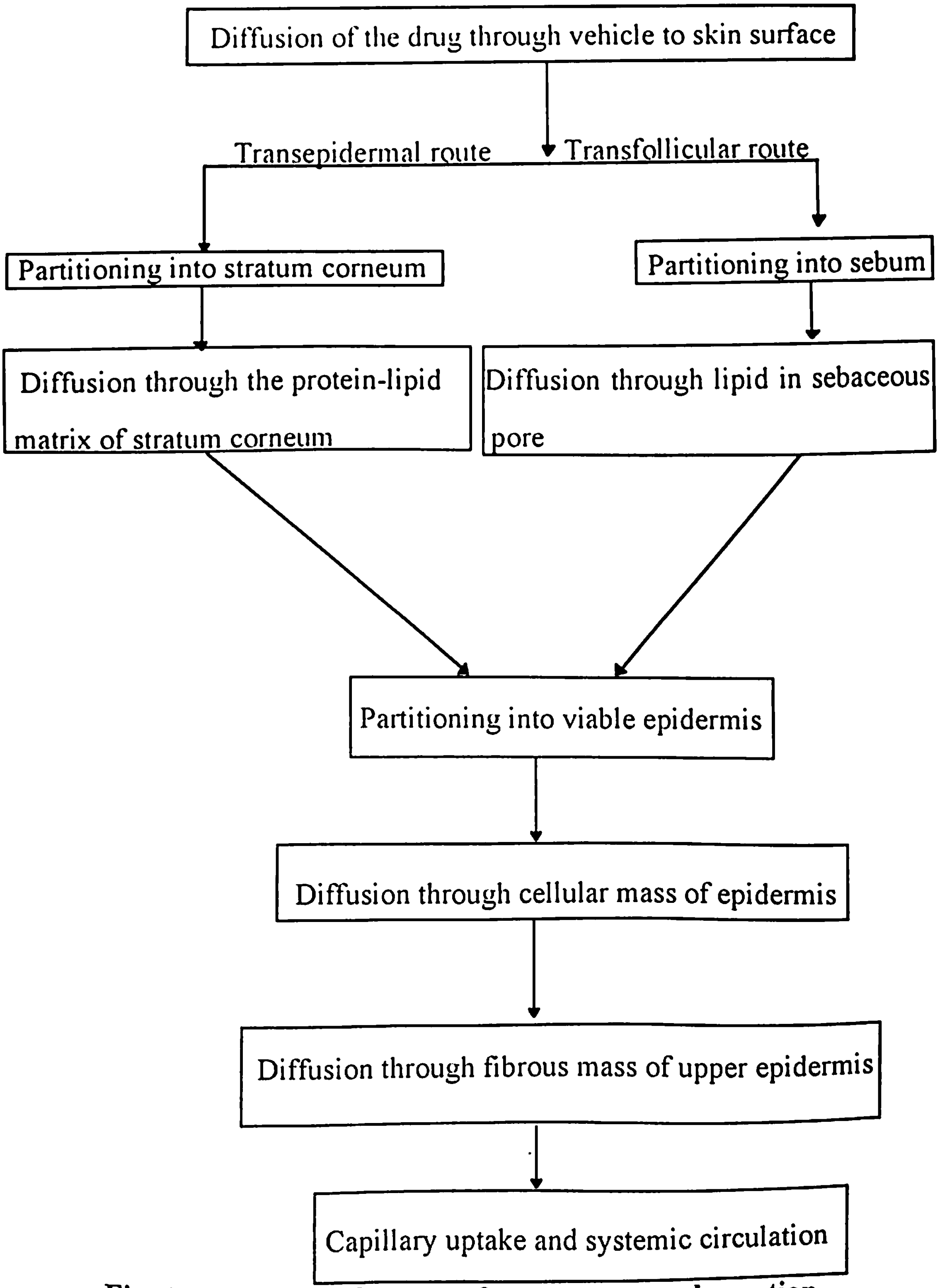


Fig. 1.3 : Scheme of events of percutaneous absorption.

From above scheme, each step is rate limiting depending on the drug and how it interacts with the skin. Two potential routes of percutaneous absorption are identified viz,

1. The transepidermal route, corresponding to diffusion directly across the stratum corneum.

2. The transfollicular route, corresponding to diffusion down the follicular route.

Though the stratum corneum is the major barrier, depending upon the drug in question and condition of the skin, whole skin may acts as a barrier.

The percutaneous absorption picture can be qualitatively clarified by considering following Figure 1.4:

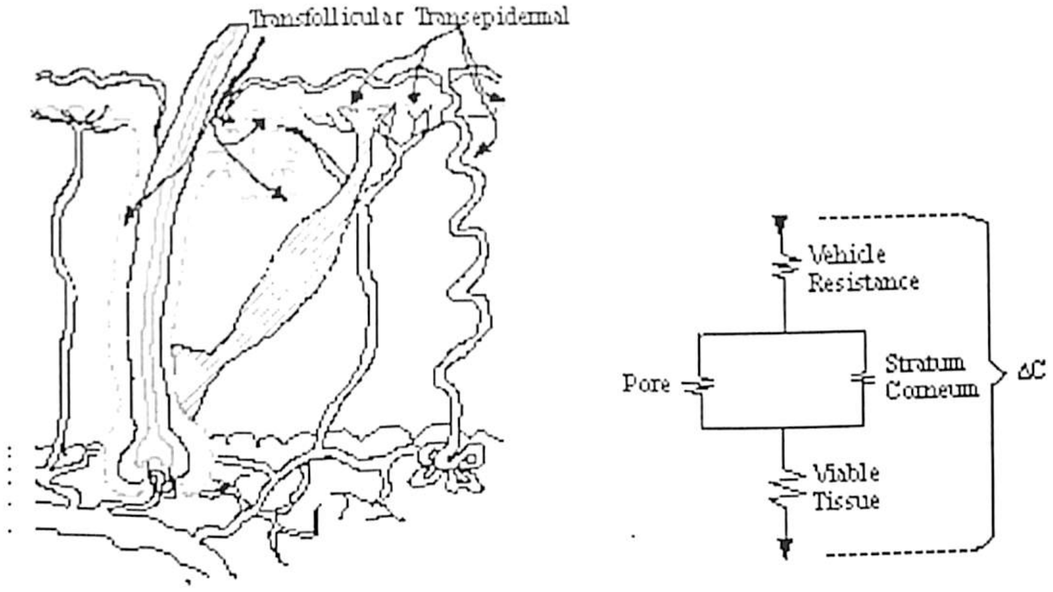


Fig. 1.4: Sketch indicating the transdermal and transfollicular pathways.

As shown in above sketch, the schematic cross section of the skin is placed, side by side, with the simple model for percutaneous absorption pattern as an electrical circuit. In case of absorption across a membrane, a current or flux is in terms of matter or molecule rather than electrons, as we would have in an electrical circuit, and the driving force is a concentration drop (technically, a chemical potential gradient) rather than voltage drop. The membrane acts as a diffusional resistor. A diffusional resistor has a resistance proportional to its thickness (h), inversely proportional to the ease of movement of matter within the membrane or to the diffusion coefficient (D), inversely proportional to the fraction or relative area of a route where there is more than one pathway (F), and inversely proportional to the relative carrying capacity of a phase as one moves from one type of matter or one stratum into the next [17]. The last mentioned parameter is simply a phase distribution or partition coefficient (K).The overall phenomenon of percutaneous absorption is traceable by recognising the resistance in series are additive and that diffusional current is parallel are additive just as they are in electrical circuit. In general, a given resistance in the set may be represented by the following equation :

$$R = \begin{pmatrix} \text{Resistance} \\ \text{of a diffusional} \\ \text{resistor} \end{pmatrix} = \frac{[\text{Thickness}]}{\begin{bmatrix} \text{Fractional} \\ \text{Area} \end{bmatrix} (\text{Diffusivity}) \begin{bmatrix} \text{Relative} \\ \text{capacity} \end{bmatrix}} = \frac{h}{FDH}$$

1.1.5. Models Of Skin Permeability:

Since the major resistance to skin permeation resides in the stratum corneum, a variety of mathematical models of the stratum corneum [18] have been proposed for predicting skin permeation of the drug. In this section, some of these models are reviewed briefly, each model has advantages and weaknesses with respect to the simplicity and rigorousness. In general, models with more parameters provide better fit with the experimental data. However, better fitting may not assure superiority over simpler models, unless the model parameters can be determined by a scientifically sound approach, either experimentally or theoretically. Before solving the skin model aimed at predicting the skin permeability of a drug in question, we must have a reliable model parameters.

These models are broadly classified into two categories :

I. Homogenous models , and

II. Heterogeneous models.

I. Homogenous Diffusion Model:

This model considers the stratum corneum as a homogenous barrier. If the concentration of the drug on the surface of the device is constant (C_d), which condition is usually the case for skin-controlled drug delivery system or in vitro experiments for the infinite dose system, the steady state rate of permeation under the sink condition is given by following equation[19,20]:

$$\left(\frac{dQ}{dt}\right)_{ss} = \frac{D_s K C_d}{h}$$

Where, K - Partition coefficient of drug between stratum corneum and donor solution

D_s - Effective diffusion coefficient across stratum corneum

h -Thickness of stratum corneum.

Following figure 1.5 shows the concentration profile at steady state:

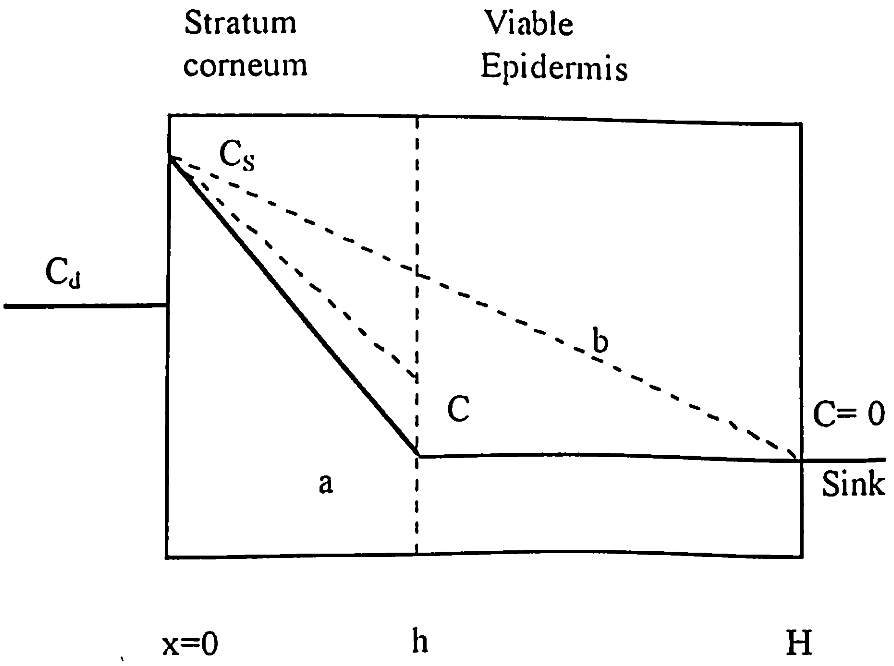


Fig. 1.5: Steady state concentration profile in the skin based on the homogenous skin model.

(a) single layer stratum corneum, (b) single layer whole skin model, (c) bilayer whole skin model.

Since the diffusivity, partition coefficient and thickness are difficult to separate for fragile membrane such as skin, the permeation data are commonly expressed by the effective permeability coefficient, defined as follows:

$$P = \frac{D_s \cdot K}{h} = \left(\frac{dQ}{dt}\right)_{ss} = \frac{1}{C_d}$$

The diffusion coefficient across the stratum corneum can be calculated from the time lag, t_d, determined in the in vitro experiments;

$$D_s = \frac{h^2}{6t_d}$$

II. Two Phase Diffusion Model:

Albery and Hadgraft propose a two phase model as shown in Fig. 1.6 for describing drug diffusion through the stratum corneum. The permeation of the drug was analysed based on Fick's second law of diffusion by assuming constant concentrations on the surface of the skin and the sink condition in the receiver.

The model considers two independent pathways :

1. Diffusion through the keratinized cells,
2. Diffusion through the intercellular route.

At steady state, the rate of permeation through each route was given by

$$\left(\frac{dQ}{dt}\right)_{ss} = \frac{C_d}{(2/\alpha k_1) + (h/\alpha\beta K_m D_s)}$$

Where, α - area fraction (unity for transcellular route and 7×10^{-3} for the intercellular route.

D_s - diffusion coefficient in stratum corneum.

K_m - partition coefficient of the drug between mineral oil and water.

β - empirical parameter.

k_1 - interfacial transfer rate constant (10^{-5} m/s)

By comparing the model solution with the experimental data of Michaels et al [24], the author suggested $\beta=5$ for the intercellular route, and $\beta=1$ for transcellular route.

By assuming parallel pathway, the total flux can be calculated by,

$$\left(\frac{dQ}{dt}\right)_t = \left(\frac{1}{(dQ/dt)_1}+\frac{1}{(dQ/dt)_2}\right)^{-1}$$

Where the subscripts 1 and 2 stands for the transcellular route and the intercellular route, respectively.

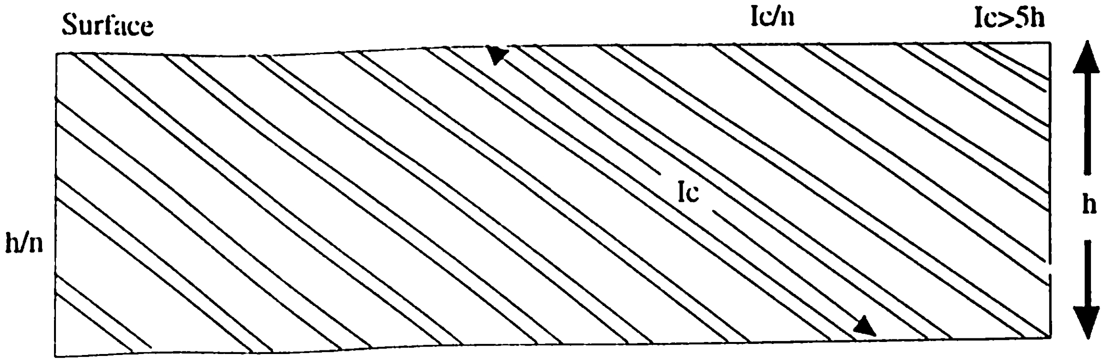


Fig. 1.6: Heterogeneous stratum corneum model (two phase).

III. Three Parallel-Pathway Model:

Berner and Cooper proposed a three phase model of the stratum corneum as shown in Fig 1.7:

This model considers three pathways:

1. nonpolar (lipid) pathway,
2. polar (aqueous) pathway, and
3. an alternating non polar-polar pathway.

The drug diffusion through each pathway occurs independently and therefore no interaction or partitioning is assumed between two adjacent routes of penetration.

Thus, J_{max}, the maximum flux of drug through the skin is proportional to C_w, the water solubility of the drug. We assume that :

1. $C^l{}_{max}$, the drug solubility in the nonpolar phase equals PC_w, where P is the octanol/water partition coefficient.
2. The partition coefficient in the fully hydrated polar pathway is unity.

The diffusion constants for the three pathways are modelled by free-volume theory. For $P \geq 1$, the water solubility C_w, is modelled by the method of Yalkowsky [118], who derived a relationship for C_w in terms of partition coefficient and melting point. C_w must be measured for $P < 1$.

The heterogeneous diffusion model is extremely complex to solve, but one can obtain an upper and lower bound to the steady-state problem by the series parallel and parallel-series approximations.

These bounds are, respectively :

$$J^u{}_{max} = \frac{2e^{[-BM]C_w}}{1} \cdot \frac{\left[A_L\ PD_L + (1-A_L)D_P{}^{(U)}\right]\left[A_P D_P{}^{(U)} + (1-A_P)PD_L{}^{(U)}\right]}{(1-A_L+A_P)D_P{}^{(U)} + (1-A_P+A_L)PD_L}$$

and

$$J^l{}_{max} = \frac{2e^{[-BM]C_w}}{1} \cdot A_L PD_L + A_p D_P + \frac{2(1-A_L-A_P)PD_L{}^{(U)}D^{(U)}{}_P}{D_P^{(U)} + PD_L^{(U)}}$$

Where,

M - molecular weight.

A_P & A_L - area fractions of the polar and nonpolar pathways, respectively.

J_{max} - maximum flux of drug through the skin.

U - upper level.

L - lower level.

D_L - diffusion coefficient of the drug in the nonpolar pathway .

D_P - diffusion coefficient of the drug in the polar pathway.

As shown in the following Fig. 1.7, this model considers three pathways :

(1) non-polar (lipid) pathway

(2) polar (aqueous) pathway, and,

(3) an alternating polar and non-polar pathways.

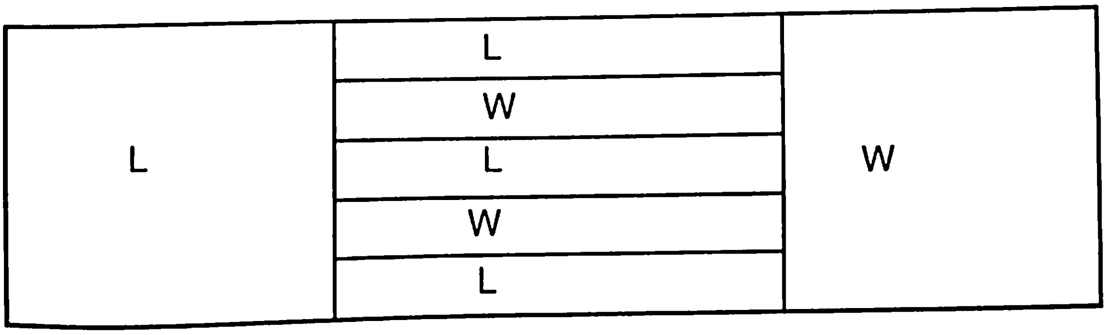


Fig. 1.7: Three parallel pathway model for stratum corneum; L, continuous lipid pathway; W-continuous aqueous pathway; L-W , lipid-aqueous multilaminate pathway.

IV. Single Compartment Model:

A single compartment model has been widely used to investigate skin pharmacokinetics for predicting the plasma concentrations following transdermal drug delivery. In a multicompartment model for skin pharmacokinetics, Guy and Hadgraft [22] assumed that the stratum corneum to be a single compartment in which the drug molecules distribute homogeneously. In addition to the stratum corneum, they considered the blood compartment, the skin surface compartment and the urine compartment (Fig 1.8).

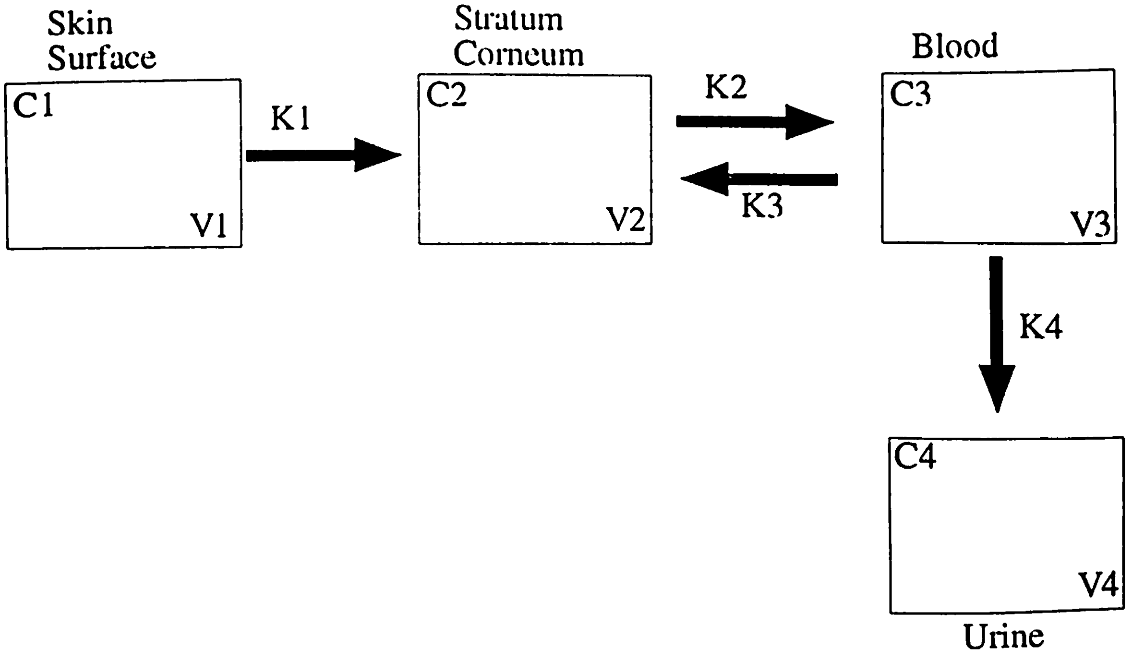


Fig. 1.8: Single compartment model for stratum corneum; C_i - Concentration; K_i - Transfer rate constant ; V_i - Volume of compartment.

By solving a set of first order differential equations together with the model parameters, the authors successfully described the plasma concentrations of various drugs. A detailed account of calculating the same using the same model has been described in Chapter 3.

1.1.5.5. Multiple Compartment Model:

Higuchi et al [23] proposed a multicompartment model of the stratum corneum as shown in Fig 1.9. In this model, the stratum corneum is divided into many compartments (elements) and arranged in series. In principle, the multi compartment model becomes identical to the diffusion model when number of elements is infinite.

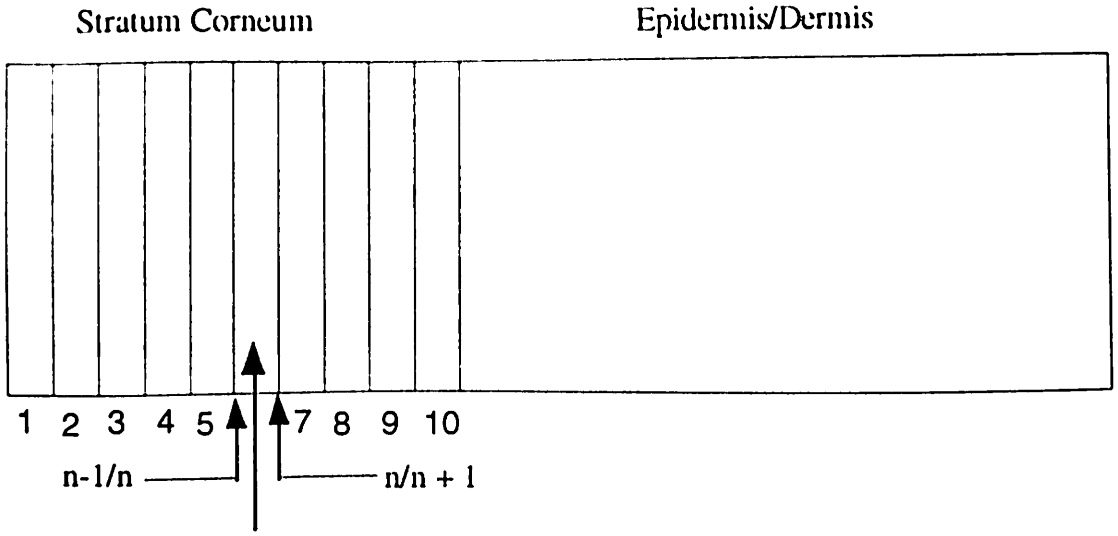


Fig. 1.9: Multicompartment model for stratum corneum.

This model is a simple but most useful model to gain a better understanding of the effect of enhancers on skin permeability.

1.1.5.6. Brick Model:

Michaels et al [24] proposed a brick model as shown in Fig 1.10, to describe the steady-state permeation rate of drugs across the stratum corneum. This model simplified the complex geometric arrangements of the stratum corneum to an ordered brick structure in which flattened protein cells (hydrophilic phase) are orderly stacked with a cement of lipid phase (continuous). The model considers two pathways,

1. lipid-protein cell in series, and
2. lipid phase only as the major route of permeation .

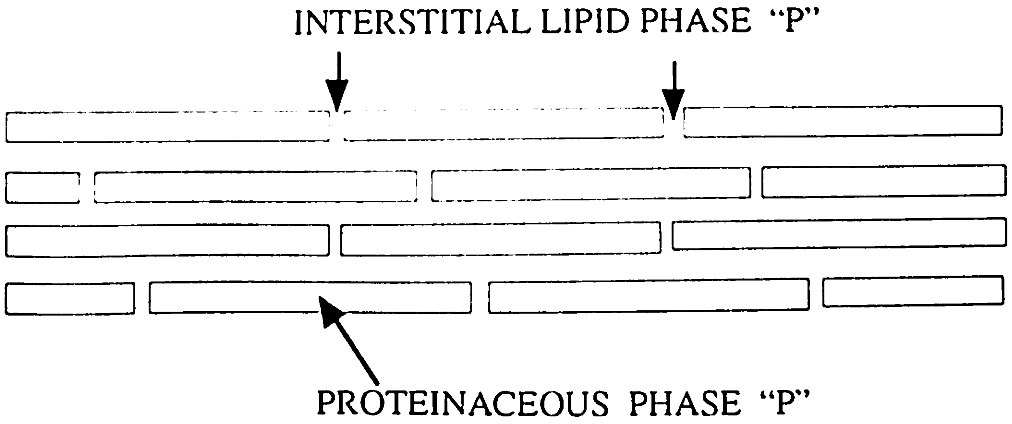


Fig. 1.10: Ordered brick model for stratum corneum.

1.2. Permeation Enhancement:

As discussed earlier, the permeation of drugs through the stratum corneum is the rate controlling barrier to transdermal permeation for both lipophilic and hydrophilic drugs. Depending on the properties of the drug, both the lipids and the proteins in the stratum corneum could offer resistance to permeation. The protein and the lipid regions form the polar and the nonpolar pathways respectively. Permeation through the polar pathway takes place by partitioning into the protein regions swollen by water. Permeation through the nonpolar pathway is more important for practical applications, since most drugs are lipophilic. Transport through this pathway takes place by dissolution and diffusion of the drug through the complex structure of the stratum corneum.

There are numerous techniques to reduce the resistance of the stratum corneum to the permeation of drug molecules. One is that the lipophilicity of the drug can be changed by preparing prodrugs [25] (e.g. from salt to base) or preparing prodrugs with a lower melting point. Sloan et al [26] prepared prodrugs of 6-thiopurines, which are very insoluble due to their high melting points and increased the permeability by preparing pivaloyloxymethyl derivatives. Valia et al [27] increased the permeability of β-Oestradiol by preparation of lower melting 17 monoester and 3,17 diester prodrugs. The other method is by altering the stratum corneum structure which can be accomplished by the use of chemical as well as physical enhancers.

1.2.1. Chemical Methods:

A chemical enhancer can be defined as a compound that, by its presence in the stratum corneum, alters the skin as a barrier to the flux of the desired drug. The definition allows for the increase in the flux due to the alteration of the stratum corneum structure or due to the increase in drug concentration in the skin.

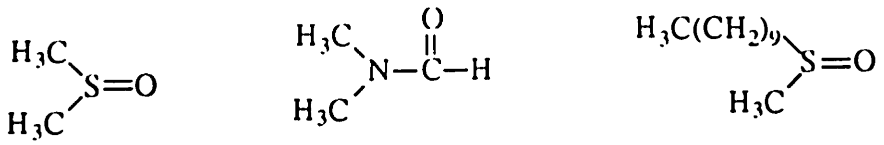
An ideal enhancer would have the following attributes [28]:

- Pharmacologically inert.
- Non-toxic, non-irritant and non-allergic.
- Onset of action should be rapid and the duration should be predictable.
- Upon removal of the enhancer, the stratum corneum should immediately and fully recover its normal barrier property.
- Chemically and physically compatible with the drugs and adjuvants to be formulated in dermatological preparations and devices.
- Inexpensive, odourless and colourless.

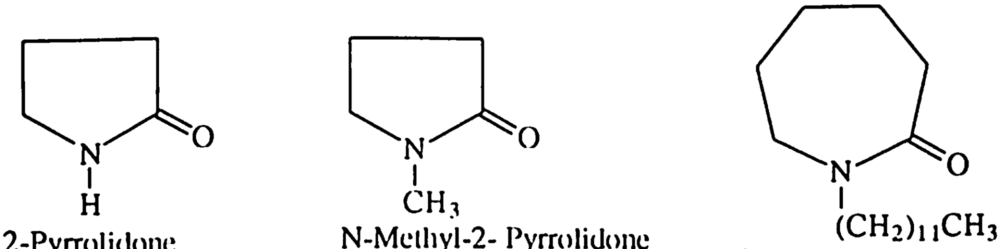
Chemical enhancers are highly diverse-solvents (such as water, alcohols) [29], dimethyl sulfoxide, dimethyl formamide and dimethyl acetamide, pyrrolidines [30,31], propylene glycol [32]), Azone and its derivatives [33-35], surfactants (anionic, cationic and nonionic) [37-40], fatty acids and alcohols, terpenes[41] and their derivatives, esters and miscellaneous materials such as urea and its long chain analogues [42], N,N-dimethyl-m-toluamide[43], calcium thioglycolate and anticholinergic agents (chemical structures are shown in Fig. 1.11 -1.12).

The approach presented here is to propose an overall scheme which explains how penetration enhancers work, known as lipid protein-partitioning (LPP) concept [44] and to select a few examples to illustrate the principles involved.

Fig. 1.11: Chemical structures of penetration enhancers and propylene glycol.

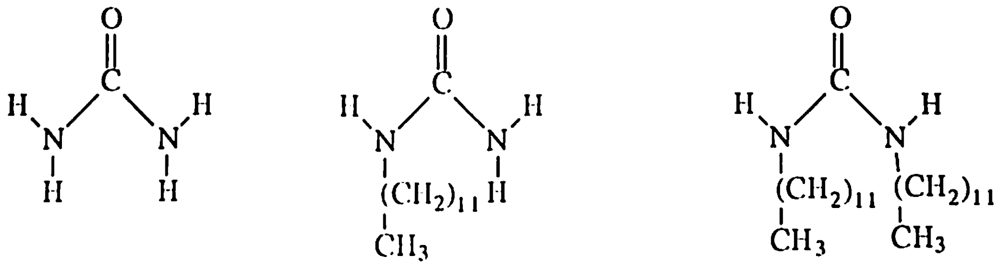


Dimethyl sulfoxide (DMSO) | Dimethyl formamide (DMF) | Decylmethyl sulfoxide (DCMS)

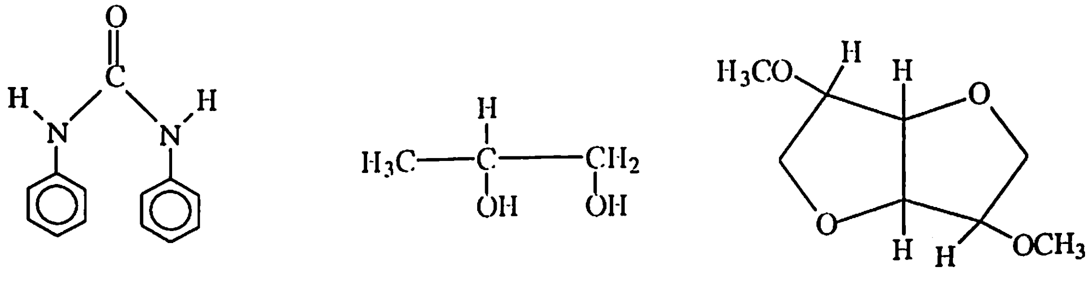


2-Pyrrolidone (2P) | N-Methyl-2- Pyrrolidone (NMP) | 1-DOdecylazocycloheptan-2-one (Azone)

Fig. 1.12: Formulas for urea based penetration enhancer and solvents.



Urea | 1-Dodecylurea | 1,3-Dodecylurea



1,3-Diphenyl urea | Propylene Glycol | Dimethylisosorbide (MDI)

1.2.2. Molecular Location For Penetration Enhancer Action:

Probably the most crucial interactions within the intercellular space of the horny layer during accelerant treatment operate at three main sites associated with the bilayer lipid structure (Fig 1.13 and Fig. 1.14).

I. Interaction At Site A:

Many accelerants should interact with the polar head groups of the lipid and alter hydrogen bonding and ionic forces. They should then modify the hydration spheres of the lipids and the subsequent modifications in head-group interactions presumably may upset the packing at the polar plane. This disruption may make the domain more disorganised and so promote the permeation of, in particular, polar penetrants.

A second response may be to allow more aqueous fluid to enter the tissue and so increase the water volume between the lipid layers or between the head groups of individual half leaflets. This swelling should provide a large fractional volume of free water as distinct from structured water and hence increase, the cross-sectional area available for polar diffusion (site B). However X-ray analysis and microscopy have provided strong evidence that the intercellular lamella do not swell with increasing hydration [45].

An important secondary feature is that disruption of interfacial structure should also alter the packing of lipid chains. The lipid hydrophobic route would thus become more disordered and more readily traversed by a lipid like penetrant (site C).

II. Direct Action At Site B:

An accelerant may alter the aqueous region in additional ways to those which change bond interactions and thereby possibly increase the water content. For example; when transdermal devices deliver solvents such as propylene glycol or ethanol [46] into the surface region of the horny layer, the solubilizing ability of the aqueous site for lipid like molecules may increase.

Fig. 1.13: Sites for penetration enhancers action within the intercellular space of the stratum corneum.

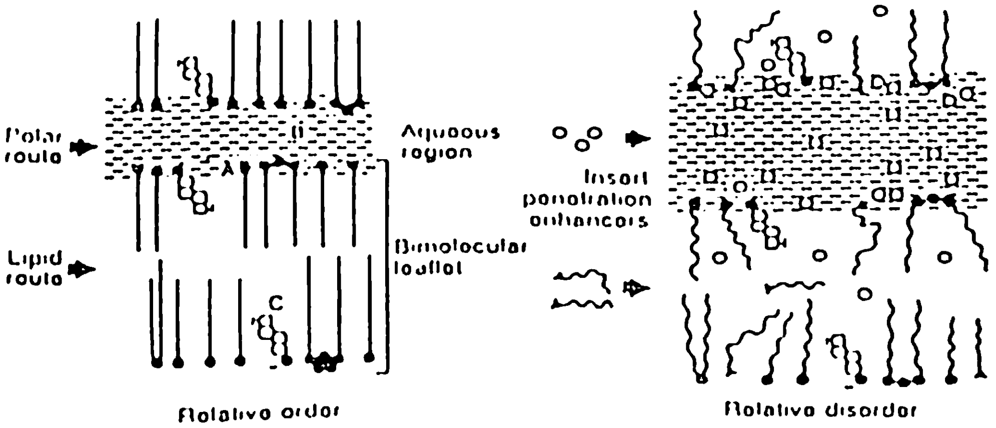
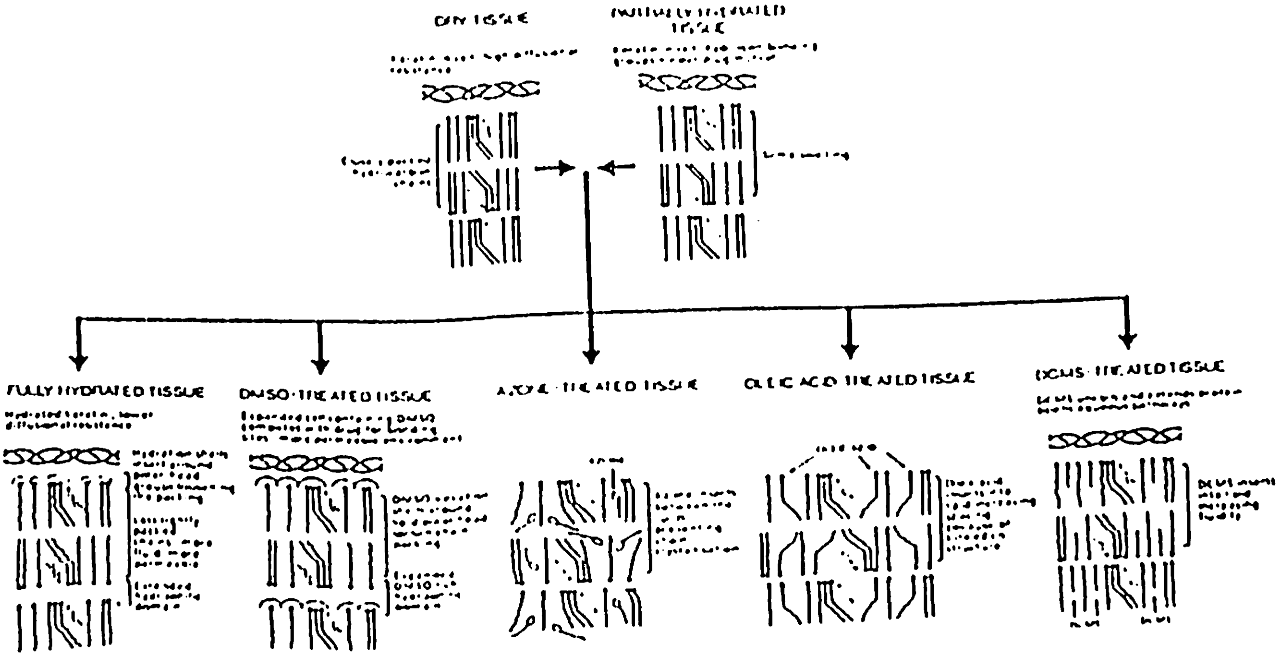


Fig. 1.14: Suggested modes of interaction of typical penetration enhancers with the components of stratum corneum.



III. Action At Site C (The Lipid Domain):

Many enhancers, because of their chemical structure should get inserted between the hydrophobic tails of the bilayer thereby modifying lipid packing, increasing lipid disorder, and thus permitting easy diffusion of drugs. This alteration in the lipid packing can reflect back to provide some disorder in the polar head-group region and so promote polar route penetration.

For many enhancers to operate maximally, the correct choice of cosolvent is important [47-51]. For such accelerants to reach site A (the polar surface of the lipid bilayer) in sufficient amounts, they may require cosolvents such as propylene glycol or ethanol. It is suggested that the cosolvent functions by increasing the lipophilicity of the aqueous region and so raising its solubilising ability for lipid like materials such as Azone and oleic derivatives, the cosolvent may also alter lipid-head group packing.

1.2.3. Enhancer Action In The Intracellular Route:

Despite much modern emphasis on the intercellular domain, it is unwise to dismiss completely the intracellular route through the corneocyte as of no consequence. If for a particular drug, the intracellular pathway provides a significant route of penetration, one should consider the keratin fibrils and the interactions that promoters such as aprotic solvents [e.g. dimethyl sulfoxide, [52], dimethyl formamide, dimethyl acetamide [53] and surfactants undergo with proteins. These mechanisms includes interactions with polar groups relaxation of binding forces and alteration in the confirmation of the helices.

1.2.4. The Lipid-Protein-Partitioning (LPP) Concept:

Using the concepts outlined above we can summarise the mode of action of penetration enhancers on the basis of the LPP theory, which concentrates on three main elements, lipid interaction, protein modification and partitioning changes in the horny layer [54-55]. Fig. 1.13 illustrates some examples of penetration enhancer action.

1. Most accelerants decrease intercellular lipid order, e.g. oleic acid [56].

2. Many enhancers interact with intracellular proteins.
3. When effect 1 and 2 combine, promoter activity may be accentuated.
4. Small polar accelerants may accumulate in the horny layer and promote partitioning of either drug or a co-enhancer, or both, e.g. dimethyl sulfoxide and its analogues, pyrrolidones, ethanol [57] and propylene glycol.
5. The diffusional resistance of the stratum corneum decreases with increased water content.

1.3. Physical Methods:

Several non-chemical methods have been proposed for enhancing the permeability of drugs through the skin. Iontophoresis and phonophoresis, the two methods most extensively used will be discussed. A third approach introduced recently involves controlled removal of stratum corneum by pulsed laser light [58-59]. When the proper wavelength, pulse-length energy and pulse rate are applied, the stratum corneum is removed without significantly damaging the underlying epidermis. When perfected, this method may be useful for the delivery of large molecular-weight proteins in a hospital environment.

1.3.1. Iontophoresis:

Iontophoresis is the administration of ionic therapeutic agents through the skin by application of an electric current.

The use of Iontophoresis goes back as long as the 19th century. Much of the early work was done on humans, later work has concentrated on different animal models and bench-top model systems using excised skin.

An Iontophoretic system or device, (Fig 1.15) has three basic components, the source of electric current, which usually consists of a battery and control electronics, an active reservoir system, which contains the ionic therapeutic agent, and an indifferent or return reservoir system, which contains an electrolyte and serves to complete the electric circuit.

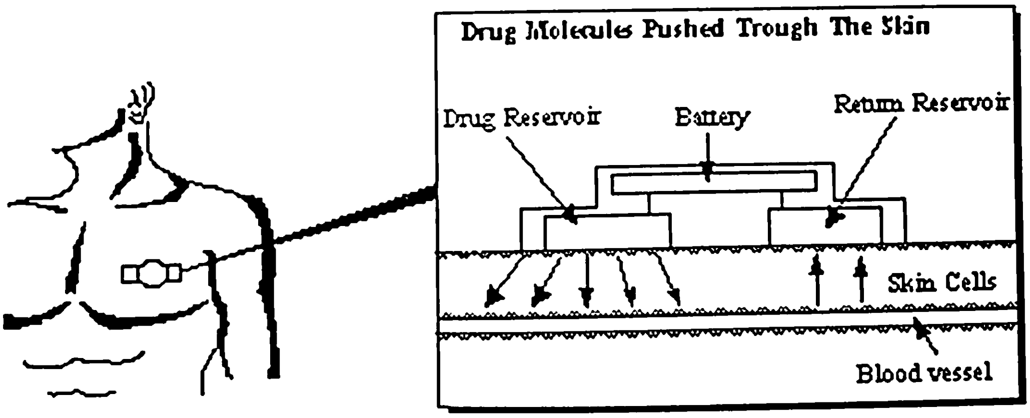


Fig. 1.15: Iontophoresis system showing the three basic components; drug reservoir, return reservoir, and power system.

When the active and indifferent reservoir systems are placed on the skin, the current source causes electronic current to flow to the active reservoir where the electronic current is transformed to the ionic current. The ionic current flows through the active reservoir, through the skin, beneath the skin towards the indifferent reservoir and back through the skin into the indifferent reservoir. At the indifferent reservoir it is transformed back into electric current. It completes its circuit at the opposite pole of the current source.

As shown in Fig. 1.15, the active reservoir is connected to the positive pole of the current source. In this case it is called the anode. If positively charged drugs are placed in the anode, they move in the direction of the skin, into the skin because of the electrical field generated in the ionic portion of the circuit. Similarly if the active reservoir is connected to the negative pole of the current source it is called the cathode. Negatively charged therapeutic ions placed in the cathode also move in the direction of and into the skin beneath the reservoir because of the electric field.

Although in most of the Iontophoresis systems described in the literature, the therapeutic agent is in one or the other of the reservoir systems (anode or cathode), the simultaneous delivery of both positively charged drug (from the anode) and a negatively charged drug (from the cathode) is possible [60].

An early clinical use was for the Iontophoresis delivery of pilocarpine for the deletion of sweat in the diagnosis of cystic fibrosis of the pancreas [61].

A wide variety of agents have been studied using Iontophoresis. Table 1.3 demonstrates that Iontophoresis has been broadly studied and that it has broad application.

Table 1.3 : Compounds that have been iontophoresised.

Model	Compounds	Indications and Applications	Ref.
In vivo Human	L H R H	Luteinizing Hormone Releasing Hormone	62, 66
	Lidocaine	Anaesthetic	63
In vitro Human (excised skin)	Propranolol	Drug administration rate	64
	L H R H	Drug administration rate	65
	Salicylate,Mannitol, Insulin.	Drug administration rate	67
	Phenylethylamine	Drug administration rate	68
In vivo animal	G R F [1-44]	Growth hormone releasing factor	69
	Tripeptide	Threonine-Lysine-Proline	70
	Insulin	Drug bioactivity	71
In vitro animal (excised skin)	Arbutamine	Drug Penetration rate	72
	Oligonucleotides	Drug Penetration rate	73
	Verapamil	Transport enhancement	74
	Arginine, Vasopressin	Transport enhancement	75
	Metoprolol	Factors affecting delivery rate of	76

1.3.2. Phonophoresis:

Phonophoresis is the application of ultrasonic energy to increase the permeation of drugs through the skin. The mechanical energy delivered by ultrasonic treatment is obtained by passing an alternating current through a piezoelectric crystal, causing it to vibrate (Fig. 1.16).

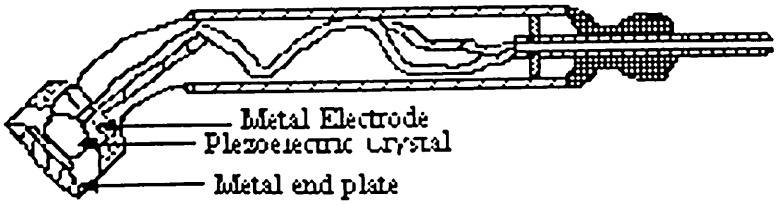


Fig. 1.16: A Schematic diagram showing the structure of an ultrasonic transducer used for phonophoresis.

In 1954 ultrasound was used in conjunction with hydrocortisone ointment to treat inflamed digital joints [76]. Since then ultrasono-phonophoresis has been widely studied for permeation enhancement of various drugs across the skin. Enhanced permeation of fluocinolone acetonide, lidocaine, prilocaine, mannitol ,insulin, physostigmine, indomethacin and ibuprofen has been demonstrated [77-80]. Kost et al [81] explored the effect of phonophoresis on the stratum corneum structure and composition using lipophilic and hydrophilic drugs. They concluded that phonophoresis alters and affects the polar as well as the nonpolar pathway.

It has been postulated that ultrasound may produce structural and functional changes by way of thermal, cavitational or other mechanical mechanisms not yet well understood. There is evidence for a small cumulative effect over a very short time base (less than 5 min.), but there is no evidence for cumulative effects on any longer scale [82].

It has also been suggested that a major factor influencing the interaction of ultrasound with living cells under experimental conditions was the occurrence of "stable" or "resonant" bubble type cavitation in the media in which cells were irradiated [83]. The mechanical sheer resulting from this cavitation leads to cell membrane disruption and disruption of other large structural cell components. Chemical changes due to free radicals appear to play a minor role in ultrasonically induced biological changes.

Julian and Zentner [84] demonstrated that the ultrasonically enhanced diffusion was a result of the decrease in the activation energy barriers within the membrane.

Although drug phonophoresis has been used in only a limited way in the past, it appears to have a potential in the future as a drug delivery method/system for specialised purposes. Additional research is required before a practical system for transdermal delivery can be evolved. A balance will have to be reached between intensity, frequency, pulse period, and duration plus the ability of the patient to tolerate the treatment.

1.4. Thermodynamics Of Skin Permeation:

The difference in the mechanisms of skin permeation between water-soluble and lipid-soluble non-electrolytes is demonstrated by very different values of activation energy E_a preexponential factor A, enthalpy ΔH and entropy ΔS (Table 1.4).

Table 1.4: Thermodynamic quantities associated with skin permeation of an alkanols.

	Thermodynamic Quantities				
	A^a	E_w	ΔF^b	ΔH	ΔS^b
Alkanols	(cm^2/sec.)	(Kcal/mol)	(Kcal/mol)	(Kcal/mol)	(Cal/mol.deg)
Ethanol	119	16.4	10.2	15.8	18.7
Propanol	502	16.5	10.2	15.9	19.2
Butanol	1760	16.7	10.4	16.1	21.2
Pentanol	3060	16.5	9.7	15.9	20.9
Hexanol	1.71	10.9	8.9	10.3	4.67
Heptanol	0.42	9.9	8.4	9.3	- 0.36
Octanol	0.076	8.7	9.4	8.1	- 4.17

a- This includes the membrane partition coefficient

b- The membrane partition coefficient was excluded from determination of F & S values (Glasstone et al , 1941.)

1.3.1. Activation Energy For Skin Permeation:

The skin permeation of non-electrolytes, like alkanols, was reported to be an energy requiring process and to follow the Arrhenius relationship.

$$J_s = A\, e^{-Ea/RT}$$

where, E_a - Activation energy

A - preexponential factor.

For hydrophilic alkanols (< C_5) the activation energy is constant and 16.5± 0.1 kcal/mol. is required for their permeation through hydrated stratum corneum (Table 1.4). On the other hand for the skin permeation of lipophilic alkanols (> C_5) a lower activation energy (10.9 kcal/mole or less) is needed at temperatures higher than 25-30°C. The observed lower energy requirement for lipophilic alkanols may be attributed to the possibility that these lipid soluble aliphatic alcohols penetrate the skin via a lipoidal pathway, at temperatures higher than 25-30 °C the lipid components in the stratum corneum become less viscous. Thus a lower energy of activation is needed for their skin permeation, a lower value of entropy, ΔS also results. The longer is the alkyl chain length, the more lipophilic the alkanols and thus the lower the activation energy and also the entropy (Table 1.4).

However both hydrophilic and lipophilic alkanols have a common feature that is a substantially higher free energy, ΔF, is needed for diffusion in the stratum corneum (9.60 ± 0.75 kcal/mole) than in the solution. This may be the underlined reason why skin permeation occurs so slowly.

1.3.2. Free Energy Of Desorption For Stratum Corneum/Vehicle Partitioning:

The stratum corneum/vehicle partitioning coefficient K_s is theoretically related to the standard Gibbs free energy of desorption F_d, i.e. the energy gained by a solute molecule upon desorption from the stratum corneum into the vehicle applied.

$$\Delta F_d = RT\, l_n\, K_s \text{ ------ } (1)$$

A large ΔF_d value implies a greater solubility in the stratum corneum, a greater K_s and consequently greater skin permeability.

If it is assumed that ΔF_d can be expressed additively in terms of the individual contribution of the polar groups (ΔF_{OH}) and the lipophilic group (ΔF_{CH2}), then

$$\Delta F_d = \Delta F_{OH} + \Delta F_{CH2} \text{ ------- } (2)$$

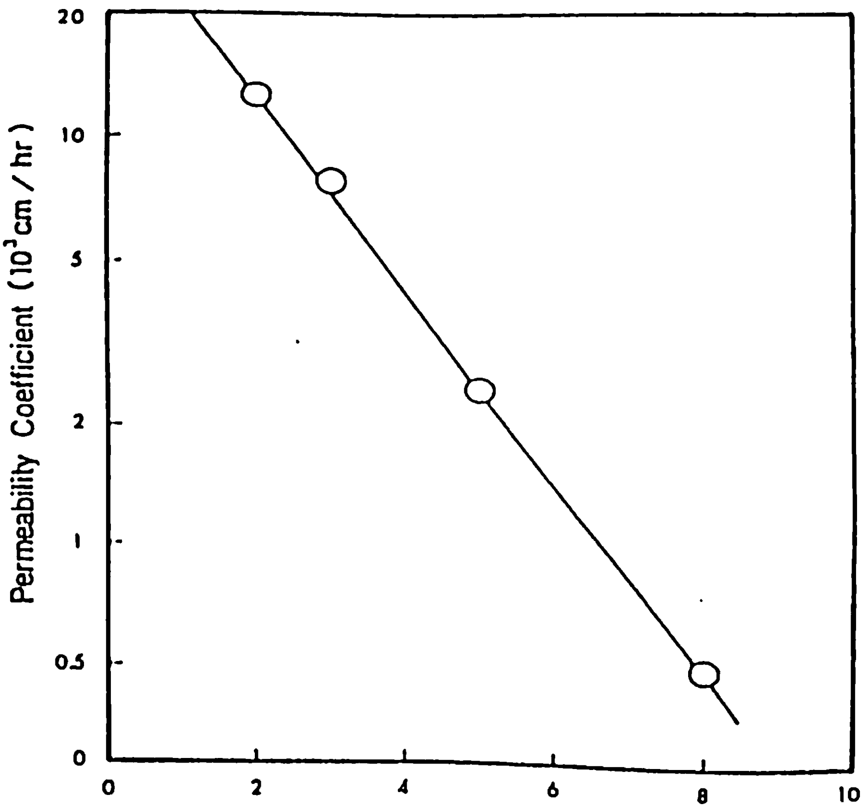
then

$$\log K_s = constant + \frac{\eta \Delta F_{CH_2}}{2.303RT} \text{ ------- } (3)$$

Following equation (1), a plot of log K_S versus η for a homologous series of alkanols should permit calculation of ΔF_{CH2}, the free energy of desorption for a single methylene group. The results suggest that the addition of a methylene group gives a ΔF_{CH2} of 241 mcal/mol for $n < 3$ and 448 mcal /mol for $n > 3$. The lower ΔF_{CH2} value observed for lower alkanols ($< C_3$) can be explained by the miscibility of these alkanols with water. Since the stratum corneum has a similar well hydrated environment, a lower value of ΔF_{CH2} results.

Equations (2) and (3) also imply that if a vehicle that has physicochemical characteristics identical to the stratum corneum is used, ΔF_d equals zero and K_S value is unity, skin permeability then becomes independent of chain length. On the other hand, if a vehicle that is more lipophilic than the stratum corneum is used, the permeability thus decreases with the increase in alkyl chain length. This behaviour is demonstrated by the use of isopropyl palmitate as the vehicle for straight chain aliphatic alcohols (Fig 1.17). A ΔF_{CH2} value of -326.5 cal/mol is estimated.

Fig. 1.17: Effect of alkyl chain length 'n' on the skin permeability coefficients of alkanols from a lipophilic vehicle of isopropyl plamitate.



With the replacement of aqueous solution by isopropyl myristate as the vehicle, the free energy of desorption of straight-chain alkanols changes from an increase of 241- 448 cal /mol to a decrease of 326.5 cal/ mol for each additional CH_2 group [85].

1.3.3. Effect Of Polar Groups On The Free Energy Of Desorption:

Scheuplein et al [86] reported that the introduction of polar groups, such as hydroxy groups reduces the permeability coefficient of steroids. The magnitude of the reduction showed a first order dependence on the number of -OH groups added to the progesterone skeleton. The relationship can be expressed as :

$$Log(P_s)_{OH} = Log(P_s)_p - (\pi)_{OH}\,(n)_{OH}$$

$$Log(P_s)_{OH} = Log(P_s)_p - (\pi)_{OH}\,(n)_{OH}$$

Where $(P_s)_P$ and $(P_s)_{OH}$ are permeability coefficients for progesterone and its hydroxyl derivatives, respectively.

$(\pi)_{OH}$ - Incremental constant for hydroxyl groups.

$(n)_{OH}$ - Number of (OH) groups.

The first order dependence of the skin permeability coefficient $(P_s)_{OH}$ on the number 'n' of OH groups can be defined alternatively by :

$$\log(P_s)_{OH} = Log(P_s)_p + \frac{\eta \Delta F_{OH}}{2.303 RT}$$

Where, $(\pi)_{OH}$ is thermodynamically equivalent to $(\Delta F_{OH})/2.303$ RT and ΔF_{OH} , the Gibbs free energy of desorption by OH groups, is related to the standard Gibbs free energy of desorption as follows :

$$\Delta F_d = \Delta F_p + \eta \Delta F_{OH}$$

Where, ΔF_p is the Gibbs free energy of desorption for the interfacial partitioning of progesterone molecule. A ΔF_{OH} value of -1.291 kcal/mol was estimated for the addition of every OH group into progesterone molecule.

1.4. Recent Developments In Transdermal Drug Delivery:

The potential of using the intact skin as the port for continuous transdermal drug delivery beyond the boundary of topical preparations has been recently recognised. The development of female syndromes in male operators working in the manufacturing areas of pharmaceutical dosage forms containing oestrogenic steroids has challenged the old theory in Biomedical Sciences that skin is basically an impermeable barrier and also has triggered the research curiosity of biomedical scientists to investigate the feasibility of using the intact skin for transdermal delivery of systemically active drugs.

The findings accumulated over the years have revolutionised the traditional belief in the impermeable skin barrier and also motivated a number of pharmaceutical scientists to develop patch type drug delivery systems for transdermal controlled administration of drugs for systemic medication.

Intensive R&D efforts over more than a decade have succeeded in the development and commercialisation of several rate controlled transdermal drug delivery systems. Some of the transdermal systems currently marketed in the world are shown in Table 1.5.

Table 1.5: Transdermal currently marketed in the world.

Sr. No.	Drug	Firm
1.	Clonidine	Boehringer-Manheim
2.	Estradiol	Ciba Besins-Iscovesco
3.	Etofenamate	Bayer
4.	Fentanyl	Janssen
5.	Isosorbide Dinitrate	Yamanouchi
6.	Nicotine	Park-Devis Ciba Marion Meriell Dow Leaderl
7.	Nitroglycerin	Key Pharmaceuticals Searl Ciba Schwartch Rugby-Schein Nippon 3M
8.	Progesterone	Besins-Iscowesco
9.	Scopolamine	Ciba Myum Moon Pharm.

The structural components of the newly developed transdermal patches and the old fashioned medicated plasters are compared in Table 1.6.

Table 1.6: Transdermal patches Vs. medicated plasters.

Structural component	Composition and functionality	
	Medicated plaster	Transdermal patches
Backing support	Non-occlusive (fabric, papers etc.)	Occlusive (drug impermeable plastic film, metallic plastic laminates)
Drug reservoir	Disorption of multiple drugs in adhesives, natural rubber, gum base.	Dispersion of single drug in liquid or solid state synthetic polymer base.
Drug release mechanism	Matrix diffusion	Membrane permeation, micro-reservoir dissolution or matrix diffusion.
Adhesive film	No.(Adhesiveness derived from gum and rubber base.)	Yes. (Surface coating with pressure sensitive adhesive polymer).
Release liner	Cellophane, gauze.	Occlusive (drug-impermeable plastic film, metallic plastic laminate with releasing surface)

Transdemal rate controlled drug delivery offers the following potential advantages :

1. Avoidance of risks and inconveniences of intravenous therapy, and of the varied conditions of absorption and metabolism associated with oral therapy.
2. Continuous drug administration, permitting the use of a drug with short biological half-life.

3. Achievement of efficacy with lower total daily dosage of drug by continuous drug input and by passing hepatic first pass elimination.
4. Less chance of over or under dosing as the result of prolonged pre-programmed delivery of the drug and the required therapeutic rate.
5. Provision of a simplified therapeutic regimen leading to better patient compliance.
6. Drugs with a narrow therapeutic range can be delivered.

It also has a few limitations :

1. Limited drug permeability of the skin.
2. Applicable for low dose drugs only.
3. Possibility of irritation and hypersensitivity reactions.
4. Adhesion problems.
5. High cost of treatment.
6. Cannot administer drugs that require blood levels in the order of micrograms.
7. Pharmacodynamic and Pharmacokinetic restrictions.

1.5. Fundamentals Of Rate Controlled Transdermal Drug Delivery:

A systemically active drug that will reach a target tissue far from the site of drug administration on the skin surface must possess some physico-chemical properties that are capable of facilitating the sorption of drug by the stratum corneum, the penetration of drug through viable epidermis, and also the uptake of the drug by the capillary network in the dermal capillary layer (Fig. 1.18).

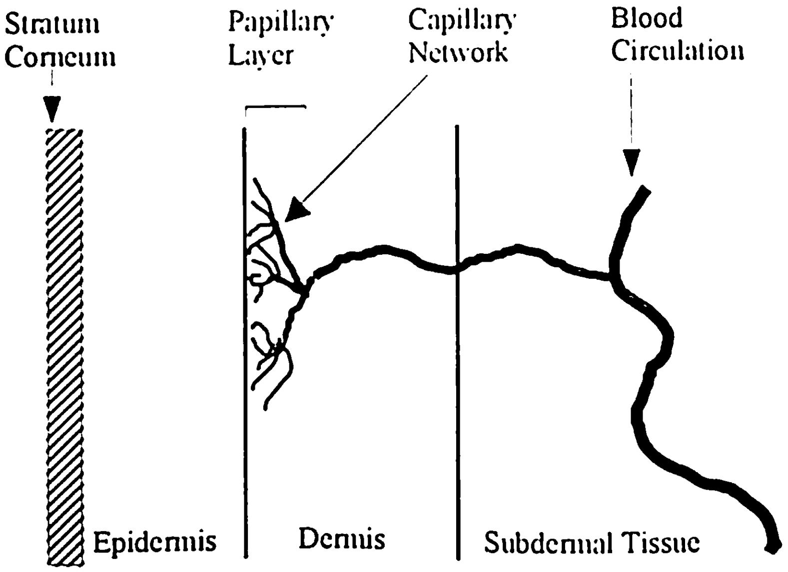


Fig. 1.18: Multilayer skin model showing the sequence of transdermal permeation of drug.

The rate of permeation, dQ/dt, across the skin tissues can be expressed mathematically by the following relationship [87] :

$$\frac{dQ}{dt} = P_s (C_d - C_r) \text{-----} (1)$$

where, C_d and C_r are the concentrations of a skin penetrant in the donor compartment (e.g. the drug concentration on the surface of stratum corneum) and in the receptor compartment (e.g. body), respectively and P_s is the overall permeability coefficient of the skin tissues to the penetrant as defined by :

$$P_s = \frac{K_s D_{ss}}{h_s} \text{-------} (2)$$

K_s - Partition coefficient for the interfacial partitioning of the penetrant molecule from solution medium or a transdermal therapeutic system onto the stratum corneum.

D_{ss} - Apparent diffusivity for the steady state diffusion of the penetrant molecule through a thickness of skin tissues.

h_s - Overall thickness of the skin tissues.

The permeability coefficient (P_s) for a skin penetrant can be considered as a constant, since K_s, D_{ss} and h_s in equation (2) are essentially constant under given conditions.

Analysis of equation (1) suggests that to achieve a constant rate of drug permeation, one needs to maintain the drug concentration on the surface of the stratum corneum (C_d) consistently and substantially greater than the drug concentration in the body (C_r) i.e. $C_d >> C_r$, under such conditions, equation (1) can be reduced to :

$$\frac{dQ}{dt} = P_s \cdot C_d$$

and the rate of the skin permeation (dQ/dt) becomes a constant, if the magnitude of C_d remains fairly constant throughout the course of skin permeation. To maintain C_d at a constant value it is necessary to make the drug release at a rate (R_r) i.e. either constant or always greater than the rate of skin uptake (R_a), i.e. $R_r >> R_a$ (Fig 1.19).

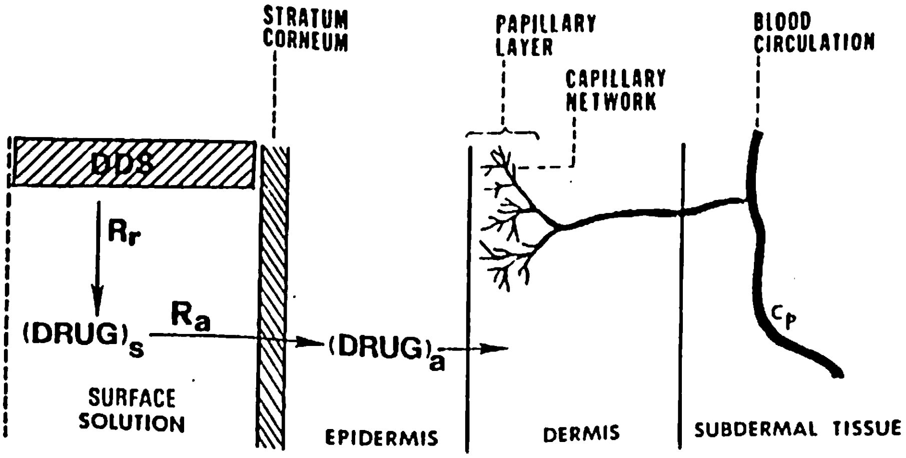


Fig. 1.18: Schematic illustration of the relationship between the rate of drug release (R_r) from a transdermal drug delivery system and rate of absorption (R_a) by the skin.

By making $R_r > R_a$, the drug concentration on the skin surface (C_d) is maintained at a level equal to or greater than the equilibrium (or saturation). Solubility of the drug in the stratum corneum(C_s^e) i.e. $C_d > C_s^e$ and a maximum rate of skin permeation $(dQ/dt)_m$ as expressed by equation (3) is thus achieved.

$$(dQ/dt)_m = P_s C_s^e \quad \text{--------} (3)$$

Apparently the magnitude of $(dQ/dt)_m$ is determined by the skin permeability coefficient (P_s) of the drug and its equilibrium solubility in the stratum corneum (C_s^e). This concept of stratum corneum limited skin permeation was investigated by depositing various doses of pure, radiolabeled nitroglycerin in a volatile organic solvent, onto a controlled skin surface area of rhesus monkey [88]. Analysis of the urinary recovery data indicated that the rate of skin permeation (dQ/dt) increases with the increase in nitroglycerin dose (C_d) applied on a unit surface area of the skin.

1.6. Pharmacokinetic Interpretation:

As with other dosage forms, transdermal delivery systems attempt to achieve optimal blood levels of the drug that maximise the intended therapeutic effect and minimise any unwanted side effects. In essence, the pharmacokinetic and pharmacodynamic activities of the drug candidate, along with its physical chemistry, are the primary considerations in designing a transdermal product. These considerations include :

1. Problems encountered with the current dosage form of the drug.
2. Physical chemistry of the drug (molecular weight, melting point, hydrophilicity etc.).
3. Blood level that provides a pharmacological response.
4. Toxic blood levels.
5. Therapeutic window.
6. Pharmacokinetic parameters of the drug such as Biological half-life and clearance rates.
7. Drug permeation rate through the skin.
8. Total amount of the drug needed to permeate through skin.
9. Skin site and system size.

Fig. 1.20 illustrate the blood level profiles of a drug administered by the intravenous, oral, multiple oral and transdermal modes.

Note that there is a blood-level region that defines an area known as the "therapeutic window" (shaded areas). In this range of blood levels the disease state can be treated with minimal side effects. If blood levels fall below or do not reach the lower level, then the drug product is therapeutically ineffective. If blood levels go beyond the higher "toxic level" then one could expect adverse side effects. Ideally a dosage form should achieve levels in the therapeutic window. Fig. 1.20a shows how an intravenous route of administration will have a very high initial blood level with little resident blood level in the therapeutic window. However an orally administered drug will start out subtherapeutically, enter the therapeutic window for a short period of time and into the toxic or side effect levels until the blood level peaks out, and then follow the intravenous decay curve (Fig 1.20b).

Fig. 1.20a-b : Blood level profiles of drug administered by i.v. (a) and oral (b) route.

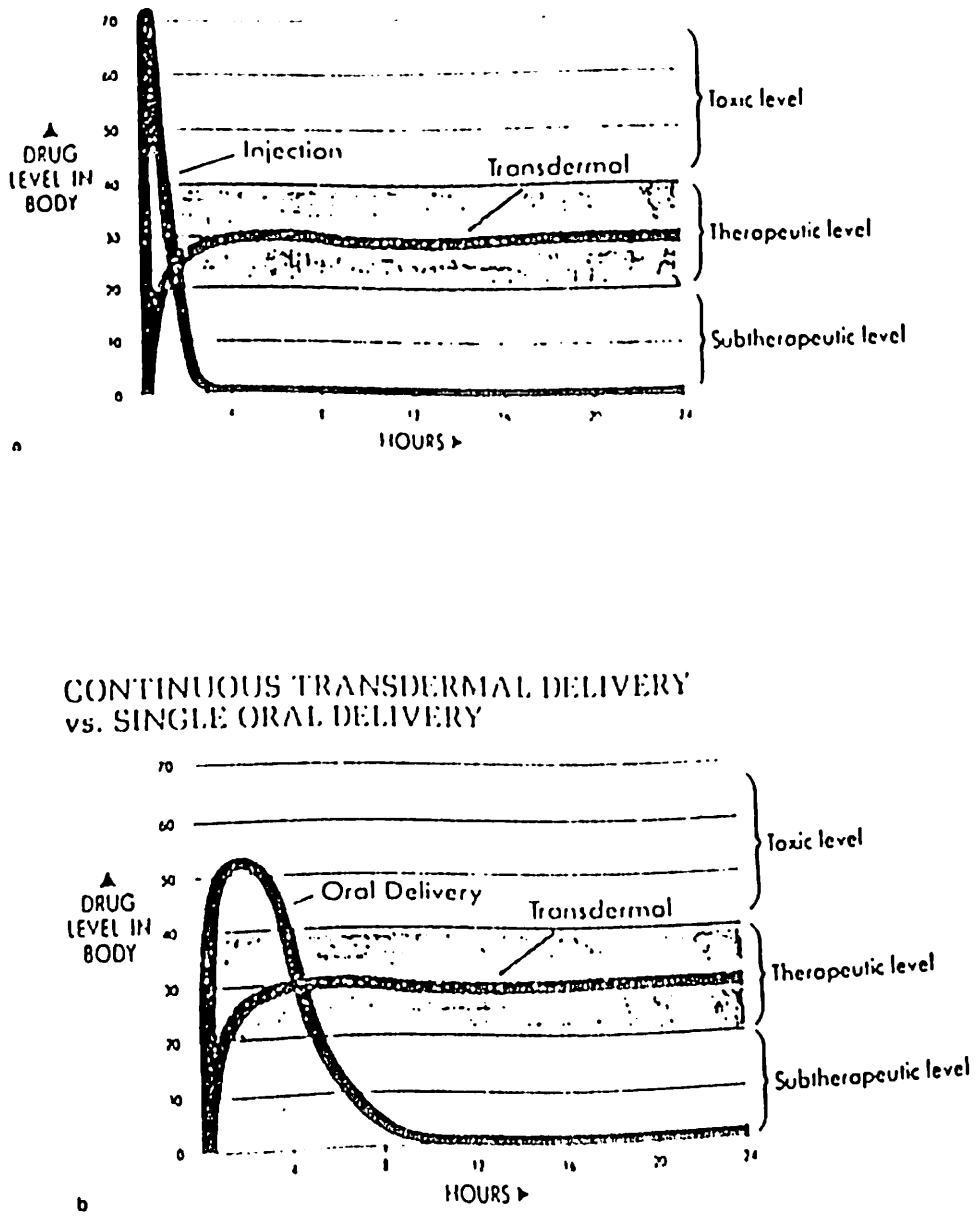
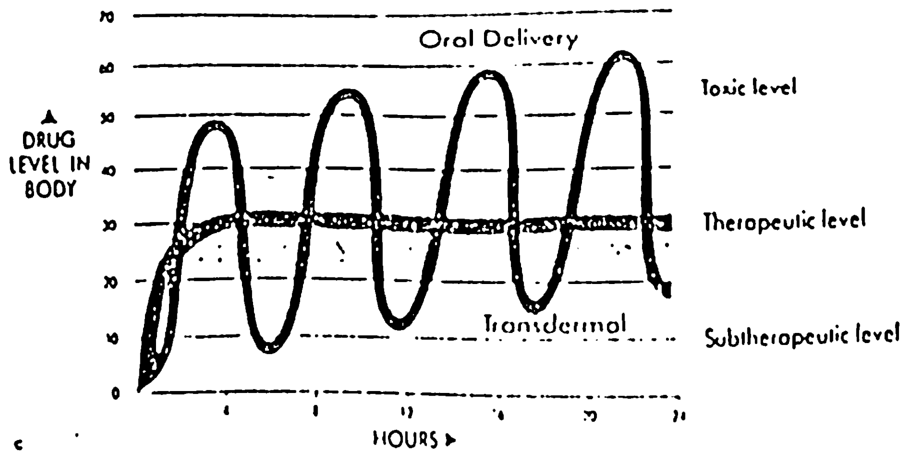
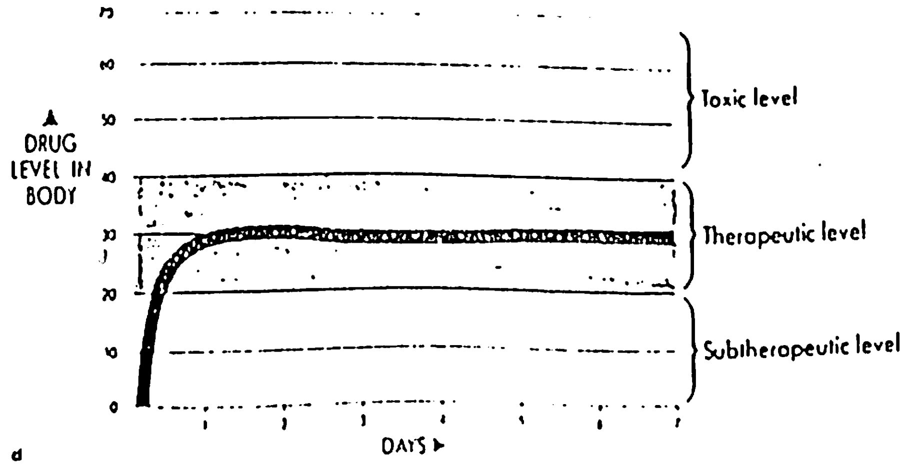


Fig. 1.20 c-d: Blood profile of drug administered by multiple oral (c) and transdermal models (d) route.

CONTINUOUS TRANSDERMAL DELIVERY vs. REPEATED ORAL DELIVERY (4X PER DAY)



LONG-TERM CONTINUOUS TRANSDERMAL DELIVERY



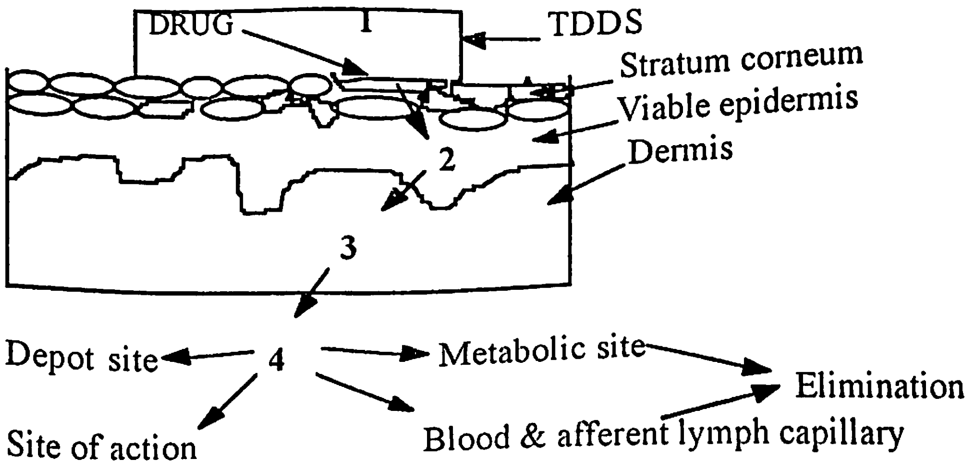
Multiple oral dosing provides multiple entries into and out of the subtherapeutic and toxic levels (Fig 1.20c). Presumably the ideal dosage form would achieve blood levels that continuously fall into the therapeutic window. Transdermal delivery systems have been able to reach this type of blood level profile (Fig 1.20d).

Not all drugs have been studied for their pharmacological effect with the continuous blood levels over long periods of time. There may be cases where constant delivery is not desirable because of tolerance of the drug's pharmacological activity. This is why the pharmacodynamic activity of the drug is important. One may be able to achieve what is thought to be appropriate blood levels as determined by intermittent dosing but chronic continuous dosing may give a less than adequate pharmacological response.

There are several steps in the process of drug permeation from a transdermal system, through the skin and into the systemic circulation. Some of these diffusion mechanism steps from the transdermal systems were described earlier. Once the drug permeates the skin and reaches the circulatory system, a new set of equations can be applied to understand the drug kinetics in the body.

Fig. 1.21 schematically illustrates how the drug transits from the transdermal system into the skin and what can happen once it reaches the circulatory system.

Fig. 1.21: Process of transdermal permeation



First, the drug molecule must be in a dissolved state before it can leave the system device, then it is able to migrate from the system and finally pass through the different layers of the skin. The drug can have an inherent skin permeation rate and skin metabolisms (regions 2 and 3 in Fig. 1.21). Once the drug molecule transits the skin and is inside the circulatory system, the drug can have the same pharmacokinetic parameters found in continuous intravenous delivery systems such as further metabolism (active or inactive metabolites), Biological half-life, depot sites (volume of distribution), and elimination (clearance rates).

The early literature treated pharmacokinetic evaluations of percutaneous absorption in terms of drugs permeating into the skin rather than through the skin. Wester and Maibach described percutaneous absorption in terms of 10 steps with examples of bioavailability [89]. In 1972, Beckett et al [90], showed that less metabolite was found in the systemic circulation when ephedrine and three of its analogues were administered transdermally compared to the oral route of administration. Time to reach peak urinary excretion rate was longer. With the transdermal, peak heights were an order of magnitude lower than peak heights for the oral route.

The work of Beckett et al with the ephedrine was later analysed by Riegelman [91] to show that the log to linear decline excretion rate of the oral dose with a 3.3 hour half-life of norephedrine indicated excretion rate properties of the intact compound. The transdermal route had an apparent half life of 8.4 hours, but by feathering data, a faster rate process was shown to have a half-life of 3.3 hours. The skin appeared to be the rate limiting step for elimination in the urine. Riegelman concluded that a steady state blood level could be obtained by changing the exposed area of contact and drug loading and by using different vehicles.

Shaw et al [92] later related ephedrine urinary excretion data to in vitro skin flux by means of two equations. The first equation :

$$J_{net} = A/f\left(1/K_{el} \cdot du_e/dt + u_e\right)$$

where ,

J_{net} - Net flux of system and skin [µg / h).

A - Area (cm^2) of the transdermal system.

f - Fraction of the drug excreted unchanged.

k_{el} - First order rate constant (hr^{-1}).

u_e - Urinary excretion at time t (µg / h).

Knowing the net flux J_{net}, and the in vitro release rate of a transdermal system into an infinite sink, J_{system} , one can calculate the skin flux in vivo, J_{skin} using a second equation:

$$1/J_{net} = 1/J_{system} + 1/J_{skin}$$

Wallace and Barnett [93] described skin penetration of drugs using compartmental models rather than the transitional Fick's law models. They attempted to describe the skin flux, lag time and rate constants using in vitro techniques without relating their data to blood levels or urinary excretion rates. Birmingham et al [94] described blood levels using a one compartment model with apparent first order absorption for salicylic acid permeating rabbit skin in vitro. Chandrashekaran et al [95] showed a two compartment model for transdermal delivery of scopolamine.

In relating lag time and steady state skin flux to excretion rates, Cooper [96] suggested that the total lag time, t_{lag}, is composed of a lag time for skin permeation, t_p, and a pharmacokinetic lag time, t_e. The pharmacokinetic lag time is the time it takes to distribute and eliminate the drug in the body. The relationship of these lag times to the excretion rates, ϕ_e (t) is expressed as:

$$\phi_e(t) = AJ_{ss}/V\left[t_{lag} - t_p - t_e\right]$$

where V = central compartment volume

A = surface area of the application site.

J_{ss} = Steady state flux.

Berner [117] describes pharmacokinetics of transdermals by combining diffusion and pharmacokinetic theories. In the case where skin is the rate-limiting barrier the lag time t_e is described as

$$t_e = 1/k + (\alpha_s^2)^2 / 6D_s$$

for a transdermal with a rate controlling membrane,

Where, k - sum of various elimination rate constants.

α_s^2 - the thickness of stratum corneum.

D_s - diffusion coefficient of the drug in the stratum corneum.

When the Transdermal is rate controlling the pharmacokinetic lag time is :

$$t_e = 1/k + (\alpha_s^2)^2 / 2D_s - 2/3D$$

α_s^2 - The thickness of transdermal membrane.

D - Diffusion coefficient of the drug in the transdermal membrane.

Guy and Hadgraft [97,98] have published a pharmacokinetic model that describes the delivery of a drug from a transdermal delivery system to the systemic region. As mentioned earlier, a detailed calculation as to how this equation can be used to predict plasma level of any drug is demonstrated for Nicardipine HCl, using this model in Chapter 3.

1.7. Approaches For The Controlled Skin Permeation:

Several technologies have been developed to provide a mechanism of rate control over the release and the skin permeation of drugs. These technologies can be classified into four approaches as outlined in the following:

A. Membrane-moderated TDDS

B. Adhesive-diffusion-controlled TDDS

C. Matrix-dispersion type TDDS

D. Micro-reservoir-type TDDS

1.7.1. Membrane-Moderated TDDS:

In this approach, the drug reservoir is totally encapsulated in a shallow compartment moulded from a drug-impermeable metallic plastic laminate and rate-controlling polymeric membrane. The drug molecules are permitted to release only through the rate controlling membrane which can be microporous or non-porous polymeric membrane with a defined drug permeability property. While inside the drug reservoir, the drug solid may be dispersed in a solid polymeric matrix or suspended in an unleachable, viscous liquid medium to form a paste like suspension.

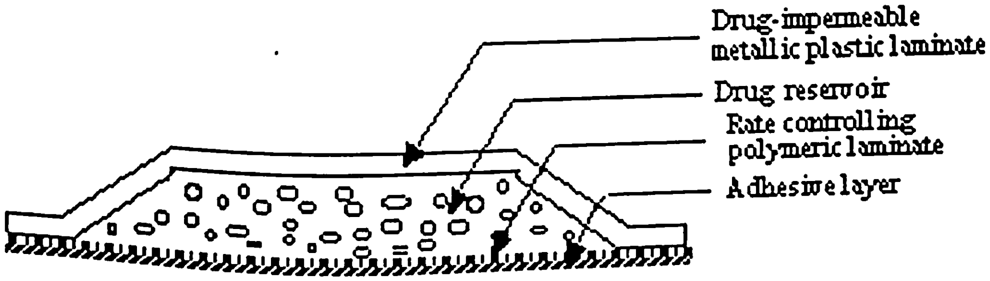


Fig. 1.22: Schematic diagram of rate controlling membrane transdermal device.

Marketed preparations of this technology are Transderm-Nitro® system (Ciba Pharmaceutical Co.) for once a day medication of angina pectoris and Transderm Scop® system for 3-day protection of motion sickness [99,100].

The intrinsic rate of drug release from this type of drug delivery system is defined by following equation :

$$\frac{dQ}{dt} = \frac{K_{m/r}K_{a/m} \bullet D_a C_R}{K_{m/r}D_m h_a + K_{a/m}D_a h_m}$$

Where,

$K_{m/r}$ & $K_{a/m}$ - are the partition coefficient for the interfacial partition of the drug from the reservoir to the membrane and from the membrane to the adhesive, respectively.

D_m & D_a - Diffusion coefficient in the rate-controlling membrane and adhesive layer respectively.

C_R - Drug concentration in the reservoir.

h_m & h_a - are the thickness of the rate-controlling membrane and adhesive layer respectively [101].

1.7.2. Adhesive-Diffusion Controlled TDDS:

In this system, drug reservoir is formulated by dispersing the drug in adhesive polymer and spreading it with the help of solvent on the drug impermeable metallic plastic backing to form a thin drug reservoir layer and on the top of this non-medicated, rate-controlling layer of adhesive polymer is applied to control the diffusion of the drug through the system (Fig. 1.23). The rate of drug release is defined by:

$$\frac{dQ}{dt} = \frac{K_{a/r} \bullet D_a \bullet C_R}{h_a}$$

Where,

$K_{a/r}$ -partition coefficient for the interfacial partitioning of the drug from the reservoir layer to the adhesive layer.

D_a - diffusion coefficient of drug through adhesive layer,

C_R - drug concentration in reservoir,

h_a - thickness of the adhesive layer.

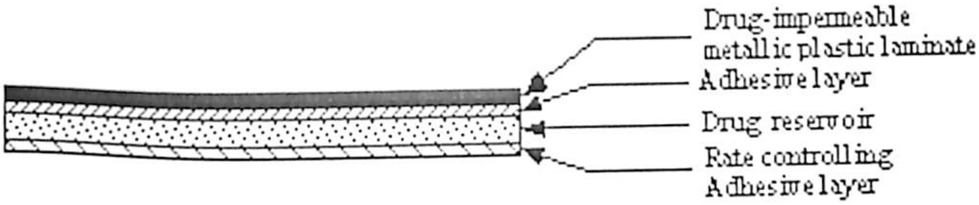


Fig. 1.23: Schematic representation of the adhesive diffusion controlled drug delivery system.

Example of this type of system is Deponit® system (Pharma-Schwartz GmbH, Germany) for once-a-day medication of angina pectoris.

1.7.3. Matrix Dispersion Type TDDS:

In this system, drug reservoir is formulated by homogeneously dispersing the drug solid in a hydrophilic or lipophilic polymer matrix and the medicated polymer is then moulded into a medicated disc with the defined surface area and controlled thickness. This drug reservoir containing polymer disc is then glued onto a occlusive baseplate in a compartment fabricated from a drug impermeable plastic backing. The adhesive polymer is applied along the circumference to form a strip of adhesive rim around the medicated disc (Fig. 1.24). The rate of drug release from this type of the system is given as bellow,

$$\frac{dQ}{dt} = \sqrt{\left(\frac{AC_pD_p}{2t}\right)}$$

Where,

A - initial drug loading dose dispersed in the polymeric matrix,

C_p & D_p - are solubility and diffusivity of the drug in the polymer respectively.

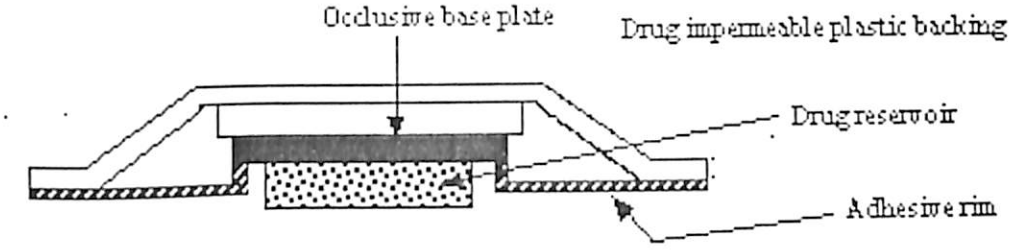


Fig. 1.24: Schematic diagram of the matrix diffusion transdermal drug delivery system.

Example of this type of system is Nitro Dur® system (Key Pharma) for once-a-day for angina pectoris.

1.7.4. Micro-Reservoir Type TDDS:

In this system the drug reservoir is formed by suspending the drug solid in an aqueous solution of water-soluble liquid polymer. The drug suspension is the homogeneous dispersion of drug in a polymer, by high-shear mechanical force, to form the thousands of unleachable microspheres of drug reservoirs. This thermodynamically unstable system is stabilised by immediately cross-linking the polymer chains in situ, which produces the polymeric disc with a constant surface area and thickness. A transdermal therapeutic system thus formed with the medicated disc positioned at the centre and surrounded by an adhesive rim (fig. 1.25).

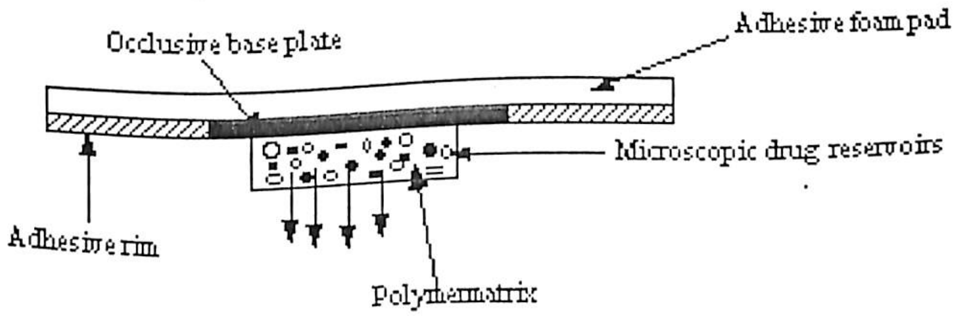


Fig. 1.25: Schematic diagram of the micro reservoir type drug delivery system.

This technology has been utilised in the developing and marketing of the Nitrodisc® system (Searle Pharmaceuticals) for once-a-day treatment of angina pectoris. The rate of the drug release is predicted by following equation,

$$\frac{dQ}{dt} = \frac{D_p D_s \alpha' K_p}{D_p h_d + D_s h_p \alpha' K_p}\left[\beta S_p - \frac{D_l S_l(1-\beta)}{h_l}\left(\frac{1}{K_l} + \frac{1}{K_m}\right)\right]$$

Where,

$\alpha' = \delta''/\beta'$.

Where, δ'' is the ratio of the drug concentration in the bulk of elution solution over drug solubility in the elution solution and β' is the ratio of the drug concentration at the outer edge of the polymer coating membrane over drug solubility in the polymer coating membrane.

D_l, D_p and D_s are the diffusivity of the drug in the liquid compartment, polymer coating membrane, and elution solution (or skin), respectively.

K_l , K_m & K_p are the partition coefficients for interfacial partitioning of drug from the liquid compartment to the polymeric matrix, from the polymeric matrix to the polymeric coating membrane, and from the polymeric coating membrane to the elution solution (or skin), respectively.

S_l and S_p are the solubility of the drug in the liquid compartment and polymeric matrix respectively.

h_l, h_p & h_d are the thickness of liquid layer surrounding the drug particles, the polymeric coating membrane around the polymeric matrix, and the hydrodynamic diffusion layer surrounding the polymeric coating membrane respectively .

β is the ratio of the drug concentration in the inner edge of the interfacial barrier over the drug solubility in the polymeric matrix [102-107].

1.8. In Vitro Evaluation Of Transdermal Drug Delivery Systems:

1.8.1. In Vitro Skin Permeation Kinetics:

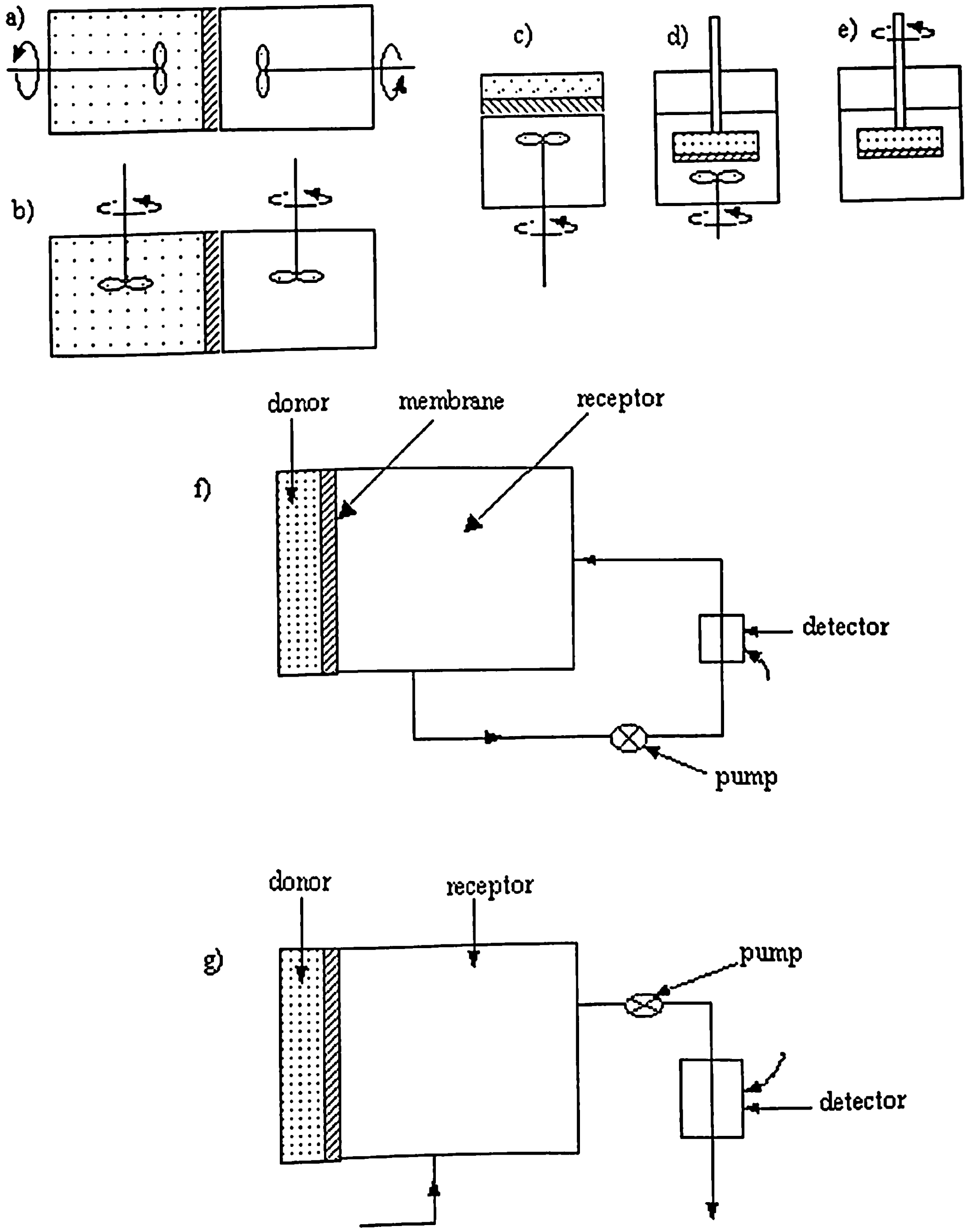
One of the approaches commonly used in the design and development of a TTS is the study, of the permeation kinetics of the drug across excised skin.

The rates of drug permeation across the skin have been measured using different kinds of in vitro skin permeation apparatus.

Ideally an in vitro system for transdermal drug delivery studies should be designed in such a way that the intrinsic rate of release or permeation, which is theoretically independent of the in vitro design, can be accurately determined. Only an in vitro skin permeation system with precisely defined hydrodynamic and thermal characteristics can provide a reliable and reproducible areas for the determination of drug release and skin permeation rates. Once the hydrodynamics of the in vitro apparatus has been well calibrated, the apparent rate of release or permeation so obtained in an experiment can be corrected for the effect of hydrodynamic diffusion layer, and the intrinsic rate of release or permeation can then be determined.

Various types of in vitro apparatus for measuring drug permeation profiles across the skin have been reported in the literature [108-109]. Several designs are illustrated in Fig. 1.26.

Fig. 1.26: In vitro membrane permeation systems. (a, b) horizontal type; (c, d, e) vertical type; (f, g) flow through type.



Generally speaking, the mechanism of agitation in the in vitro diffusion cell should be designed to maintain hydrodynamic condition such that the thickness of the diffusion boundary layer is at a minimum while the primary convective flow toward the drug releasing surface also is minimised. It is therefore more desirable to have the in vitro diffusion cell designed in such a way that the agitation element will rotate at a plane parallel to the drug releasing surface (Fig 1.26 a, c, d). If this is not the case, the intensity must be controlled within an appropriate range.

On the other hand, the effect of the hydrodynamic diffusion layer on the rate of skin permeation may not be subtracted, since the principle resistance to drug transport in the transdermal delivery system is often the drug permeation process through the stratum corneum. Therefore, the agitation in this skin limited the permeation study is important only for maintaining uniform temperature and drug distribution through out the skin permeation cell.

Several designs of the in vitro membrane permeation apparatus whose hydrodynamic characteristics have been fully investigated as shown in the Fig. 1.27 (a-e) and will be discussed in the sections as follows:

1. Horizontal-Type Skin Permeation System, Small Cell Volume:

The skin permeation system (Fig 1.27a) developed by Valia-Chein [110] has been extensively used for studying the skin permeation kinetics of drugs, using either human cadaver skin or freshly excised animal skin.

This cell design has a solution compartment of relatively small volume (3.5 ml) in each half-cell, and a membrane area of 0.64 cm^2. Both donor and receptor compartments are agitated, under a totally enclosed system, by a matched set of star-head magnets (diameter 8 mm.) which are rotated at a synchronised speed of 600 rpm. The temperature of the system can be controlled at isothermal or nonisothermal conditions by circulating thermostatic water through the water jacket surrounding the solution compartment.

Fig. 1.27: In vitro membrane permeation systems with fully-investigated characteristics.

(a) Valia-Chien (V-C) skin permeation cell.

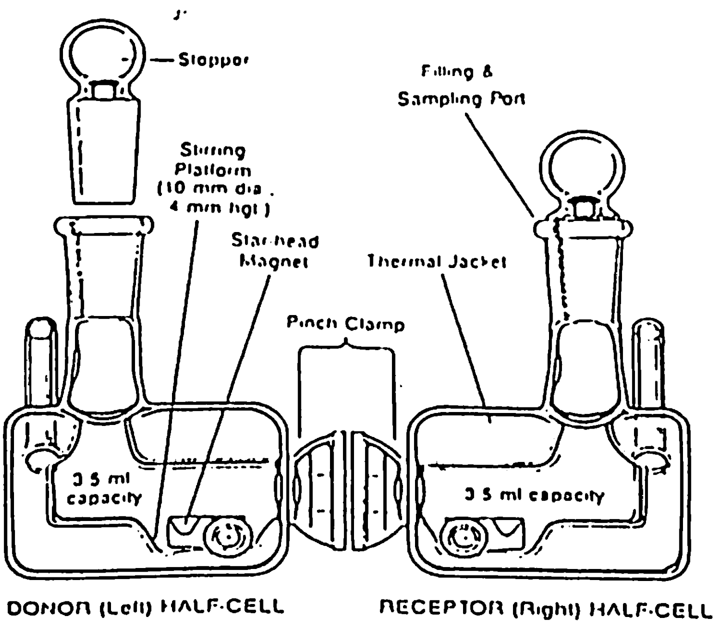


Fig. 1.27b: Ghananam-Chien (G-C) membrane permeation cell.

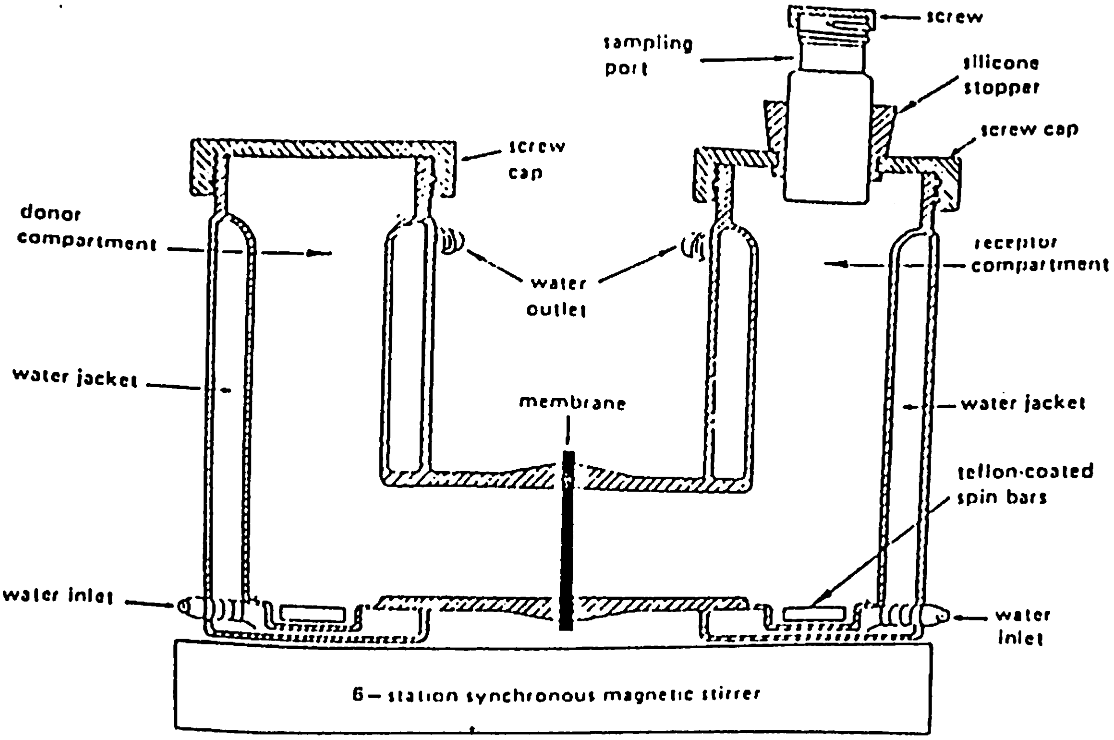
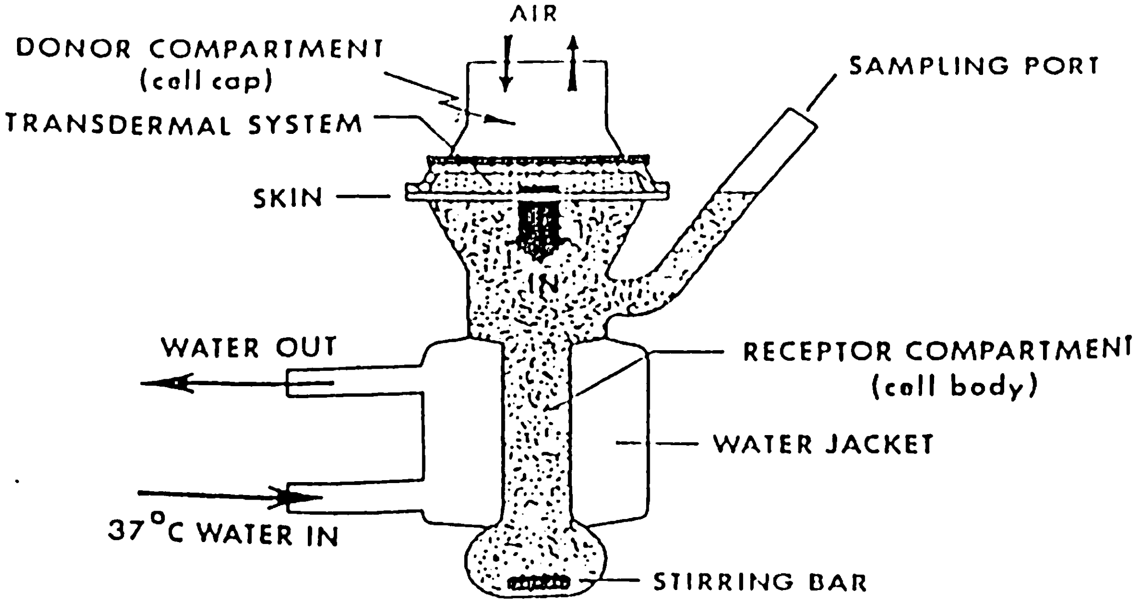


Fig. 1.27c: Franz diffusion cell.



(d) Keshary-Chien (K-C) skin permeation cell.

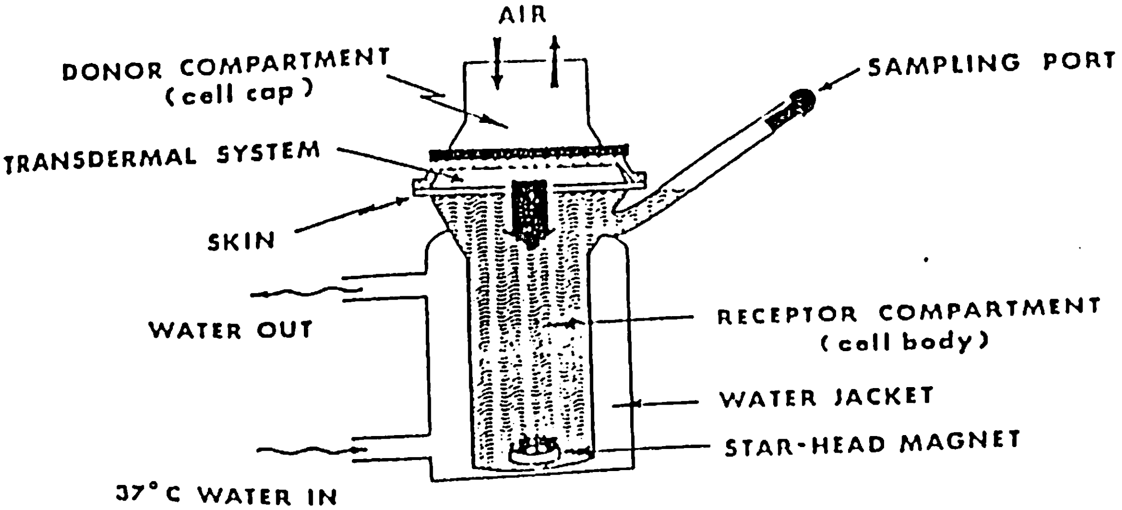
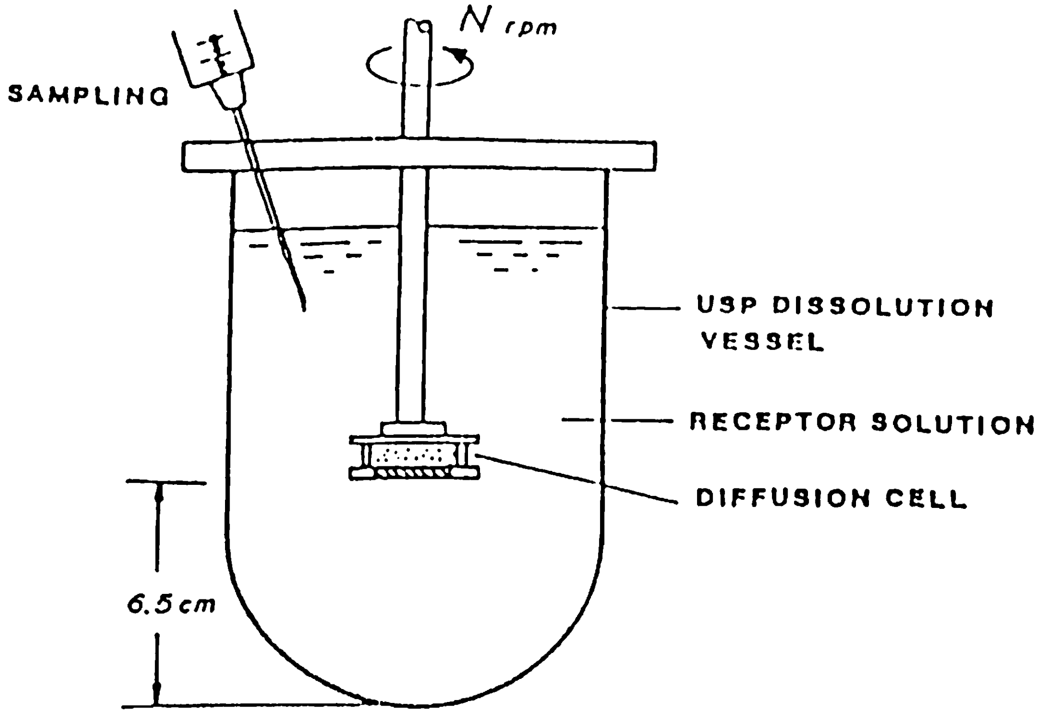


Fig. 1.27e: Jhawer-Lord (J-L) rotating-disc system.



2. Horizontal-Type Membrane Permeation System, Large Solution Volume:

This cell is similar to the above mentioned V-C cell but has a compartment volume of 140-250 ml. The magnets for agitation are 2.54 cm. long. Each pair of half-cells has a large effective membrane area for permeation (13.3 cm^2). The rotation speed of the magnets can be controlled at a constant level of 60-1000 rpm. In this case the donor and the receptor compartments are also thermostatic . This diffusion cell (G-C cell) was developed by Ghananam and Chien (Fig 1.27b).

3. Franz Diffusion Cell:

The vertical-type skin permeation system (Fig 1.27c) developed by Franz and commercialised by Crown Glass has been frequently used for studying the kinetics of percutaneous absorption [111, 112].

The cell has a receptor compartment with an effective volume of approximately 12 ml. and an effective surface area for permeation varying from 1.57 to 4.71 cm^2. The solution in the receptor compartment is stirred by a rod-shaped magnet driven by a 3-W synchronous motor. The stirring magnet rotates at 600 rpm in low viscosity solution such as saline. The receptor compartment is jacketed, thus the solution can be thermostated. However, the temperature near the upper opening, at which the skin will be positioned, varies as the surrounding temperature varies.

4. Modified Franz Diffusion Cell:

Another vertical-type skin permeation cell (Fig 1.27d) was developed in response to the observation that the Franz cell (Fig 1.27c) has rather poor solution hydrodynamics as a result of insufficient mixing. The results are a significant temperature gradient in the diffusion cell and non-homogeneous drug concentration in the receptor solution. Recognising these deficiencies, Keshary and Chien [113] set forth to modify the Franz diffusion cell to improve its efficiency of fluid mixing.

The modified cell (K-C cell) has an effective receptor solution volume of 12 ml. and a skin surface area of 3.14 cm^2. The receptor solution is stirred by a star-head magnet rotating at a constant speed of 600 rpm.

5. Rotating-Disc-Type Membrane Permeation Cell:

One of the advantages of the rotating disc cell is that the hydrodynamic diffusion boundary layer on the surface of the rotating disc has been well established theoretically [113].

If the rotating disc is assumed to be sufficiently large, that the edge effects are negligible, the thickness of diffusion boundary layer is given by :

$$\delta = 1.61 \cdot \left(D_f \rho / \mu\right)^{1/3} \cdot \left(\mu / \rho_w\right)^{1/2}$$

where, D_f - Drug diffusivity in the fluid

ρ - density of the fluid

μ - viscosity of the fluid

w - angular velocity. (= π Nd).

In the rotating-disc diffusion cell, if the disc is not large enough, the flow pattern in the cell is easily influenced by the wall of the vessel. Then the diffusion layer determined experimentally is usually thicker than that calculated from above equation due to dissipation of additional energy in the vessel wall.

Jhawar [114] developed a rotating-disc-type membrane permeation cell (J-L cell) for studying the release of drug from suppositories (Fig 1.26e). The rotating disc has an affective membrane area of 12.6 cm^2 and can be used together with the one-litre USP dissolution vessel as the receptor compartment.

1.8.2. Skin Models Used In The In Vitro Permeation Studies:

Though freshly excised human skin or human cadaver skin are preferred membranes for in vitro permeation studies, yet it is not easily available to most investigators.

Among the animal skins commonly used in the in vitro studies are in particular the hairless mouse skin, with hairless and fuzzy rat, guinea pig, rabbit and miniature swine skins.

Although there exists a number of similarities, there is as yet no animal skin that completely mimics the penetration characteristics of human skin.

Another alternative to skin are artificial membranes, designed to mimic the barrier properties of the skin, which have been used with some success. Nacht and Yeung [115] used a multilaminate membrane consisting of alternate hydrophobic and hydrophilic laminates. They concluded that the choice of materials and number of laminates directly affected penetration kinetics and were unable to mimic accurately the penetration of test compounds such as salicylic acid, hydrocortisone, or water through human skin in vitro. In contrast Kazmi et al [116] used ointment jars covered with a cellulose membrane, 1000 mol. wt. pore size immersed in phosphate buffer to measure release of indomethacin from various vehicles . This was then correlated with in vitro rabbit TDD studies from the same vehicles. Not surprisingly, a good correlation between the in vitro release characteristics and TDD in vivo was observed.

The experiments indicates that the skin is far more complex than a simple hydrophobic/ hydrophilic membrane of fixed pore size. Its rate limiting characteristics are some what poorly understood, as evidenced by many attempts to draw simple correlations between penetration kinetics and factors such as mol. wt., partition coefficients and molecular size.

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Fig. 1.28: Paddle over disc (All measurements are expressed in milimetres unless noted otherwise.)

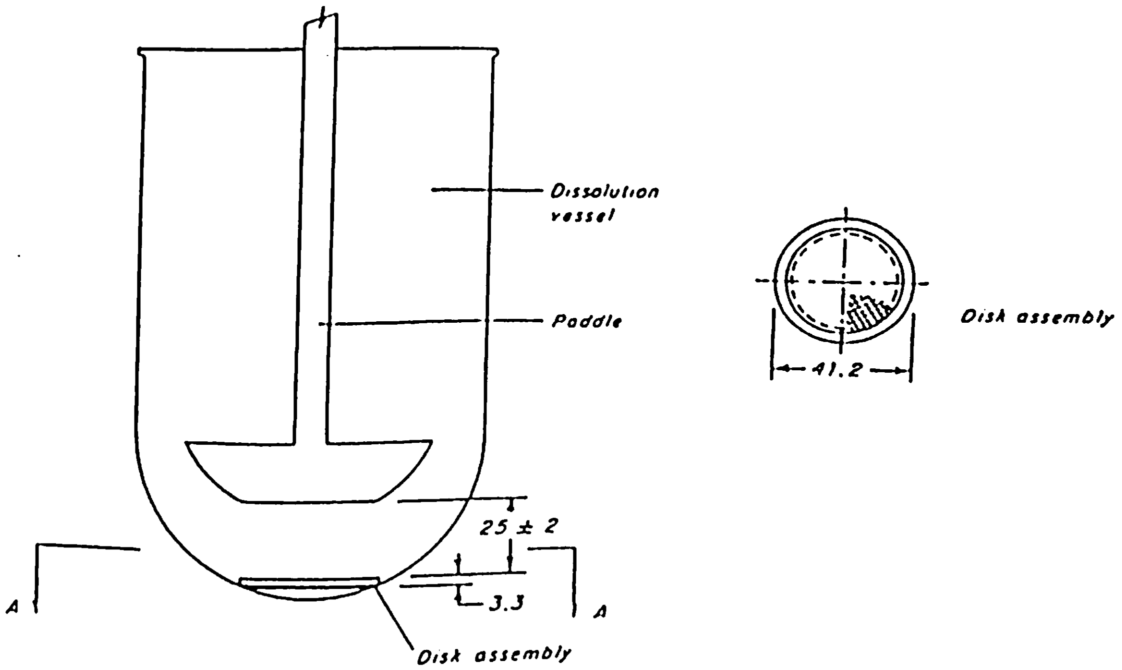
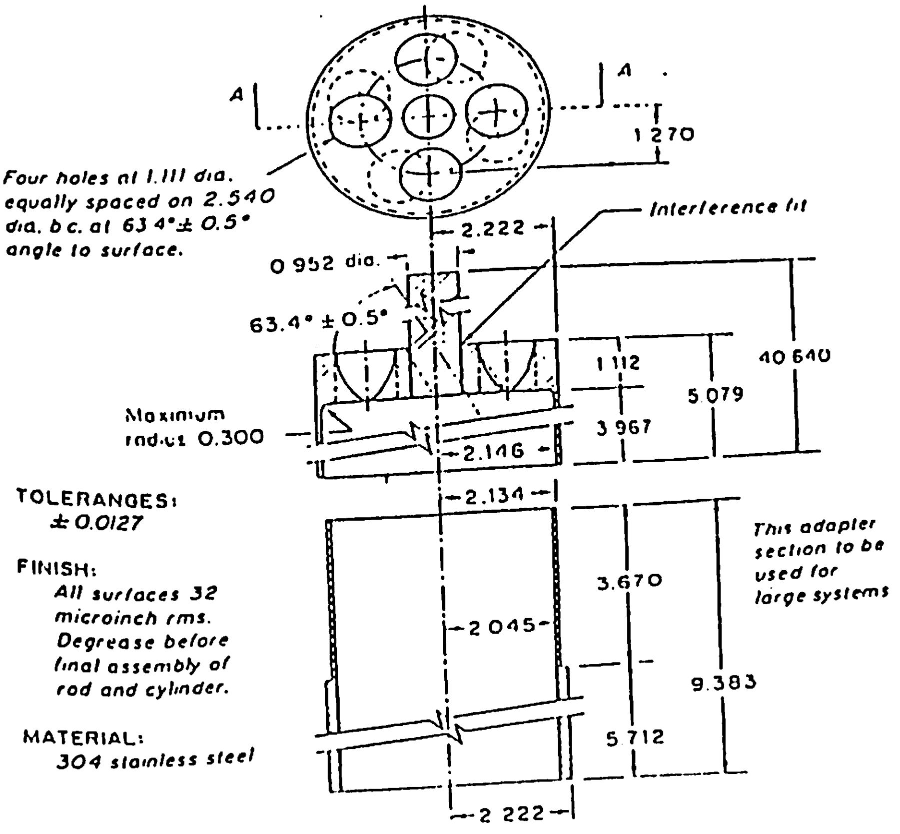


Fig. 1.29: Cylinder stirring (All measurements are expressed in centimetres unless noted otherwise).



1.8.3. In Vitro Release Kinetics Of Transdermal Therapeutic Systems [117]:

1. Paddle Over Disk Method: (Apparatus 5; USP XXIII)

Similar to the paddle and vessel assembly from Apparatus II, with the addition of a stainless steel disk assembly designed for holding the transdermal system at the bottom of the vessel. The temperature is maintained at 32±0.5 °C. A distance of 25±2 mm. between the paddle blade and the surface of the disk assembly is maintained during the test. The vessel may be covered to minimise evaporation (Fig 1.28).

2. Cylinder Method: (Apparatus 6; USP XXIII)

The use of the vessel assembly from USP Apparatus II, except to replace the basket and shaft with a stainless steel cylinder stirring element and to maintain the temperature at 32± 0.5 °C during the test. The shaft and cylinder components of the stirring element are fabricated of stainless steel to the specifications shown in Fig 1.29.

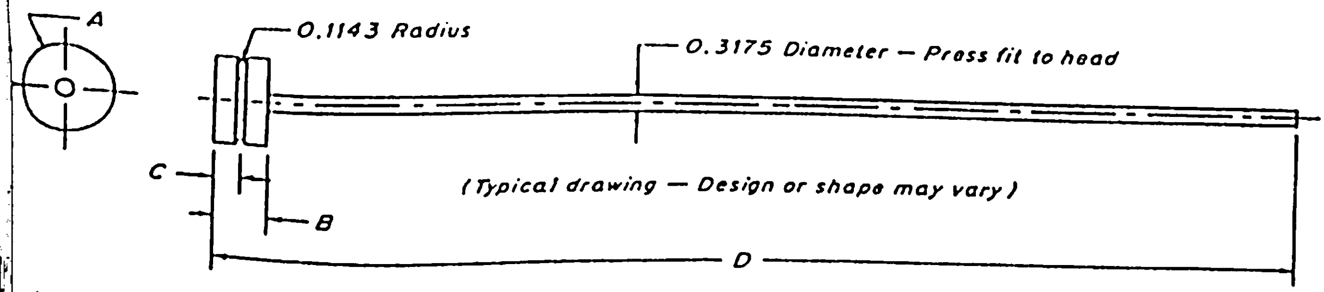
The dosage unit is placed on the cylinder at the beginning of each test. The distance between the inside bottom of the vessel and the cylinder is maintained at 25±2 mm during the test.

3. Reciprocating Disc: (Apparatus 7; USP XXIII)

[Note- This apparatus may also be specified for use with solid oral dosage forms.]

The assembly (Fig 1.30) consists of a set of volumetrically calibrated or tarred solution containers made up of glass or other suitable inert materials, a motor and a drive assembly to reciprocate the system vertically and to index the system horizontally to a different row of vessels automatically if desired, and a set of disk shaped sample holders.

Fig. 1.30: Reciprocating disc sample holder .

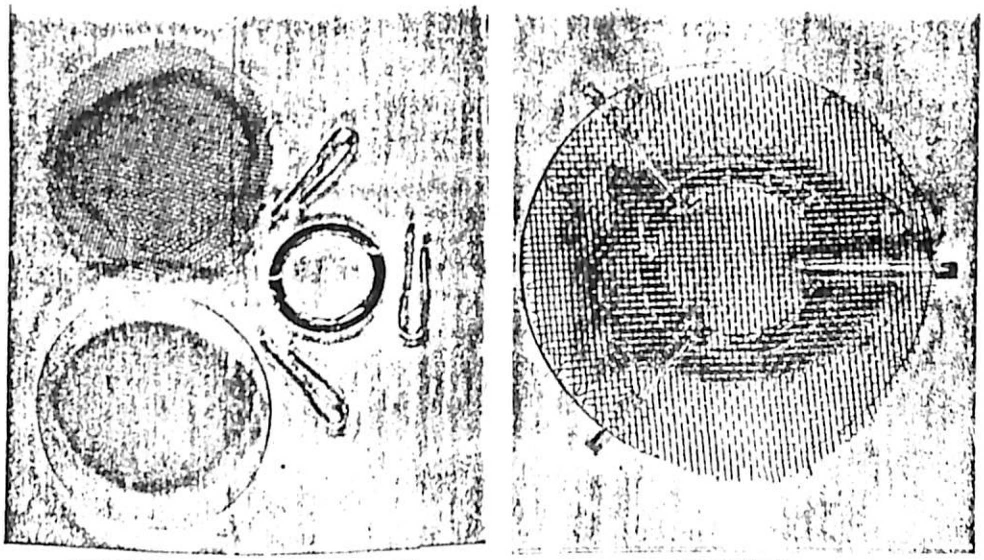


nsions are in centimeters.

System[a]	HEAD				ROD		O-RING
	A (Diameter)	B	C	Material[b]	D	Material[c]	(not shown)
1.6 cm^2	1.428	0.9525	0.4750	SS/VT	30.48	SS/P	Parker 2-113-V884-75
2.5 cm^2	1.778	0.9525	0.4750	SS/VT	30.48	SS/P	Parker 2-016-V884-75
5 cm^2	2.6924	0.7620	0.3810	SS/VT	8.890	SS/P	Parker 2-022-V884-75
7 cm^2	3.1750	0.7620	0.3810	SS/VT	30.48	SS/P	Parker 2-124-V884-75
10 cm^2	5.0292	0.6350	0.3505	SS/VT	31.01	SS/P	Parker 2-225-V884-75

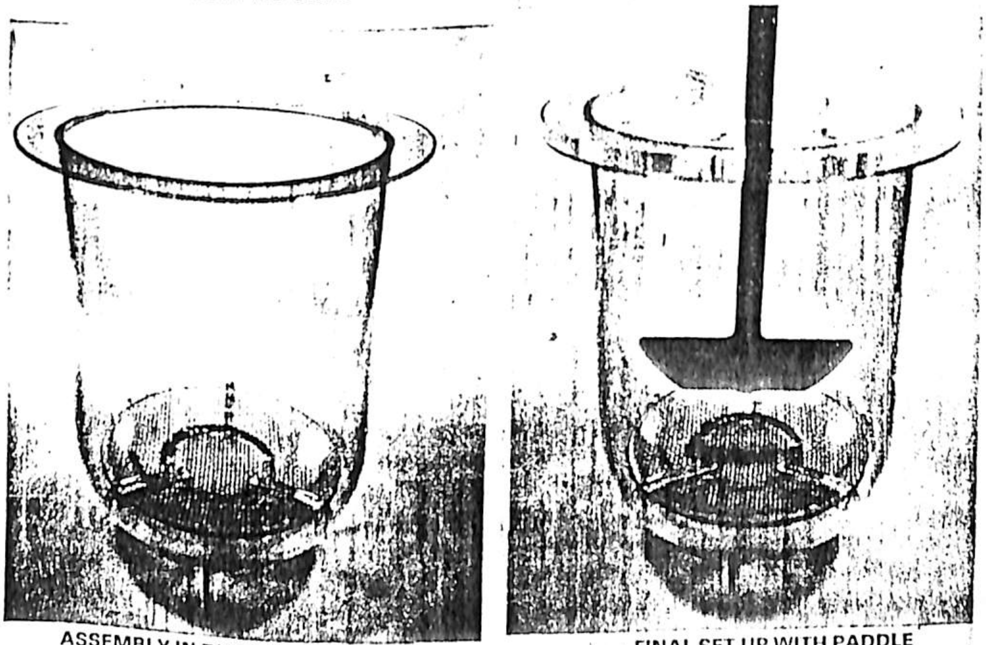
[a] Typical system sizes.
[b] SS/VT = Either stainless steel or virgin Teflon.
[c] SS/P = Either stainless steel or Plexiglas.

Fig. 1.31: Experimental set up for transdermal patch dissolution studies.



WATCHGLASS, PATCH, WIREMESH SCREEN AND CLIPS

ASSEMBLY



ASSEMBLY IN DISSOLUTION FLASK

FINAL SET UP WITH PADDLE APPARATUS

The solution containers are partially immersed in a suitable water bath of any convenient size that permits maintaining the temperature inside the container at 32 ± 0.5 °C during the test. The system is attached to a suitable size sample holder with a suitable O-ring such that the back of the system is adjacent to and centred on the bottom of the sample holder.

4. **FDA Procedure:**

Similar to USP Apparatus II, except that the method was modified by sandwiching the transdermal system between a watch glass and an aluminium wire screen (Fig 1.31), held in place by clips. The assembly is placed at the bottom of the dissolution flask.

Then the drug release profile from the device was determined using the same specifications as paddle over disk method.

1.9. In Vivo Evaluation Of Transdermal Drug Delivery:

In the development of a transdermal drug delivery system (TDDS) two major questions are to be answered in respect to in vivo studies :

1. Does the drug proposed for transdermal delivery penetrate the skin in vivo, and if so, to what extent?
2. How is a useful indicator of in vivo skin penetration obtained and, hence, how feasible is the transdermal route of drug administration ?

Thus for the estimation of a potential TDDS in vivo, there are 3 available options :

- Measure the skin penetration and subsequent bio-disposition in an intact animal model.
- Evaluate percutaneous absorption directly in human volunteers, and the third, simple but perhaps the less readily acceptable alternative.
- Formulate a biophysical model for TDDS and after validation, use the simulation to predict the feasibility of drug administration via the skin.

Animal Models:

Although the most relevant data pertaining to TDDS are obtained in humans, this desirable approach is not always possible, since considerable time and resources are required to conduct a safe and meaningful percutaneous absorption study in man. Consequently one must be prepared to use an in vivo animal model.

The available information on in vivo animal models points to some very simple conclusions:

1. Small "hairy" animals (e.g. rat, rabbit) invariably yield penetration values much greater than those seen in man. Their usefulness as predictive models for human in vivo TDDS is questionable.
2. The use of the rhesus monkey, if available, appears to be the most reliable and well validated of the existing models for human skin penetration.
3. The weanling pig (although perhaps not the most convenient and readily available species) and the human skin grafted nude mouse, offer new, alternative models of much promise and potential.

But eventually, in the development of a TDDS system, the drug must be applied to human subjects and pharmacokinetic and pharmacodynamic data must be collected. Specific and sensitive assays for the parent drug and active metabolite needed to be developed, and a suitable pharmacological response, which can be quantified , will have been identified.

SCOPE OF WORK :

PART I. NITROGLYCERIN PATCHES :

Since Nitroglycerin is available in the form of patches in almost all Western and European countries and one of the largest commercially explored molecule for the transdermal dosage form in virtually all the type of theoretical models . It was decided to undertake the development of patches in atleast two forms namely, Adhesive-matrix dispersion system and Membrane-controlled drug delivery system.

To manufacture these kinds of products, the following steps are necessary for achieving this objective,

1. Synthesis of acrylate adhesives and analysis there of.
2. Development of laboratory scale and scaling up machines involved and preparing batches suiting our need.
3. Selection of packaging materials.
4. Establishing bioequivalence of the product manufactured in atleast one animal model with the marketed preparations of similar kind.

PART II. DICLOFENAC PLASTERS:

Diclofenac is one of the most widely used drugs for inflammation and pain controlled in various conditions. About 7 % of the population taking this drug experiences severe gastro-intestinal disorders, sometime serious enough to warrant discontinuation. Though Diclofenac in the form of diethyl ammonium salt is available from a leading multinational company in the form of a gel, the literature survey revealed availability of the another salt Diclofenac Hydroxy Ethyl Pyrrolidone (DHEP) in the form of plasters for treatment of rheumatoid arthritis, osteoarthritis and ankylosing spondylitis and other minor sport injuries. The objective of our work was to study physicochemical properties of Diclofenfac Diethyl Ammonium (DDEA) salt versus DHEP and after having established the similarity as well as

superiority, the work was undertaken to develop Diclofenac plasters containing DDEA, involving the following steps ,

1. Development of suitable adhesive system with or without the inclusion of aqueous acrylate adhesives.
2. Development of aqueous adhesives, compatible with other ingredients.,
3. Establish at least similar release rates in vitro and in vivo compared to DHEP plaster.
4. Develop the machines to produce the plaster on commercial scale.

PART III. NICARDIPINE HCl PATCHES :

Nicardipine HCl is Ca^{++} channel antagonist widely used for stable angina and hypertension and undergoes extensive first pass metabolism, has a fair enough profile for considering a transdermal formulation with the reasonable dosage. The objective of this study was to,

1. Predict the plasma levels utilising the Guy and Hadgraft's proposed mathematical model and also the conventional mathematical models.
2. Develop the transdermal systems with the adhesives already developed for nitroglycerin and diclofenac diethyl ammonium salt and establish at least the in vitro release rates for the same.

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Chapter II.

LITERATURE SURVEY

CHAPTER II. PROFILE OF LITERATURE SURVEY

2.1. NITROGLYCERIN (NTG):

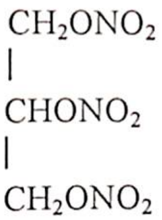
2.1.1. Name, Formula and Molecular weight:[1]

- Synonyms: Glyceryl trinitrate; Trinitroglycerol; 1,2,3-propanetriol trinitrate.

- Molecular Formula : $C_3H_5N_3O_9$

- Molecular weight : 227.09

- Chemical Structure:



$$\begin{array}{l} CH_2ONO_2 \\ | \\ CHONO_2 \\ | \\ CH_2ONO_2 \end{array}$$

2.1.2. Appearance, Colour, Odour:

Pale yellow , colourless, oily liquid with a sweet burning taste.

2.1.3. Physical properties:

2.1.3.1. UV Spectrum:

Single maxima was found at 207 nm using absolute alcohol as a solvent and UV spectrum is shown on Page 211 in Chapter 5.

2.1.3.2. IR Spectrum:

The principal peaks were found at wave numbers 850,1280 and 1650 cm^{-1}. The IR spectrum of NTG as shown on the Page 211 in Chapter 5.

2.1.3.3. NMR Spectrum:

NMR spectrum of the sample, isolated from the lactose by solvent extraction, in $CDCl_3$ showed the multiplet at 4.8 ppm is assigned for four protons at the 1 and 3 carbon atoms and that at 5.5 ppm is assigned for at C2 position. The NMR spectrum is shown in Chapter 5 on Page 212.

2.1.3.4. Melting Point And Crystal Properties: [2]

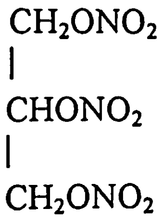
At low temperature, NTG exists in two crystal forms. It freezes to form a stable dipyramidal polymorph which melts at 13.2 °C. Under some

CHAPTER II. PROFILE OF LITERATURE SURVEY

2.1. NITROGLYCERIN (NTG):

2.1.1. Name, Formula and Molecular weight:[1]

- Synonyms: Glyceryl trinitrate; Trinitroglycerol; 1,2,3-propanetriol trinitrate.
- Molecular Formula : $C_3H_5N_3O_9$
- Molecular weight : 227.09
- Chemical Structure:



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The principal peaks were found at wave numbers 850,1280 and 1650 cm^{-1}. The IR spectrum of NTG as shown on the Page 211 in Chapter 5.

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NMR spectrum of the sample, isolated from the lactose by solvent extraction, in $CDCl_3$ showed the multiplet at 4.8 ppm is assigned for four protons at the 1 and 3 carbon atoms and that at 5.5 ppm is assigned for at C2 position. The NMR spectrum is shown in Chapter 5 on Page 212.

2.1.3.4. Melting Point And Crystal Properties: [2]

At low temperature, NTG exists in two crystal forms. It freezes to form a stable dipyramidal polymorph which melts at 13.2 °C. Under some conditions, an unstable triclinic crystal (m.p. 2.2 °C) may form. This labile polymorph will convert in to the more stable form on standing.

2.1.3.5. Solubility:

NTG has an aqueous solubility of 1.73 and 2.46 mg/ml at 20 °C and 60 °C respectively [1,3].

2.1.4. Methods Of Analysis :

Various methods have been cited in the literature for the determination of NTG in raw material, pharmaceutical dosage forms and plasma, most of which used ECD detector. HPLC procedures are also defined using C-18 and phenyl bonded gel columns. GC and HPLC parameters described by some of the references are mentioned below.

2.1.4.1. GC Parameters For NTG:

Table 2.1 : GC Methods Of Analysis For NTG.

Reference	Column	Detector	Temperature (°C) 1. Injector port 2. Column 3. Detector	Sample Analyzed	Sensitivity (ng/ml)
4.	3.5% QF-1 on 60-80 Gas Chrom Q	ECD	1.160 2.120 3.180	5 ml human plasma	0.5
5.	3% SP-2401 on 100-120 Supelcoport	ECD	1.160 2.140 3.180	0.2 ml rat/ human plasma	0.1
6.	3% SE-30 on 50-60 Ankrom AB15	TCD	1.130 2.------ 3.192	Tablet extract	—
7.	0.4% OV-17 on 60-80 glass beads	ECD	1.150 2.120 3.150	2 ml human blood or urine	0.1-2
8.	3% SE-30 on 100 - 120 Gas Chrom Q	ECD	1.200 2.150 3.175	3 ml human blood	?
9.	10% OV-101 on 100-120 Chromosorb W-HP	ECD	1.150 2.130 3.210	5 ml human plasma	?
10.	30% SE-30 80-100 on Chromosorb W-HP	ECD	1.150 2.130 3.210	5 ml human plasma	≈ 0.5
11.	3.8 % OV-101 on 80-100 Gas chrom Q ; 2.5% OV-210	FID	1.70 2.70-220 @ 6 °C/min 3.225	Nitrocellulose propellants	--
12.	3% XE-60, 3.5% QF-1 on 60-80 Gas Chrom Q	FID ECD	1.160 2.150 3.200 1.160 2.120 3.200	solvent mixture	--

2.1.4.2. HPLC Conditions For Assay Of NTG:

Table 2.2: HPLC Method Of Analysis For NTG.

Sr. No.	Parameter	Ref. 13	Ref. 14
1.	Column	C18 microparticulate	Alkyl phenyl bonded to silica gel.
2.	Mobile phase	60% MeOH	Acetonitrile-Tetrahydrofuron- Water (26:64:10).
3.	Flow rate (ml/min)	2.0	2.0
4.	Detection	U.V. 200 nm	U.V. 218 nm
5.	Detection limit	30 ng on column	50 ng on column
6.	Retention time	4 min	10 min.

2.1.4.3. HPTLC Methods:

In addition, Crew and DiCarlo [15] have reported TLC conditions for NTG and its metabolites as follows:

Table 2.3: Thin layer chromatography of NTG.

Sr. No.	Parameter	Specification	
1.	TLC plates	250 silica gal G bound with calcium sulphate	
2.	Solvent	Benzene : Ethyl acetate : Acetic acid (16:4:1)	
3.	Rf Values	Nitro glycerine	0.60
		Glyceryl- 1,3-dinitrate	0.45
		Glyceryl-1,2-dinitrate	0.30
		Glyceryl-1-mononitrate	0.10
		Glyceryl-1-mononitrate	0.10
		Glycerol	0.00

2.1.5. Stability:

2.1.5.1. Chemical Stability:

The stability of NTG in alcoholic solution as a function of pH has been studied by Amshler [18]. This compound is relatively stable in neutral and weakly acidic solutions but degrades very rapidly in the presence of alkali. [16,17].

Acid catalysed hydrolysis of NTG was found to occur at a much slower rate than that of alkaline hydrolysis [15,19]. Incubation of NTG at 37 °C for 15 min. in 4N NaOH resulted in essentially complete degradation, while in 4N HCl, NTG was degraded only 28% after 6 hrs.[15]. Under acidic conditions, twice as much Glyceryl-1,2-dinitrate is formed compared to Glyceryl-1,3-dinitrate [20], suggesting that the initial reaction site is on the primary nitrate.

2.1.5.2. Physical Stability :

Instability of NTG in pharmaceutical dosage forms can generally be attributed to two processes, viz.: 1) vaporization leading to loss of drug to the atmosphere, and 2) sorption of drug to plastics [21,22].Drug loss due to sorptive phenomenon has been implied when NTG tablets are stored in plastic containers and unit dose strip packages [23,24] .

Extensive loss of NTG from intravenous solutions stored in plastic I.V. bags can be attributed to sorption [25,26,27], since intact drug can be recovered from the container [28]. Plastic tubing used for the administration of intravenous NTG solution also causes drug loss due to sorption [29,30]. High density polyethylene tubing, however is non-adsorptive [30].

2.1.6. Metabolic Fate :

Upon oral administration of 10 mg/Kg of 1,3-^{14}C NTG to rats [31], 20% of the labeled dose was expired as carbon dioxide with an equal amount of the radioactivity excreted in the urine at the end of 4 hrs. TLC-radio chromatographic analysis revealed that the cumulative urinary excretion consisted of 7% glycerol, 1% Glyceryl-1,2-dinitrate, 0.5% Glyceryl-1,3-dinitrate, 4% Glyceryl mononitrate and 8% of unidentified water soluble metabolites.

In a more recent study, Hodgson and Lee [32] administered a very high dose, 180 mg/Kg (LD 10%), of NTG to rats. Radioactive CO_2 accounted for 26% of the dose and 40% of the label was eliminated in the urine within 24 hrs. These authors showed that the major urinary metabolites are Glyceryl dinitrate glucuronide (14% of the dose), Glyceryl mononitrate (11%) and glycerol (7%). This study was the first which showed that condition plays a major role in the metabolism of NTG.

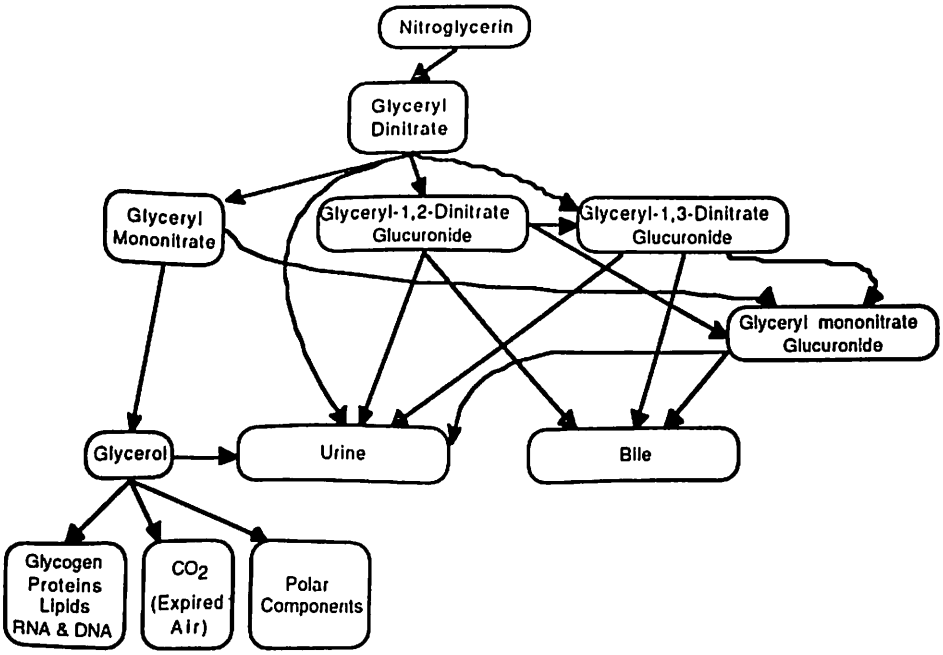


Fig. 2.1: Metabolic fate of NTG.

2.1.7. Pharmacology:

2.1.7.1. Absorption, Distribution, Metabolism and Excretion :

NTG is readily absorbed from sublingual mucous. For sublingual absorption of NTG requires presence of saliva [33], Blumenthal et al [34], have reported plasma level as low as 0.1 ng/ml after administration of 6.5 mg sustained release oral capsule and also after application of NTG ointment 2%

(corresponding to 16 mg NTG). NTG skin absorption has been shown [35,36] to depend on the area of application, cutaneous blood flow at the site of application, the rate of evaporation and the dose.

NTG is rapidly and extensively distributed in the body. Dicarlo et al. [37], have found that liver and superficial tissues are the major sites of distribution where as only a small quantity of the drug is found in heart, lungs, kidney and spleen. Needleman and co-workers [38] found that apparent distribution phase of unchanged NTG had half life of less than 20 Sec.

NTG degrades primarily in liver by a partial denitration process mediated by *glutathione organic nitrate reductase* [39]. The denitration products namely 1,2- and 1,3-Glycerol dinitrates and mononitrates are also reported to be active [40]. Part of the NTG is also believed to be metabolised in blood.

2.1.7.2. Pharmacokinetics:

Some of the important pharmacokinetic parameters of NTG are :

1. Concentration at steady state, Css = 2.3 ng/ml [41]
2. Bioavailability F = 45%[42]
3. Volume of distribution Vd = 3.7 Lit./Kg [42]
4. Half life $t_{1/2}$ = 1.77 hrs.[40]
5. Protein binding = 60 %
6. Clearance rate Cl = 2.2 ml/min./Kg.[42]

2.1.7.3. Side Effects:[43]

Severe and persistent headache, cutaneous flushing, dizziness, and weakness are commonly associated symptoms. Headache should be treated with mild analgesics. In case of untreatable headache alone, the dose should be reduced. Occasionally, drug rash or exfoliate dermatitis may occur. The responses may disappear with a decrease in dosage. Although (uncommon) hypotension and an increase in heart rate may occur. In case, this occurs and persist for longer time, dosage must be reduced. Occasionally, contact dermatitis has been reported with continuous use of topical NTG. Such incidence may be reduced by changing the site of application or by using topical corticosteroid.

2.1.7.4. Indications: [43]

Based on the reviews of NTG and related drugs, publications of National Academy of Sciences, National Research Council and other information, FDA has classified the indications as follows:

"Possibly effective" for the management, prophylaxis or treatment of angina pectoris.

2.1.7.5. Contraindications: [43]

Acute or recent myocardial infarction, severe anaemia, close angle glaucoma, postural hypotension, increased intracranial pressure and idiosyncrasy to NTG.

2.1.7.6. Mechanism Of Action:[43]

The mechanism of action of NTG in the relief of angina pectoris is not yet known. However, it's main pharmacological action is to relax the smooth muscle, principally in the small blood vessels, thus dilating arterioles and capillaries especially in the coronary circulation. In therapeutic doses, NTG is thought to increase the blood supply to myocardium, which may, in turn, relieve myocardial ischaemia, the possible functional basis for the pain of angina pectoris.

2.1.8. Proprietary Preparations:

Some of the marketed preparations of NTG are given below:

Table 2.4: Proprietary preparation of NTG in market.

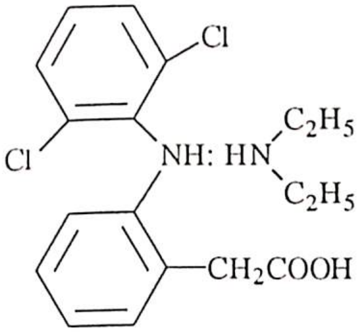
Sr. No.	Product	Manufacturer
1.	Transderm Nitro	Summitt
2.	Nitro disc	Searle
3.	Nitro Dur	Key
4.	Minitran	3M Pharma
5.	Deponit	Schwarz
6.	Nitrodisc	Roberts Pharma

2.2. DICLOFENAC DIETHYL AMMONIUM:

Diclofenac is a potent non-steroidal anti inflammatory agent . It is used in the treatment of rheumatoid arthritis and other rheumatic disorders [44]. Inspite of good bioavailability after oral administration contraindicative manifestations are associated with diclofenac therapy. It is extensively metabolised in the liver and mainly excreted in the urine. It has a narrow therapeutic index. It has a short half life and has to be given frequently [45].

2.2.1. Name, Formula, Molecular weight :

- Diclofenac Diethyl ammonium
- $C_{18}H_{22}Cl_2N_2O_2$
- Molecular weigh: 369.3.
- Chemical structure:



2.2.2. Physical properties :

2.2.2.1. Appearance:

White to buff coloured free flowing powder.

2.2.2.2. Acidity/Alkanality :

6.5-8.5

2.2.2.3. Melting point :

149-153 °C.

2.2.2.4. Solubility :

Freely soluble in methanol, sparingly soluble in acetone, water solubility approximately 1.2%, octanol solubility approximately 15%.

2.2.2.5. UV Spectrum:

Two maxima were observed at 211 nm and 284 nm in pure methanol. UV spectrum is shown on Page 213 in Chapter 5.

2.2.2.6. I. R. Spectrum:

Comparative IR was recorded with standard substance using Shimadzu FTIR 4200. The principal peak observed at wave numbers 806, 1377, 1557, 1577, 1630, 2370, 2957, 3250 cm^{-1}. I.R. spectrum is shown on Page 213 in Chapter 5.

2.2.2.7. NMR Spectrum :

NMR spectrum of Diclofenac DEA was taken in CDCl3 using TMS as an internal standard on Bruker 200 MHz. In the spectrum chemical shifts observed were at 6. and 7.5 ppm for aromatic protons while at 1.2, 2.6, 3.7 and 8.2 ppm for other aliphatic protons. NMR spectrum recorded is shown on Page 214 in Chapter 5.

2.2.2.8. DSC:

DSC was performed using Mettler TA4000 and a single endotherm was observed at 152.8 °C. The thermograph is shown on Page 215 in Chapter 5.

2.2.3. Method Of Analysis :

Various methods have been cited in the literature for the determination of diclofenac in raw materials, pharmaceutical dosage forms like gels, tablets, injections, human and animal plasma. Some of which are enumerated below :

Table 2.5 : HPLC analytical methods for diclofenac.

Sr. No.	Method	Description.	Reference
1.	HPLC	Gradient system, photodiode array detector and column 250 x 4 mm , Urosphere 100, C18, 5μ. Mobile phase:acetonitrile:tetrahydrofuron: phosphate buffer pH 7. (Composition not mentioned)	46
2.	GC-ECD	Detector ECD, Column 30 x 0.3 mm (ID), soft glass capillary column layered with Barium carbonate an and coated with carbowax 40 M. Splitless injection, carrier gas: Helium.	47
3.	HPLC	Automated column switching, Detector UV using two column C18, first 3.5 cm x 4.6 mm, second 150 x 4.6 mm, mobile phase 22 mM sod. acetate pH 7.1, acetonitrile:methanol (52:23:25)	48
4.	GC-MS	Column 2000 x 3 mm packed with 1.5% silicone OVA 17 on Shimalite WAW DMCS 80-100 mesh, pulse rate 10 KHz.	49
5.	HPLC	Column C10 reverse phase, mobile phase: acetonitrile : methanol : Pic B6 (25:25:50)	50
6.	GC-MS	Column capillary 12.5x0.2 mm, film thickness 0.33μm,DB1, Helium, split mode, using single ion monitor mode.	51

2.2.4. Pharmacodynamics:

Diclofenac suppresses acute and chronic inflammation, pain and hyperthermia in various animal models which proved Diclofenac is more potent weight by weight, than aspirin, ibuprofen, neproxen, phenylbutazon while less potent than piroxicam and similar to indomethacin. These animal models included rat paw oedema induced by caraginin, kaolin, and ultraviolet radiation induced erythema in guinea pig. It had also inhibited primary and secondary inflammation in adjuvant arthritis. It was shown to inhibit Ethacrynic acid and induced acetic acid inducing writhing in rats. It also reduces yeast induced fever in rats [52-55].

2.2.5. Mode Of Action:

Most of the pharmacological effects of diclofenac as with other NSAIDs are believed to be mediated by inhibition of prostaglandin synthesis. Diclofenac is a potent inhibitor of *Cyclo-oxygenase*. It markedly reduces synthesis of prostaglandin, prostacyclin and thromboxin production in sheep's seminal vesicles [56]. It also reduces formation of *Lipoxy oxygenase* pathway products such as leukotrines [56,57]. In general, with other NSAIDs it is a potent reversible inhibitor of platelets aggregation in vitro and in vivo. However, in usual therapeutic dosages, it has very little effect on spontaneous platelet aggregation [44]. Diclofenac has also been postulated to probably inhibit number of liposide responses such as lisosomal enzyme release and superoxide production which appear to play a crucial role in pathogenesis of rheumatic diseases and degradation of connective tissues and joints [58,59].

2.2.6. Therapeutic Studies:

Diclofenac is recommended for relief of pain and inflammation in following conditions :

Rheumatoid arthritis, osteoarthritis, ankylosing spondylitis, acute gout, renal colic pain, surgical conditions like tooth extraction, tonsillectomy, lower back pain and musculoskeletal disorder such as periarthritis (frozen shoulder, tendinitis, tenosinovitis, brusitis, sprain and strain of body muscles). It has been an effective analgesic in various pain and conditions such as those in dysmenorrhoea, postpartum pain, post surgery pain, biliary and renal colic pain. It is also an effective antipyretic [60-64].

2.2.7. Pharmacokinetics:

Diclofenac is rapidly and effectively absorbed after oral dosing and undergoes first pass metabolism with about 60% drug reaching systemic circulation achieving C_{max} within 1.5-2.5 hours. It is extensively bound to serum proteins (albumin mainly). The drug gets widely distributed into tissues like liver, bile, kidney, heart and lungs. The principal metabolite is 4-hydroxy diclofenac (about 40-50%). The amount excreted in urine accounts for 20-30% of the dose and the three other metabolites each amounting to 10-20% of the dose excreted in the urine and small amounts excreted in bile have also been identified. Glucuronide and sulphate conjugates account for 5-10% of the dose recovered in urine [65]. The terminal elimination half life of the drug from plasma is about 1.2-1.8 hours.

2.2.8. Adverse Effects:

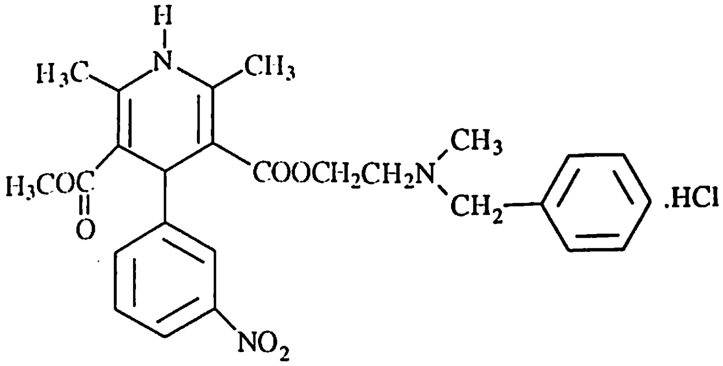
The major side effects are gastro intestinal effects including epigastric pain, nausea, vomiting, diarrhea, some incidences of headache, dizziness, drowsiness and insomnia.

2.3. NICARDIPINE HCl :

Nicardipine HCl is a calcium channel blocker which inhibits the transmembrane influx of calcium ions into cardiac muscles and smooth muscles without changing the serum calcium concentration. The contractile processes of cardiac muscles and vascular smooth muscle are depends upon the movement of calcium ion into these cells through specific ion channels (Ca^{++} channels). The effect of Nicardipine HCl is more selective in vascular smooth muscles than cardiac muscles. It is mainly indicated in chronic stable angina and hypertension [66].

2.3.1. Name, formula and molecular weight: [67]

- **Chemical Name:** 2-(N-Benzyl-N-methylamino)ethyl methyl 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-3,5-pyridine dicarboxylate monohydrochloride.
- **Molecular Formula:** $C_{26}H_{29}N_3O_6$ .HCl
- **Molecular weight:** 515.99.
- **Chemical Structure :**



2.3.2. Appearance : Crystalline powder

2.3.3. Colour : Greenish-yellow

2.3.4. Odour : Odourless

2.3.5. Physical Properties :.

2.3.5.1. Melting point: 169 °C.

2.3.5.2. U.V. spectrum:

U.V. spectrum recorded using absolute alcohol showed three maxima at 207, 237 and 349.6 nm. The UV spectrum is shown on Page 216 in Chapter 5.

2.3.5.3. Infrared Spectrum:

Principal peaks observed are at 118.8, 1354, 1491, 1533, 1622, 1705, 2951, 3071 cm^{-1}. The IR spectrum is shown on Page 216 in Chapter 5.

2.3.5.4. NMR Spectrum:

NMR spectrum recorded on Bruker 200 MHz NMR using $CDCl_3$ and TMS as internal standard shows principal chemical shifts at 1.2, 2.6, 3.7, 6.5, 7.5 and 8.2. NMR spectrum is shown on Page 217 in Chapter 5.

2.3.5.5. DSC:

The thermograph recorded on Mettler TA-4000 showed a single endotherm at 165.3 °C. The thermograph is shown on Page 218 in Chapter 5.

2.3.5.6 Solubility :

Freely soluble in chloroform, methanol and glacial acetic acid; sparingly soluble in anhydrous ethanol; slightly soluble in n-butanol, water, 0.01M potassium hydrogen phosphate, acetone and dioxane, very slightly soluble in ethyl acetate and practically insoluble in benzene, ether and hexane.

2.3.6. Method of Analysis:

2.3.6.1. HPLC Methods:

1. Detector U.V. at 237 nm; Column ODS, (Symmetry, Waters), (5 µm particle size, 4x150 mm). The mobile phase is consisting of Acetonitrile:Perchloric acid (43 in 50,000) (2:3) and the flow rate is 1 ml/min [68].
2. Detector U.V. at 275 nm; column C-18 Novopac cartridge (5 µm particle size, 8x100 mm). The mobile phase is consisting of

acetonitrile:10 mM phosphate buffer at pH 4.5 (70:30) and the flow rate is 2 ml/min [69].

2.3.7. Pharmacokinetics:

Following intravenous infusion of Nicardipine HCl, plasma concentration of Nicardipine decline triexponentially with rapid early distribution phase (α half life of 2.7 minutes), an intermediate phase (β half life of 44.8 min.) and slow terminal phase (γ half life of 14.4 hours) which can be detected after long term infusion. Total plasma clearance is 0.41 L/hrs.kg and the apparent volume of distribution is 8.3 L/kg. The pharmacokinetics of the Nicardipine was studied with the dosage range of 0.5 to 40 mg/hr. infusion. The steady state is achieved at 24-48 hrs.[70]. Nicardipine is highly protein bound (>95 % in human plasma over a wide concentration range). Pharmacokinetic studies in animal and man has shown that after oral administration Nicardipine is rapidly and completely absorbed [71]. In human plasma, peak plasma concentration reached between 20 min. to 120 mins. after oral administration thereafter decline via first order rate kinetics [72-74] . The highest concentration of the drug recovered from the liver, kidney, lungs, heart and spleen. There are two main classes of metabolites:

i. those where the Dihydropyridine ring is intact, and,

ii. those where oxidation has occurred resulting in a pyridine analogue metabolite. Although several metabolites resulting from sequential metabolism of the N-benzyl side chain have been identified. The extent of oxidation occurring in vivo has not been clearly determined. Major metabolites appearing in the urine are the glucuronide conjugates of the alcohol metabolites [75,76]. As Nicardipine HCl is extensively metabolised and unchanged drug is essentially absent from the urine, it is considered that the clearance is mainly due to its metabolism by the liver.

The other important pharmacokinetic data collected from literature is given below in Table 2.6 and subsequently utilized for theoretical modeling later in Chapter 3:

Table 2.6: Estimated parameters and in vitro permeation data [77].

Sr. No.	Description	Values
01.	MEC (ng/ml)	20
02.	CL (L/hr.)	23
03.	J required (μg/hr.)	460
04.	Solubility (mg/ml) water octanol M.E. (mg)	 12.7 1.31 319
05.	Log $K_{o/w}$	- 0.99
06.	J_{max} (μg/hr.cm^2) rat :M.E. :Water Human : water : M.E.	 2690 1.24 0.12 3400
07.	E.R. : rat : Human	2169 2833
08.	Log P (cm/sec.) rat :water : M.E.	 - 7.6 -5.6
09.	A required (cm^2)	0.14

Table 2.7: Relevant parameters used to formulate a therapeutic transdermal system [78].

Sr. No.	Parameter	Values
01.	D_o (mg^a)	60.00
02.	D_t (mg^b)	14.10
03.	X_{24} (mg^c)	11.21
04.	D_{ss} (ng/ml^d)	11.21
05.	C_{ss} (g/ml^e)	13.90
06.	C_t (ng/ml^f)	6-40
07.	CL (L/hr.)^g	33.60

[a] Oral daily dose. [b] Theoretical daily transdermal dose. [c] Predicted permeated amount during the first 24 hrs. [d] predicted permeated

amount in steady state conditions. [e] Predicted plasma levels at steady state. [f] therapeutic plasma concentrations. [g] Clearance.

2.3.8. Mechanism of Action :

Nicardipine is a calcium channel blocker which inhibits the transmembrane influx of Ca^{++} into the cardiac muscles and smooth muscle without changing serum calcium concentrations.

2.3.9. Indications:

1. Stable angina
2. Hypertension

2.3.10. Contraindications :

It is contraindicated in patient with hypersensitivity to the drug and to the patients with advanced aortic stenosis.

2.23. Preparations :

Following are the preparations available in the world,

1. Cardene
2. Karden
3. Loxen
4. Nicapress.

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80

Chapter III.

INTRODUCTION TO EXPERIMENTAL

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PART I. NITROGLYCERIN:

3.1. Preliminary Experiments:

3.1.1. The Extraction Of Nitroglycerin From Lactose Triturate:

The nitroglycerin (NTG) is available in the form of 10% lactose adsorbed triturate and 1% alcoholic solution. For the formulation purposes, concentrated solution (about 40%) is needed. Hence, instead of concentrating the alcoholic solution, it is practically convenient to extract it from the lactose triturate. For this purpose, the lactose triturate was dissolved in water so that lactose dissolves in water to give a separate layer of NTG which was then extracted into ethyl acetate. To remove water traces from the ethyl acetate extract, it was treated with anhydrous sodium sulphate. The ethyl acetate solution of nitroglycerin was stored in refrigerator in glass bottle till used.

3.1.2. Synthesis of Pressure Sensitive Adhesive:

3.1.3. Introduction:

A pressure sensitive adhesive is generally defined as a material that will adhere to a substrate when a light pressure is applied and will leave no residue when removed [1]. These type of adhesives have high degree of tackiness as compared to the other type of adhesives. As a result, they bound almost instaneously when slight contact pressure is applied to force the two mating surfaces together. This type of adhesive shows more cohesiveness rather than adhesiveness. Hence, this can be removed from the surface, after adhesion, without leaving appreciable quantity on the surface. Thus, it is more suitable candidate for application to the skin surface [2].

The major pressure sensitive adhesives that have been evaluated for potential medical applications are,

1. polyisobutylenes;
2. acrylics, and
3. silicones.

3.1.4. Polyisobutylene-Type Pressure Sensitive Adhesive:

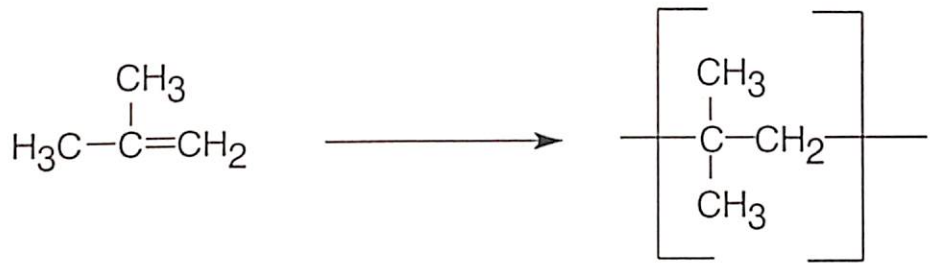


Fig. 3.1. Polymerisation of isobutylene.

They are the homopolymers of isobutylene, polymerised using the Lewis acid type catalyst, such as aluminium chloride. The hydrocarbon backbone is relatively long and straight, with only terminal unsaturation. Because of very less unsaturation, they are more stable and resistant to the effect of weathering, ageing and heat. They have good resistant to vegetable and animals oils as well as to the chemicals. Polyisobutylenes have little tendency to recrystallize and hence their amorphous character imparts an internal mobility that leads to the flexibility, permanent tack and resistance to the shock. Due to lack of polarity, they show weak adhesion to the substrate even though it shows high tack. This problem can be overcome by the addition of resins and other tackifiers [3].

3.1.5. Acrylic-Type Pressure-Sensitive Adhesives:

Acrylic-type pressure-sensitive adhesives are produced by the co-polymerisation of acrylic esters with acrylic acid and the other functional monomers [3,4,5].

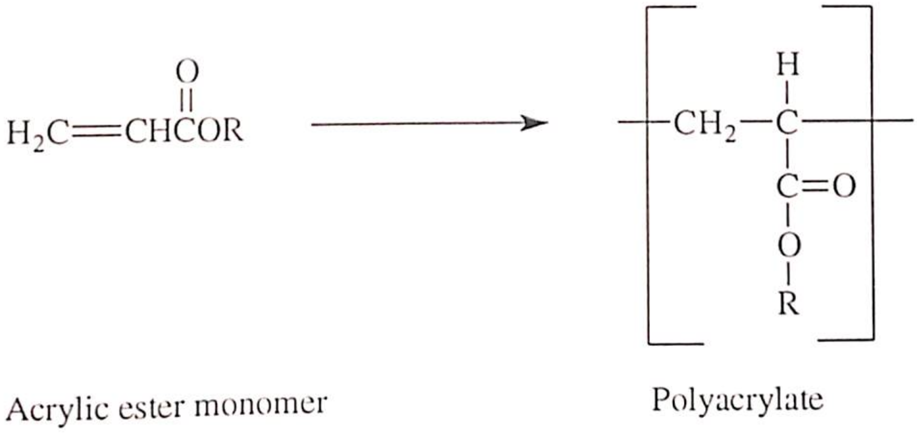


Fig. 3.2: Polymerisation of Acrylic acid.

The ester group of the monomer is attached in a pendant fashion from the polymer backbone and provides a unique method of tailoring the adhesive properties of the polymer. Akin to polyisobutylene, due to less unsaturation these are resistant to oxidation. The polymers are moderately polar, due to their acrylic ester structure, and, thus, have a degree of moisture permeability [4].

Polyacrylates can be synthesized through either emulsion or solution polymerisation, using free radical initiated processes. The most commonly used monomers are ethyl acrylate, butyl acrylate, and 2-ethylhexyl acrylate. Incorporation of the ester groups with longer chain lengths increases the randomness of the polymer, which reduces the recrystallinity and lowers the glass transition temperature. The resultant polymers are softer and more flexible, and have increased tackiness [5].

Advantage of this single-component adhesive system includes its low level of lower molecular weight species and impurities that may migrate to the surface of the adhesive polymer and affects its adhesion [5].

3.1.6. Silicone-Type Pressure-Sensitive Adhesives:

Silicone-type adhesive are synthesized by the reaction of the linear polydimethylsiloxane fluid with the solvent-soluble, low molecular weight silicate resin. The linear polydimethylsiloxane fluid possesses a backbone of alternating silicone and oxygen bonds

$(-\overset{|}{\underset{|}{Si}}-O-)$ and is terminated by the silanol group $(-\overset{|}{\underset{|}{Si}}-OH)$. Each silicon atom is also attached with two methyl group [4,5].

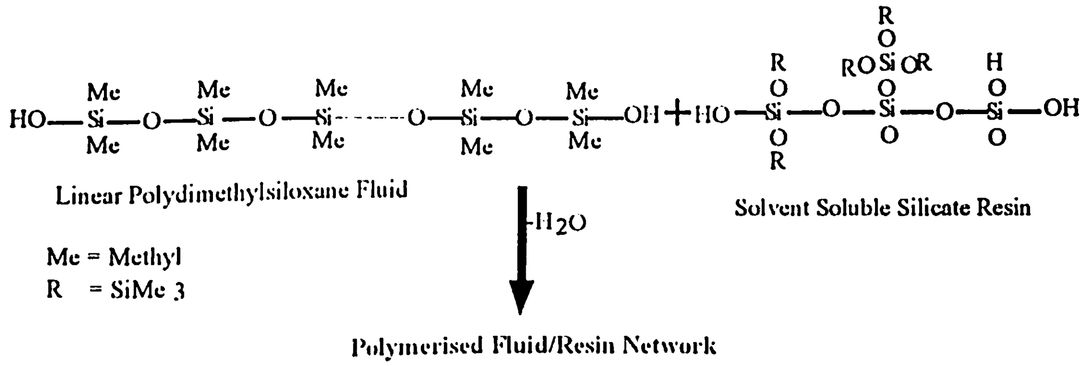


Fig. 3.3: Polymerisation of Polydimethyl Siloxane.

The polymer has very low glass transition temperature and hence a great degree of flexibility. The chain mobility also produces openings in the network to create free volume for molecular diffusion, hence, it has high permeability to the moisture and oxygen. They possess good resistance for wide range of temperature, moisture and chemicals [6,7].

3.2. General Methods of Synthesis of Adhesives by Polymerisation:

3.2.1. Solution Polymerisation:

The monomer or monomer mixture is dissolved in a solvent which is relatively inert to free radicals (examples: ethyl/butyl acetate, benzene, toluene, petroleum solvents or ketones), then the polymerisation is effected at elevated temperature in presence of an initiator such as organic peroxide or an azo compound which is soluble in the solvent. The type of solvent used has a great influence on the reaction and the molecular weight of the resulting polymer in solution, because of different chain transfer activities of different solvents. Thus, the viscosity of the polymethyl acrylate solution and the molecular weight of the polymer decreases in the following order:

benzene, ethyl acetate, ethylene dichloride, butyl acetate, methyl isobutyl ketone and toluene.

The molecular weights of solution polymer are normally lower than those of emulsion polymers [6].

3.2.2. Suspension Polymerisation :

In this method, the monomer or monomer mixture is suspended in the form of droplets by agitation. To prevent coalescence of droplets, suspending agent such as water soluble polymer e.g. polyvinyl alcohol or a finely divided water insoluble organic compound e.g. kaolin, tricalcium phosphate, aluminium hydroxide is added. A monomer soluble initiator is used. Heat transfer from these droplets is good and heat can be removed by cooling coils or jacket cooling.

Disadvantage of this process is that the suspending agent can cause contamination of the polymer with the deleterious effect on clarity and colour [7].

3.2.3. Emulsion Polymerisation:

Acrylate monomers are polymerised by a typical anionic-nonionic emulsifier system using a redox initiator system of ammonium persulfate and sodium metabisulfite, adding part of the initiator and part of the monomer mixture by gradual addition.

The water-soluble initiator (inorganic persulfate) generates free radicals in aqueous phase. When an initiator radical encounters a micelle/droplet, it diffuses into it and initiates the polymerisation. The termination occurs only when the next radical diffuses in. Thus, very high molecular weight products can be obtained at high rate of polymerisation.

Disadvantage is the contamination of polymer by surface active agents.

3.3. Ideal Characteristics Of An Adhesive For Dermatological Application [8]:

1. It should not show any chemical interaction with the drug on prolonged storage.

2. Protective peel strip over the adhesive must be easily removed and none of the adhesive should stay on the skin.
3. It must hold the device securely in the place for periods as long as a week, yet the removal must not be so painful so as to discourage the patient. When removed, all adhesive must remain on the strip leaving no residue on skin.
4. It should not have dermal toxicity, skin irritation or allergic reactions.
5. It should be air and water vapour permeable.

3.4. Synthesis Of Adhesives:

3.4.1. Acrylic Adhesive (Solvent Based):

An extensive survey of the manufacturers of adhesives of either rubber based or acrylate-based was carried out and to our big surprise, there were many manufacturers of acrylate based adhesives but were not willing to disclose the process or the monomers employed and even the analytical procedure to be followed for analysis besides vehemently refusing to supply any information on toxicity. As a result of literature survey and personal discussion with Dr. Lalla, it was decided to pursue the work which was done earlier on the same subject with one of his earlier student, Ms. Bapat, where some of the formulations synthesized had shown some promise of being potential candidates for future exploitation. The following formulations are suggested in the literatures to be of some promise.

Table 3.1: Composition of PSA -S1.

Batch Size : 100 ml

Reaction Time : 5 Hrs.

Sr. No.	Ingredient	Quantity
1.	Vinyl acetate	6.93 ml
2.	Acrylic acid	0.365 ml
3.	2-ethylhexyl acrylate	29.2 ml
4.	Azoiso butyronitrile (AIBN)	0.03 gms.
5.	Ethyl acetate	56.57 ml

Table 3.2: Composition of PSA -S2.

Batch Size : 100 ml

Reaction Time : 20 Hrs.

Sr. No.	Ingredient	Quantity
1.	Butyl acrylate	16.80 ml
2.	2- ethyl hexyl acrylate	16.90 ml
3.	AIBN	0.030 gms.
4.	Dioctyl Maleate	25.0 ml
5.	Ethyl acetate	41.10 ml

3.4.2. Acrylic Adhesive (Aqueous) :

As expected, neither market survey nor the literature survey could take us anywhere near to start any meaningful work which could lead us to prepare meaningful formulations. Hence, it was decided to pursue work done by Dr. Lalla. Therefore, initially some experiments mentioned by Ms. Bapat in her thesis were repeated in this laboratory and an extensive modification of the same were made to get a suitable grade of adhesive for initial experimental trials. Following is the formula obtained from Ms. Bapat's thesis, the technology involved here is emulsion polymerisation.

Table 3.3: Composition of PSA-A1.

Batch Size: 100 gms. Reaction Time: 20 Hrs.

Sr. No.	Ingredient	Quantity
1.	Monomer phase: Composition : 1. Vinyl acetate : 14.6 ml 2. Acrylic acid : 0.73 ml 3. 2- Ethyl hexyl acrylate : 57.67 ml	75 ml
2.	Initiator system:	
	1. Solution of Sod. metabisulfate.	1.5 ml
	2. 10% solution of ammonium persulfate	3 ml
	3. Nonyl phenyl with 10 moles of ethylene oxide	1.0 ml
	4. Indion P	6 ml
3.	Distilled water upto	77 ml

3.5. Evaluation Of The Adhesive Polymers:

The adhesive polymers were subjected to following physico-chemical evaluation: appearance, odour, solid content, determination of molecular weight, UV spectrophotometry, IR spectrophotometry, peel strength testing and finally the chemical analysis. The chemical analysis includes analysis of free monomers (by bromine number, mercaptan method or instrumental method) and determination of other additives and contaminants.

Only those evaluation procedure having special significance in the present context are discussed here.

3.5.1. Free Monomers:

Free monomers in the adhesive solution may create bio-incompatibility and irritation problems along with other additives and contaminants. In case of polyacrylates and polymethylacrylates, the content of free monomers are determined by chemical methods based on addition of the iodine, bromine and mercaptans. However, only HPLC methods for this purpose have been employed [9,10,11].

3.5.2. Moisture Content:

Moisture in polyacrylates and polymethacrylates is determined by titration with Karl Fisher reagent.

3.5.3. Determination Of Solid Content:

The easiest but approximate way of finding out concentration of the polymer in polymer solution is determination of its solid content. The free monomers form only a small fraction of the total mass and the initiator, even if present in trace amount, is decomposed and driven off along with the solvent in the drying process. Therefore, the concentration of the polymer so determined is close approximation of the real value.

3.5.4. Determination Of Molecular Weight Of Polymers:

Polymerisation is a random process and molecules given within a polymer mass will have different molecular weight, and for this reason, molecular weights of the polymers are described in terms of average molecular weight. The most common molecular weights are the average molecular weight, M_w and the viscosity average molecular weight , M_v. [12,13].

A knowledge of molecular weight of the polymer is important because there is a definite relationship between polymer molecular weight and polymer properties. Thus, at very low molecular weight, the polymer has essentially no useful mechanical properties but as the molecular weight increases, the magnitude of mechanical property of interest also increases.

Molecular weight can be determined by osmometry, dilute solution viscometry, end group analysis, elevation of boiling point, depression in freezing point, light scattering and gel permeation chromatography [14].

Gel permeation is another very popular method for determining the molecular weight of the polymer. In this procedure, a dilute polymer solution is pumped through a series of columns containing porous beads of different pore sizes but about same dimension as the polymer molecule. The smallest polymer molecule will be able to penetrate all pore and will emerge first and the larger polymer will penetrate slowly and emerge last. The detail account of procedure is given in experimental .

3.5.5. Determination Of Molecular Weight By Gel Permeation Chromatography:

The determination of molecular weight was done using combination of four μ-styragel columns of 100 Å, 500 Å, 10^3 Å, and 10^4 Å using THF as a solvent, flow rate 1.5 ml/min, using RI detector and Waters System. Sample size chosen was 0.5 ml of 0.05% concentration of standard polystyrenes procured from Waters. The system is equipped with the software which automatically determines the average mol. wt. (M_w) and average mol. number (M_n).

3.6. Methods Of Analysis Of Nitroglycerin:

In the literature, several methods are reported for the analysis of nitroglycerin. Nitroglycerin was mainly analysed by UV spectrophotometry and HPLC.

I. UV Method:

In this method, the UV absorption of the diluted test solution was compared with that of standard at 220 nm.

II. HPLC Method:

The HPLC method for analysis was developed in the laboratory and utilised for the analysis of nitroglycerin solution, nitroglycerin -lactose triturate, nitroglycerin extracted solution and nitroglycerin blend for application as well as final patches. This method is also utilised for the analysis of the in vitro samples. Following are details for the HPLC analysis:

1. *HPLC pump* : Jasco Intelligent 980
2. *Detector* : Jasco UV 975
3. *Detection* : 220 nm
4. *Integrator* : Borwin Chromatography, version 1.21.
5. *Column* : Bondapack, ODS, C18, 10μ, 3.9 X 300 mm.
6. *Operating Temperature* : Room Temperature
7. *Solvent system* : Methanol: water (1:1)
8. *Flow rate* : 1 ml/min.

3.7. Formulation Development:

Approach to the development of the TDDS depends upon type of the delivery system to be selected (discussed in the Chapter 1). For this, two type of drug delivery systems for nitroglycerin patches were selected :

1. Adhesive-drug dispersion delivery system.
2. Membrane controlled drug delivery system.

3.7.1. Adhesive Matrix Dispersion TDDS:

Initially, after looking at the commercial samples, it was decided to duplicate the type of system used in Nitro Dur® patches. For laboratory trials, one machine was developed in the laboratory which is totally mechanical in design for the application of the adhesive-matrix dispersion on the backing membrane. This machine has provision for adjustment of application layer thickness and capacity to produce 200 patches of 10 cm^2 in single application. However, this was followed by pilot scaling process, after some modification in the machine design (available with KMK College of Pharmacy, Mumbai), was achieved to be able to coat about 1 kg. material at a time. This was essentially achieved by changing the trough design so that roller can pick up the mass easily and to avoid material wastage. Some minor changes were also made in the formulation to suit the application process.

For the development of this type of TDDS, following major components were considered :

1. Backing membrane
2. Adhesive- drug matrix system
3. Release liner
4. Application techniques.

3.7.2. Backing Membrane:

The backing membrane used in this system was siliconised pterephthalte (PET) film of 50 µm. The backing membrane should be essentially,

1. Inert to the system components,
2. Non toxic,
3. Free from allergenic reactions,
4. Should have sufficient strength to withstand the operating tension.
5. Should be impermeable to moisture.

3.7.3. Adhesive-Drug Matrix System:

In this type of TDDS, drug solution along with the other excipients and solvents were mixed into the polymer and was applied to form a layer. NTG solution (ethyl acetate extract) was mixed with solvent and polymeric adhesive in varied quantities to get the desired release pattern of the drug.

3.7.4. Release Liners:

The release liner used was siliconised PET of 125 µm thickness and should be,

1. Inert to the TDDS components,
2. Impermeable to the drug and moisture.

3.8. Application Techniques :

There are several techniques used for the application of the drug at laboratory and pilot scaling process.

A. Laboratory Coating Techniques:

3.8.1. Mayor's Rod :

In this type of coating, the solution to be coated was held on one end of the backing membrane and it was spread with the help of a rod covered with the uniform coil of wire. The diameter of the wire controls the thickness of the layer to be applied.

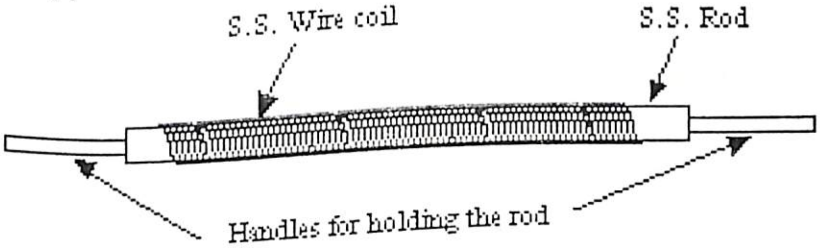


Fig. 3.4: Mayor's rod for laboratory application.

Disadvantage of this applicator was non-uniform application of drug adhesive mixture on the backing liner.

3.8.2. Applicator :

Due to the problem of non-uniform application associated with the Mayor's rod, an applicator was fabricated which consists of a reservoir trough for application mixture holding and an opening at one side of the bottom. The uniform slit opening results into uniform thickness of the application. The application amount can be controlled by varying the opening from 0.1-2 mm by simple mechanical device.

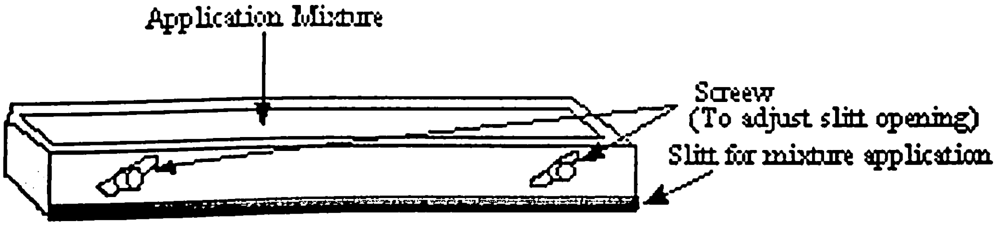


Fig. 3.5: Modified applicator for laboratory development.

B. Scale- Up Techniques:

The pilot scale trials have been taken on semiautomatic machines developed for the coating and laminating of the patches. The control over the amount of coating and its overall uniformity depends on the coating system used. Following are different techniques utilised in the coating:

3.8.3. Graver And Reverse Direct Graver [18]:

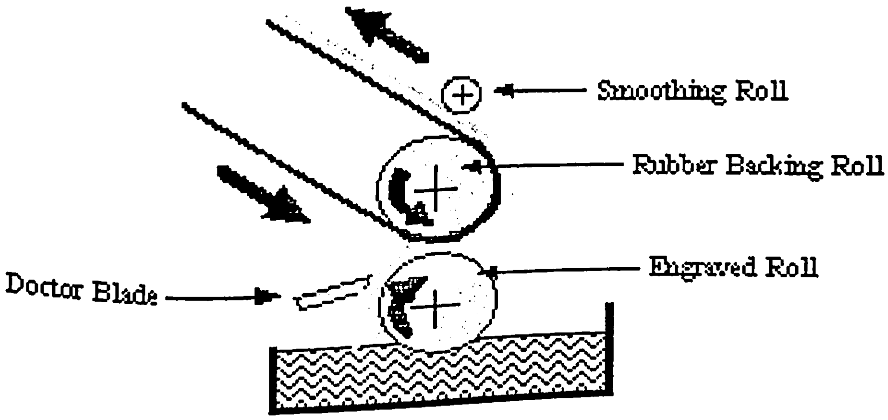


Fig. 3.6: Schematic diagram of Graver & Reverse direct Graver.

This technique is utilised for the low coating weight and smooth finish. In this, engraved roll picks the mass while doctor blade scraps excess mass to give desired thickness of the layer to be applied. It is transferred on the backing liner with the help of backing roll and then applied layer is slightly pressed by smoothing roll to give a smooth and uniform layer.

3.8.4. Wire Rod Coating [18]:

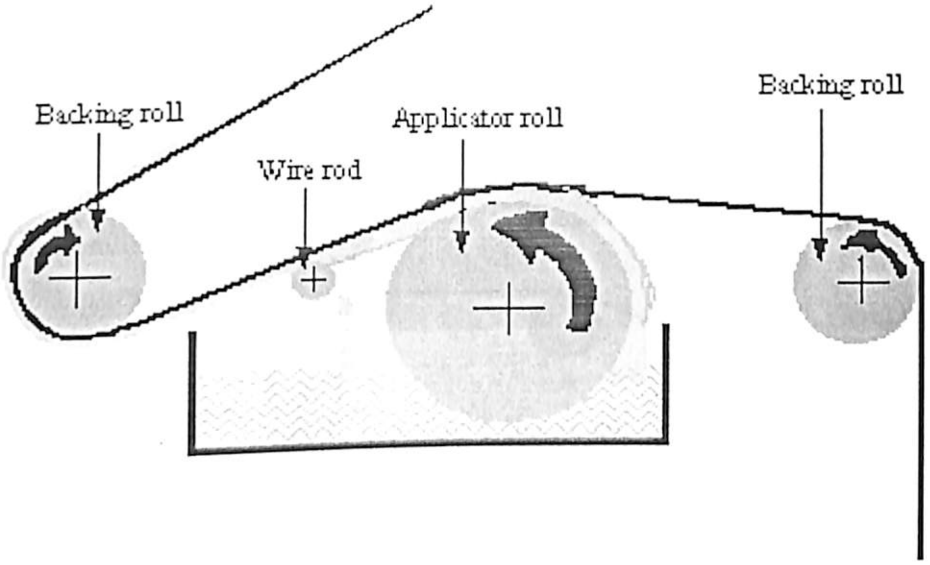


Fig. 3.7: Schematic diagram of wire rod coating.

This type of system is extremely useful in the fast application with smooth coating.

3.8.5. Air Knife Coating [18]:

For the coating of aqueous emulsion with low shear requirement and specific viscosity ranges, this system is used.

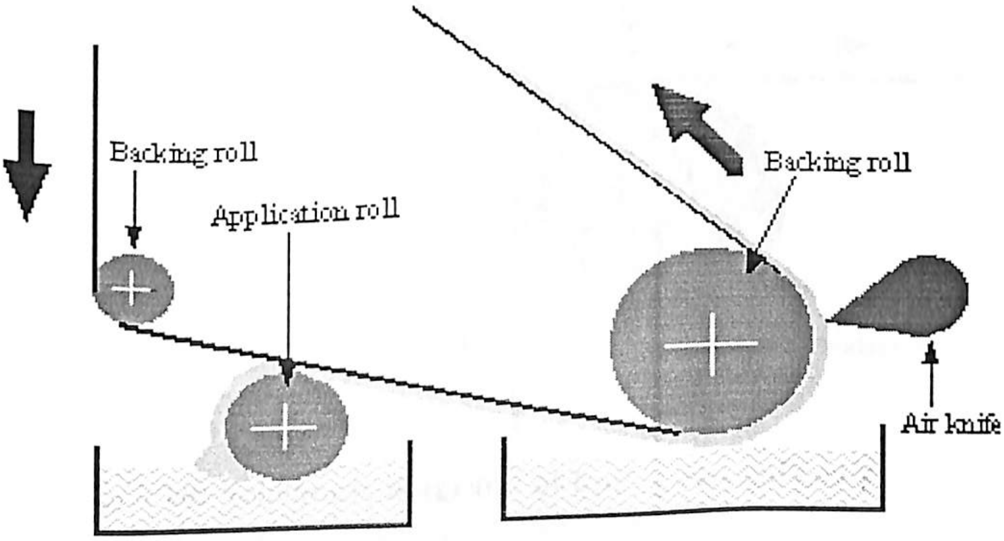


Fig. 3.8: Schematic diagram of Air Knife coating.

3.8.6. Slot-Orifice Coating [18]:

This method is useful for the hot melt compounds.

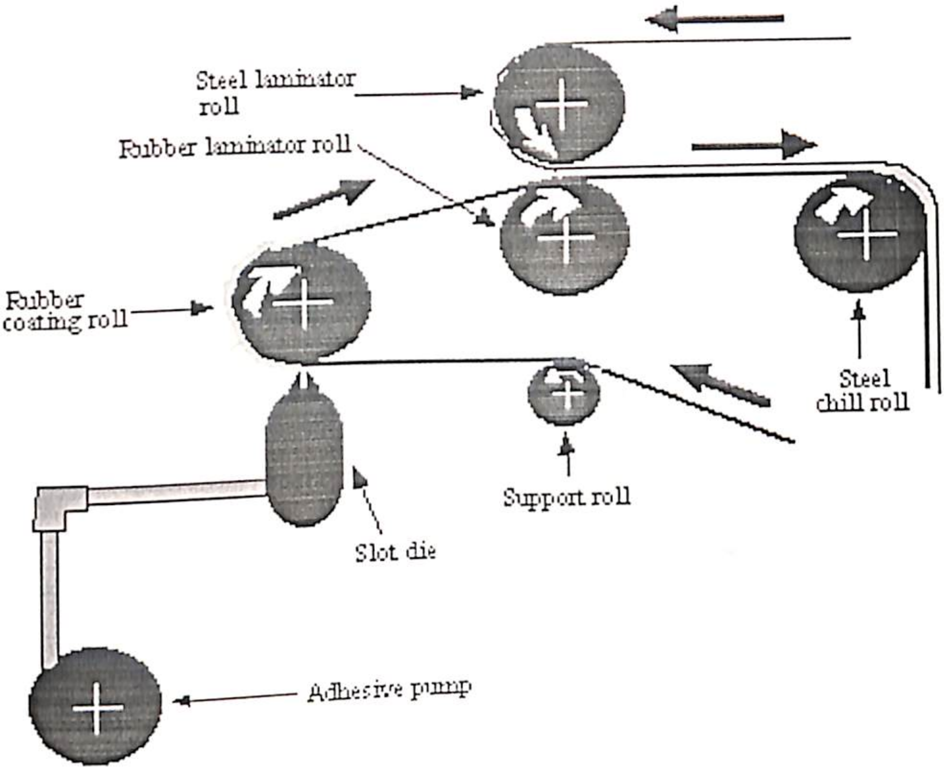


Fig. 3.9: Schematic diagram of slot orifice coating process.

3.8.7. Knife Over Roll Coater [18] :

This technique is used for the versatile strip coating and higher viscous material.

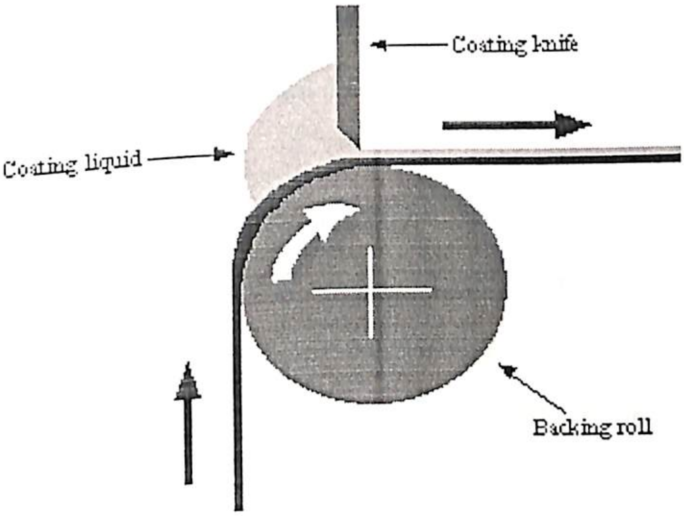


Fig. 3.10: Schematic diagram of knife over roll coater.

3.8.8. Reverse Roll Coating [18]:

This technique is best for coating a variable or uneven capillary substrate.

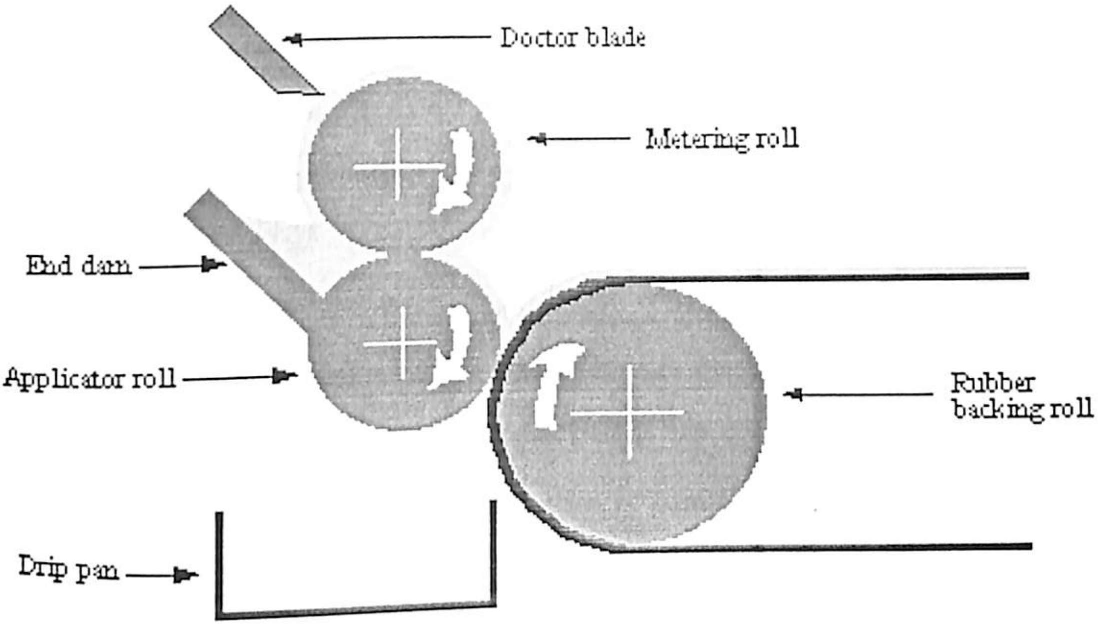


Fig. 3.11: Schematic diagram of reverse roll coating.

3.9. Evaluation Of NTG Patches:

3.9.1. Analysis Of Drug Content In Drug-Dispersion And Patches:

Drug content in both was analysed using HPLC method as described in Section 3.6II. For this, drug-dispersion/patch was extracted in ethanol, centrifuged and the area response of sample was compared with standard and only after confirmation of the NTG content further evaluation of the patches was done.

3.9.2. In-Vitro Release Study Of Nitroglycerin TDDS:

The in vitro permeation release was done on the synthetic membranes, goat skin, pig skin and human cadaver skin using modified Franz cell for 24 hrs. The methodology is described in the experimental section in Chapter 4.

3.9.3. In-vivo Release Study:

The in vivo study of the patches was carried out on six rabbits in a cross over design using Nitro Dur® as a reference patch. The details of experiments are discussed in the experimental section in Chapter 4.

3.9.4. Skin-irritation test:

The skin irritation test was carried on rabbits by applying the patches. The details have been discussed in the experimental section in Chapter 4.

3.10. Membrane Controlled Drug Delivery System:

Nitroderm TTS® made by Ciba Geigy was chosen as the model to develop this type of system in this laboratory. On critical examination, this system was found to consist of a backing membrane, adhesive/drug dispersion, rate controlling membrane impregnated with adhesive and the release liner. The nearest possibility of making such a system was to develop a form-fill-seal machine but the major problem, after discussion with several machine fabricators, was found to be the application of adhesive on rate controlling membrane. Hence it was decided to develop this machine indigenously if possible for both laboratory work as well as pilot scale process. This was achieved after long time with some innovative work and the final model of the machine developed in laboratory can produce 200-500 patches a day and it essentially consists of,

1. Die cavity which can form a cavity of required depth, size and shape by application of vacuum.
2. A dosing system which can deliver a predetermined dose into the cavities with the help of peristaltic pump.
3. Sealing facilities to seal rate controlling membrane to metallised polyester film on periphery alone embedding the drug dispersion.
4. Application of adhesive on the rate controlling membrane with the help of inbuilt spray gun.
5. Facilities to laminate the release liner.

While for cutting of the patches into desired size and shape, separate slitting dies were developed.

The components of this kind of system are as follows:

1. Backing membrane,

2. Drug polymer dispersion system,
3. Rate controlling membrane,
4. Adhesive,
5. Release liner,
6. Secondary packaging materials.

3.10.1. Backing Membrane:

Backing membrane used for this type of system was metallised-polyester film procured from 3M Silicones. It is heat sealable and occlusive in nature.

3.10.2. Drug-Polymer Dispersion:

Drug polymer dispersion was made with Hydroxy Propyl Cellulose solutions in varying ratios and different of solvents.

3.10.3. Adhesive:

10 % aqueous solution of Plastoid E15 (methacrylic acid polymer, Rhom Pharma) was used to coat the rate controlling membrane for the adhesion of the release liner.

3.10.4. Rate Controlling Membrane:

9% Ethylene Vinyl Acetate (EVA) film was used as rate controlling membrane for this TDDS system procured from 3M Silicones.

3.10.5. Secondary Packaging:

The release liner used for this system was clear fluoropolymer coated polyester film of 50 μm thickness made by 3M corporation. While the patches were packed into the alupoly pouches which were thermally sealed.

3.11. Evaluation Of NTG Patches:

3.11.1. NTG Content In Drug-Dispersion And Patches:

Drug content in both was analysed using HPLC method which is described in the Section 3.6II. And the methodology used was same as described in Section 3.9.1. for matrix-dispersion system and only after confirmation of NTG content further evaluation of the patches was done.

3.11.2. In-Vitro Release Study Of NTG TDDS:

The in vitro permeation studies of the patches were done through synthetic membranes and pig skin using modified Franz cell for 24 hrs. The methodology is described in the experimental section.

PART II. DICLOFENAC DIETHYL AMMONIUM :

3.12. The Partition Coefficient:

The partition coefficient of the Diclofenac diethyl ammonium was determined by using a reported method [19]. It consists of dissolving the drug at 25 °C in water and n-octanol till saturation and storing for 7 days with frequent shaking. The solutions were centrifuged and analysed by UV spectrophotometry. The details of the experiments are discussed in experimental section.

3.13. Measurement Of Critical Micelle Concentration :

To ten solutions containing diclofenac diethyl ammonium 10-50 mM, few crystals of Orange OT were added, an azo dye insoluble in water and solubilizable in micelles. After a week of gentle shaking, the solutions were filtered through 0.2 μm cellulose acetate membranes (Millipore) and the absorbance values of the solubilized dye was plotted against the concentrations of the DDEA solubilization started above 40 mM which is considered as the critical micelle concentration [19].

3.14. Interactions With Asolecithin [19]:

A system containing Asolecithin (is a commercial balanced mixture of phophatidyl inositol and phosphatidyl ethanolamine, Fluka) and DDEA in the weight ratio of 1:1 was prepared in water at a concentration of 40 mM DDEA . The mixture was stirred till a homogeneous i.e. a milky emulsion was formed. The transmission of the system was measured at 580 nm increased after the addition of the prefixed value of 40 mM DDEA solution. System containing almost pure Phosphotidyl choline and DDEA in the weight ratio of 1:1 was stable upto 10 % w/w concentration in water .

3.15. Method Of Analysis For DDEA:

The methods for analysis of the DDEA (raw material) has been developed in the laboratory. It is a chemical method and consists of titration of free Diclofenamic acid with Perchloric acid using crystal violet as an indicator. Each ml of 0.1N Perchloric acid is equivalent to 0.036913 gms of Diclofenac diethyl ammonium.

3.16. Synthesis of Adhesive:

For the development of the Diclofenac plaster , the adhesive should be water-miscible. Hence as discussed in Section 3.4.2 on 114, emulsion polymerisation technique was used for the synthesis of the adhesive using 2-ethylhexyl acrylate as a monomer. The details of the synthesis of the adhesives is discussed in the experimental section.

3.17. Formulation Development:

This TDDS consists of drug dispersed in thermoreversible polymeric adhesive which in hot condition is applied on the suitable backing membrane and after cooling, it is laminated with suitable release liner and cut into the pieces of 10 x 15 cm. Apart from being devoid of skin-irritation and allergenic reaction, the other requirements of Diclofenac plaster are:

1. The entire area of knees and elbow should be covered as it is essentially meant for arthritis.
2. Adhesion of the plaster should be strong enough to withstand movements.
3. It must be easily removable and leave no adhesive mass on removal.
4. The backing membrane must be breathable and stretchable.

During formulation development of the Diclofenac plaster following points have been considered :

3.17.1. Vehicle for the Diclofenac Diethyl Ammonium:

The drug is slightly soluble in the water and hence other solvents are also used not only to solubilise the drug in the system but to keep the other excipients like gelatine and pectin in solution form. During trials, solvents like glycerine, propylene glycol, polyethylene glycol as well as surfactants like Polysorbate 80, Sodium lauryl sulphate were also tried.

3.17.2. Drug- Adhesive Dispersion:

Bulk of the plaster contains polymers like Sodium carboxymethylcellulose, Hydroxypropyl methyl cellulose (higher viscosity grade), Hydroxypropyl cellulose, Veegum, Xanthan gum, Alginic acid, Gelatine and Pectin along with aqueous adhesive and water.

3.17.3. Packaging Material:

Since the backing liner has to be breathable and stretchable, only nylon jersey cloth which fulfils this need was largely tried in addition to cotton cloth, meeting B.P. specifications.

For release liner, siliconised PET (120 µm) and siliconised high density polyethylene (HDPE) films were used.

The final plasters samples were packed into duplex board cartoons and pouch made of LDPE/metallised polyester (150 gauge/100 µm).

3.17.4. Process Development:

The most crucial part of this TDDS is application of the drug adhesive mixture to the backing membrane. For the laboratory purpose, the applicator fabricated (discussed in Part I. section 3.8.2 on Page 119) was utilised, while for scaling up, a special machine was developed which consists of a trough fitted over a plain platform in such a way that from the bottom backing material can be continuously taken from unwinding roll with the help of controlled motor drive, while drug-adhesive dispersion contained in the trough is applied from the top. The application weight is controlled with the help of microgauges through a slit opening. After achieving desired drying conditions, the plaster is laminated and slit into desired size pieces. Following is the schematic diagram, fig. 3.12 for pilot scale process machine:

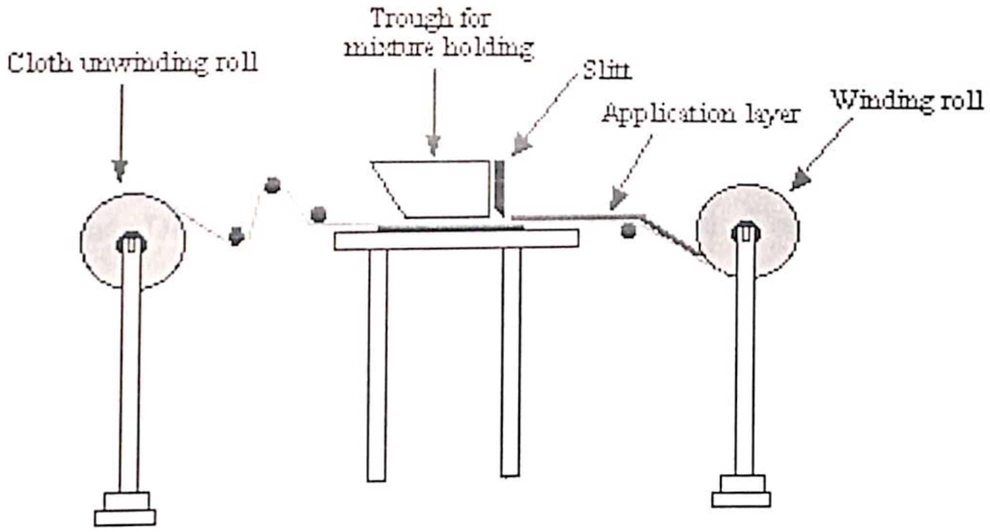


Fig. 3.12: Schematic diagram of scale up applicator.

3.18. DDEA Content of Adhesive Mix And Plasters:

The assay of the plaster was done by HPLC. The adhesive mix/plaster was extracted in mobile phase with sonication at 60 °C, centrifuged and injected. The area response of sample was compared with standard and only after confirmation of DDEA content further evaluation of the patches was done. The Following are the parameters of HPLC method :

- *HPLC pump* : Jasco Intelligent 980
- *Detector* : Jasco UV 975
- *Detection* : 275 nm
- *Integrator* : Borwin chromatography version 1.21
- *Column* : Shandon, Hypersil, C18, 10µ, 4.6 X 250 mm.
- *Operating Temperature* : Room Temperature
- *Solvent system* : Methanol: water : acetonitrile : Glacial acetic acid (1:1: 0.5 : 0.00015)
- *Flow rate* : 1.5 ml/min.

3.18.1. In vitro Permeation Study:

The in vitro release study of the plaster was done on the pig skin and human cadaver skin. The method used was same as above. The details of the study have been described in the experimental.

3.18.2. In vivo Study:

The in vivo study of plaster was carried out with six rabbits using a cross over design, comparison being made with internationally available plaster of Diclofenac Dihydroxy Ethyl Pyrrilidone (DHEP) salt (Flector Tissue Gel ®).

PART III. NICARDIPINE HCl:

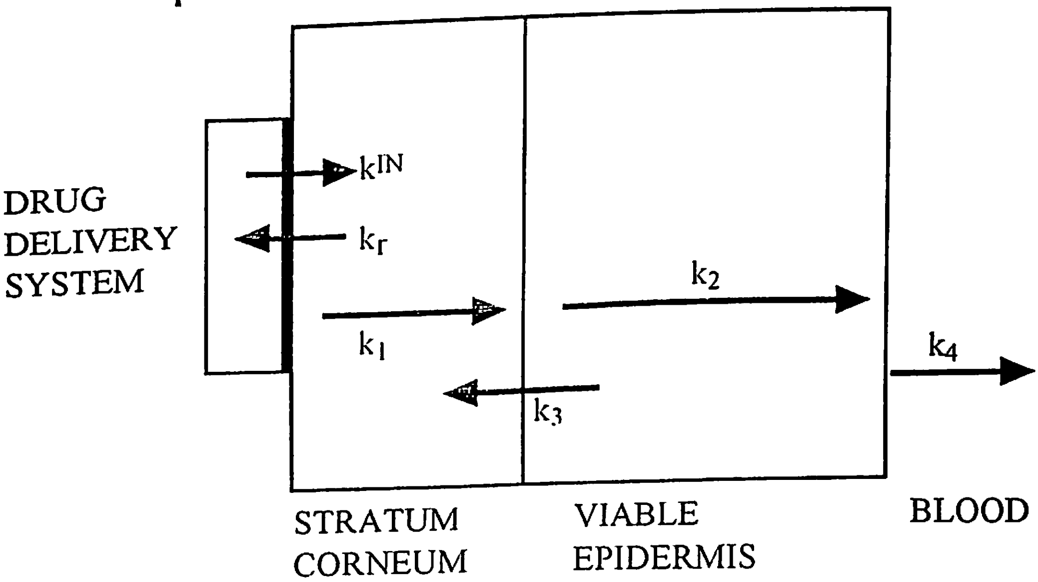
The development of the Nicardipine patch was followed after ascertaining the suitability by theoretical modelling which clearly indicated that Nicardipine is a suitable candidate for the TDDS. A detailed account of the theoretical modelling is given below :

3.19. Theoretical Modelling of Nicardipine HCl:

Guy and Hadgraft [20] have described a pharmacokinetic model for predicting plasma levels of a drug from a transdermal therapeutic system. They have cited practical examples of Clonidine and Nitroglycerin in which the predicted plasma profiles correlate well with the actual in vivo plasma profiles for the drug.

Drug input from a membrane controlled transdermal drug delivery system is illustrated in the following figure.

Figure 3.13. Kinetic model for transdermal drug delivery system and percutaneous absorption.



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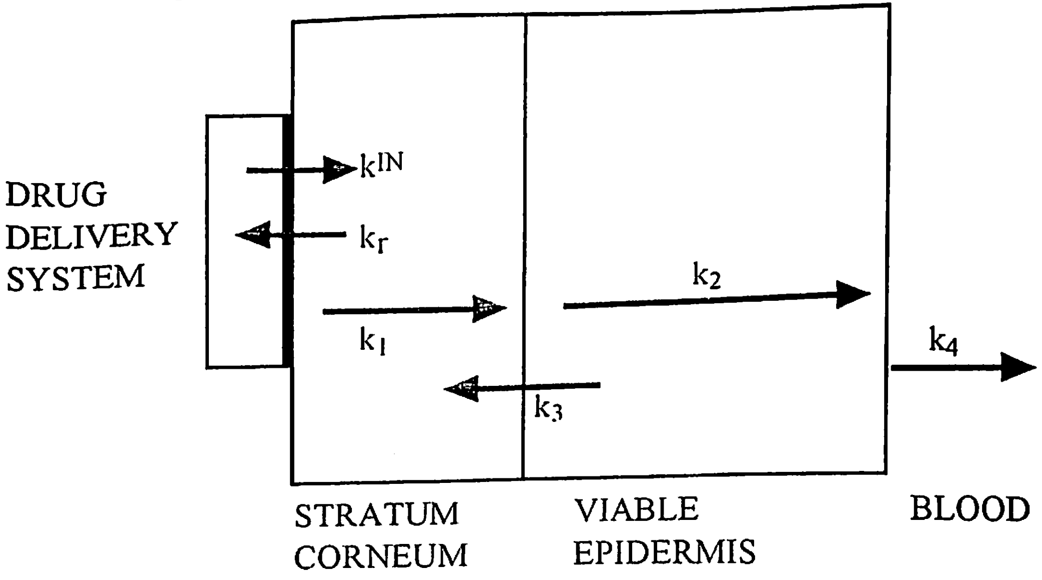
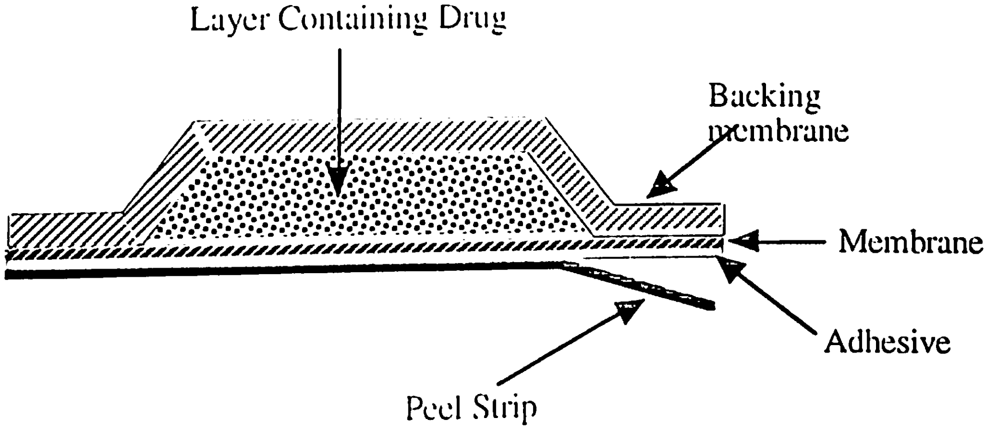


Input kinetics from the device are described by $f(k^{IN})$ for so called membrane controlled patch (e.g. Clonidine containing Catapres-.TTS).

Figure 3.14. : Schematic diagram of membrane controlled drug delivery system



If k^{IN} consists of a first order component (k^{I}) accounting for drug release from the contact adhesive and a zero-order contribution (k^{O}) representing the membrane determined flux of drug from the reservoir.

k_r reflects the fact that there will be competition for the drug between the patch and the stratum corneum; if the system is well designed then k_r will be small.

k_1 and k_2 are first order rate constants describing drug transport across the stratum corneum and viable tissue respectively. k_1 and k_2 are therefore proportional to the corresponding diffusion coefficients through these layers of skin and may be simplistically related to the penetrant molecular weight (M) via equation (1).

$$D = C.M^{-1/3} \qquad \text{----------(1)}$$

where, D is the molecular weight and C is a constant. (The validity of equation (1) is assumed for diffusion in the skin.) For benzoic acid (BA), k_1

and k_2 values have been established and hence, may be used in equations (2) and 3 to calculate k_1 and k_2 parameters for other penetrants.

$$k_1 = k_1^{BA}\left(M^{BA}/M\right)^{1/3} \quad ----(2)$$

$$k_2 = k_2^{BA}\,(M^{BA}/M)^{1/3} \quad ----(3)$$

The k_3 rate constant describes the affinity of the penetrant for the stratum corneum compared to the viable epidermis. k_3 compensates for the estimation of k_1 and allows for greater interaction between penetrant and stratum corneum (thereby producing slower rates of transport out of the horny layer). The ratio k_3/k_2 may be viewed as an effective partition coefficient between stratum corneum and viable epidermis; the larger the ratio, the longer the penetrant transit time across the outermost skin layer. It has been shown, for most of the compounds analysed with the kinetic approach, that k_3/k_2 appears to be linearly correlated with the corresponding octanol/water partition coefficient (K), and the relationship:

$$k_3/k_2 = K/5 \quad -----------(4)$$

describes this dependence adequately. Thus, if K is known, equations 2 to 4 can be used to estimate k_1, k_2 and k_3 for any penetrant on the basis of physicochemical properties alone.

Finally, k_4 is the elimination rate constant of the drug from the blood. More complicated excretion behaviour can be used, if necessary. k_4 cannot be predicted but must be measured following I.V. administration of the penetrant.

A series of differential equations characterise the transdermal absorption of drug from the device into the body according to the scheme shown in fig. 3.13. Solution of the kinetic expressions in such a situation then leads to the following equation for the concentration of drug in the blood (c);

$$c=\left\{\frac{Ak^{0}k_1k_2}{V}\left[\begin{array}{l}1/\alpha\beta\varepsilon-\exp(-\alpha t)/(\alpha(\alpha-\beta)(\alpha-\varepsilon))-\exp(-\beta t)/(\beta(\beta-\alpha)(\beta-\varepsilon))\\-\exp(-\varepsilon t)/\varepsilon((\varepsilon-\alpha)(\varepsilon-\beta))\end{array}\right]\right\}+$$

$$\left\{\frac{M_{ad}k^{1}k_1k_2}{V}\left[\begin{array}{l}\exp(-\alpha t)/((\beta-\alpha)(\alpha-\omega)(\alpha-\mu))\\-\exp(-\beta t)/((\alpha-\beta)(\beta-\omega)(\beta-\mu))\\-\exp(-\omega t)/((\alpha-\omega)(\omega-\beta)(\omega-\mu))\\-\exp(-\mu t)/((\alpha-\mu)(\mu-\beta)(\mu-\omega))\end{array}\right]\right\}----(5)$$

The first series of terms in curly brackets is the zero order contribution, while the second is the first order component. In equation (5), A is the surface area of the delivery system; M_{ad} is the amount of drug in the "priming" contact adhesive; V is the volume of distribution of the drug; and α, β, ω and μ are defined by equations (6) to (8).

$$(\alpha+\beta)=k_2+k_3+k_4\ ;\ \alpha\beta=k_2k_4 \quad \ldots\ldots\ldots\ldots(6)$$

$$\varepsilon\ \omega=k_1+k_r \quad \ldots\ldots\ldots\ldots\ldots(7)$$

$$(\omega+\mu)=k^{1}+k_r+k_1\ ;\omega\mu=k^{1}k_1 \quad \ldots\ldots\ldots\ldots(8)$$

Nicardipine HCl was evaluated as a candidate drug for transdermal delivery using the above model.

Parameters for Nicardipine HCl

⇒ Molecular weight : 516

⇒ $K_{o/w}$: 0.1023 [21]

⇒ V (Volume of distribution) : 52.5 litres [22]

⇒ A (cm^2) : 16 cm^2

⇒ k^0 : (1)75 $mg/cm^2/hr$

⇒ 31.25 $mg/cm^2/hr$

⇒ k_1 : 0.1113 hr^{-1}

⇒ k_2 : 1.7932 hr^{-1}

⇒ k_3 : 0.03676 hr^{-1}

⇒ k_4 : 0.5507 hr^{-1}

Calculation Of Different Parameters:

I. V (Volume Of Distribution):

Volumes of distribution of Nicardipine in healthy volunteers have ranged from 0.6 litres/kg to 63 litres [22]. An average value of 52.5 litres was therefore considered here.

II. k^0 (mg/cm^2 /hr) (Input Rate Of Drug Required From Patch):

Intravenous Nicardipine infusion is given at 3 different infusion rates of 0.5 mg/hr, 1.2 mg/hr and 2.2 mg/hr corresponding to 20 mg in 8 hrs., 30 mg in 8 hrs. and 40 mg in 8 hrs. orally, respectively [23].

The plasma concentration at steady state (C_{SS}) following an intravenous infusion is given by the following equation :

$$C_{SS} = \text{Infusion rate/ Clearance} \quad \text{-------} (9)$$

The plasma clearance of Nicardipine hydrochloride is reported to be 0.4 L/hr/kg [22]. An average body weight of 70 kg is considered. Thus, the total clearance is,

Cl = 0.4 x 70 = 28 L/hr.

Using equation (9), C_{SS} of 17.85 ng/ml and 42.85 ng/ml are obtained for infusion rates of 0.5 mg/hr and 1.2 mg/hr.

For a patch of 16 cm^2 area, which can deliver IN= 0.5 mg/hr and IN= 1.2 mg/hr. of drug, k^0 is calculated as follows:

$$IN = k^0 \times A. \text{------}(10)$$

Therefore, k^0 is 31.25 $\mu g/cm^2/hr$ and 75.00 $\mu g/cm^2/hr$, when IN = 0.5 mg/hr and IN = 1.2 mg/hr respectively.

$$k_1 = k_1^{BA}\left(M^{BA}/M\right)^{1/3} = 0.18(122/516)^{1/3} = 0.1113 hr^{-1}$$

$$k_2 = k_2^{BA}(M^{BA}/M)^{1/3} = 2.90(122/516)^{1/3} = 1.7932 hr^{-1}$$

Using values of $M=516$, $M^{BA} = 122$, $k_1^{BA} = 0.18$ hr^{-1} and $k_2^{BA}= 2.9$ hr^{-1}[25]

$k_1 = 0.1113$ hr^{-1} and $k_2 = 1.7932$ hr^{-1}.

$$k_3 = \frac{K_{o/w}}{5} \cdot k_3 = \frac{0.1023}{5} \cdot 1.7932 = 0.03676 hr^{-1}$$

k_4 (elimination rate constant from the body)

$$k_4 = 0.693 / t_{1/2} \quad \text{-------(11)}$$

$t_{1/2}$ for Nicardipine hydrochloride has been reported to be 44-107 minutes [21]. An average value of 75.5 minutes i.e. 1.258 hours has been used to calculate k_4. Thus,

$k_4 = 0.693/ 1.258 = 0.5507$ hr^{-1}.

Note :

k_r i.e. the tendency for the drug to diffuse from the stratum corneum to the patch is assumed to be negligible.

Only the zero order component of release is considered for predicting the plasma profiles of the drug; the first order component helps in reaching the steady state faster. However, consideration of the only the zero order release is good enough for predicting blood levels since a monolithic system does not contain rate controlling membrane and for membrane-controlled delivery system drug loading on rate controlling membrane can be avoided.

Thus, using the parameters, the values for α,β and ε were calculated,

$$(\alpha+\beta)=k_2+k_3+k_4=1.7932+0.03676+0.5507=2.38066$$

$$\alpha\beta=k_2k_4=1.7932\times0.5507=0.9875$$

$$\frac{0.9875}{\beta}+\beta=2.38066$$

$$\beta^2-2.38066\beta+0.9875=0$$

Solving the quadratic equation, following values are obtained,

$\alpha = 1.84558$, $\beta = 0.53508$

$\varepsilon = k_1 + k_r = 0.1113$

The equation for predicting plasma profiles of the drug is:

$$c=\left\{\frac{Ak^0k_1k_2}{V}\left[\begin{array}{l}1/\alpha\beta\varepsilon-\exp(-\alpha t)/(\alpha(\alpha-\beta)(\alpha-\varepsilon))-\exp(-\beta t)/(\beta(\beta-\alpha)(\beta-\varepsilon))\\-\exp(-\varepsilon t)/\varepsilon((\varepsilon-\alpha)(\varepsilon-\beta))\end{array}\right]\right\}$$

$$1/\alpha\beta\omega=1/1.84558\times0.53508\times0.1113=9.098153.1$$

$$1/\alpha(\alpha-\beta)(\alpha-\omega)=1/1.584558\times1.3105\,1.73428=0.238402$$

$$1/\beta(\beta-\alpha)(\beta-\varepsilon)=1/0.53508\times(-1.3105)\times(0.42378)=-3.3651$$

$$1/\varepsilon(\varepsilon-\alpha)(\varepsilon-\beta)=1/0.1113\times(-0.42378)\times(-1.73428)=1222489$$

$$-\frac{Ak^0k_1k_2}{V}=\frac{16\times31.25\times0.1113\times1.7932}{525}=1.9008\,wherk^0=31.5$$

$$\frac{Ak^0k_1k_2}{V}=\frac{16\times7500\times0.1113\times1.7932}{525}=4.562\,wherk^0=7500$$

Thus,

$$c=1.9008\left[9.09815-0.2384e^{-1.84558t}+3.3651e^{-0.53508t}-1222489e^{-0.1113t}\right]$$

when Input rate is IN = 0.5 mg/hr

and

$$c=4.562\left[9.09815-0.2384e^{-1.84558t}+3.3651e^{-0.53508t}-1222489e^{-0.1113t}\right]$$

when Input rate is IN = 1.2 mg/hr.

Table 3.4: Calculated values for exponential terms versus the time mentioned.

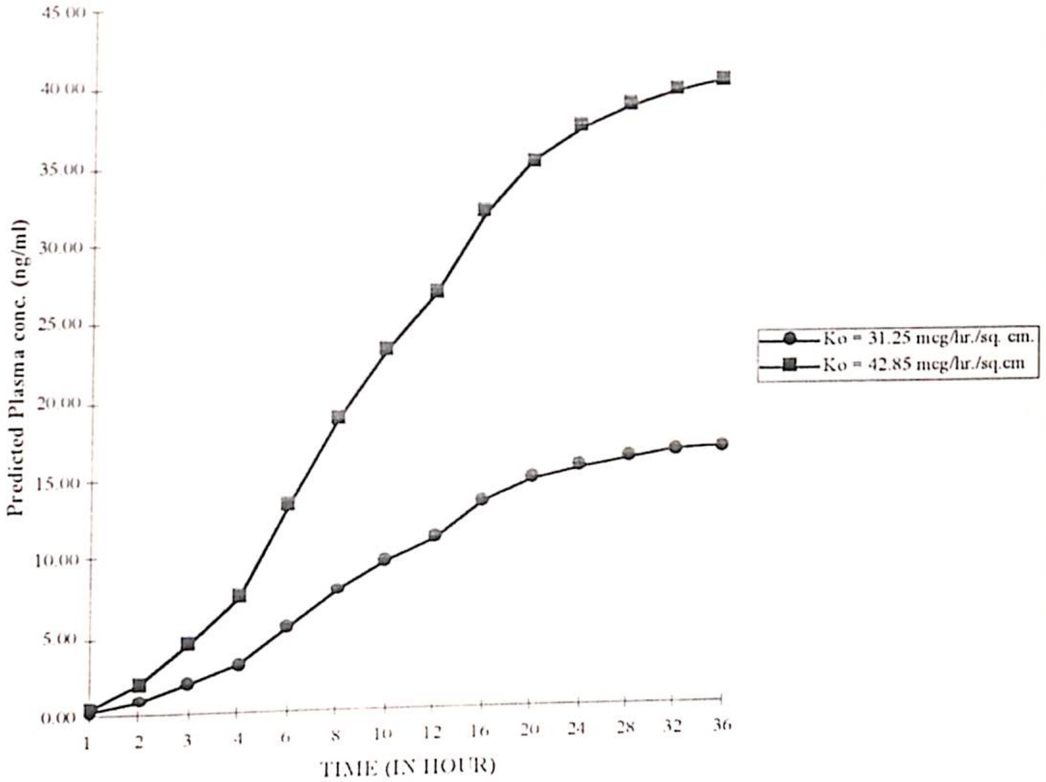
Time t(hr)	$e^{-1.84558.t}$	$0.2384 \times e^{-1.84558.t}$	$e^{-0.53508.t}$	$3.3651 \times e^{-0.53508.t}$	$e^{-0.1113.t}$	$12.22489 \times e^{-0.1113.t}$	Total in brackets
1	0.1579	0.037651	0.58562	1.9706	0.8946	10.9371	0.093999
2	0.0249	0.0059	0.3429	1.1540	0.8004	9.7851	0.46115
3	0.0039	0.0009	0.2008	0.6758	0.7161	8.7544	1.01865
4	0.0006	0.00014	0.1176	0.3957	0.6406	7.8323	1.66141
6	0.0000	0.0000	0.0403	0.1357	0.5128	6.2693	2.96455
8			0.0138	0.0465	0.4104	5.0181	4.12655
10			0.0047	0.0159	0.3285	4.0167	5.09735
12			0.0016	0.0054	0.2630	3.2151	5.88845
16			0.00019	0.0006	0.1685	2.0599	7.03885
20			0.00002	0.0000	0.10795	1.3197	7.77845
24					0.0691	0.8455	8.25265
28					0.0443	0.5417	8.55645
32					0.0284	0.3471	8.75105
36					0.01819	0.2223	8.87585

Table 3.5: Plasma levels after substituting the values from Table 3.4.

Time t(hrs)	Predicted plasma levels when $k^0 = 31.25$ µg/hr/cm^2 (ng/ml)	Predicted plasma levels when $k^0 = 42.85$ µg/hr/cm^2 (ng/ml)
1	0.3396	0.4288
2	0.8765	2.0137
3	1.9362	4.6471
4	3.1580	7.5793
6	5.6350	13.5242
8	7.8437	18.8253
10	9.6890	23.2541
12	11.1927	26.8631
16	13.3794	32.1112
20	14.7852	35.4852
24	15.6866	37.6485
28	16.2641	39.0345
32	16.6339	39.922
36	16.8712	40.49

75·0

FIG. No. 3.15: Predicted plasma levels of Nicardipine HCl when Ko = 31.25 and 42.85 mcg/sq. cm./hr.



A patch of 16 cm^2 area which delivers 31.25 $\mu g/cm^2/hr$ will deliver 12 mg in 24 hours. Assuming only 20% of the drug permeates through, the dose of Nicardipine Hydrochloride required in the patch = 60 mg.

Similarly, a patch of 16 cm^2 area which delivers 42.85 $\mu g/ cm^2$ /hr will deliver 16.454 mg in 24 hours. Assuming only 20% of the drug permeates through, the dose of Nicardipine Hydrochloride required in the patch = 82.27 mg.

The therapeutic plasma concentrations of Nicardipine are reported to be in the range 6 - 40 ng/ml [25]. If a target plasma level of 20 ng/ml is considered to be ideal then a transdermal therapeutic system (TTS) which can deliver 1.2 mg/hr of Nicardipine Hydrochloride will achieve the desired levels in 6-8 hrs. However a TTS which can deliver 0.5 mg/hr also achieves therapeutic levels in 6-8 hrs.

Conclusion:

It is feasible to consider Nicardipine HCl as a candidate for transdermal drug delivery.

Alternative Equations For Calculation Of Plasma Concentrations:

Simple equations to calculate flux, amount permeated, lag time are [26].

$$\frac{dM}{dt} = \frac{DSK(C_d - C_r)}{h} \text{ ---------(1)}$$

Where dM/dt is the rate of permeation of the drug, S is the cross-sectional area of the membrane, K is the distribution or the partition coefficient, h is the thickness of the membrane, C_d and C_r are concentration of the drug on the donor side and the receptor side of the membrane respectively.

If sink conditions hold in the receptor compartment, $C_r \equiv 0$,

$$\frac{dM}{dt} = \frac{DSKC_d}{h} = PSC_d \text{ ------------(2)}$$

in which $$P = \frac{DK}{h} \text{ --------- (3)}$$

P is known as the permeability coefficient and has units cm/sec. One can then obtain P from the slope of a linear plot of M (amount permeated) vs t (time):

$$M = PSCdt \text{ --------(4)}$$

In the case of a time lag, equation 4 gets modified to:

$$M = \frac{SDKC_d(t - tL)}{h} \text{-------------(5)}$$

The lag time tL is given by:

$$tL = \frac{h^2}{6D} \text{ ----------(6)}$$

and its measurement provides a means of calculating the diffusivity D, presuming a knowledge of the membrane thickness, h. Also knowing P the thickness can be calculated from :

$$t_L = \frac{h}{6P} \text{------------}(7)$$

Flux:

The amount M of material flowing through a unit cross section, S, of a barrier in unit time, t, is known as the flux , J.

$$J = \frac{dM}{S.dt} \text{ ---------}(8)$$

In some cases it is not possible to determine D, K, or h independently and thereby to calculate P. It is a relatively simple matter to measure the rate of barrier permeation, to obtain the surface area S, and concentration C_d in the donor phase and the amount of permeant M in the receiving sink. One can then obtain P from the slope of a linear plot of M versus t.

From the slope, flux can be calculated as follows:

$$Slope = \frac{DSKC_d}{h} \text{ ----------}(9)$$

$$J = \frac{M}{S.t} = \frac{DKC_d}{h} = \frac{Slope}{S} \text{----------}(10)$$

As an example, the flux (amount permeated in 24 hours) was calculated for Nicardipine using equations (5) and (10) and data given in Ref. 25 (shown in Chapter 2, Page 101). From the plot of permeated amounts (mg) versus time (hours), the slope was calculated using the equation :

$$Slope = \frac{y_2 - y_1}{x_2 - x_1} = \frac{10 - 5}{53.75 - 35} = \frac{5}{18.75} = 0.2666$$

using $\{(x_1,y_1) = (35,5)\}$ and $\{(x_2,y_2) = (53.75,10)\}$

The authors have used skin of cross sectional area 3.142 cm^2 . Therefore, the flux is :

$$J = \frac{Slope}{S} = \frac{0.2666}{3.142} = 0.0848 \ mg.hr^{-1}cm^{-2}$$
$$= 8.48 \ mg.hr^{-1}cm^{-2} \cdot 10^2$$

From the plot, the lag time is found to be 16 hours. Amount permeated in 24 hours is calculated using equation (5).

$$M = slope(t - tL) = 0.2666(24 - 16) = 2.1328\ mg.$$

Diez et al [25] have used a non-linear least squares computer program (MULTI) for calculating the different parameters. However, the above equations which have been used here give similar values thus establishing the validity of simple equations used above.

Table 3.6: Pharmacokinetic parameters of Nicardipine HCl .

Parameter	Values calculated using above equations	Values reported in Ref. 25
Flux $mg.hr^{-1}cm^{-2} . 10^2$	8.48	8.76 ± 2.61
Amount permeated in 24 hours (mg)	2.1328	2.63 ± 0.755

Diez et al [25] have also given four equations for calculating daily transdermal dose (DT), the theoretical permeated amount in 24 hours (X24), the theoretical daily permeated amount in steady state conditions (Dss), and the theoretically predicted plasma steady-state concentrations (C_{ss}).

$$DT = DO . [100 - E(\%)] / 100 \quad ....(11)$$

Where DO is the daily oral dose and E(%) is the percent extraction ratio;

$$X24 = Q24 . TTS\ area / A.3 \quad ....(12)$$

Where Q24 is the amount permeated in 24 hours, "TTS area" is the surface of the theoretical TTS (Diez et al considered 16 cm^2 as a reasonable limit), A is the surface area of cell permeation (Diez et al considered 3.142 cm^2) and 3 is the correction factor between the penetration rates of rat and human skin;

$$D_{ss} = J. TTS\ area. t/3 \quad .....(13)$$

Where J is the flux, t is the time (as D_{ss} is the daily permeated dose, t equals 24 hr) and 3 is the above mentioned correction factor of permeability;

$$C_{ss} = J. TTSarea . t/3\ CL \quad ......(14)$$

Using equation (11), (12), (13) and (14) Diez et al [25] have calculated DT, X24, C_{ss} and D_{ss}. The values are given in the following table:

Table 3.7: Pharamcokinetic parameters (calculated) for Nicardipine HCl.

Do (mg) Oral daily dose	60 mg
DT (mg) Theoretical daily transdermal dose	14.10 mg
X24(mg) Predicted permeated amount during 1st 24 hours.	4.459 mg
D_{ss}(mg) Predicted permeated amount in steady state condition	11.21 mg
C_{ss} (ng/ml) Predicted plasma levels in steady state	13.90 ng/ml
CT (ng/ml) Therapeutical plasma concentrations	6 - 40 ng/ml
CL (L/hr) Clearance	33.6 L/hr.

From the table, it can be seen that predicted plasma levels at steady state (13.90 ng/ml) are within therapeutical levels. Therefore, Nicardipine HCl may be considered to be a candidate for transdermal delivery.

3.20. Matrix-Dispersion Controlled System:

Since the major amount of work was done on Nitroglycerin with this type of system, same expertise was used here except that the drug was dissolved in ethyl alcohol instead of ethyl acetate and mixed with solvent based adhesive, due to insolubility in ethyl acetate. Initial release rates being very poor, hence aqueous based polymer used for diclofenac was tried and some release rates were established using different excipients as described in experimental section.

3.21. Components Of The Systems:

3.21.1. Backing Membrane:

The backing membrane tried for this system was siliconised PET film 50 µm thick.

3.21.2. Release Liners:

The material used for this system was siliconised PET film of 150 µm.

3.22. Formulation Development :

After several trials with different types of polymer, thickening agents, auxiliary solvents (like Propylene glycol, Glycerine etc.), the formulation containing emulsion based polymers, Gelatine, Glycerine , Propylene glycol and preservatives containing 60 mg of Nicardipine HCl in 16 sq. cm. area and having weight of 1.87 gm was finally selected for further studies.

3.23. Evaluation Of Patches:

3.23.1. Analytical Methods:

3.23.2. Nicardipine HCl Content in Adhesive-Dispersion And Patches:

The assay of the patch was done by HPLC method. For this, the patch/adhesive-dispersion was extracted with absolute ethanol, centrifuged and injected. The area response was compared with that of standard. After confirmation of Nicardipine content in the patches further evaluation was done. The HPLC parameters are given below:

- *HPLC pump* : Jasco Intelligent 980
- *Detector* : Jasco UV 975
- *Absorption* : 275 nm
- *Integrator* : Borwin chromatography version 1.21
- *Column* : Symmetry (waters), ODS, 5μ, 4 X 150 mm.
- *Operating Temperature* : Room Temperature
- *Solvent system* : Perchloric acid (43 in 50,000) : Acetonitrile (3:2)
- *Retention Time* : 5.7 min.

3.23.3. In Vitro Permeation Study:

The in vitro study was conducted on synthetic membrane followed by pig skin same as described in the experimental section.

3.24. Membrane Controlled Drug Delivery System:

The development work of this type of system consists of selection of all the components viz. rate controlling-membrane, backing membrane, release liners and adhesives. This is essentially similar to the one used for nitroglycerin except the change in the matrix composition.

3.24.1. Evaluation of patches:

Drug content and in vitro study was carried out in similar fashion as described above for matrix system.

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Chapter IV.

EXPERIMENTAL

4. Synthesis Of Adhesive:

4.1. Synthesis Of Acrylic Adhesives (Solvent Based):

4.1.1. Preliminary work:

As reported by Dr. Lalla (personal communication, M. Pharm. Thesis of Ms. Varda Bapat), adhesives therein were initially synthesized. However, these did not meet desired tackiness and viscosity. Hence, the procedure was slightly modified by increasing the concentration of AIBN, inclusion of small amount of vinyl acetate monomer and decreasing the concentration of Dioctyl Maleate. The final compositions of the reactants as well as reaction conditions were optimised after several trials.

4.1.2. Final experiment:

Based on preliminary experiments, the final composition and method used for the manufacturing is given below,

Table 4.1: Composition of PSA -S3.

Batch Size: 1.0 kg.

Reaction Time: 20 Hrs.

Sr. No.	Ingredients	Quantity
1.	Ethyl acetate	500 gms.
2.	2- ethyl hexyl acrylate	300 gms.
3.	AIBN	3.0 gms.
4.	Dioctyl Maleate	20 gms.
5.	Butyl acrylate	200 gms.

Procedure:

1 250 gms. of ethyl acetate was placed in resin kettle and allowed to reflux.

2 Butyl acrylate, 2-ethylhexyl acrylate, Dioctyl Maleate and AIBN (1.5 gms.) were added, in the sequence mentioned to 250 gms. of ethyl acetate separately and stirred for 10 mins.

3 The reaction mixture, obtained in step 2, was added in a thin stream over a period of 30 mins. to ethyl acetate under refluxation in step 1.

4 As the reaction was exothermic, the resin kettle was immersed in an ice bath, the temperature was not allowed to rise above 70°C. The heating was continued while maintaining the temperature at 70 °C for 15 hrs on water-bath at 80 rpm.

5 The reaction was terminated by adding the remaining 1.5 gms of AIBN and continued the heating for another 3 hrs. on hot water-bath increasing the temperature gradually upto 90 °C.

6 After cooling, the polymer was collected in a glass container and stored in refrigerator till its use.

4.2. Synthesis Of Acrylic Adhesives (Aqueous Based):

4.2.1. Preliminary Work:

As reported by Dr. Lalla (personal communication, M. Pharm. Thesis of Ms. Varda Bapat), work done by Ms. Bapat on aqueous based acrylic adhesive was pursued and the quality required was obtained after several trials and which involved various changes such as inclusion of Hydroxy ethylcellulose, Formalin, Dibutyl phthalate, sodium benzoate and potassium persulfate. The final formulation emerged out of all these trials was found to be suitable for experimental work.

4.2.2. Final experiment:

The composition and method for final adhesive selected and used for the formulating the patches is given in Table 4.2,

Table 4.2: Composition of PSA-A2.

Batch Size: 1.0 KG

Sr. No.	Ingredients	Quantity (In gms.)
1.	Vinyl acetate	200.0
2.	Dioctyl Maleate	200.0
3.	2- Ethyl hexyl acrylate	110.0
4.	Hydroxy ethyl cellulose	20.0
5.	Ammonium persulfate	5.0
6.	Potassium persulfate	5.0
7.	Dibutyl phthalate	20.0
8.	Sodium benzoate	1.0
9.	Formalin	1.0
10.	Nonyl phenyl ethylene oxide condensate	15.0
11.	water	qs 1.0 Kg.

Procedure:

1. Vinyl acetate, dioctyl maleate, and 2- ethylhexyl acrylate were taken in a resin kettle along with Nonyl phenyl ethylene oxide condensate and mixed.
2. In another suitable glass container, hydroxy ethyl cellulose was dispersed in water under constant stirring for 0.5 hr. To this, potassium persulfate, ammonium persulfate and Dibutyl phthalate were added and mixed for 20 minutes and transferred to resin kettle, in step 1. The resultant mixture was allowed to stir continuously for 4 hrs. in ice-bath after which the temperature was gradually raised to 60 °C. After establishing the proper tack, the temperature was raised to 85 °C and stirring was continued for additional 2 hrs.
3. The final solution was transferred to a suitable container and stored in the refrigerator till used.

4 .3. Evaluation Of Polymers:

4.3.1. Acrylic Adhesive (Solution Based):

Physical Appearance:

Clear viscous solution.

Solid Content:

Solid content of polymer was determined by evaporating the solvents at 80°C to constant weight. (49-51 %).

Analysis Of Free Monomers In Polymer:

The analysis of free monomers was done by HPLC. Following are the details of the method:

- *HPLC pump*: Jasco Intelligent 980
- *Detector*: Jasco UV 975
- *Detection* : 240 nm
- *Integrator*: Borwin Chromatography, version 1.21.
- *Column*: Shandon, C18, Hypersil, 10μm, 4.6x250 mm.
- *Operating Temperature*: Room Temperature
- *Solvent system*: Methanol
- *Flow rate*: 1 ml/min.
- *Retention time* : 3.3 min.

The content of free monomers mainly 2-ethylhexyl acrylate was determined by comparing with standard solution of 2-ethylhexyl acrylate (99.9% pure) of approximately same concentration and was found to be less than 0.2 %.

Viscosity:

Viscosity was found to be 50±5 cps. (done in triplicate), measured using Brookfield RVT model, spindle 4 at 10 rpm and at 30°C.

Determination Of Molecular Weight:

The analysis of average molecular weight (M_w) was using Gel Permeation

Chromatography (GPC). The equipment is equipped with software with the facility to calculate average mol. wt. automatically from the standards used. Following are the details of the method:

- *HPLC pump*: Waters
- *Detector*: Waters, Refractive Index.
- *Integrator*: Waters, 745B.
- *Column*: 1. 100 Å, 250 x 10 mm SS; 2. 500 Å , 250 x 10 mm SS; 3. 10^3 Å 250 x 10 mm SS ; 4. 10^4 Å, 500 x 22 mm SS.
- *Operating Temperature*: Room Temperature.
- *Solvent system* : Tetrahydrofuron.
- *Flow rate*: 1.5 ml/min.
- *Sample Size & Standards* : 0.5 ml of 0.05 % concentration of polystyrene pure standard (Waters).

The Avg. Mol wt. obtained was 62,985.

4.3.2. Acrylic Adhesive (Aqueous Based):

Physical Appearance:

Milky white viscous solution.

Solid Content:

Determined by evaporating the solvents at 80°C to constant weight (56±1 %).

Analysis Of Free Monomers:

Analysis was carried out as described in Section 4.3.1 on Page 150.

Viscosity:

Viscosity was found to be 90±5 cps. (done in triplicate), measured using Brookfield RVT model, spindle 4 at 10 rpm and at 30°C.

Determination Of Molecular Weight:

Methodology followed was it as in Section 4.3.1 as given above.

The Avg. mol. wt. found was 73,490.

4.4. Formulation Development:

4.4.1. NITROGLYCERIN PATCHES:

4.4.2. Laboratory Scale Development:

4.4.3. Preliminary Work:

Several formulations using different combinations of adhesives and NTG (extracted from lactose triturate) solutions were made in different solvents such as petroleum ether, hexane, ethyl acetate and chloroform and coated on backing membrane using the laboratory applicator (described in Section 3.8.2. on Page 119). Subsequently the patches were subjected to primary skin irritation on rabbits for comparison of in vitro release profile with Nitro Dur®, using synthetic membrane and pig skin. After establishing safety and efficacy of the patch developed, having comparative in vitro release rates as Nitro Dur® of the same area using modified Franz cell, following is the composition of patch finalised:

Table 4.3: Composition of NTG patch 40 mg. B. No. NTG-A40 -01.

Sr. No.	**Ingredients**	**Quantity (In ml)**
01.	NTG solution (35 % in ethyl acetate)	150 ml (≡52.5 gms. of pure drug)
02.	Solution Polymer PSA-S3	225 gms.
03.	Ethyl acetate	75 ml.

Procedure:

1. Ethyl acetate was mixed with NTG solution for 10 min. in closed vessel followed by addition of solution polymer PSA-S3 and mixing for 1 hr. NTG content in the mass was analysed.
2. With predetermined clearance of the machine, the solution was applied on the PET backing membrane with the help of laboratory applicator and the mass was allowed to dry for 24 hrs. in dehumidified room provided with air circulation.

3. The film was laminated with the release liner and the patches were cut into round pieces of 10 cm^2 each with a central complete cut on the release liner (for the ease of removing the liner).

The patches were individually packed in alupoly (gauge 40/150 µm) hand made pouches and kept in the refrigerator till the completion of the studies.

4.5. Evaluation Of Patches:

4.5.1. In Vitro Release Of NTG Patches:

The in vitro release of NTG patches was carried out on the synthetic membrane, pig skin, and cadaver skin.

Synthetic Membrane (Cellulose Acetate, 0.45 µm Pore Size):

In vitro release through cellulose acetate membrane 0.45 µm was carried out.

For reference Nitro Dur® 40 mg was used.:

(Note- All solvents used were of HPLC grade and the reagents were of GR grade. Phosphate buffer pH 7.4 used was prepared as per USP XXIII, Page 2049-2050). Following was the methodology used for analysis:

- The modified Franz cells were mounted on the synchronised magnetic stirrer plate (Photographs of cells and assembly are shown on Page 236 in Chapter 5) and water circulation was provided to maintain the temperature of the cells at 32 °C (± 0.5 °C).
- The cells were filled upto neck with phosphate buffer USP pH 7.4 and allowed to reach the equilibrium temperature by putting bar magnets in the cells and continuous stirring at 60-70 rpm. Care was taken to avoid vortex formation.
- The cellulose acetate membranes were cut into circular pieces, to fit over the opening of the cells and were placed over the cells. Care was taken to avoid formation of voids at the interface.
- The membranes were allowed to equilibrate at 32 °C by continuing the stirring for 1 hr.

- After removing the release liners, patches were applied on each cellulose acetate membrane with minimum disturbance to the membrane and care was taken to ensure firm adherence of the patches to the membrane.
- 0.2 ml samples were collected at 0, 1, 2, 3, 4, 6, 8, 12 and 24 hrs while every time 0.2 ml of fresh Phosphate buffer pH 7.4 was replaced.
- The samples collected were directly injected.

  The HPLC parameters used are given below:

  - *HPLC pump* : Jasco Intelligent 980
  - *Detector* : Jasco UV 975
  - *Detection* : 220 nm
  - *Integrator* : Borwin Chromatography, version 1.21.
  - *Injection volume* : 20 µl.
  - *Column* : Bondapack, ODS, 10µ, 3.9 X 300 mm.
  - *Operating Temperature* : Room Temperature
  - *Solvent system* : Methanol: water (1:1)
  - *Flow rate* : 1 ml/min.

The results are given in Chapter 5 on Page 180-181 for NTG patch 40 mg B. No. NTG-A40-01 and Nitro Dur®, respectively. A typical chromatogram is also show on Page 221 in Chapter 5.

Validation of the HPLC method :

The method was validated for precision, accuracy, limit of detection (DL) and limit of quantitation (QL) and the method adopted was as follows:

Precision:

For measuring the precision, a known concentration (20 µg/ml) of standard NTG solution was injected six times and relative standard deviation (std. dev.) of not more than 2% was considered as acceptable limit. The results are given in Table 5.27 on Page 207 of Chapter 5 and representative chromatogram is shown on Page 219.

Accuracy by linearity:

Accuracy of method was carried out by establishing linearity of different concentrations of NTG from 20-100 μg/ml. A correlation coefficient of 0.999 was accepted as the limit. The area response to the different concentrations is given in Table 5.28 on Page 207 and typical chromatogram of 30 μg/ml is shown on Page 220 of Chapter 5.

Limit of Detection:

DL was established using the following methodology:

From the linearity curve obtained above and response to different concentrations in terms of height, slope was calculated. The noise level on that particular day was calculated by putting the system on and measuring the response in μvolts for 30 seconds. For limit of detection (DL) following equation is used;

DL = Slope x Noise x 3

Limit of quantitation (QL):

QL was calculated in a same way as described above except the equation used is as follows;

QL = Slope x Noise x 10.

The results of DL and QL are shown on Page 207 in Table 5.28 in Chapter 5.

In vitro Permeation Study Through Pig Skin :

Analysis was carried using HPLC and parameters are given in section 4.5.1. All the materials used for the analysis were similar to as mentioned in Section 4.5.1. for in vitro analysis using cellulose acetate membrane.

Preparation And Mounting Of Pig Skin On Modified Franz Cell:

- Freshly excised pig skin (from abdomen region of the same pig) was collected from a known source and the fatty layer was removed carefully to avoid damage to epidermal layer. Hairs were removed with the help of scissors.

- The Franz cells were mounted on the synchronised magnetic stirrer plate and water circulation was provided to maintain the temperature of the cells at 32 °C (± 0.2 °C).
- The cells were filled upto neck with Saline Phosphate Buffer pH 7.4 and allowed to reach the equilibrium temperature by putting bar magnets in the cells and continuous stirring at 60-70 rpm. Care was taken to avoid vortex formation.
- The cleaned skin was cut into circular pieces to fit over the opening of the cell and were placed over the cells. Care was taken to avoid the formation of voids at the interface.
- The mounted skin was allowed to equilibrate at 32 °C ± 0.5 °C by continuing stirring for 24 hr.
- After removing the release liners, patches were applied on each cell with minimum disturbance to the skin and care was taken to ensure the firm adherence.
- 0.2 ml samples were collected at 0, 1,2 ,3, 4, 6, 8, 12 and 24 hrs while replacing with fresh 0.2 ml of Phosphate buffer USP pH 7.4.
- The samples collected were stored in refrigerator till analysis .

The results are shown in Chapter 5 on Page 182-183 for NTG patches B. No. NTG-A40-01 and Nitro Dur® 40 mg, respectively.

4.5.2. Primary Skin Irritation Study:

The aim of this experiment was to find out the topical irritation and the local tolerance of the transdermal patch B. No. NTG-A40-01 on rabbits.

- The experiment was conducted on six rabbits (3 males and 3 females) weighing between 1.8 and 2.0 kg.
- Before commencement of the experiment, the skin of every rabbit was thoroughly examined for any abnormality and only those having no structural abnormality of the skin were included in the experiment.

Table 4.4: Composition of NTG patch 40 mg. B. No. NTG-A40 -02.

Sr. No.	Ingredients	Quantity (In ml)
1.	NTG solution (35 % in ethyl acetate)	300 ml (≡105 gms. of pure drug)
2.	Solution Polymer PSA-S3	500 gms.
3.	Ethyl acetate	100 ml

The procedure followed for the preparation of mass was same as used for the laboratory scale given in Section 4.4.3. The application was carried out after adjusting the application amount with the help of application roll and kept for drying (solvent evaporation) for 6 hrs. with the help of blower at room temperature.

The batch was finally packed similarly in alupoly foils as described earlier .

4.7. Evaluation Of NTG Patches:

4.7.1. In Vitro Release:

The in vitro release was performed on pig skin of same pig and cadaver skin on one occasion while using Nitro Dur® as reference standard while the methodology used was the same as described in Section 4.51.

The results are shown in Chapter 5, from Page 185 to 186 for NTG patch and Nitro Dur® for pig skin while on Page 187 that for cadaver skin, respectively .

4.7.2. In Vivo Release:

The in vivo experiments were carried out to compare NTG release of the NTG patch B. No. NTG-A40-02 with Nitro Dur® 40 mg as standard.

Following are the details of the procedure:

- The experiment was conducted on six rabbits (3 males and 3 females) weighing between 1.8 and 2.0 kg using cross over design and one week wash out period was given in between cross over experiments.

- Before commencement of the experiment, the skin of every rabbit was thoroughly examined for any abnormality and only those having no structural abnormality of the skin were included in the experiment.
- The skin about 6 cm. in diameter was shaved on one side of vertebral column of each rabbit and care was taken to avoid the damage to the skin during shaving.
- Before application of the patch, rabbits were kept for 24 hrs. to subside the excitation of the skin which might be caused due to shaving process and were fasted during this period.
- On the shaved surface, of the rabbits, patches were applied.
- 2 ml of blood was withdrawn from the ear vein of the rabbit at 0, 4, 8, 12 and 24 hrs. interval. Due to small size of the animal and potential damage to the ear vein, it was not possible to withdraw the blood for more than above mentioned intervals.
- After the blood was withdrawn, 0.5 ml of 10% Sodium EDTA was added and mixed thoroughly to avoid coagulation of blood.
- Blood samples were centrifuged for 30 min. at 8000 rpm and plasma was separated.

The separated plasma was kept in refrigerator till analysis.

Sample preparation:

- 1 ml of plasma was extracted with 5 ml of n-pentane twice and n-pentane layer was collected.
- The n-pentane extract collected was evaporated to dryness with gentle flow of nitrogen gas.
- The residue was again reconstituted with 0.2 ml of n-pentane and injected for establishing the response.

Preparation of standard curve:

- 1 ml of standard NTG solution (prepared from 1% NTG solution in alcohol, 99.9 % pure) in n-pentane containing 50, 100, 150, 200 and 500 pg/ml of NTG were placed in five test tubes and evaporated to dryness using nitrogen. 1 ml of rabbit plasma was added to each test tube and mixed thoroughly on cyclone mixer.
- The plasma mixture was extracted with 5 ml of n-pentane twice and n-pentane layer was collected.
- The n-pentane extract collected was evaporated to dryness with gentle flow of nitrogen gas.
- The residue was again reconstituted with 0.2 ml of n-pentane and injected for establishing linearity, accuracy and response of NTG.
- The analysis was carried out using GC-ECD and following were the parameters :

◆ *GC* : Netel Chromatograph Ltd.

◆ *Detector* : Ni^{66} electron capture detector

◆ *Integrator* : Netel Chromatograph

◆ *Column* : Bonded phase, fused silica (BP5, nonpolar); film thickness 3 µm, internal diameter 0.53 mm, outer diameter 0.66 mm; length 6 meters.

◆ *Injection volume* : 2 µl, splitless mode.

◆ *Carrier Gas* : Nitrogen Iolar grade, 7 ml/min . Make up gas 30 ml/min.

◆ Temperature Conditions:

⇒ *Injection port* : 150 °C

⇒ *Column* : 250 °C isothermal.

⇒ *Detector* : 270 °C

- *Retention time* : 7.8 min.

The results are shown on Page 188-190 in chapter 5 for NTG patches B. No. NTG-A40-02, Nitro Dur 40 mg patches and comparison of both, respectively. A typical chromatogram is shown on Page 224 in Chapter 5.

Validation Of GC-ECD Method :

The method was validated for precision, accuracy, limit of detection (DL) and limit of quantitation (QL) and the method adopted was as follows:

Precision:

For measuring the precision, a known concentration (50 pg/ml) of standard NTG solution was injected six times and relative std. dev. of not more than 2% was considered as acceptable limit. The results are given in Table 5.29 on Page 207 and representative chromatogram is shown on Page 222 in Chapter 5.

Accuracy By Linearity:

Accuracy of method was carried out by establishing linearity of different concentrations of NTG from 50-500 pg/ml. A correlation coefficient of 0.999 was accepted as the limit. The area response to the different concentrations is given in Table 5.30 on Page 208 and typical chromatogram 50 pg/ml is shown on Page 223 in Chapter 5.

Limit of Detection:

DL was established using the following methodology:

From the linearity curve obtained above and response to different concentrations in terms of height, slope was calculated. The noise level on that particular day was calculated by putting the system on and measuring the response in µvolts for 30 seconds. For DL following equation is used;

DL = Slope x Noise x 3

Limit Of Quantitation (QL):

QL was calculated in a same way as described above except the equation used is as follows;

QL = Slope x Noise x 10.

The results of DL and QL are shown in Table 5.30 on Page 208 in Chapter 5.

4.8. Stability Study Of Nitroglycerin Patches:

The transdermal patches B. No. NTG-A40-02 was subjected to both long-term and short-term accelerated stability studies.

I. Storage Conditions:

a. Refrigeration - 4-5 °C for 6 months (control)
b. Room temperature - 30 ± 2 °C for 6 months
c. Elevated temperature - 37 ± 2 °C for 3 months

II. Storage interval and sample withdrawal for analysis:

a. Refrigeration - 0, 1, 2, 3, 4, 5 and 6 months
b. Room temperature - 0, 1, 2, 3, 4, 5 and 6 months
c. Elevated temperature - 0, 15, 30, 45, 60, 75 and 90 days.

IV. Method of analysis :

1. Change in appearance
2. Drug content

V. Results:

The results are shown on Page 191 in Chapter 5.

(Note: For the stability studies, each patch was individually packed in heat-sealed foil pouches.)

4.9. Development Of Membrane-Controlled TDDS:

After several initial trials with aqueous acrylate adhesive, Hydroxypropyl cellulose, Carbopol etc., a final formulation which showed encouraging release versus Nitroderm TTS® is shown below.

Table 4.5: Composition of NTG membrane-controlled drug delivery system B. No. NTG-M25-01.

Sr. No.	Ingredients	Quantity for 100 patches
1.	NTG solution 16% in ethyl acetate	15.625 ml (≡2.5 gms. NTG)
2.	Hydroxy Propyl Cellulose (HPC) (10 % solution in propylene glycol)	120 gms.

As described in the Chapter 3 and in Section 3.10, machine developed was utilised. The methodology followed is given below:

- Preparation of 10% HPC Solution:

  12 gms of finely powdered HPC was dispersed in 108 gms. of hot propylene glycol (60 °C) and was dissolved completely by stirring for 3 hrs. and was allowed to cool to room temperature followed by addition of 16% NTG solution and mixing for 30 min.
- Backing liner (metallised polyester) was placed over the die and vacuum was applied to allow the cavity formation.
- With the help of dosing pump, 1.32 gms of drug dispersion was delivered to each of 6 dies.
- Rate controlling membrane (EVA film) was placed over it and was heat sealed at 160 °C with a dwell time of 4 seconds.
- With the help of spray gun (1 mm nozzle), 10% Plastoid E15M solution in water was sprayed at a rate of 15 ml/min. at 7 Kg/cm^2 pressure. The adhesive was allowed to dry completely for 8 hrs and release liner (PET film, 125 µm thickness) was applied. Finally, the patches were die cut to the desired size.
- The patches were packed in alupoly pouches.

Schematic diagram of manufacturing of the same is given in Fig. 4.1, while the photograph of the machine is shown on Page 235 in Chapter 5.

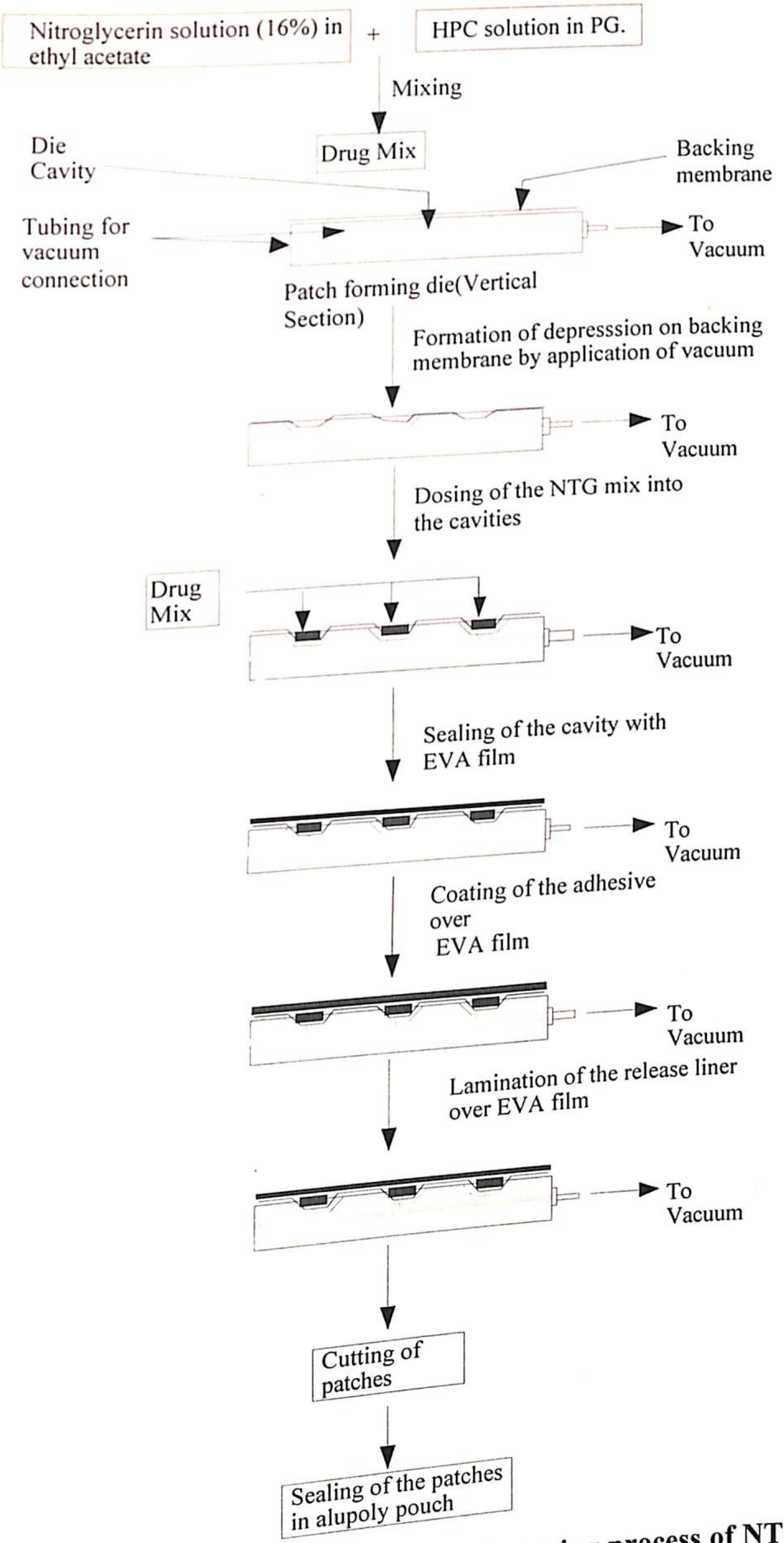


Fig. 4.1: Schematic diagram of manufacturing process of NTG membrane-controlled release patches.

4.10. Evaluation Of The Patches:

4.10.1. NTG In Vitro Release:

In vitro evaluation of the patches using pig skin was performed and the method used was same as for the matrix system described in the Section 4.7.1.

The experiments were performed on NTG patches B. No. NTG-M25-01 and Nitroderm TTS ® 25 mg and results are shown on Page 192-193 of Chapter 5, respectively.

4.11. DICLOFENAC PLASTER:

4.11.1. Partition Coefficient:

DDEA was dissolved in 100 ml each of water and n-octanol till saturation at 25 °C and stored for seven days with frequent shaking. The solutions were centrifuged and the DDEA concentration in the solutions were analysed by UV spectrophotometry at 275 nm and comparing the same with that of standard solution.

4.11.2. Critical Micelle Concentration:

To ten solution containing 5-50 mM of DDEA, few crystals of Orange T (azo die insoluble in water and soluble in micelle) were added. After few weeks of gentle shaking, the solutions were filtered through 0.2 μm cellulose acetate membrane (Millipore) and absorbance value of the solubilised dye was plotted against the concentrations of the DDEA solubilisation started above 40 mM which is considered as the critical micelle concentration.

4.11.3. Interaction With Asolecithin:

A system containing Asolecithin (mixture of phospotidyl inositol and phosphatidyl ethanolamine, Fluka) and DDEA in the weight ration of 1:1 was prepared in water at concentration of 40 mM of DDEA. The mixture was stirred till a homogenous i.e. a milky emulsion is formed. The transmission of the system was measured at 580 nm and was increased after addition of prefixed value of 40 mM of DDEA. System containing pure

Phosphotidyl choline and DDEA in the weight ratio of 1:1 was stable upto 10 % w/w concentration in water.

4.11.4. Preliminary Work:

The first few materials, which we thought, could contribute to the bulk and enhance the tack and adhesion were solubilised Starch, Sodium CMC, Gelatine, Pectin, Alginic acid, HPMC, PVP, PVA, combination of the PVA-PVP etc. were tried to achieve the tack as well as bulk of plaster. During trials, varying compositions of HPMC, Sodium CMC, PVP, PVA , Gelatine were mixed with acrylic adhesive. The adhesive mix was applied with the help of applicator (as described in Section 3.8.2. on Page 119). Few formulations containing Gelatine, Pectin, Sodium CMC and aqueous acrylic adhesives were finalised.

4.11.5. Final Experiments:

On the basis of above preliminary work, following three formulations were selected for the final choice from the point of view of aesthetic looks, adhesiveness to body particularly knees, easy removal after 12 hrs. application on body surface and non- irritation.

Table 4.6: Composition of Diclofenac plasters

Sr. No.	Ingredients	DDA-01	DDA-02	DDA-03
1.	Diclofenac Diethyl Ammonium	1.4%	1.4 %	1.4%
2.	Pectin	5 %	6%	7%
3.	Gelatine	5%	5%	6%
4.	Sodium CMC	1%	1%	1%
5.	Glycerine	12%	12%	12 %
6.	Propylene Glycol	8%	8%	8%
7.	Acrylic adhesive PSA-A3	10%	11%	12%
8.	Methyl Paraben	0.1%	0.1%	0.1%
9.	Propyl Paraben	0.02%	0.02%	0.02%
10.	Distilled Water upto	100%	100%	100%

Out of above formulations, formulation No. DDA-3 emerged as the best formulation and all further work, in the laboratory and pilot scale, was extension of the same.

Table 4.7: Composition of Diclofenac plaster B. No. DDA- 4.

Batch Size : 1.0 kg.

Sr. No.	Ingredients	Content
1.	Diclofenac Diethyl Ammonium	14 gms.
2.	Pectin I.P.	70 gms.
3.	Gelatine I.P	60 gms.
4.	Sodium CMC (High Viscosity) I.P.	10 gms.
5.	Glycerine I.P.	120 gms.
6.	Propylene Glycol I.P.	80 gms.
7.	Acrylic adhesive PSA-A3	120 gms.
8.	Methyl Paraben I.P.	1 gms.
9.	Propyl Paraben I.P.	0.2 gms.
10.	Distilled Water upto	1 kg.

Following is the procedure used for the formulation of the plasters,

- Gelatine was soaked in 60 gms. of glycerine and 40 gms. of propylene glycol for 4 hrs.
- Pectin was soaked in 60 gms. of glycerine for 4 hrs.
- Diclofenac diethyl ammonium, methyl paraben and propyl paraben were dissolved in 40 gms. of propylene glycol.
- Sodium CMC was dispersed in 100 ml of water and kept aside.
- 300 ml of water was heated to 80°C and into it soaked gelatine was added with thorough mixing, followed by pectin and Sodium CMC, in 2L capacity Kenwood mixer, the temperature was maintained at 60 °C for 4 hrs., and acrylic adhesive was added and mixing was continued for 1 hr., not allowing temperature to drop below 50°C.
- The hot mass, with the same laboratory applicator (described in the Section 3.8.2. of Chapter 3), was applied on surgical grade cotton cloth

and jersey cloth at 50 °C such that 10x15 cm^2 area should contain 180 mg of DDEA and allowed to cool overnight in a ventilated room.

The system was laminated with siliconised HDPE film (40 µm) and packed into alupoly pouches (40 µm/ 150 gauge PE).

To confirm the reproducibility of process, batch manufacturing was repeated three times on different occasions.

4.12. Evaluation Of The Plasters:

4.12.1. In Vitro Permeation Study:

In vitro permeation experiments of the plaster B. No. DDA-4 and Flector Tissue Gel was carried out on the pig skin using two sizes of the modified Franz cells, in order to understand the effect of change in surface area. The first cell was 73.40 mm in diameter and having volume of 460 ml (photograph is shown on Page 237 in Chapter 5) while another one was 37.14 mm and having volume of 60 ml, the experimental procedure and parameters were same for both type of cells used.

The methodology used for the in vitro evaluation was same as described for NTG patches except the HPLC parameters which are described as below:

HPLC parameters :

- *HPLC pump* : Jasco Intelligent 980
- *Detector* : Jasco UV 975
- *Absorption* : 275 nm
- *Integrator* : Borwin chromatography version 1.21
- *Column* : Shandon, Hypersil, C18, 10µ, 4.6 X 250 mm.
- *Operating Temperature* : Room Temperature
- *Solvent system* : Methanol: water : acetonitrile : Dilute Glacial acetic acid (1:1:0.5 : 0.00015)
- *Retention Time* : 9 min.
- *Injection Volume* : 20 µl.

The results of DDEA plaster B. No. DDA-4 and Flector are given on Page 194-195 using big cells while for small cell are given on Page 196-197 in Chapter 5, respectively. A typical chromatogram is shown on Page 227.

Validation Of Analytical Method:

The method was validated for precision, accuracy , limit of detection (DL) and limit of quantitation (QL) and the method adopted as follows:

Precision:

For measuring the precision, a known concentration (5 μg/ml) of standard DDEA solution was injected six times and relative std. dev. of not more than 2% was considered as acceptable limit. The results are shown in Table 5.31 on Page 208 and representative chromatogram of is shown on Page 225 of Chapter 5.

Accuracy By Linearity:

Accuracy of method was carried out by establishing linearity of different concentrations of DDEA from 5-25 μg/ml. A correlation coefficient of 0.999 was accepted as the minimum limit. The area response to the different concentrations is shown in Table 5.32 on Page 208 and typical chromatogram 10 μg/ml are shown on Page 226 in Chapter 5.

Limit of Detection:

DL was established using the following methodology:

From the linearity curve obtained above and response to different concentrations in terms of height, slope was calculated. The noise level on that particular day was calculated by putting on the system and measuring the response in μvolts for 30 seconds. For limit of detection (DL) following equation is used;

DL = Slope x Noise x 3

Limit of quantitation (QL) : QL was calculated in a same way as described above except the equation used is as follows;

QL = Slope x Noise x 10.

The results of DL and QL are shown on Page 208 in Table 5.32 of Chapter 5.

4.13. Scaling Up Of The Plaster:

4.13.1. Preliminary Work:

Since the formulation developed on laboratory scale was slightly viscous and dark in colour, some changes were made such as alteration in quantities of glycerine and propylene glycol, reducing the amount of Sodium CMC and inclusion of Titanium dioxide for scaling up purpose and machine suitability.

4.13.2. Final Experiment:

The final composition of the formula is as follows:

Table 4.8: Composition of diclofenac plaster B. No. DDA-5 .

Sr. No.	Ingredients	Content Per batch of 10 kg
1.	Diclofenac Diethyl Ammonium	140 gms.
2.	Pectin I.P.	700 gms.
3.	Gelatine I.P	600 gms.
4.	Sodium CMC (High Viscosity)I.P.	500 gms.
5.	Glycerine I.P.	800 gms.
6.	Propylene Glycol I.P.	800 gms.
7.	Acrylic adhesive (PSA-A3)	120 gms.
8.	Methyl Paraben I.P.	10 gms.
9.	Propyl Paraben I.P.	2 gms.
10.	Titanium Dioxide	40 gms.
11.	Distilled Water upto	10 kgs.

The manufacturing procedure followed is given below,

- Gelatine was soaked in a mixture of 600 gms. of glycerine and 400 gms. of propylene glycol and kept aside for 4 hrs.
- Pectin was soaked in 800 gms of glycerine and was kept aside for 4 hrs.
- DDEA, methyl paraben and propyl paraben were dissolved in 400 gms. of propylene glycol. Titanium Dioxide was dispersed in this solution.
- Sodium CMC was dispersed in 1 litre of water and kept aside.

- 3.0 Litres of water was heated to 80°C in which soaked gelatine, pectin and Sodium CMC were added and mixed thoroughly in 15L capacity planetary mixer and the temperature was maintained at 60 °C for 4 hrs. using vacuum to avoid air entrapment .
- Acrylic adhesive (PSA-A3) was added and mixing was continued for one hr. maintaining the temperature below 50 °C.
- The mass so obtained was applied, with the help of machine developed for scaling up (described in the Section 6.4 of Chapter 3), at 50 °C on surgical grade cotton cloth/ jersey cloth and allowed to cool overnight in a well ventilated room.
- The plaster was laminated with siliconised HDPE film (40µ) and packed into alupoly pouches (40µ/ 150 gauge PE) after cutting into 10x15 cm pieces.

4.14. Evaluation Of The Plasters:

4.14.1. In Vitro Release Study:

This was also carried out on B. No. DDA-5 using the same method as described before in the Section 4.12. on small cells .

The results of DDEA plaster and Flector are shown in Chapter 5 on Page 198-199, respectively.

4.14.2. In Vivo Release Study:

The comparative in vivo experiments of the DDEA plaster B. No. DDA-5 with Flector were carried out in a cross over design with six rabbits using HPLC procedure and ketoprofen as an internal standard. Following are the details of the methodology and the analytical procedure used.

- The experiment was conducted on six rabbits (3 males and 3 females) weighing between 1.8 to 2.0 kg using cross over designs and after a period of 1 week the sequence of application of patches was reversed .

- Before commencement of the experiment, skin of every rabbit was thoroughly examined for any abnormality and only those having no structural abnormality of skin were included in the experiment.
- The skin area of around 11 x 16 cm. was shaved, covering both the side of vertebral column of each rabbit and care was taken to avoid damage to skin during shaving.
- Before application of the plaster, rabbits were kept for 24 hrs. under observation for any untoward effect of shaving to the skin and were on fasted over this period.
- On the shaved surface, plasters (Diclofenac and Flector) were applied.
- 2 ml blood was withdrawn from the ear vein of each rabbit at 0, 4, 8, 12 and 24 hrs. interval. Due to small size of the animal and damage to the ear vein it was not possible to withdraw the blood for more than above mentioned intervals.
- After the blood was withdrawn, 0.5 ml of 10% Disodium EDTA was added and mixed thoroughly to avoid coagulation of blood.
- Blood samples were centrifuged for 30 mins. at 8000 rpm and plasma was separated.
- The separated plasma was kept in refrigerator till analysis.
- To each of 30 test tubes, 1 ml of 200 ng/ml solution of Ketoprofen in methanol was added and evaporated to dryness using nitrogen and to each one of them 1 ml of plasma collected at different time interval from each rabbit was added and mixed thoroughly with the help of cyclone mixer.
- 0.6 ml of 10% Perchloric acid solution was added to each of the tube and thoroughly mixed on cyclone mixer for 2 min.

- 5 ml of extraction solvent mixture (4.5 ml of n-hexane + 0.5 ml of isopropranol) was added in above step tubes and mixed on cyclone mixer for 20 mins.
- The organic layer was separated and evaporated to dryness with the help of gentle stream of nitrogen.
- The residue was dissolved in 0.4 ml of methanol and injected.

Preparation Of Standard Curve:

- Stock solution of 20, 50, 100 and 200 ng/ml DDEA standard were prepared in methanol.
- Stock solution of 200 ng/ml of ketoprofen was prepared in methanol.
- To all the test tubes containing 1 ml of DDEA in step 1, 1 ml of ketoprofen solution was added and methanol was evaporated to dryness with the help of Nitrogen and to each of 5 test tubes 1 ml of rabbit plasma was added and mixed thoroughly on cyclone mixer.
- 0.6 ml of 10% Perchloric acid solution was added to each of the tube and thoroughly mixed on cyclone mixer for 2 min.
- 5 ml of extraction solvent mixture (4.5 ml of n-hexane + 0.5 ml of isopropranol) was added in each test tube and mixed on cyclone mixer for 20 min..
- The organic layer was separated and evaporated to dryness with the help of gentle stream of nitrogen.
- The residue was dissolved in 0.4 ml of methanol and 20 μl of this solution was injected.
- Standard curve was plotted with concentrations of DDEA versus ratio of peak area of diclofenac to ketoprofen. For the estimation of DHEP from Flector, standard curve was prepared with DHEP instead of DDEA.

HPLC Method Parameters:

The HPLC method used for the analysis was same as for the in vitro permeation in the Section **4.19**

The results of DDEA plaster, Flector and comparison of both are given in Chapter 5 from Page 200-202, respectively.

Validation Of Analytical Method:

The method was validated for precision and accuracy, the method adopted was as follows:

Precision:

For measuring the precision, a known concentration (100 ng/ml) of standard DDEA and ketoprofen (1000 ng/ml) *solution was injected six times* and ratio of peak areas of DDEA/ketoprofen were calculated and relative std. dev. of not more than 15% was considered as acceptable limit. The results are shown in Table 5.31 on Page 208 and representative chromatogram of is shown on Page 228 in Chapter 5.

Accuracy by linearity:

Accuracy of method was *carried out in similar* manner as above and establishing the correlation coefficient by plotting concentration of DDEA verses the peak ratios of DDEA/ketoprofen and correlation coefficient of 0.98 was considered acceptable. The peak area ration w.r.t. the different concentrations are shown on Page 209 in Table 5.34 and typical chromatogram 50 ng/ml of DDEA is shown on Page 229 of Chapter 5.

4.15. Primary Skin Irritation Study:

Primary skin irritation carried out was same as described in Section 4.7 except area used, for application, was similar to the size of plaster.

4.16. Stability Study Of Diclofenac Plaster:

The diclofenac plasters B. No. DDA-5 were subjected to both long-term and short-term accelerated stability studies.

I. Storage Conditions

a. Refrigeration - 4-5 °C - 6 months (control)

b. Room temperature - 30 ± 2 °C - 6 months

c. Elevated temperature - 45 ± 2 °C - 3 months

II. Storage interval and sample withdrawal for analysis

a. Refrigeration - 0, 1, 2, 3, 4, 5 and 6 months

b. Room temperature -0, 1, 2, 3, 4, 5 and 6 months

c. Elevated temperature - 0, 15, 30, 45, 60, 75 and 90 days.

IV. Method of analysis :

1. Change in appearance

2. Drug content

V. Results

The results are shown on Page 203 in Chapter 5.

(Note: For the stability studies, each patch was individually packed in heat-sealed foil pouches.)

4.17. Nicardipine HCl Patches:

For Nicardipine HCl we have developed two types of systems :

A. Matrix Adhesive Dispersion System

B. Membrane Controlled Release System.

4.18. Matrix Dispersion System:

4.18.1. Preliminary Experiments:

The work done on Nicardipine HCl initially was an extension of work done earlier on NTG. However, using the same formula as used for NTG except the change of solvent did not give any in vitro release even using cellulose acetate membrane (0.45 μm pore size). In absence of any release rates, aqueous based acrylic adhesive in combination with other excipients such as gelatine were tried and fairly good release rates were obtained on synthetic membrane.

4.18.2. Final Experiment:

Following is the formulation of the matrix dispersion system :

Table 4.9: Composition of the Nicardipine patch B. No. NIC-MD-01

Sr. No.	Ingredients	Amount for 200 patches
1.	Nicardipine HCl	12 gms.
2.	Sodium CMC	20 gms.
3.	Gelatine	30 gms.
4.	Glycerine	40 gms.
5.	Propylene Glycol	35 gms.
6.	Aqueous Acrylic adhesive (PSA-A3)	80 gms.
7.	Propyl Paraben	0.3 gms.
8.	Methyl paraben	0.06 gms.
9.	Distilled water	250 gms.

Procedure:

1. Nicardipine HCl, methyl paraben and propyl paraben were dissolved in propylene glycol.
2. Sodium CMC and gelatine were dispersed and soaked overnight in 200 gms. of distilled water and were added to Nicardipine solution followed by addition of glycerine and aqueous acrylic adhesive PSA-A3 was added. The mixture was heated gradually to 50 °C to obtain a clear solution.
3. The adhesive-DDEA mass so obtained was applied on 36 x 137 cm area of PET film (siliconised from other side) to get 60 mg of Nicardipine per 16 cm^2, using laboratory applicator as described in Section 4.1.2. of Chapter 3.
4. The mass was allowed to dry at room temperature for 10 hrs. and was laminated with 125 μm siliconised PET film.
5. The patches were cut into 16 cm^2 circular pieces.

4.19. Evaluation Of The Patches:

4.19.1. In Vitro Release Study:

The in vitro release rates of the patches were determined using same procedure as for diclofenac plaster described in Section 4.12. except the HPLC parameters and which are given below:

HPLC parameters :

- *HPLC pump* : Jasco Intelligent 980
- *Detector* : Jasco UV 975
- *Absorption* : 275 nm
- *Integrator* : Borwin chromatography version 1.21
- *Column* : Symmetry (waters), ODS, 5μ, 4 X 150 mm.
- *Operating Temperature* : Room Temperature
- *Solvent system* : Perchloric acid (43 in 50,000) : Acetonitrile (3:2)
- *Retention Time* : 5.7 min.

The in vitro analysis was carried on both synthetic as well as pig skin. Results are given in the Chapter 5 on Page 204-205 and Fig. 5.24 and 5.25 and a typical chromatogram on Page 233.

Validation Of Analytical Method:

The method was validated for precision, accuracy , limit of detection (DL) and limit of quantitation and the method adopted as follows:

Precision:

For measuring the precision, a known concentration (11.2 μg/ml) of standard Nicardipine HCl solution was injected six times and relative std. dev. of not more than 2% was considered as acceptable limit. The results are shown in Table 5.35 on Page 209 and representative chromatogram is shown on Page 231 in Chapter 5.

Accuracy by linearity:

Accuracy of method was carried out by establishing linearity of different concentrations of NTG from 7-16.8 µg/ml. A correlation coefficient of 0.999 was accepted as the limit. The area response to the different concentrations are given in Table 5.36 on Page 210 and typical chromatogram of 10 µg/ml is shown on Page 232 in Chapter 5.

Limit of Detection:

DL was established using the following methodology:

From the linearity curve obtained above and response to different concentrations in terms of height, slope was calculated. The noise level on that particular day was calculated by putting on the system and measuring the response in microvolts for 30 seconds. For limit of detection (DL) following equation is used;

DL = Slope x Noise x 3

Limit of quantitation (QL) : QL was calculated in a same way as described above except the equation used is as follows;

QL = Slope x Noise x 10.

The results of DL and QL are shown on Page 210 in Table 5.36 of Chapter 5.

4.20. Membrane Controlled Release System:

4.20.1. Preliminary Experiments:

The experiments done for NTG i.e. HPC drug mix was first tried to develop this kind of systems. However, no release was obtained in in vitro study and since the amount of propylene glycol was very high, inclusion of penetration enhancers was considered.

4.20.2. Final experiments:

Following is the composition of the membrane-controlled drug delivery system:

Table 4.10: Composition of Nicardipine patch B. No. NIC-MC-01

Sr. No.	Ingredients	Total Amount for 200 patches
01.	Nicardipine HCl	12 gms.
02.	Propylene Glycol	80 gms.
03.	Methyl diisosorbide	80 gms.

Methodology :

1. Nicardipine HCl was dissolved in propylene glycol and Methyl diisosorbide was mixed with continuous stirring.
2. As described in Section 4.13 the patches were made using same backing membrane, release liner and rate controlling membrane and machine, developed in this laboratory. Fill weight was 850 mg/ patch containing 60 mg of Nicardipine HCl.

4.21. Evaluation Of Nicardipine HCl Patches:

4.21.1. In Vitro Release Of Nicardipine HCl In Patches:

The in vitro release of patches was studied on pig skin and the method used for this was same as for Nicardipine matrix dispersion controlled release system described in Section 4.20.

The results are shown on Page 206 and Fig. 5.26 in Chapter 5.

Chapter V.

RESULTS

Table 5.1: In vitro release of NTG from NTG patches (40 mg) B. No. NTG-A40-01 through cellulose acetate membrane (0.45 µm pore size).

Sr. No.	Time (hrs.)	Cumulative Release (mg/cell)				STD. DEV. (±)
		NTG1	NTG2	NTG3	NTG AVG.	
1.	0	0.000	0.000	0.000	0.000	0.000
2.	1	2.362	1.251	0.155	1.256	1.104
3.	2	3.350	2.568	2.145	2.688	0.611
4.	3	4.545	2.710	2.420	3.225	1.152
5.	4	4.708	3.652	3.154	3.838	0.793
6.	6	7.462	4.185	3.215	4.954	2.226
7.	8	8.101	5.254	4.985	6.113	1.727
8.	10	8.604	5.459	5.366	6.476	1.843
9.	12	9.802	6.214	6.310	7.442	2.044
10.	24	11.821	10.987	11.586	11.465	0.430

Fig. 5.1 : IN VITRO RELEASE OF NTG FROM NTG PATCHES B.No. NTG-A40-01.

Membrane: Cellulose acetate (0.45 micron pore size). Cell Volume: 60 ml.
Dissolution medium: Phosphate buffer USP pH 7.4. RPM: 70+/-10
Temperature: 32 deg. C.

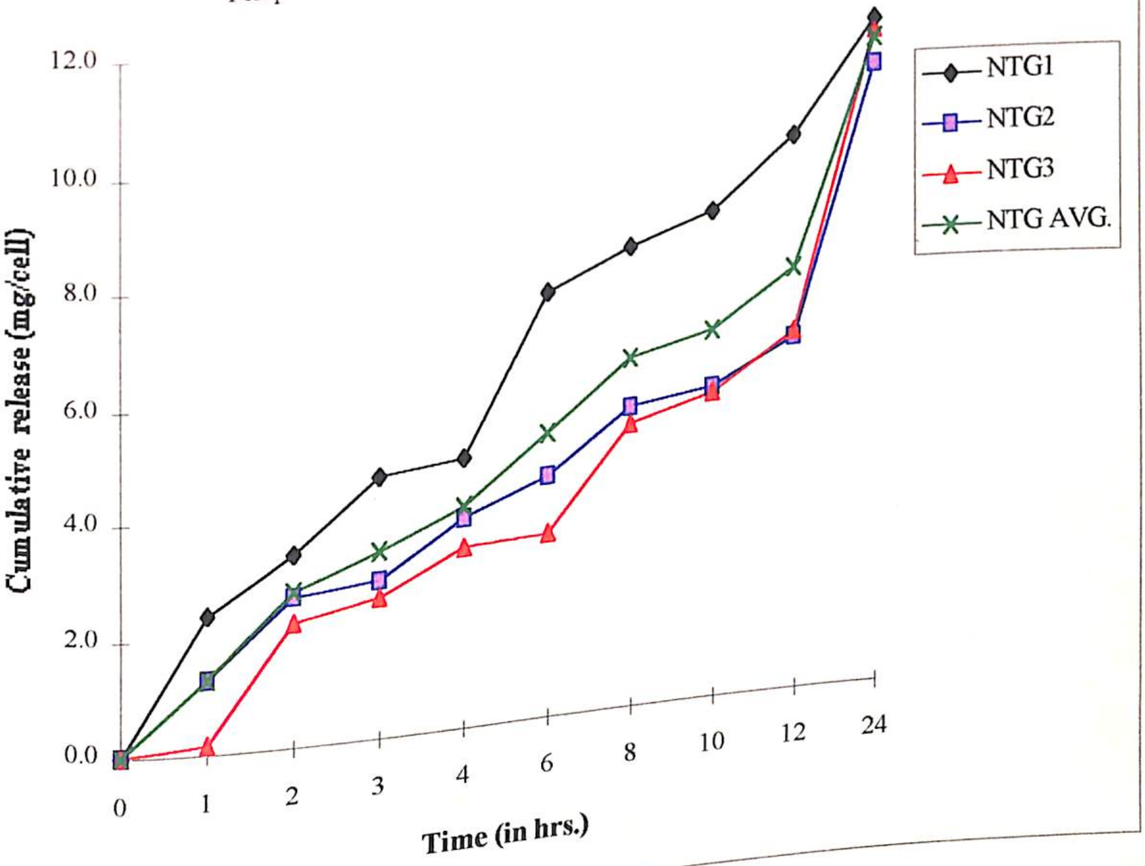


Table 5.2: In vitro release of NTG from the Nitro Dur® (40 mg) through cellulose acetate membrane (0.45 µm pore size).

Sr. No.	Time (Hours)	Cumulative Release (mg/cell)				STD. DEV. (±)
		ND1	ND2	ND3	ND AVG.	
1.	0	0.00	0.00	0.00	0.00	0.00
2.	1	4.51	1.88	2.72	3.04	1.34
3.	2	7.43	3.39	6.99	5.94	2.22
4.	3	10.34	4.98	9.28	8.20	2.84
5.	4	11.66	5.84	11.51	9.67	3.32
6.	6	13.62	8.25	13.00	11.62	2.94
7.	8	14.25	9.41	13.58	12.41	2.62
8.	10	14.90	9.97	14.65	13.17	2.78
9.	12	14.90	10.56	15.10	13.52	2.56
10.	24	14.90	13.49	16.20	14.86	1.36

Fig. 5.2: IN VITRO RELEASE OF NTG FROM NITRODUR 40 mg.

Membrane: Cellulose acetate (0.45 micron); Cell volume: 60 ml.

Dissolution medium: Phosphate buffer USP pH 7.4. RPM=70+/-10.

Temperature: 32 deg. cel.

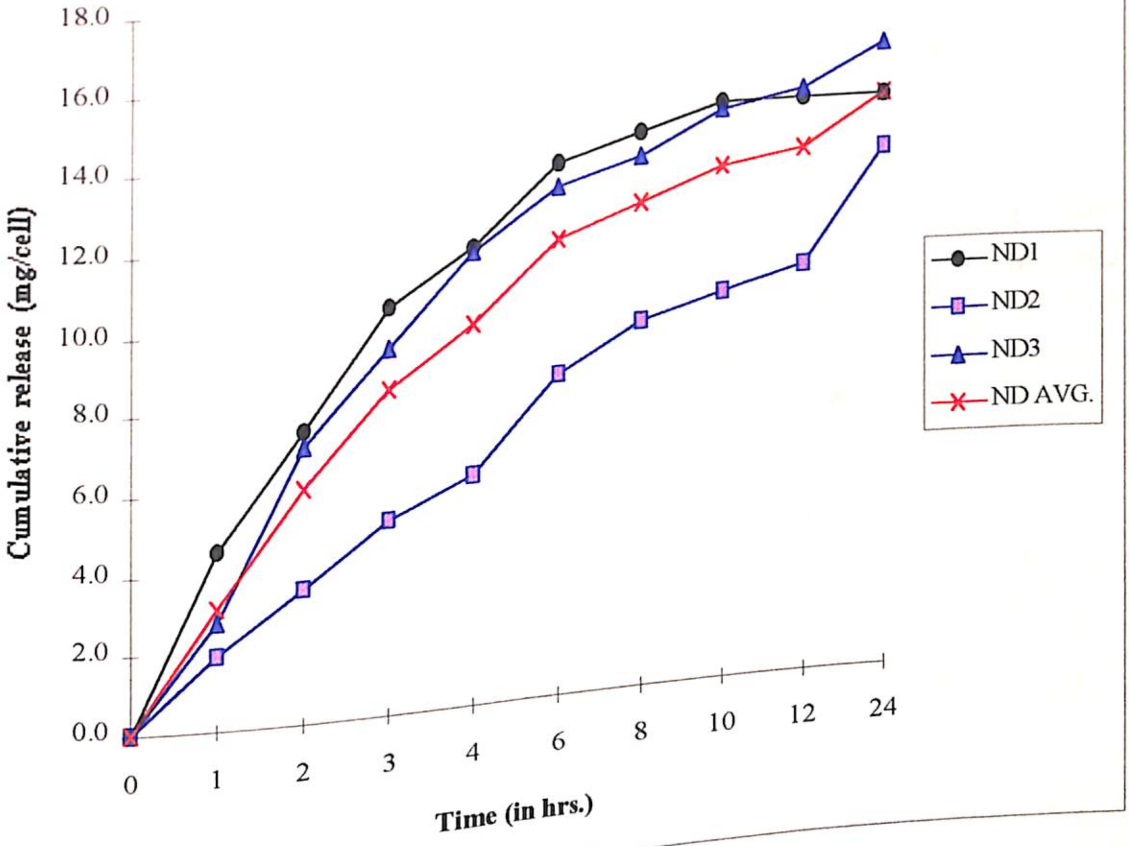


Table 5.3: In vitro release of NTG from NTG patches (40 mg) B. No. NTG-A40-01 through pig skin.

Sr. No.	Time (hrs.)	Cumulative Release (mg/cell)				STD. DEV. (±)
		NTG1	NTG2	NTG3	NTG AVG.	
1.	0	0.000	0.000	0.000	0.000	0.000
2.	1	0.106	0.235	0.270	0.203	0.086
3.	2	0.280	0.559	0.655	0.498	0.195
4.	3	0.711	0.718	0.855	0.762	0.081
5.	4	1.236	1.155	1.335	1.242	0.090
6.	8	4.012	2.583	4.556	3.717	1.019
7.	10	2.026	3.221	4.967	3.404	1.479
8.	12	2.833	4.293	5.334	4.153	1.256
9.	24	5.818	6.794	7.885	6.832	1.034

Fig. 5.3: IN VITRO RELEASE OF NTG FROM NTG PATCH 40 mg B. No. NTG-A40-01.

Membrane: Pig skin. Cell volume: 60 ml. RPM: 70+/-10.
Dissolution medium: Phosphate buffer USP pH 7.4.
Temperature: 32 deg. cel.

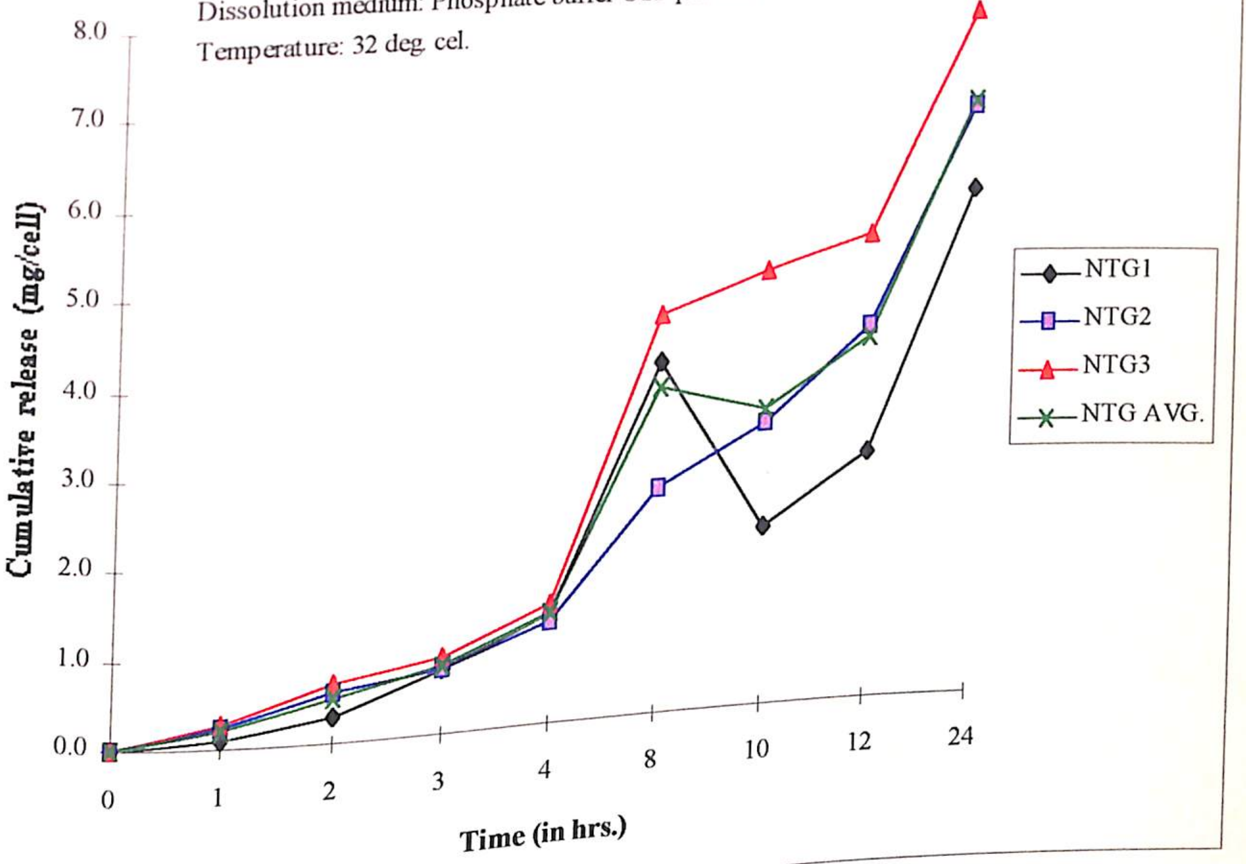


Table 5.4 : In vitro release of NTG from the Nitro Dur® patches (40 mg) through pig skin.

Sr. No.	Time (hrs.)	Cumulative Release (mg/cell)				STD. DEV. (±)
		ND1	ND2	ND3	ND AVG.	
1.	0	0.000	0.000	0.000	0.000	0.000
2.	1	0.184	0.000	0.102	0.095	0.092
3.	2	1.052	0.191	0.887	0.710	0.457
4.	3	1.354	0.290	1.056	0.900	0.549
5.	4	2.139	0.417	1.567	1.374	0.877
6.	8	3.099	1.166	2.670	2.312	1.015
7.	10	4.042	1.613	3.200	2.952	1.233
8.	12	4.911	2.041	3.556	3.503	1.436
9.	24	7.687	4.204	5.670	5.853	1.749

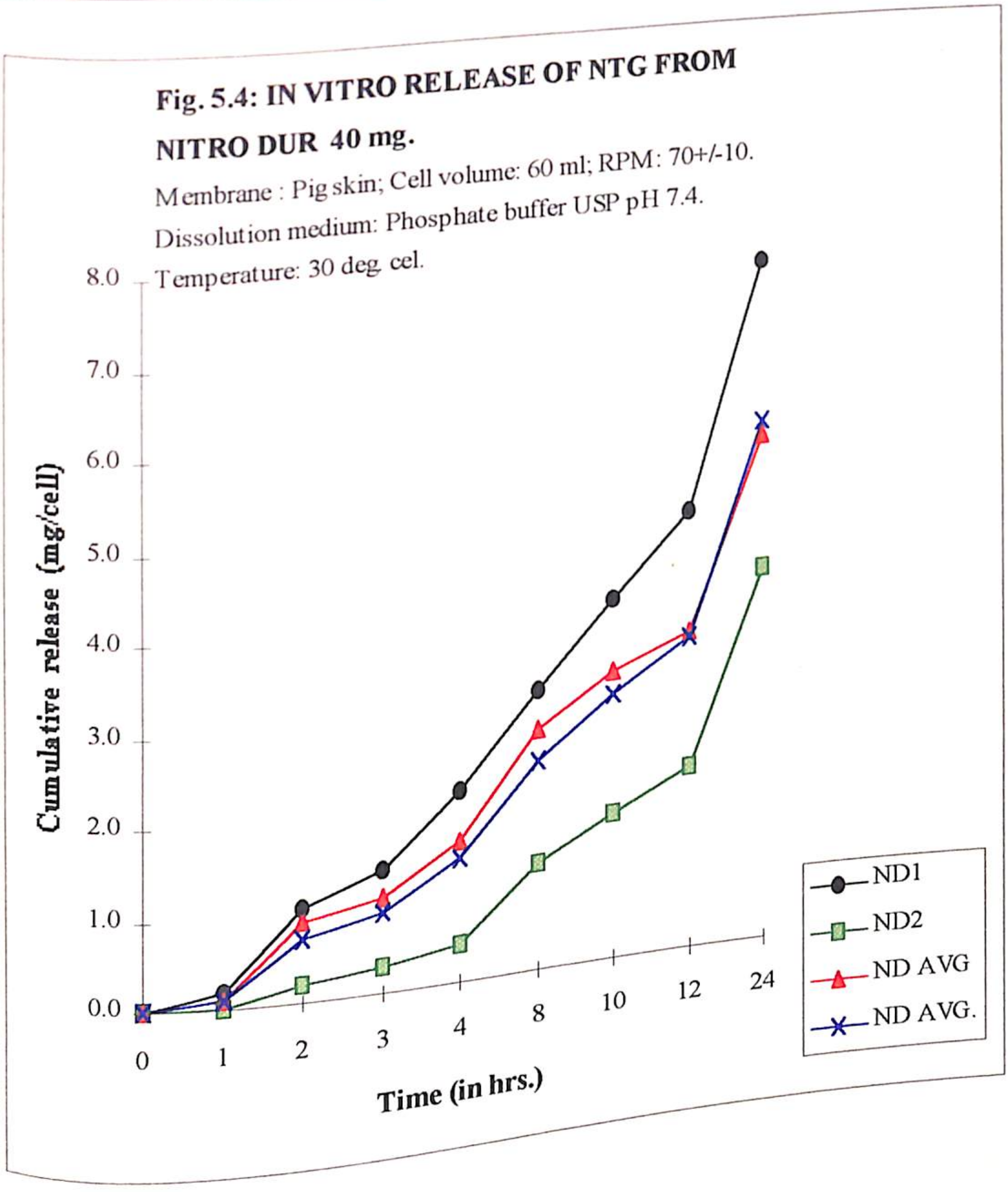


Fig. 5.4: IN VITRO RELEASE OF NTG FROM NITRO DUR 40 mg.

Membrane : Pig skin; Cell volume: 60 ml; RPM: 70+/-10.

Dissolution medium: Phosphate buffer USP pH 7.4.

Temperature: 30 deg. cel.

Table 5.5: Primary Skin Irritation Test of NTG patches B. No. NTG-A40-01 in rabbits.

Rabbit No.	Etiology				
	Erythema	Oedema	Ulceration	Necrosis	Pigmentation
1.	-	-	-	-	-
2.	-	-	-	-	-
3.	-	-	-	-	-
4.	-	-	-	-	-
5.	-	-	-	-	-
6.	-	-	-	-	-

- Absent ; + Present

Table 5.6: In vitro release of NTG from NTG patches (40 mg) B. No. NTG-A40-02 through pig skin.

Sr. No.	Time (in hrs.)	Cumulative Release (mg/cell)				STD. DEV.(±)
		NTG1	NTG2	NTG3	NTG. AVG.	
1.	0	0.000	0.000	0.000	0.000	0.000
2.	1	0.000	0.000	0.000	0.000	0.000
3.	2	0.256	0.198	0.394	0.283	0.101
4.	3	0.465	0.350	0.411	0.409	0.058
5.	4	0.946	1.058	1.156	1.053	0.105
6.	8	3.456	3.245	2.987	3.229	0.235
7.	10	3.987	3.798	3.564	3.783	0.212
8.	12	4.886	4.487	3.989	4.454	0.449
9.	24	6.689	6.456	6.017	6.387	0.341

Fig. 5.5: IN VITRO RELEASE OF NTG FROM NTG PATCHES 40 mg B. No. NTG-A40-02.

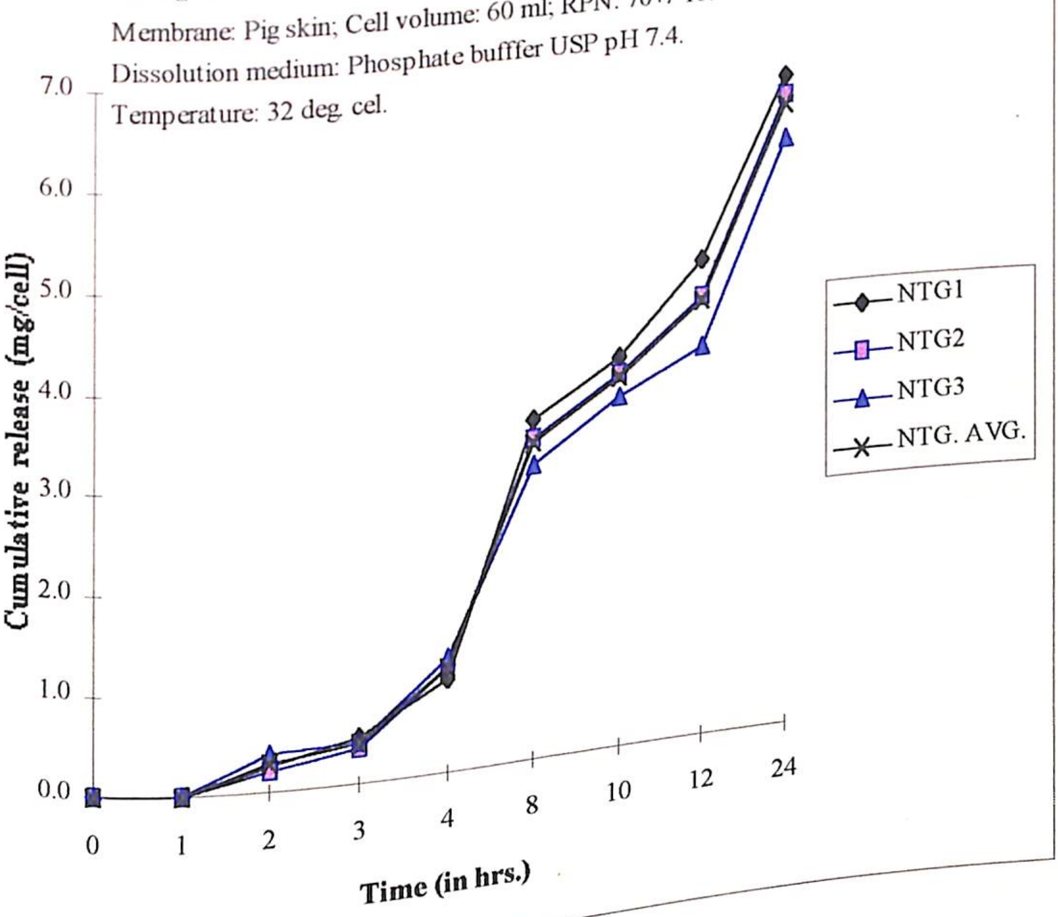


Table 5.7: In vitro release of NTG from Nitro Dur® patches (40 mg) through pig skin.

Sr. No.	Time (hrs.)	Cumulative Release (mg/cell)				STD. DEV. (±)
		ND1	ND2	ND3	ND AVG.	
1.	0	0.000	0.000	0.000	0.000	0.000
2.	1	0.110	0.330	0.260	0.233	0.112
3.	2	0.390	0.460	0.680	0.510	0.151
4.	3	0.680	0.880	0.670	0.743	0.118
5.	4	1.640	1.350	1.800	1.597	0.228
6.	8	4.490	2.890	5.050	4.143	1.121
7.	10	4.890	3.890	2.801	3.860	1.045
8.	12	5.250	3.258	3.378	3.962	1.117
9.	24	6.220	7.020	7.390	6.877	0.598

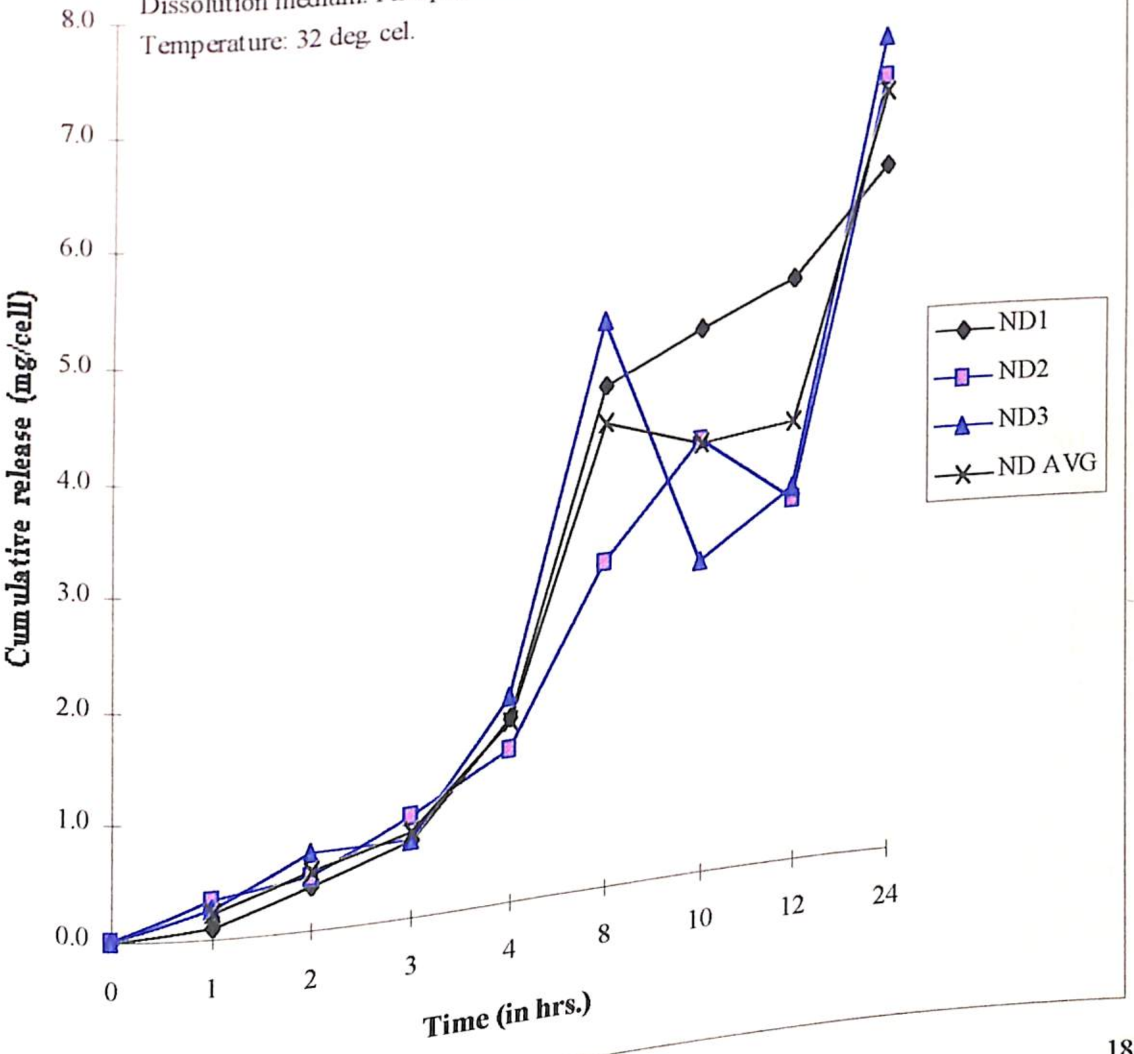


Fig. 5.6: IN VITRO RELEASE OF NTG FROM NITRODUR 40 mg.

Membrane: Pig skin; Cell volume: 60 ml; RPM: 70+/-10.
Dissolution medium: Phosphate buffer USP pH 7.4.
Temperature: 32 deg. cel.

Table 5.8: In vitro release of NTG from Nitro Dur® (40 mg) and NTG patch (40 mg) B. No. NTG-A40-02 through cadaver skin.

Sr. No.	Time (hrs.)	Cumulative Release (mg/cell)	
		ND	NTG
1.	0	0.000	0.000
2.	1	0.000	0.000
3.	2	0.035	0.015
4.	3	0.041	0.021
5.	4	0.049	0.025
6.	6	1.024	0.900
7.	8	1.235	1.305
8.	10	1.256	1.113
9.	12	1.311	1.289
10.	24	1.875	1.460

Fig. 5.7: IN VITRO RELEASE OF NTG FROM NTG PATCH 40 mg B. No. NTG-A40-02 AND NITRO DUR 40 mg .

Membrane : Cadaver skin; Cell volume: 60 ml; RPM: 70+/-10.

Dissolution medium: Phosphate buffer USP pH 7.4.

Temperature: 32 deg. cel.

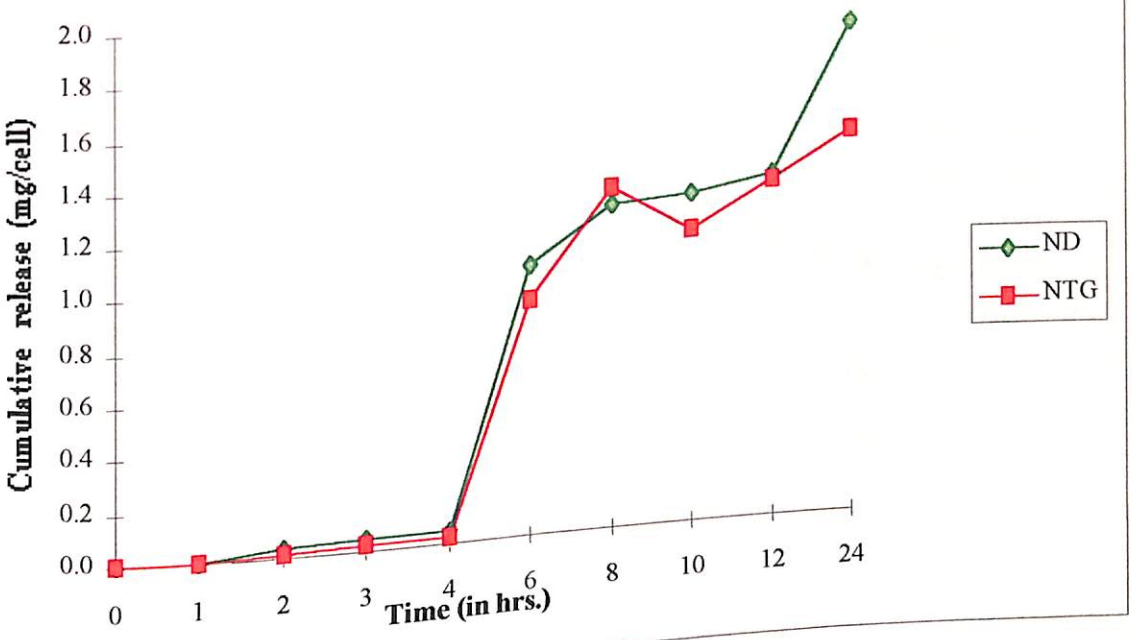


Table 5.9: In vivo release of NTG from NTG patches (40 mg) B. No. NTG-A40-02 in rabbits in a cross over design.

Time (in hrs.)	Plasma Concentration (pg/ml)							STD. DEV. (±)
	NTG1	NTG2	NTG3	NTG4	NTG5	NTG6	NTG AVG.	
0	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
4	34.003	6.750	5.197	5.614	9.492	14.513	12.595	11.038
8	27.440	22.810	22.430	24.918	22.894	36.413	26.151	5.369
12	29.367	48.928	58.780	28.579	24.592	21.596	35.307	14.966
24	24.718	18.050	13.094	17.605	14.330	17.823	17.603	4.048

Fig. 5.8: IN VIVO RELEASE OF NTG FROM NTG PATCHES B. No. NTG-A40-02 USING RABBITS IN A CROSS OVER DESIGN.

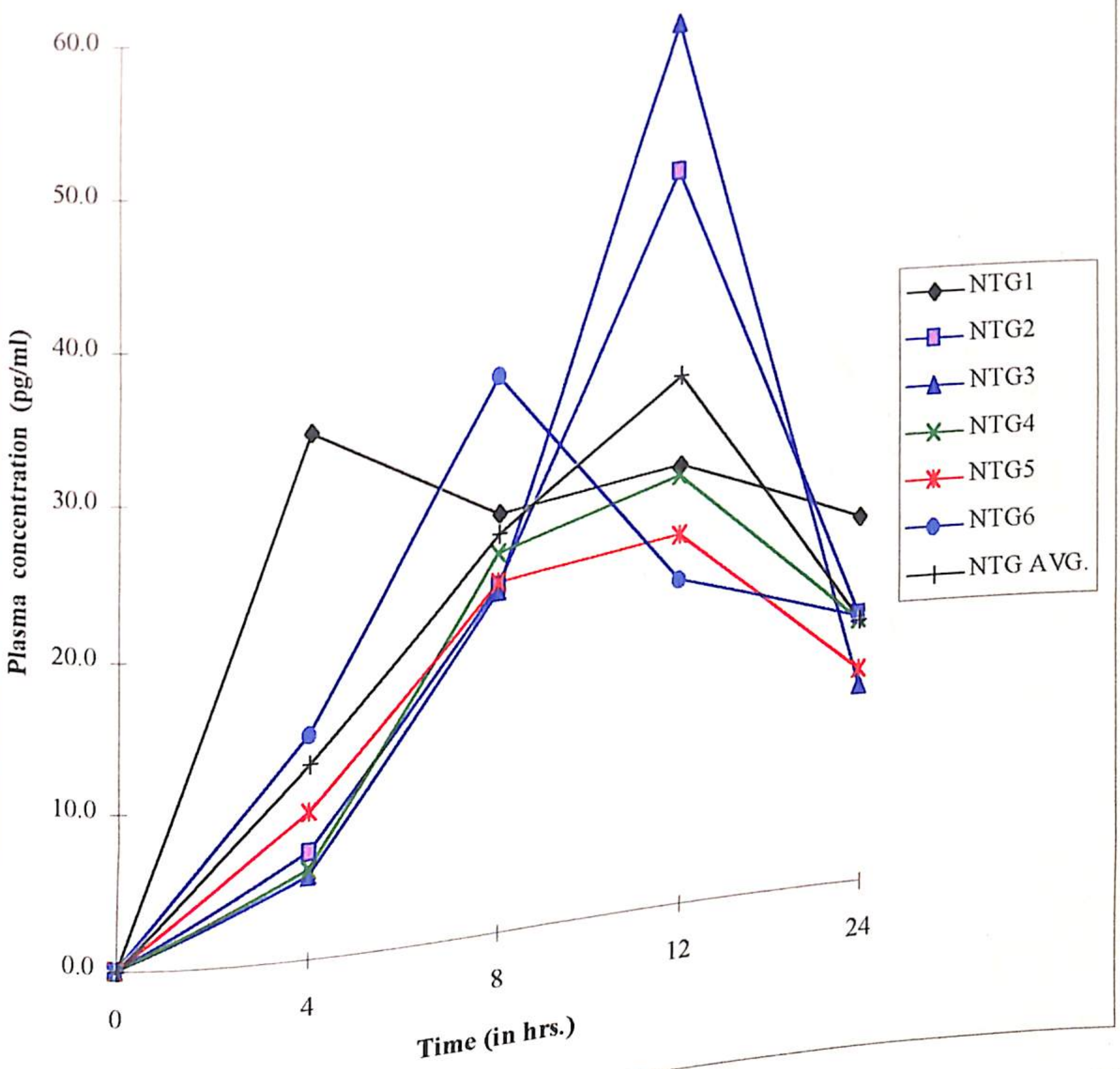


Table 5.10: In vivo release of NTG from Nitro Dur® patches (40 mg) in rabbits in a cross over design.

Time (in hrs.)	Plasma concentration (in pg/ml)							STD. DEV. (±)
	ND1	ND2	ND3	ND4	ND5	ND6	ND AVG.	
0	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
4	33.380	24.279	10.622	30.375	39.936	29.739	28.055	9.959
8	24.499	19.603	28.579	26.995	18.500	19.444	22.937	4.330
12	14.622	40.692	31.581	31.290	36.103	30.911	30.867	8.815
24	25.597	17.605	8.326	22.520	19.619	18.043	18.618	5.869

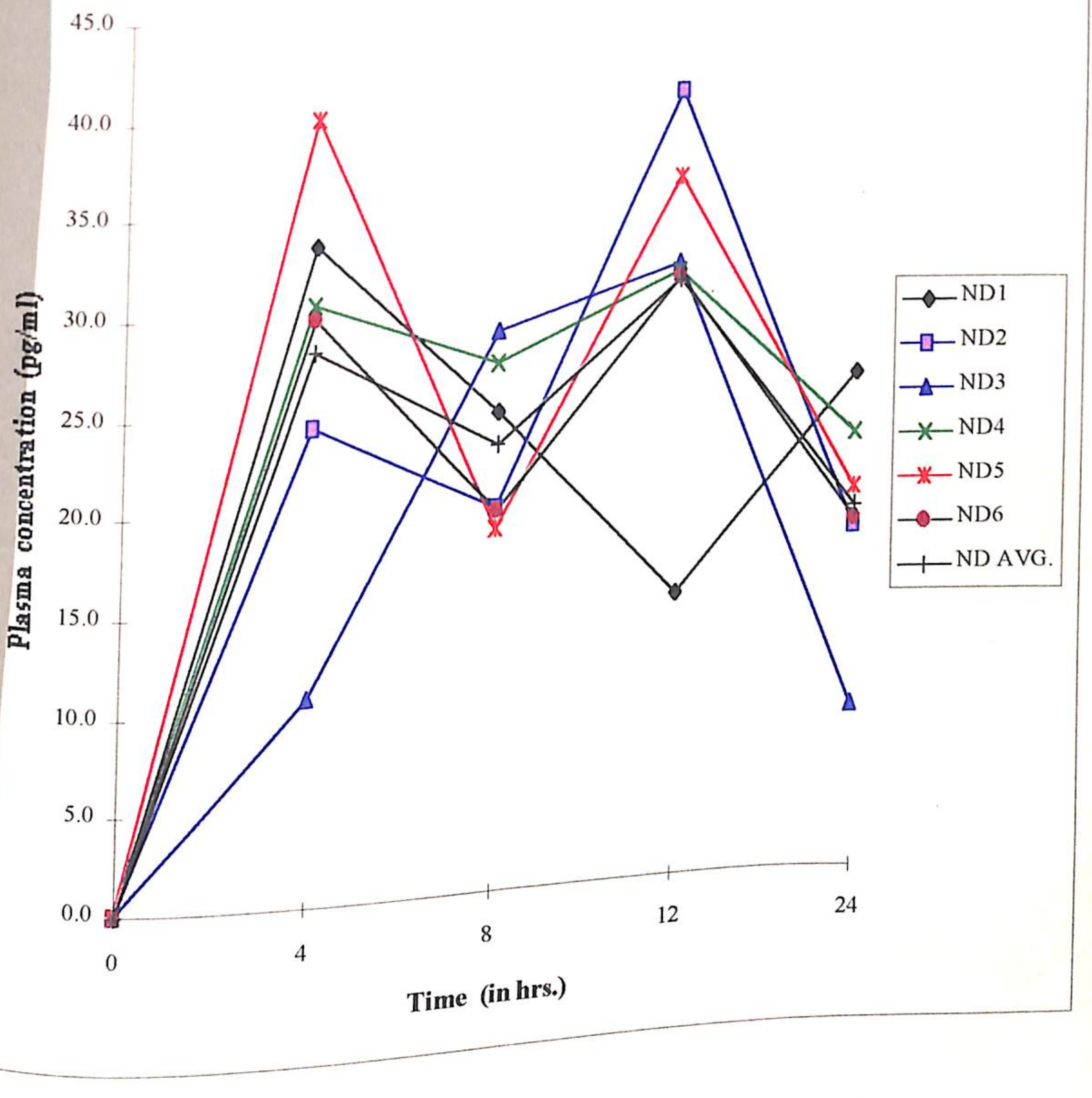
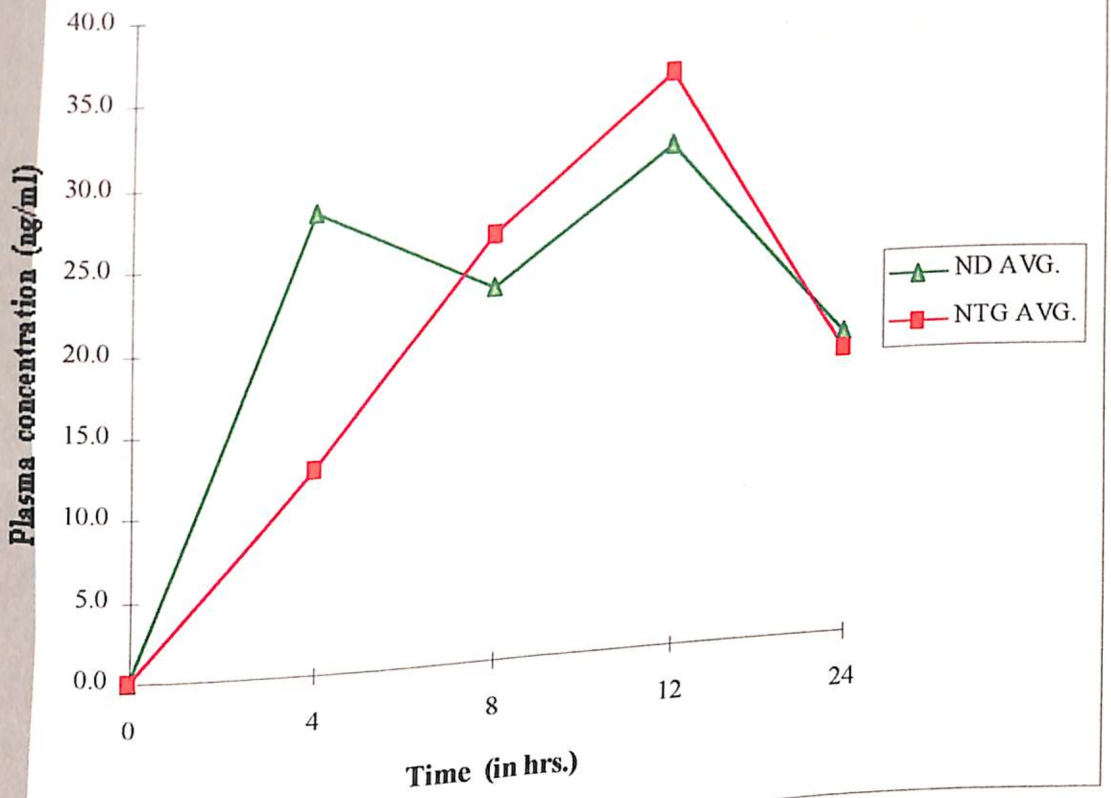


Fig. 5.9: IN VIVO RELEASE OF NTG FROM NITRO DUR 40 mg USING RABBITS IN A CROSS OVER DESIGN.

Table 5.11 : Comparison of in vivo release of NTG from Nitro Dur® (40 mg) with that of NTG patches B. No. NTG-A40-02 in rabbits in a cross over design.

Time (in hrs.)	Plasma Conc. (pg/ml)	
	ND AVG.	NTG AVG.
0	0.000	0.000
4	28.055	12.595
8	22.937	26.151
12	30.867	35.307
24	18.618	17.603

Fig. 5.10: COMPARISON OF THE IN VIVO RELEASE OF NTG FROM NITRO DUR (ND) AND DEVELOPED NTG PATCH B. No. NTG-A40-02.



Appearance		

Note: No change in the physical appearance under any of the conditions.

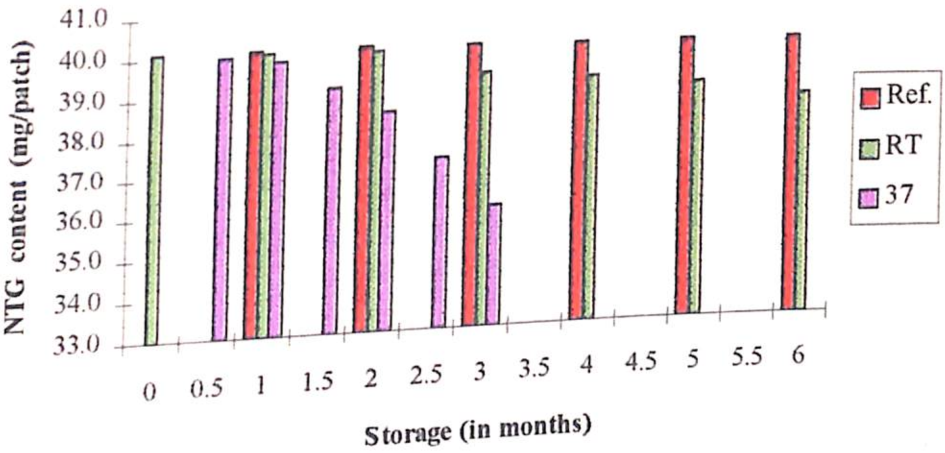


Fig. 5.11: Stability study of NTG patches B. No. NTG-A40-02 for NTG content.

able 5.14: In vitro release of NTG from Nitroderm TTS® (25 mg) through pig skin.

Sr. No.	Time (hrs.)	Cumulative Release (mg/cell)				STD. DEV. (±)
		NTTS1	NTTS2	NTTS3	NTTS AVG.	
1.	0	0.000	0.000	0.000	0.000	0.000
2.	1	0.146	0.115	0.095	0.119	0.025
3.	2	0.365	0.214	0.198	0.259	0.092
4.	3	1.155	0.785	1.025	0.988	0.187
5.	4	2.570	1.985	2.235	2.263	0.293
6.	8	4.012	4.013	4.315	4.113	0.175
7.	10	5.899	4.896	4.757	5.184	0.623
8.	12	6.001	5.148	5.954	5.701	0.480
9.	24	12.556	11.146	12.001	11.901	0.710

Fig. 5.13: IN VITRO RELEASE OF NTG FROM NITRODERM TTS 25 mg .

Membrane: Pig skin; Cell volume:60 ml; RPM : 70+/-10.

Dissolution medium: Phosphate buffer USP pH 7.4.

Temperature: 32 deg. cel.

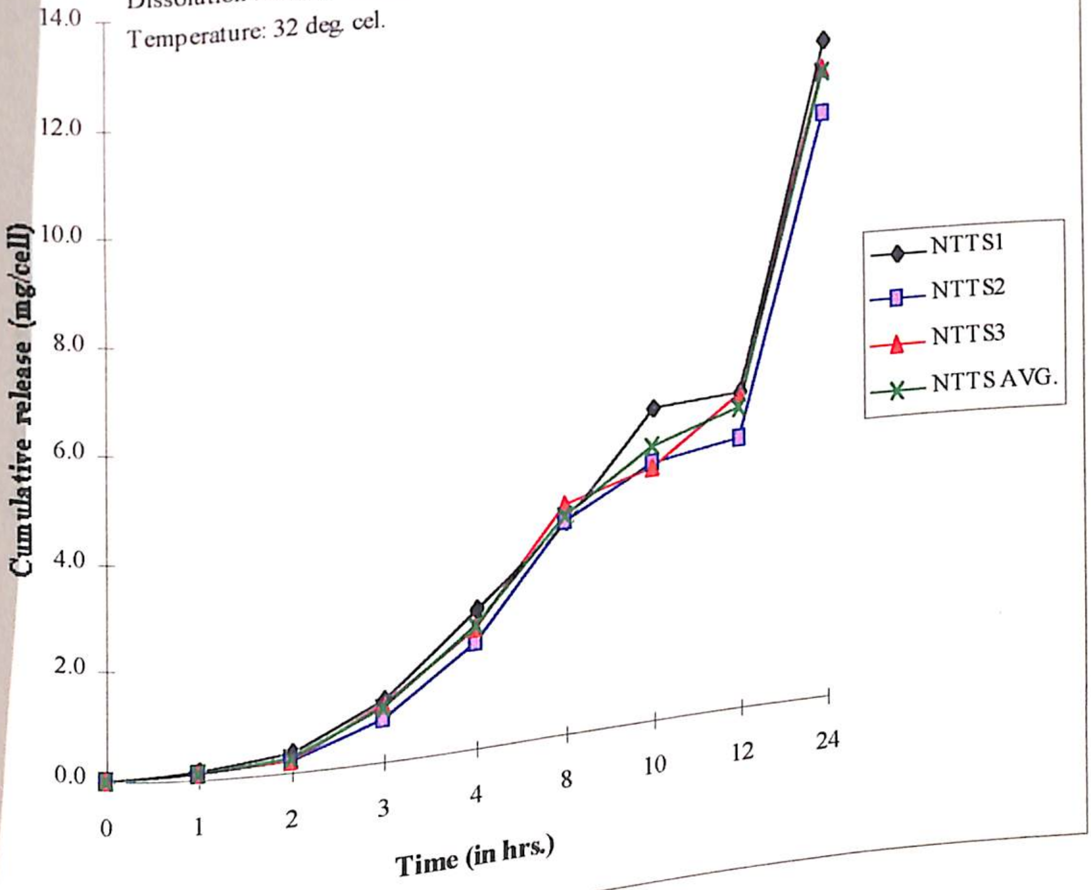


Table 5.15: In vitro release of DDEA from diclofenac plaster B. No. DDA-4 in big cells through pig skin.

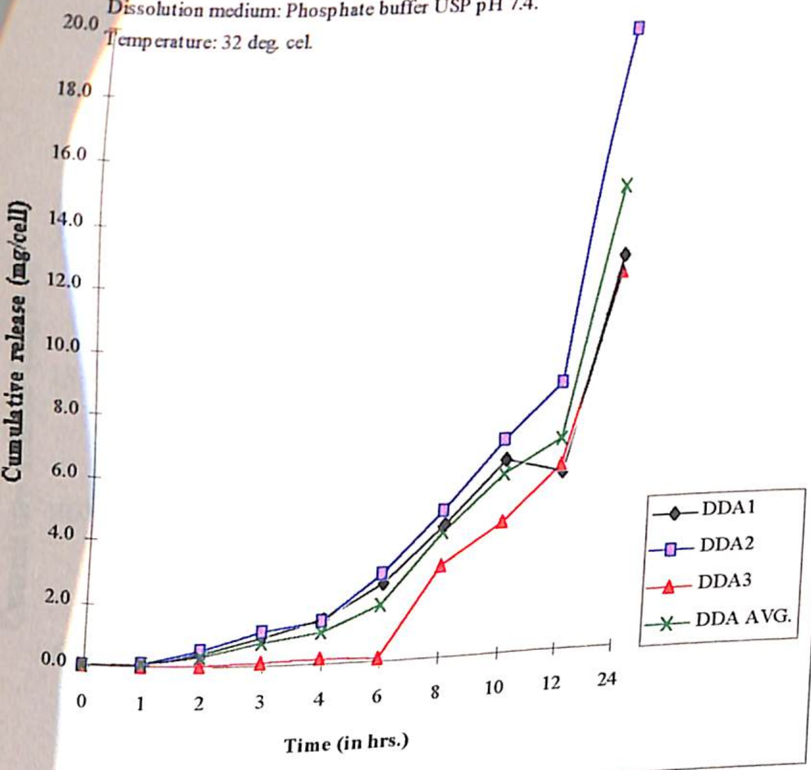
Sr. No.	Time (hrs.)	Cumulative Release (mg/cell)				STD. DEV. (±)
		DDA1	DDA2	DDA3	DDA AVG.	
1.	0	0.000	0.000	0.000	0.000	0.000
2.	1	0.082	0.089	0.009	0.060	0.044
3.	2	0.439	0.472	0.023	0.311	0.250
4.	3	0.907	1.052	0.076	0.678	0.527
5.	4	1.406	1.363	0.113	0.961	0.734
6.	6	2.438	2.730	0.075	1.748	1.456
7.	8	4.207	4.664	2.861	3.911	0.937
8.	10	6.211	6.792	4.126	5.709	1.402
9.	12	5.683	8.592	5.874	6.717	1.627
11.	24	12.607	19.75	12.04	14.799	4.297

Fig. 5.14: IN VITRO RELEASE OF DDEA FROM DICLOFENAC PLASTER B. No. DDA-4 IN BIG CELL.

Membrane: Pig skin; Cell volume:460 ml; RPM:50+/-10.

Dissolution medium: Phosphate buffer USP pH 7.4.

Temperature: 32 deg. cel.

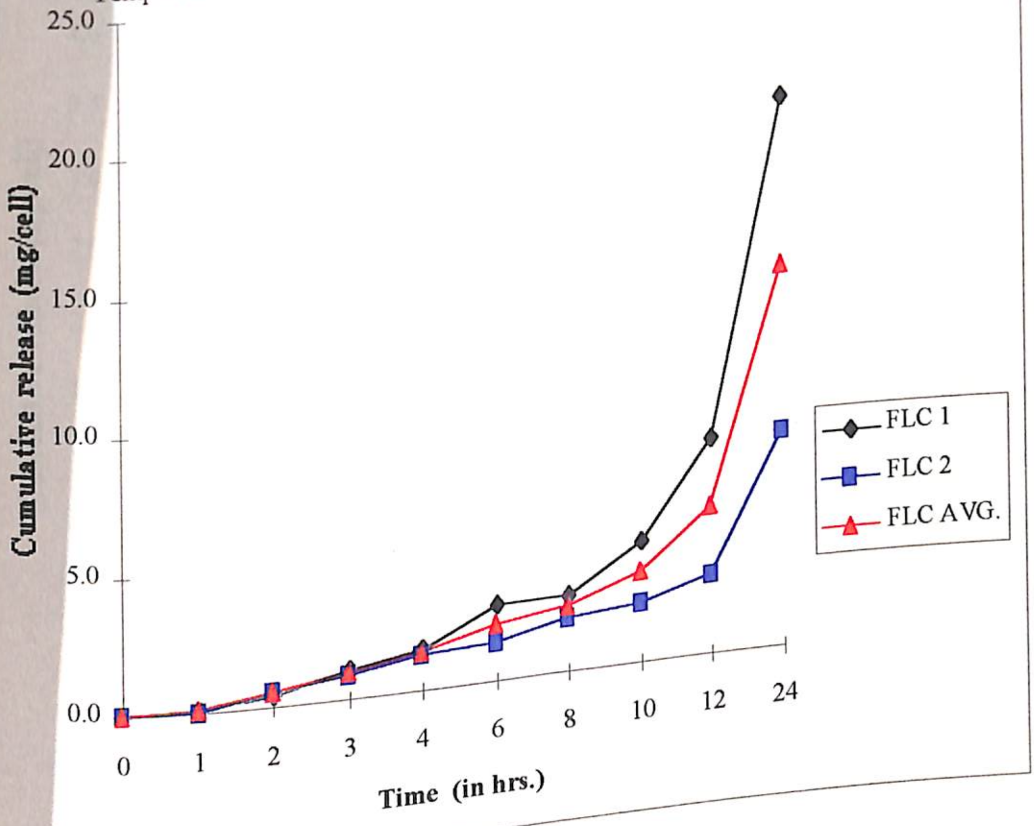


ble 5.16: In vitro release of DHEP from Flector plaster in big cells through pig skin.

. No.	Time (hrs.)	Cumulative Release (mg/cell)			STD. DEV.(±)
		FLC 1	FLC 2	FLC AVG.	
1.	0	0.048	0.000	0.024	0.034
2.	1	0.128	0.000	0.064	0.090
3.	2	0.415	0.568	0.492	0.108
4.	3	1.052	0.865	0.958	0.132
5.	4	1.472	1.265	1.369	0.147
6.	6	2.730	1.351	2.041	0.975
7.	8	2.730	1.865	2.298	0.612
8.	10	4.397	2.103	3.250	1.622
9.	12	7.895	2.891	5.393	3.538
11.	24	20.204	7.930	14.067	8.679

Fig. 5.15 : IN VITRO RELEASE OF DHEP FROM FLECTOR IN BIG CELL.

Membrane: Pig skin; Cell volume: 460 ml; RPM: 50+/-10.
Dissolution medium: Phosphate buffer USP pH 7.4.
Temperature: 32 deg. cel.



e 5.17: In vitro release of DDEA from Diclofenac plaster B. No. DDA-4 in small cells through pig skin.

Sr. No.	Time (hrs.)	Cumulative Release (mg/cell)				STD. DEV. (±)
		DDA1	DDA2	DDA3	DDA AVG.	
1.	0	0.000	0.000	0.000	0.000	0.000
2.	1	0.022	0.035	0.025	0.027	0.007
3.	2	0.010	0.190	0.179	0.126	0.101
4.	3	0.085	0.331	0.587	0.335	0.251
5.	4	0.126	0.563	0.924	0.538	0.399
6.	6	0.862	1.237	1.335	1.145	0.250
7.	8	1.235	1.689	1.378	1.434	0.232
8.	10	1.459	1.870	1.834	1.721	0.228
9.	12	1.562	2.024	2.200	1.928	0.329
10.	24	3.610	3.028	3.625	3.421	0.341

Fig. 5.16: IN VITRO RELEASE OF DDEA FROM DDEA PLASTER B. No. DDA- 4 IN SMALL CELLS.

Membrane: Pig skin; Cell volume: 60 ml; RPM:70+/-10.
Dissolution medium: Phosphate buffer USP pH 7.4.
Temperature: 32 deg. cel.

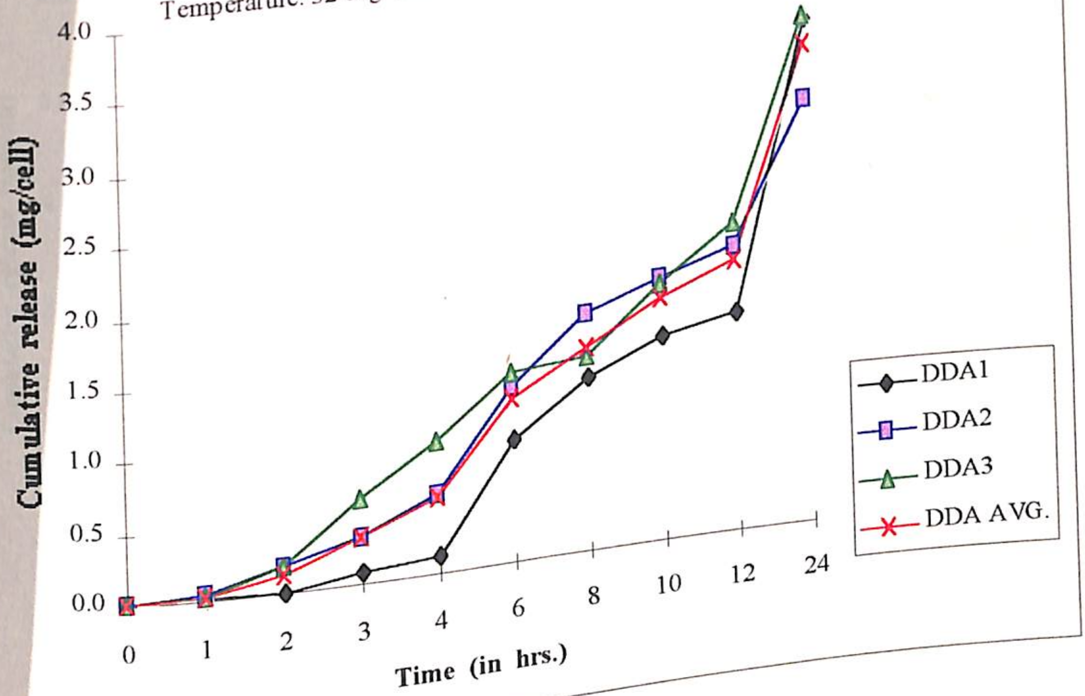
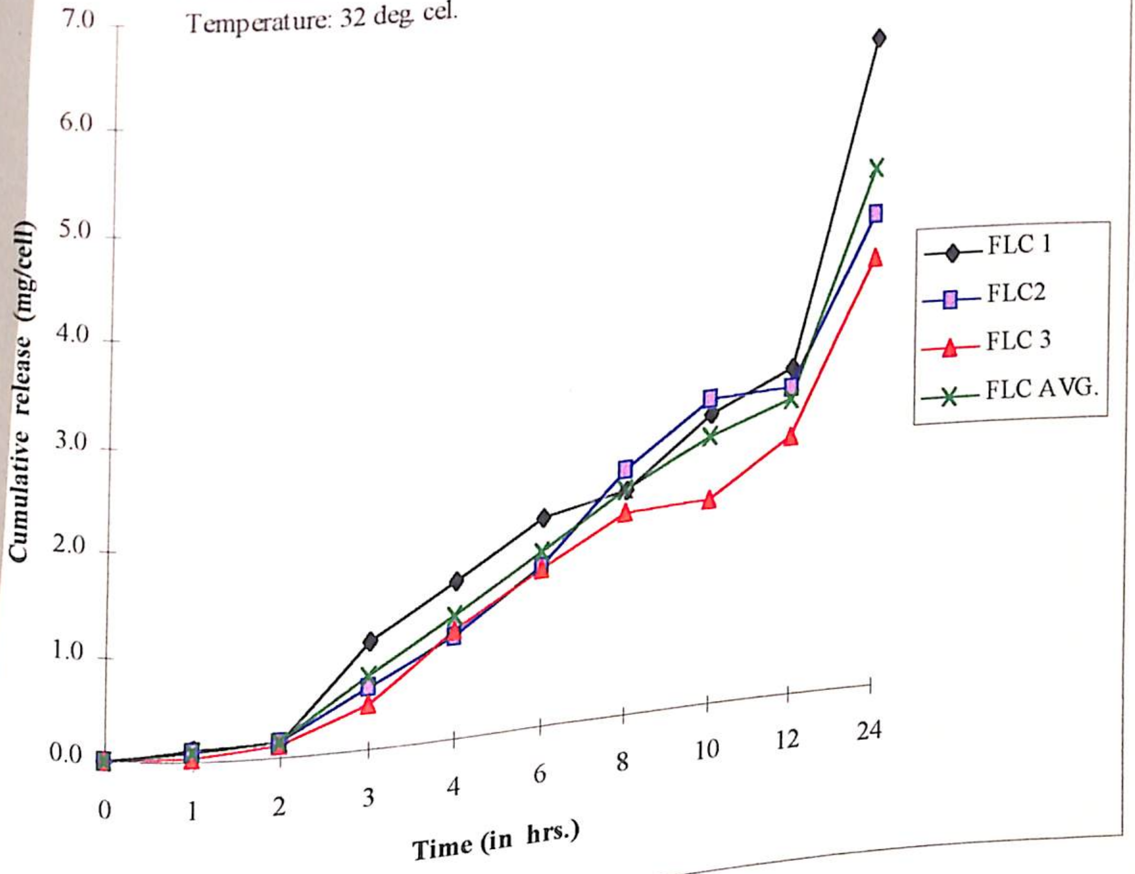


Table 5.18: In vitro release of DHEP from Flector in small cells through pig skin.

Sr. No.	Time (hrs.)	Cumulative Release (mg/cell)				STD. DEV. (±)
		FLC 1	FLC2	FLC 3	FLC AVG.	
1.	0	0.000	0.000	0.000	0.000	0.000
2.	1	0.111	0.095	0.037	0.081	0.039
3.	2	0.150	0.143	0.110	0.134	0.021
4.	3	1.050	0.593	0.433	0.692	0.320
5.	4	1.531	0.985	1.033	1.183	0.302
6.	6	2.019	1.562	1.523	1.701	0.276
7.	8	2.211	2.412	1.967	2.197	0.223
8.	10	2.862	2.993	2.011	2.622	0.533
9.	12	3.215	3.012	2.536	2.921	0.348
10.	24	6.412	4.670	4.221	5.101	1.157

Fig. 5.17: IN VITRO RELEASE OF DHEP FROM FLECTOR IN SMALL CELL.

Membrane: Pig skin; Cell volume: 60 ml; RPM: 70+/-10.
Dissolution medium: Phosphate buffer USP pH 7.4.
Temperature: 32 deg. cel.



5.19: In vitro release of DDEA from Diclofenac plaster B. No. DDA-5 in small cells through pig skin.

	Time (hrs.)	Cumulative Release (mg/cell)				STD. DEV.(±)
		DDA1	DDA2	DDA3	DDA AVG.	
.	0	0.000	0.000	0.000	0.000	0.000
2.	1	0.010	0.005	0.050	0.022	0.025
3.	2	0.019	0.031	0.095	0.048	0.041
4.	4	0.289	0.165	0.195	0.216	0.065
5.	6	1.002	1.552	1.755	1.436	0.390
6.	8	1.022	1.965	2.065	1.684	0.575
7.	10	1.456	2.235	2.364	2.018	0.491
8.	12	1.882	2.365	2.564	2.270	0.351
9.	24	4.521	5.435	5.765	5.240	0.644

Fig. 5.18: IN VITRO RELEASE OF DDEA FROM DDEA PLASTER B. No. DDA-5 IN SMALL CELLS.

Membrane: Pig skin; Cell volume: 60 ml; RPM: 70+/-10.
Dissolution medium: Phosphate buffer USP pH 7.4.
Temperature: 32 deg. cel.

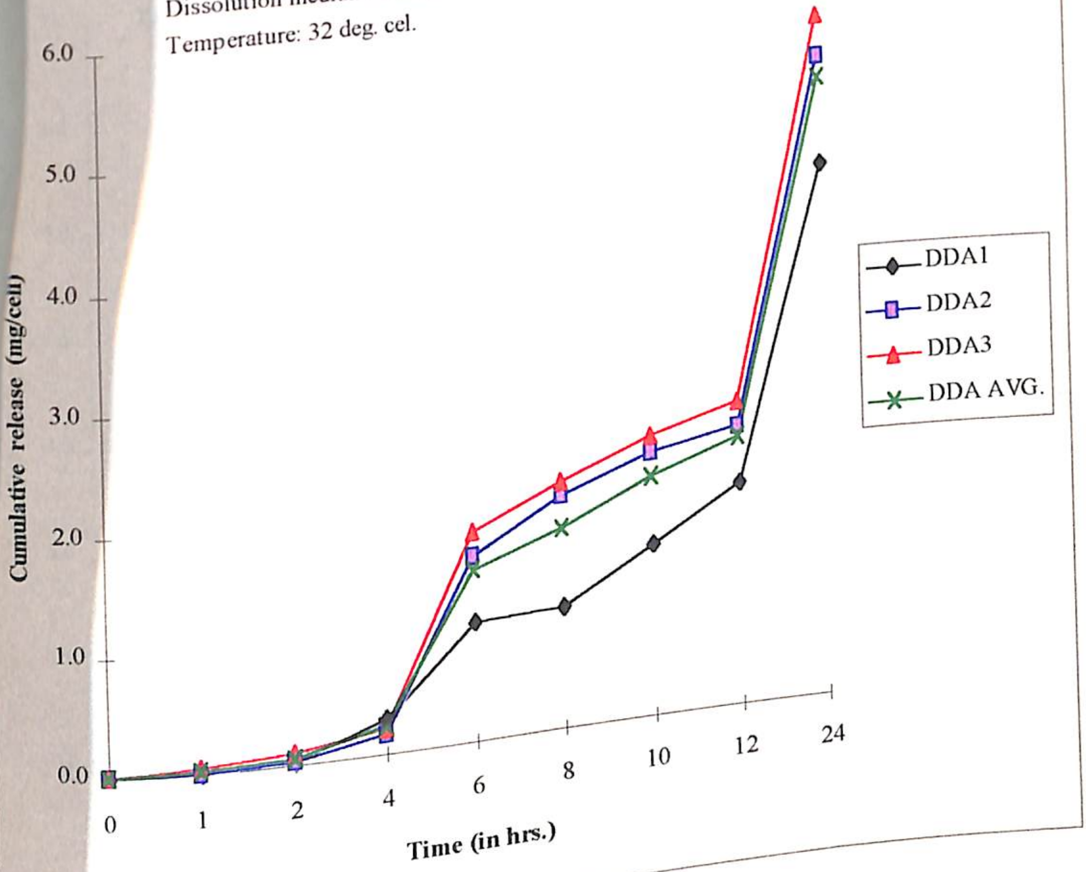


Table 5.20: In vitro release of DHEP from Flector in small cells through pig skin.

S. No.	Time (hrs.)	Cumulative Release				STD. DEV. (+/-)
		FLC 1	FLC2	FLC 3	FLC AVG.	
1	0	0.000	0.000	0.000	0.000	0.000
2	1	0.150	0.085	0.045	0.093	0.053
3	2	0.185	0.156	0.156	0.166	0.017
4	3	1.256	0.688	0.556	0.833	0.372
5	4	1.865	0.998	1.126	1.330	0.468
6	6	2.265	1.455	1.665	1.795	0.420
7	8	2.650	2.356	2.056	2.354	0.297
8	10	3.015	2.886	2.421	2.774	0.313
9	12	3.337	3.156	2.768	3.087	0.290
10	24	6.668	5.867	4.985	5.840	0.842

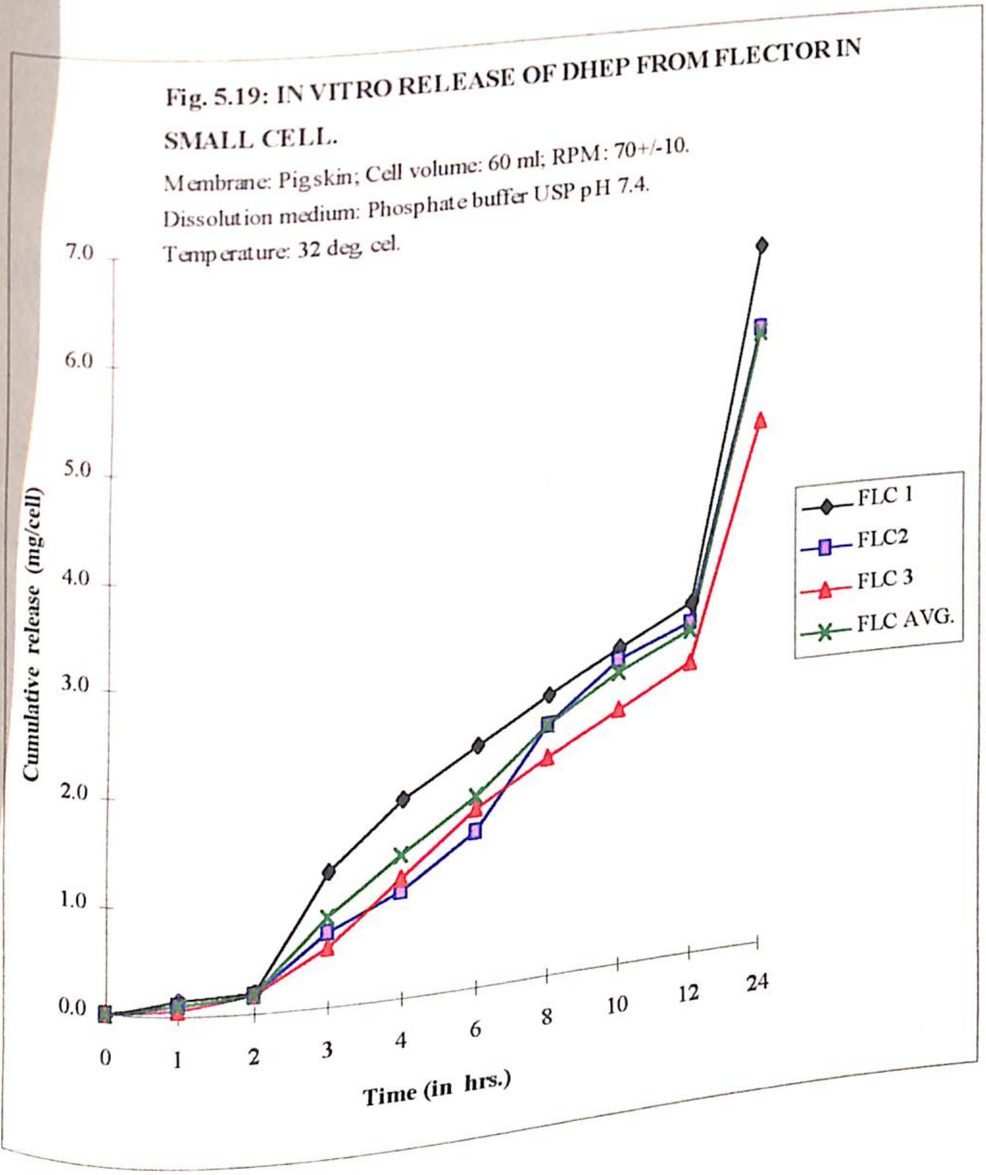


Fig. 5.19: IN VITRO RELEASE OF DHEP FROM FLECTOR IN SMALL CELL.

Membrane: Pigskin; Cell volume: 60 ml; RPM: 70+/-10.
Dissolution medium: Phosphate buffer USP pH 7.4.
Temperature: 32 deg. cel.

Table 5.21: In vivo release of DDEA from Diclofenac plaster B. No. DDA-5 through cross over design.

Sr. No.	Time (hrs.)	Plasma concentration of diclofenac (mcg./ml)							STD. DEV.(±)
		DDA 1	DDA 2	DDA 3	DDA 4	DDA 5	DDA 6	DDA AVG.	
1.	0	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
2.	4	0.183	0.093	0.136	0.104	0.164	0.185	0.144	0.040
3.	8	0.209	0.392	0.183	0.466	0.222	0.314	0.298	0.113
4.	12	0.152	0.112	0.152	0.034	0.210	0.215	0.146	0.067
5.	24	0.302	0.453	0.227	0.345	0.318	0.284	0.322	0.076

Fig. 5.20: IN VIVO RELEASE OF DDEA FROM DDEA PLASTER B. No. DDA-5 IN RABBITS IN A CROSS OVER DESIGN.

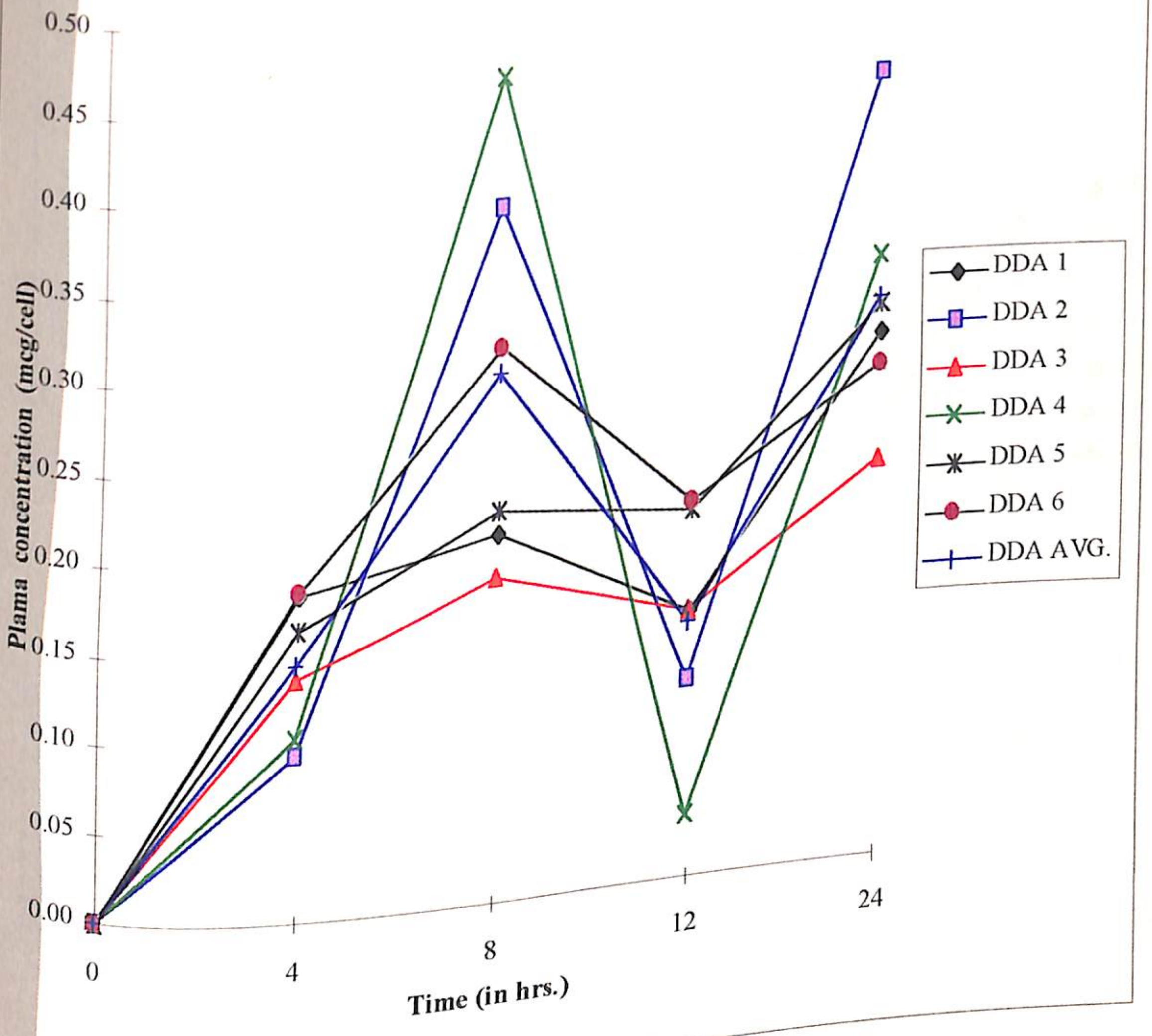


Table 5.22: In vivo release of DHEP from Flector in a cross over design.

Sr. No.	Time (hrs.)	Plasma concentration of diclofenac (mcg./ml)							STD. DEV.(±)
		FLC1	FLC 2	FLC 3	FLC 4	FLC 5	FLC 6	FLC. AVG.	
1.	0	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
2.	4	0.164	0.195	0.104	0.183	0.093	0.207	0.158	0.048
3.	8	0.222	0.189	0.466	0.209	0.392	0.155	0.272	0.126
4.	12	0.210	0.119	0.034	0.152	0.112	0.186	0.136	0.062
5.	24	0.318	0.152	0.345	0.302	0.453	0.325	0.316	0.097

Fig. 5.21: IN VIVO RELEASE OF DHEP FROM FLECTOR IN RABBITS IN A CROSS OVER DESIGN.

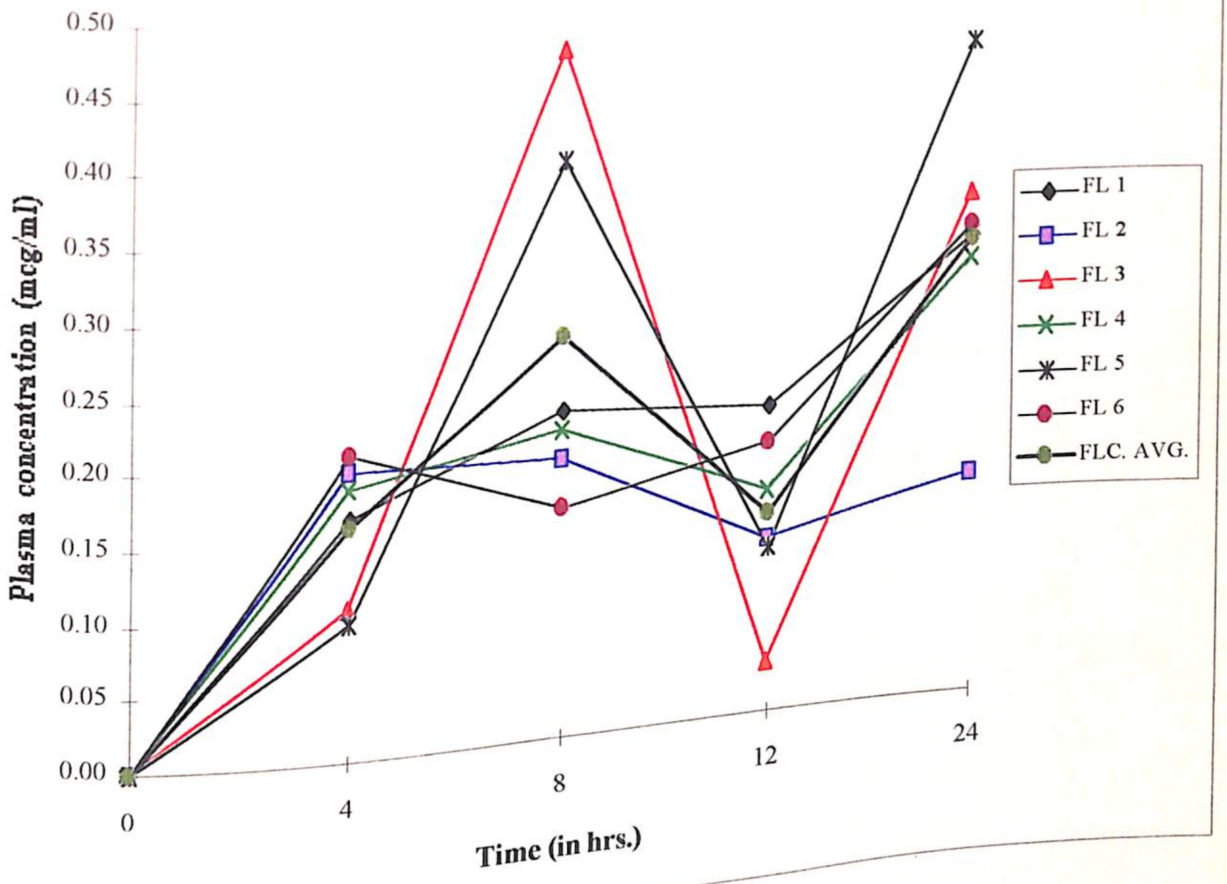


Table 5.22: Comparison of in vivo release of Diclofenac from DDEA plaster B. No. DDA-5 with that of Flector in a cross over design.

Sr. No.	Time (hrs.)	Plasma conc. (mcg./ml)	
		DDA AVG.	FLC. AVG.
1.	0	0.000	0.000
2.	4	0.144	0.158
3.	8	0.298	0.272
4.	12	0.146	0.136
5.	24	0.322	0.316

Fig. 5.22: COMPARISON OF IN VIVO RELEASE OF DDEA FROM DICLOFENAC PLASTER B. No. DDA-5 WITH THAT OF FLECTOR IN RABBITS IN A CROSS OVER DESIGN.

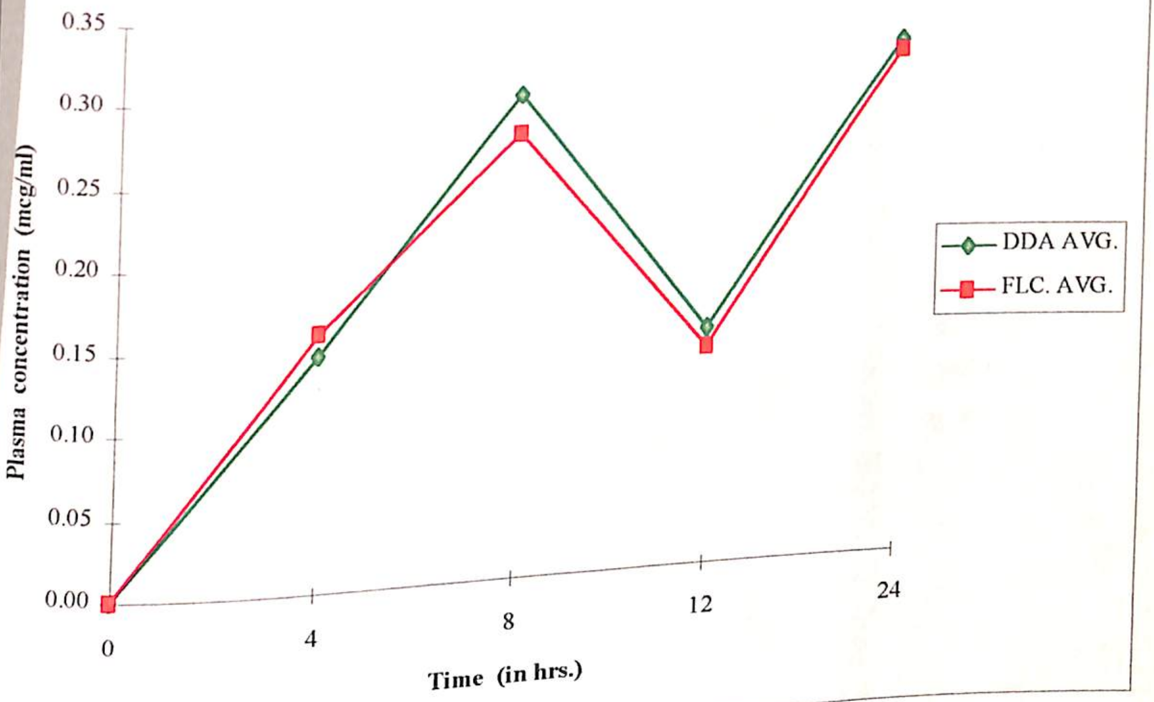


Table 5.23: Stability study of Diclofenac plaster B. No. DDA-5 for DDEA content and physical appearance.

B. No.	Parameter	Storage Temperature	Time Elapsed (in months)									
			0	0.5	1.0	1.5	2.0	2.5	3.0	4.0	5.0	6.0
DDA-5	Drug Content (mg/plaster)	RT(30±2°C)	182.19	-	182.09	-	182.0	-	181.94	181.21	181.09	181.98
		Ref.(4-8°C)	-	-	181.13	-	182.09	-	182.01	180.0	181.98	181.89
		45°C	-	181.82	181.0	180.05	179.21	179.09	178.56	-	-	-
	Physical Appearance		*									

* Note- No change in the physical appearance under any of the condition.

Fig. 5.23: Stability study of DDEA plaster B. No. DDA-5 for DDEA content.

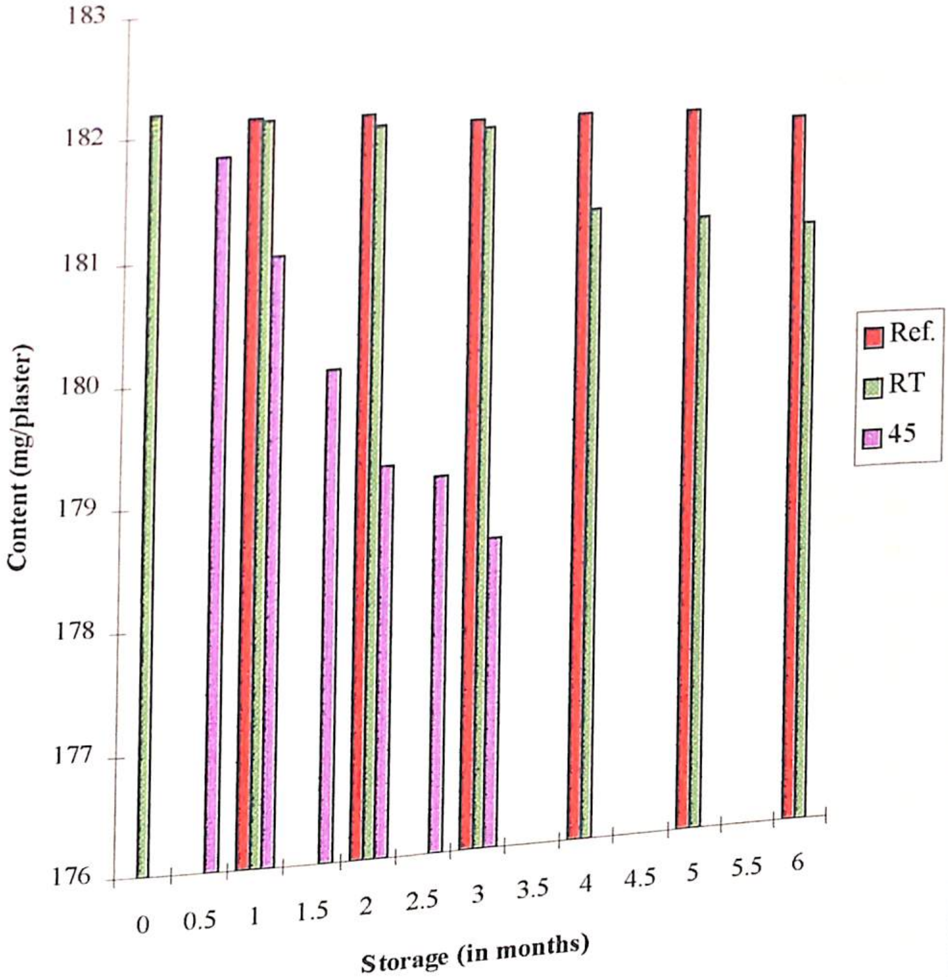


Table 5.24: In vitro release of Nicardipine HCl from Nicardipine patches (Matrix Adhesive Dispersion) B. No. NIC-MD-01 through cellulose acetate membrane (0.45 μm pore size).

Sr. No.	Time (hrs.)	Cumulative Release (mg/cell)				STD. DEV. (±)
		NIC.1	NIC.2	NIC.3	NIC. AVG.	
1.	0	0.000	0.000	0.000	0.000	0.000
2.	1	0.055	0.085	0.060	0.067	0.016
3.	2	0.126	0.100	0.968	0.398	0.494
4.	3	0.866	1.235	1.123	1.074	0.190
5.	4	1.337	1.245	1.355	1.312	0.059
6.	6	1.986	1.467	1.675	1.709	0.261
7.	8	2.024	1.865	1.775	1.888	0.126
8.	10	2.086	1.875	1.965	1.976	0.106
9.	12	2.124	1.998	2.101	2.074	0.067
10.	24	2.237	2.189	2.354	2.260	0.085

Fig. 5.24: IN VITRO RELEASE OF NICARDIPINE HCl FROM PATCH (MATRIX SYSTEM) B. No. NIC-MD-01.

Membrane: Cellulose acetate (0.45 micron pore size).

Cell volume: 60 ml; RPM:70+/-10.

Dissolution medium: Phosphate buffer USP pH 7.4.

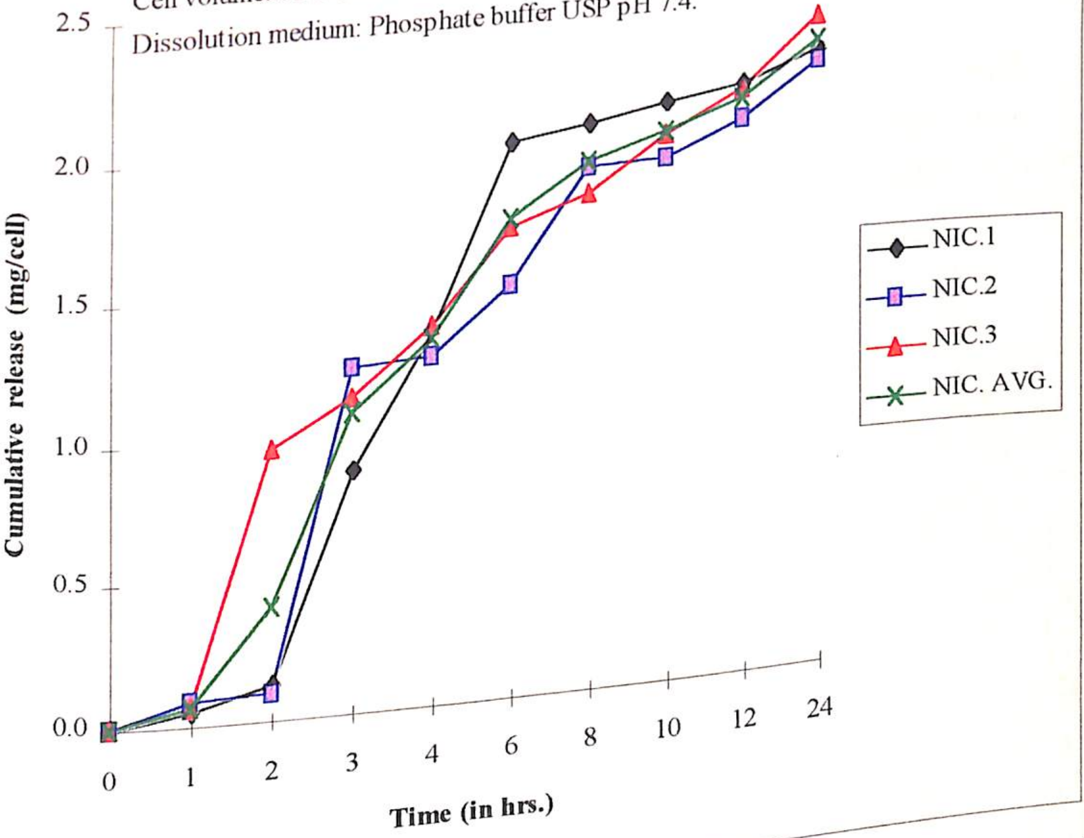
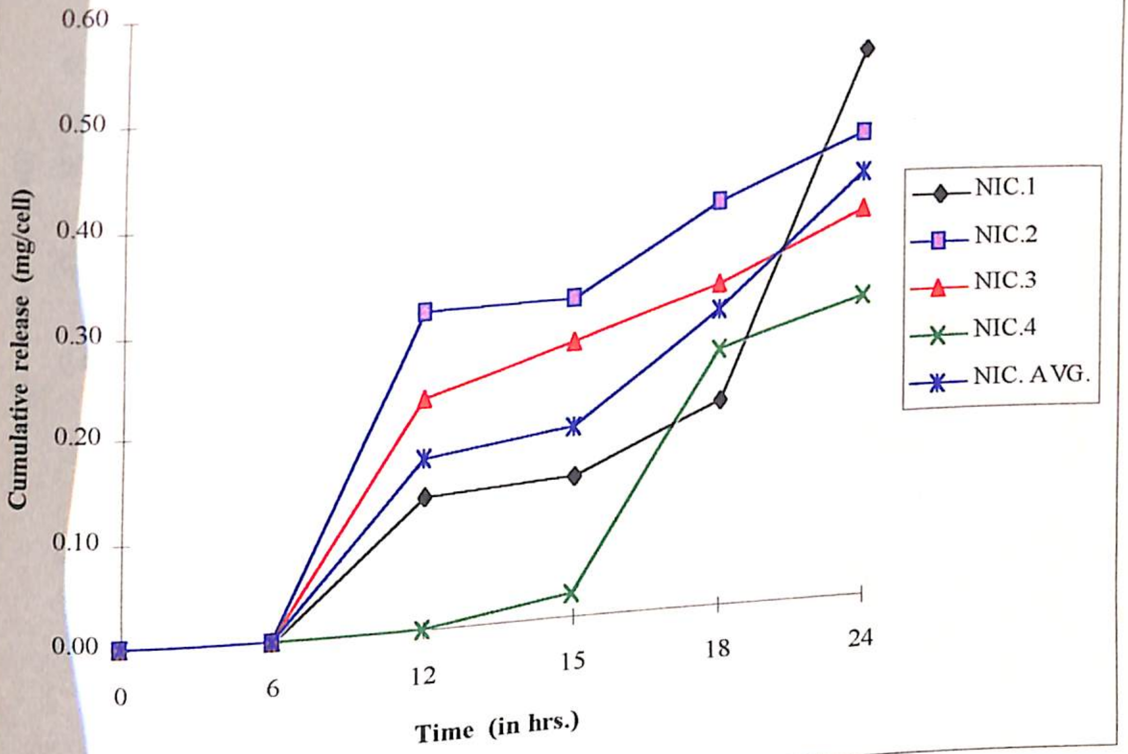


Table 5.25: In vitro release of Nicardipine HCl from Nicardipine patches (Matrix Adhesive Dispersion) B. No. NIC-MD-01 through pig skin.

Sr.No.	Time (in hrs.)	Cumulative Release (mg/cell)					STD. DEV. (±)
		NIC.1	NIC.2	NIC.3	NIC.4	NIC. AVG.	
1.	0	0.000	0.000	0.000	0.000	0.000	0.000
2.	6	0.000	0.000	0.000	0.000	0.000	0.000
3.	12	0.127	0.307	0.222	0.000	0.164	0.132
4.	15	0.137	0.309	0.267	0.022	0.184	0.130
5.	18	0.200	0.395	0.314	0.252	0.290	0.084
6.	24	0.541	0.458	0.383	0.297	0.420	0.104

Fig. 5.25: IN VITRO RELEASE OF NICARDIPINE HCl FROM PATCH B. No.NIC-MD-01 (MATRIX SYSTEM).

Membrane: Pig skin; Cell volume: 60 ml; RPM:70+/-10.
Dissolution medium: Phosphate buffer USP pH 7.4.
Temperature: 32 deg. cel.



.26: In vitro release of Nicardipine HCl from Nicardipine patches (Membrane Rate Controlled System) B. No. NIC-MC-01 through pig skin.

	Time (hrs.)	Cumulative Release (mg/cell)				STD. DEV.(±)
		NIC.1	NIC.2	NIC.3	NIC. AVG.	
.	0	0.000	0.000	0.000	0.000	0.000
.	6	0.000	0.000	0.000	0.000	0.000
.	12	0.001	0.000	0.002	0.001	0.001
4.	15	0.020	0.030	0.025	0.025	0.005
5.	18	0.224	0.312	0.256	0.264	0.044
6.	24	0.238	0.323	0.258	0.273	0.044

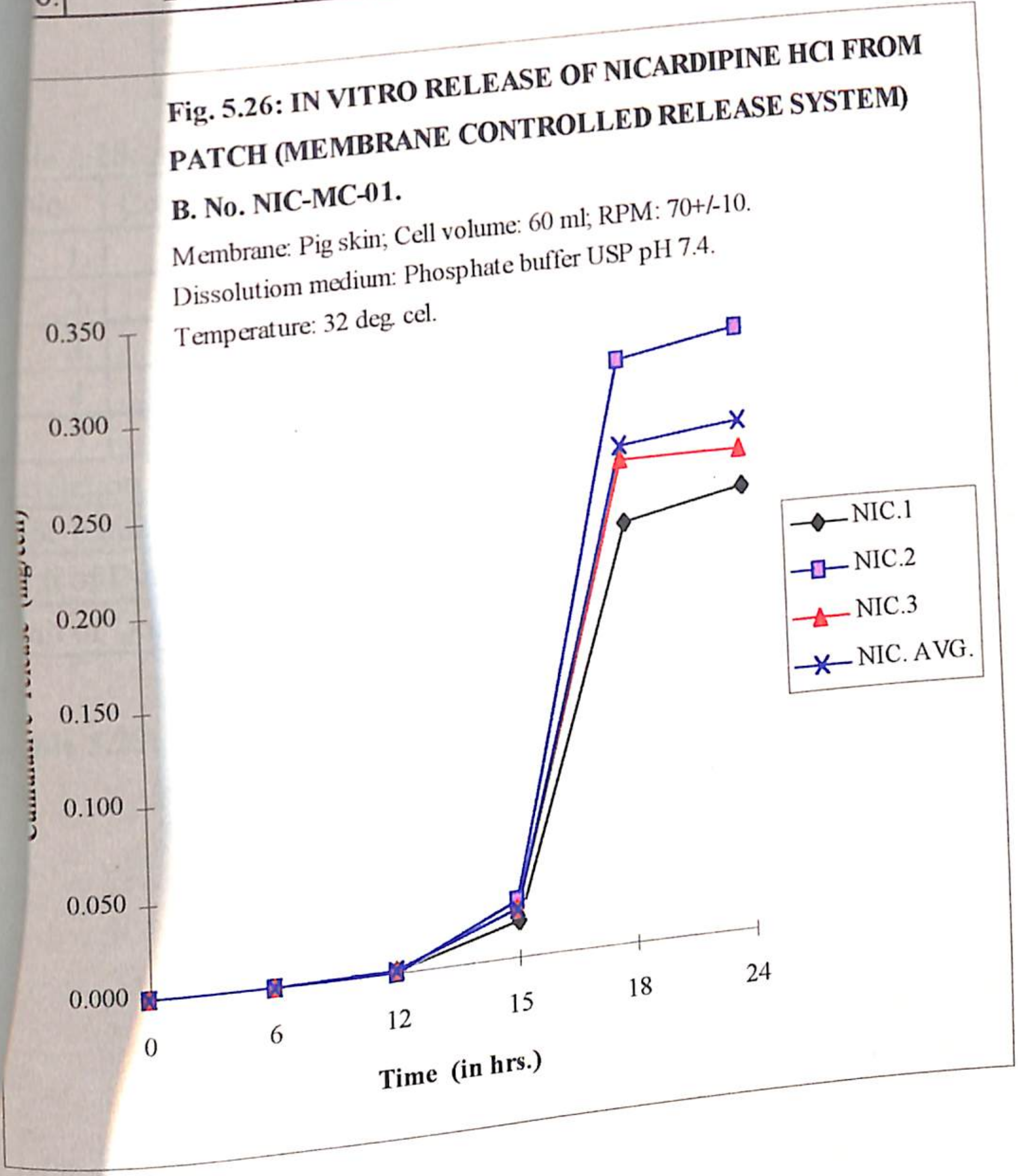


Fig. 5.26: IN VITRO RELEASE OF NICARDIPINE HCl FROM PATCH (MEMBRANE CONTROLLED RELEASE SYSTEM) B. No. NIC-MC-01.

Membrane: Pig skin; Cell volume: 60 ml; RPM: 70+/-10.

Dissolutiom medium: Phosphate buffer USP pH 7.4.

Temperature: 32 deg. cel.

Table 5.27: Precision of NTG in vitro.

Sr. No.	Peak Area
1.	193774.18
2.	196864.00
3.	195584.50
4.	196658.84
5.	198556.77
6.	192556.25
Avg. 195665.75	
Std. Dev. 2192. 4199	
% RSD 1.1205	

Table 5.28: Accuracy by linearity of NTG in vitro.

Sr. No.	Concentration (μg/ml)	Peak Area	Peak Height (mV)
1.	20.0	196864	7771
2.	30.0	340154.5	14726
3.	40.0	448285.5	19136
4.	50.0	549565.25	25789
5.	100.0	1141568.74	45698
Correlation coeff. 0.99954			
Slope 0.00213965			
Limit of Detection 0.29821089 μg/ml			
Limit of Quatitation 0.99403629 μg/ml			

Table 5.29: Precision of NTG in vivo.

Sr. No.	Peak Area
1.	48556525
2.	49556589
3.	48965875
4.	50456896
5.	48012589
6.	49554584
Avg.	49183843
Std. Dev. 61385.659	
% RSD 1.1751	

Table 5.30: Accuracy by linearity of NTG in vivo.

Sr. No.	Concentration (μg/ml)	Peak Area	Peak Height (mV)
1.	50.0	63005324	86458
2.	100.0	109478104	96736
3.	150.0	167399792	157714
4.	200.0	268152912	300856
5.	500.0	541994323	146629
Correlation coeff. 0.9936			
Slope 0.00045			
Limit of Detection 0.05355309 pg/ml			
Limit of Quatitation 0.17851029 pg/ml			

Table 5.31: Precision of DDEA in vitro.

Sr. No.	Peak Area
1.	196763.3
2.	198432.3
3.	195570.1
4.	194233.5
5.	195547.1
6.	196325.8
Avg. 196130.35	
Std. Dev. 1421.471	
% RSD 0.7247	

Table 5.32: Accuracy by linearity of DDEA in vitro.

Sr. No.	Concentration (μg/ml)	Peak Area	Peak Height (mV)
1.	5.0	198432.2	8409.7
2.	10.0	388468.8	16013.1
3.	15.0	555906.8	22975.8
4.	20.0	774042.0	32349.5
5.	25.0	958456.5	40856.8
Correlation coeff. 0.99934			
Slope 0.00054			
Limit of Detection 0.14476 μg/ml			
Limit of Quantitation 0.48253 μg/ml			

Table 5.33: Precision of DDEA in vivo.

Sr. No.	Peak Area ratio of DDEA/ketoprofen
1.	0.1201
2.	0.0906
3.	0.1056
4.	0.1154
5.	0.1246
6.	0.1102
Avg.	0.1110833
Std. Dev.	0.012108
% RSD	10.900

Table 5.34: Accuracy by linearity of DDEA in vivo.

Sr. No.	Conc. of DDEA (ng/ml)	Peak Area ratio of DDEA/ketoprofen
1.	50.0	0.03708
2.	100.0	0.1201
3.	150.0	0.1354
4.	200.0	0.2313
5.	250.0	0.2935
Correlation coefficient 0.984824		

Table 5.35: Precision of Nicardipine HCl in vitro.

Sr. No.	Peak Area
1.	781123
2.	782937
3.	786588
4.	785546
5.	781125
6.	784415
Avg.	783622.333
Std. Dev.	2283.7257
% RSD	0.2914

Table 5.36: Accuracy by linearity of Nicardipine HCl in vitro.

Sr. No.	Concentration (μg/ml)	Peak Area	Peak Height (mV)
1.	7.0	487456	22154
2.	8.4	586698	23546
3.	11.2	782937	24693
4.	14.0	952369	25921
5.	16.8	1178985	26874
Correlation coeff. 0.99905			
Slope 0.00210			
Limit of Detection 0.25226 μg/ml			
Limit of Quantitation 0.84089 μg/ml			

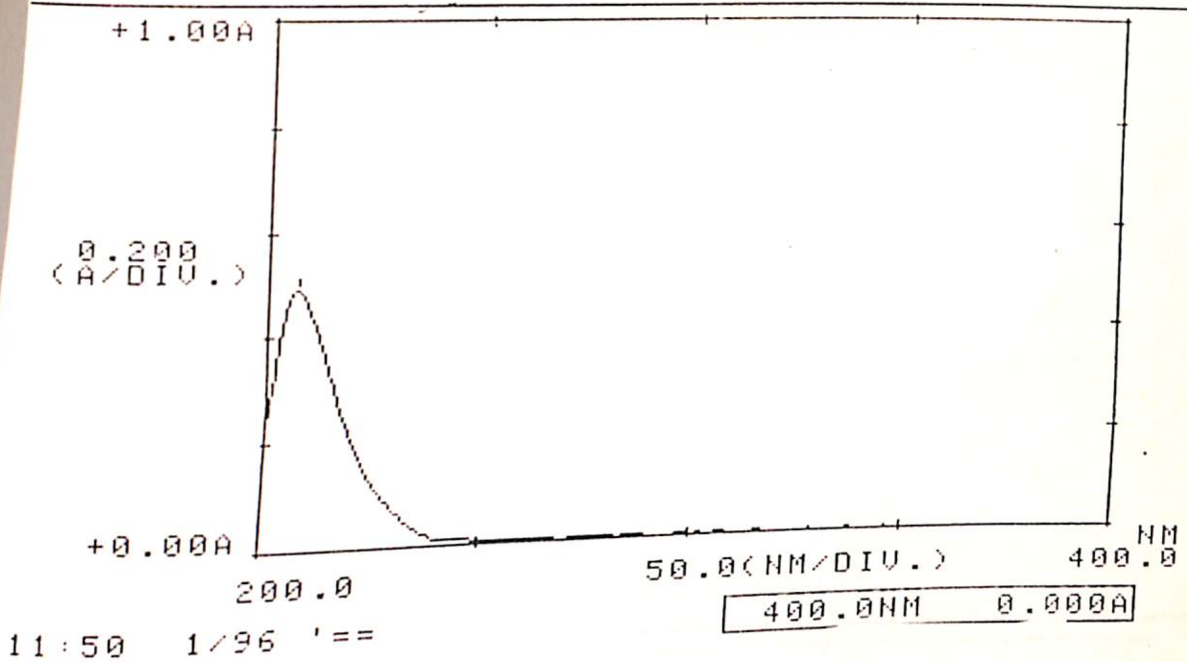


Fig. 5.27: UV spectrum of Nitroglycerine.

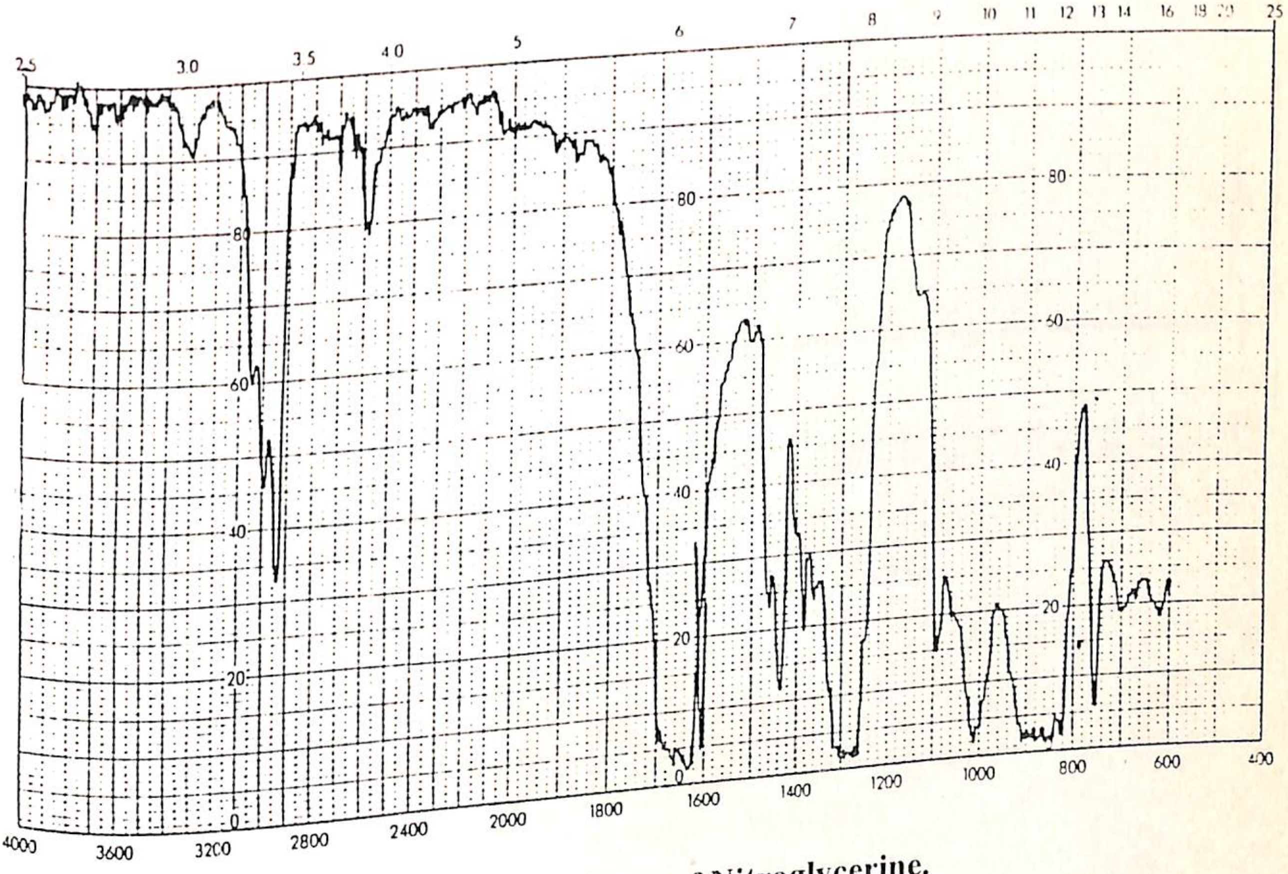


Fig. 5.28: IR spetrum of Nitroglycerine.

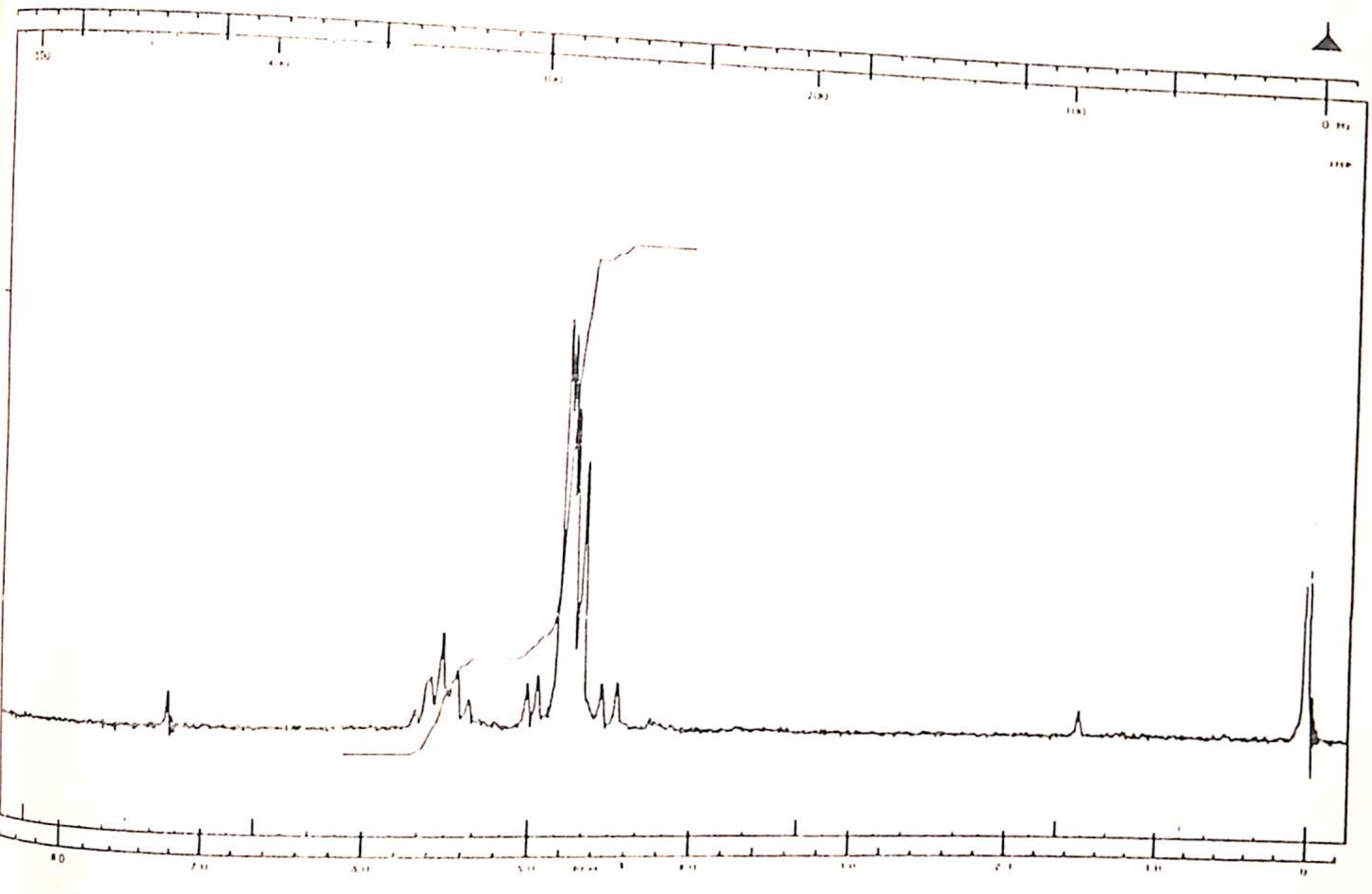


Fig. 5.29: NMR spectrum of Nitroglycerin.

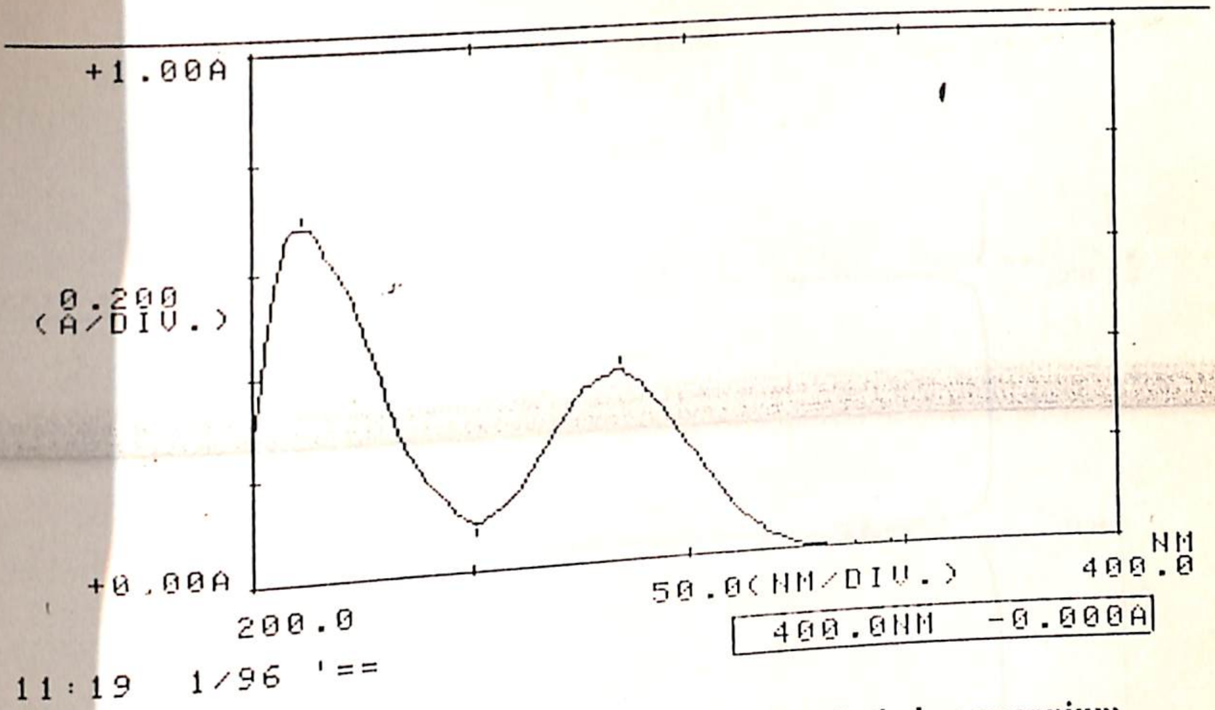


Fig. 5.30: UV spectrum of Diclofenac diethyl ammonium.

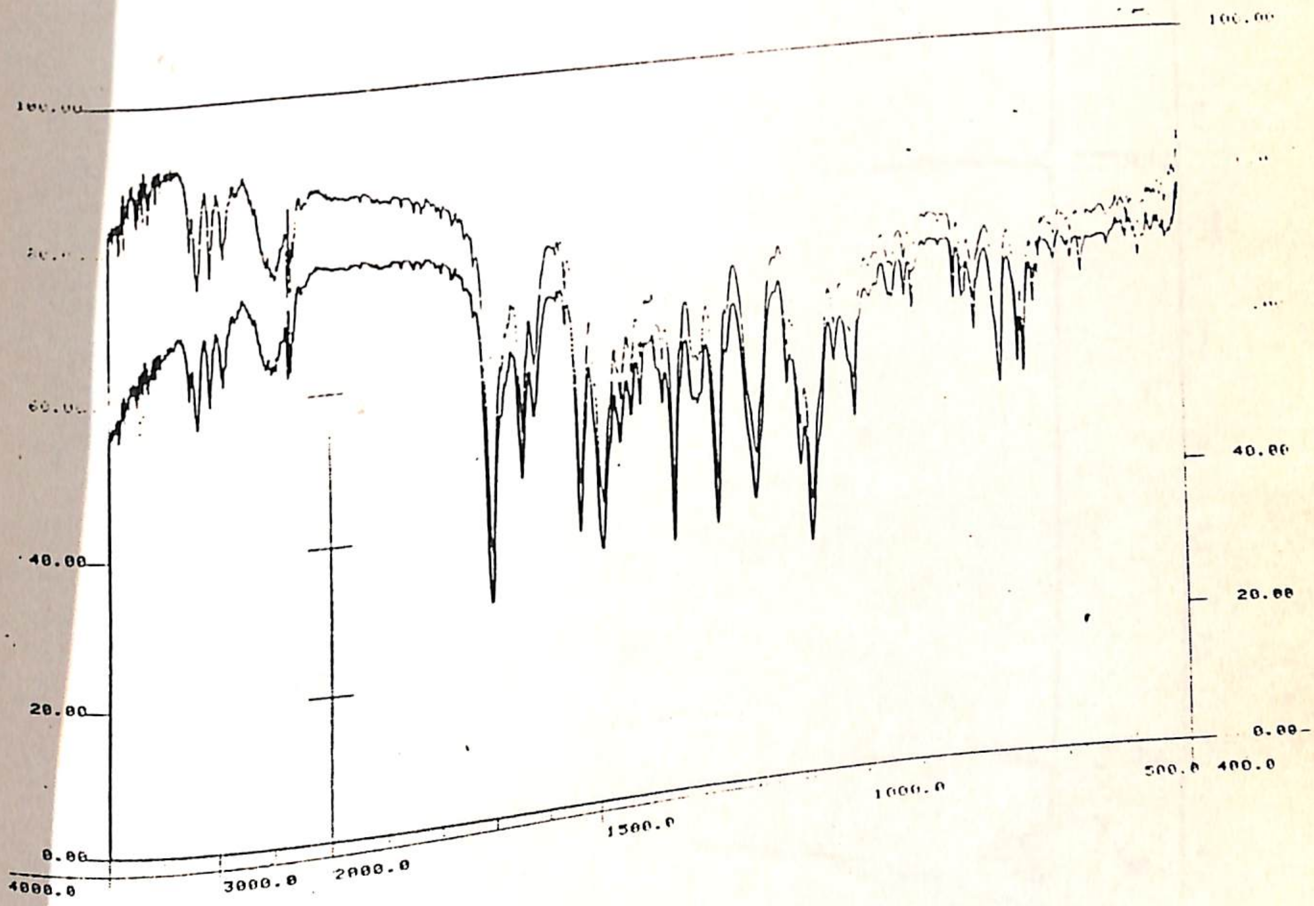
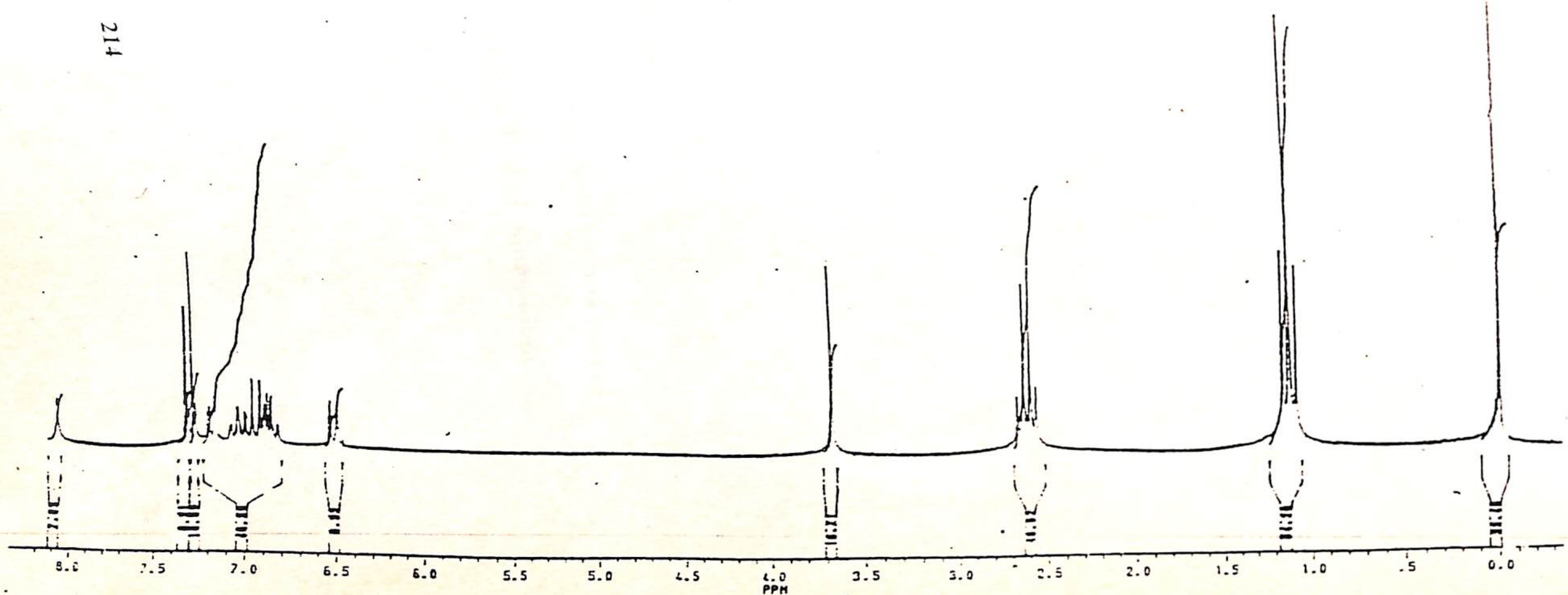


Fig. 5.31: IR spectrum of Diclofenac diethyl ammonium.

Fig. 5.32: NMR spectrum of Diclofenac diethyl ammonium.



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DATE 1-11-9:

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SI  32768
TD  32768
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HZ/PT     .119

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RD        1.000
AQ        8.389
RG       40
NS       32
TE      297

FW    2500
O2        0.0
DP   63L PO

LB        0.0
GB        0.0
CX       35.00
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HZ/CM   49.697
PPM/CM    .248
SR     2338.13
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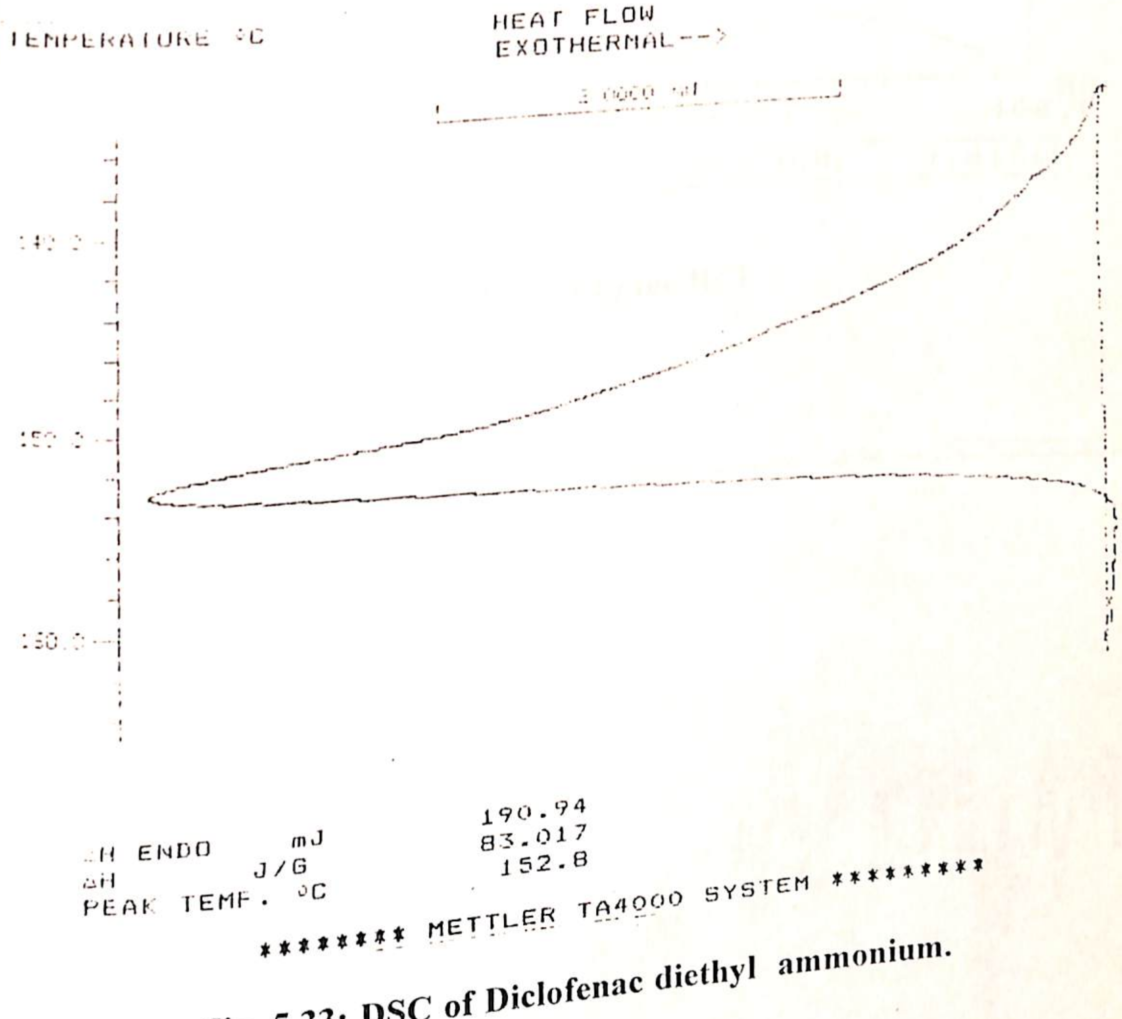

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ΔH           J/G              152.8
PEAK TEMF.  °C
          ******** METTLER TA4000 SYSTEM *********
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Fig. 5.33: DSC of Diclofenac diethyl ammonium.

c·c

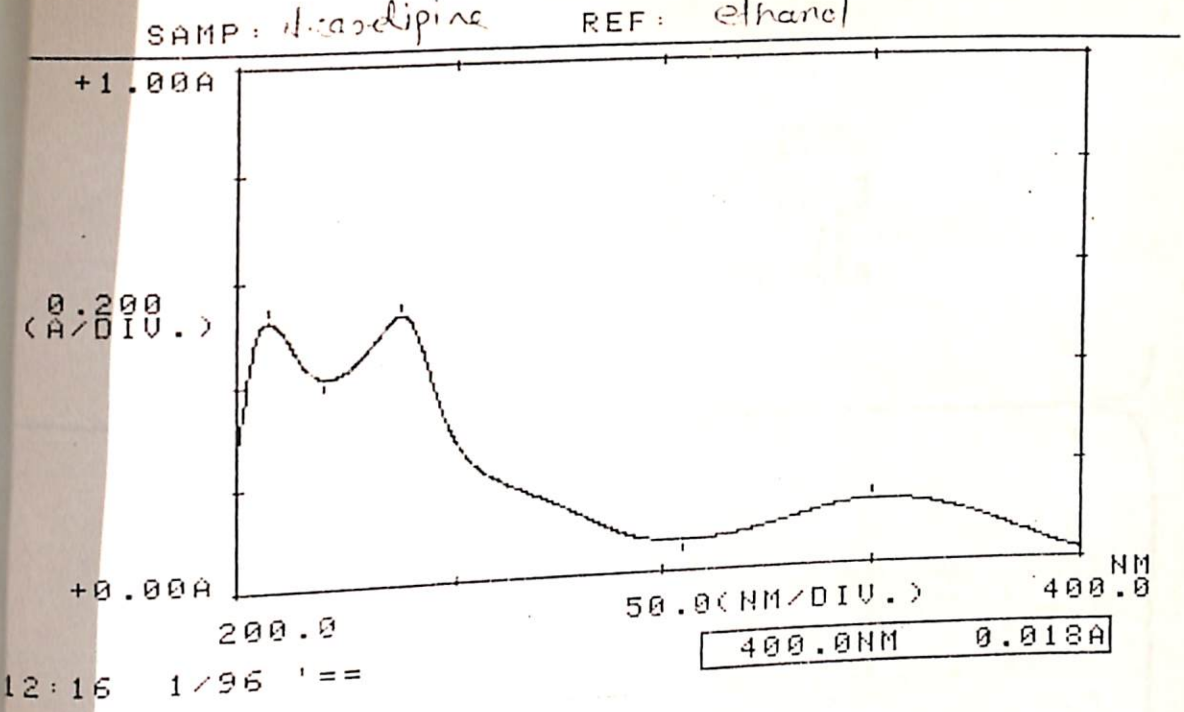


Fig.5.34: UV spectrum of Nicardipine HCl.

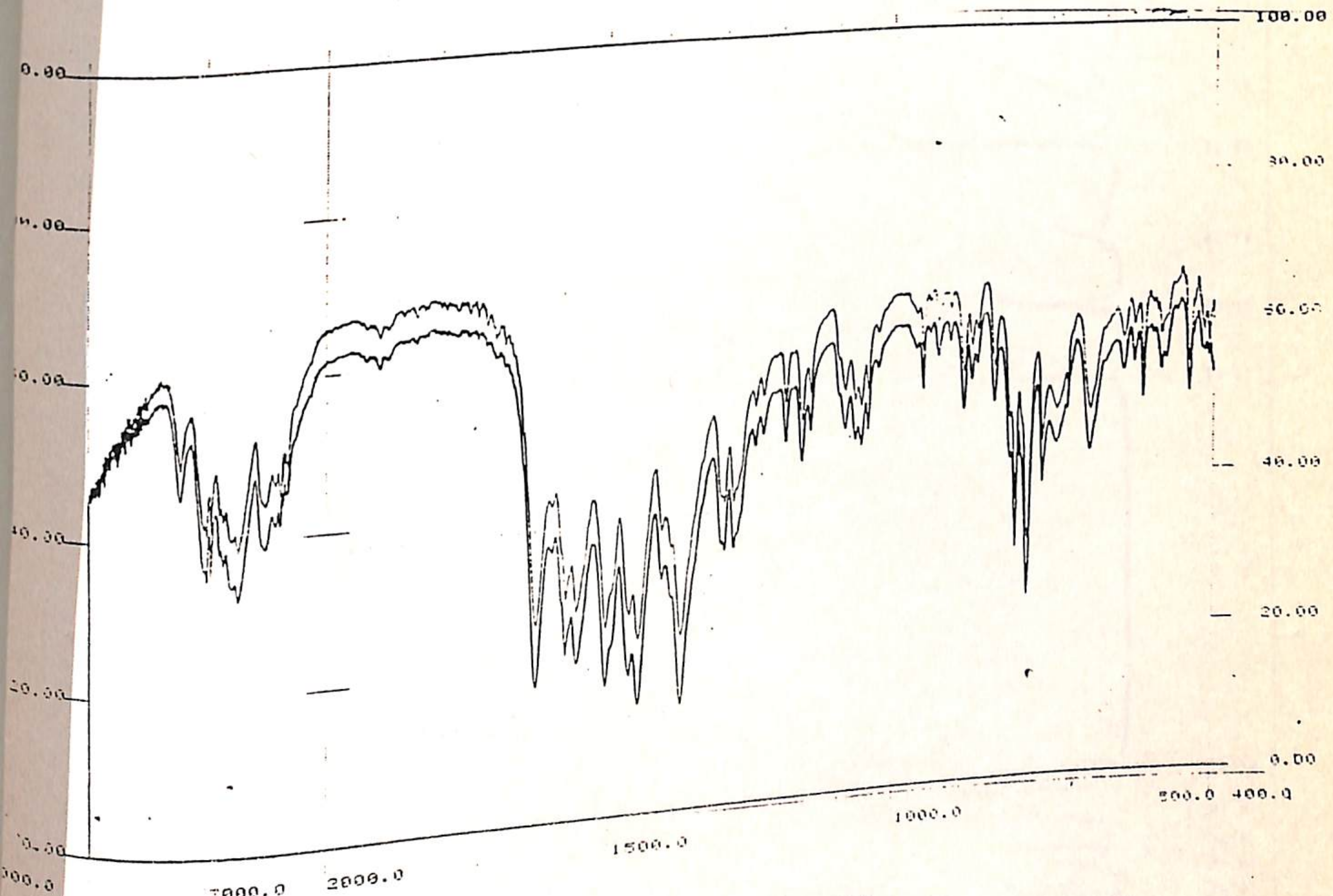
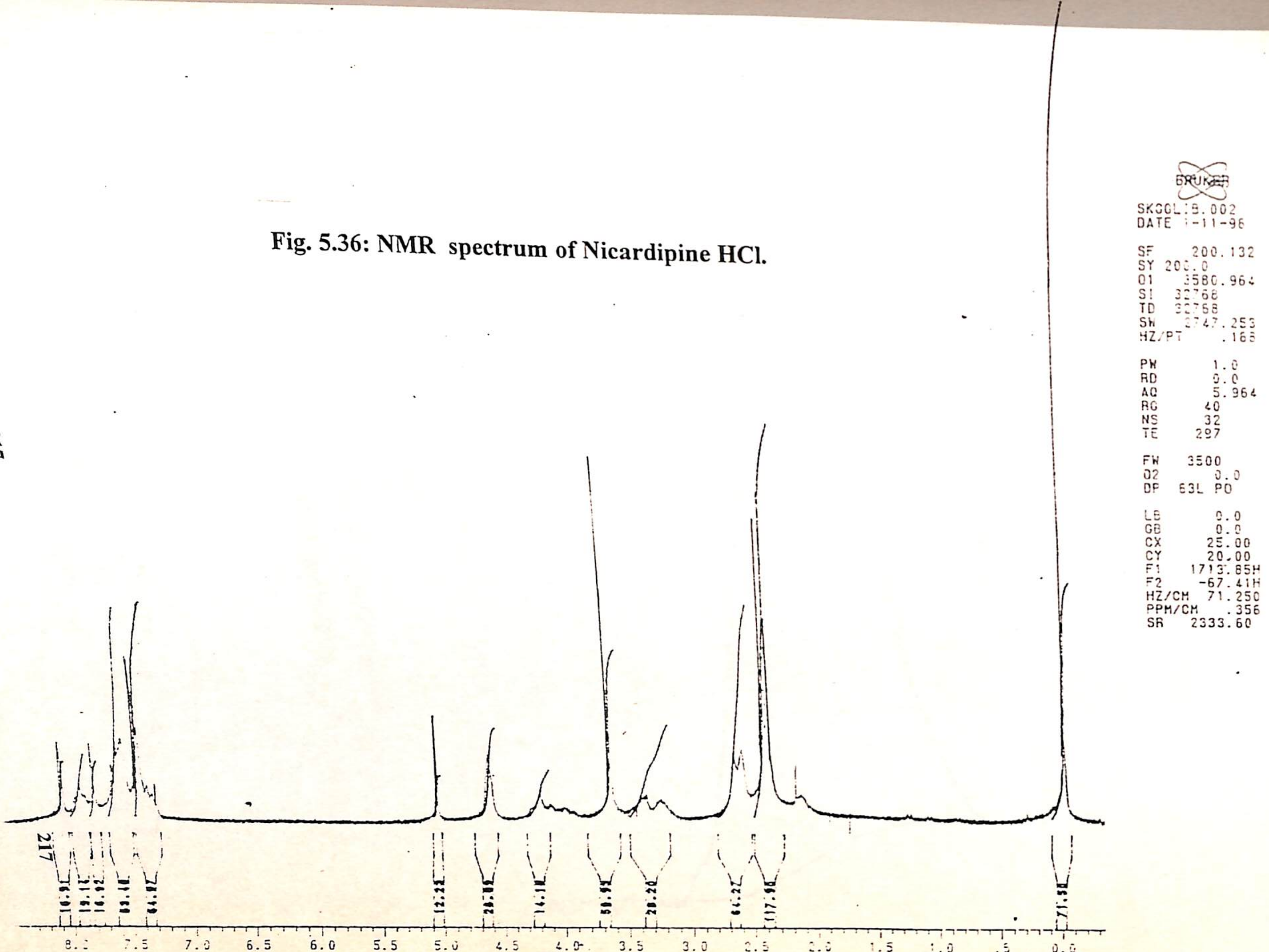


Fig. 5.35: IR spectrum of Nicardipine HCl.

Fig. 5.36: NMR spectrum of Nicardipine HCl.



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DATE 1-11-96

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RD        0.0
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NS       32
TE      297

FW    3500
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LB        0.0
GB        0.0
CX       25.00
CY       20.00
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PPM/CM    .356
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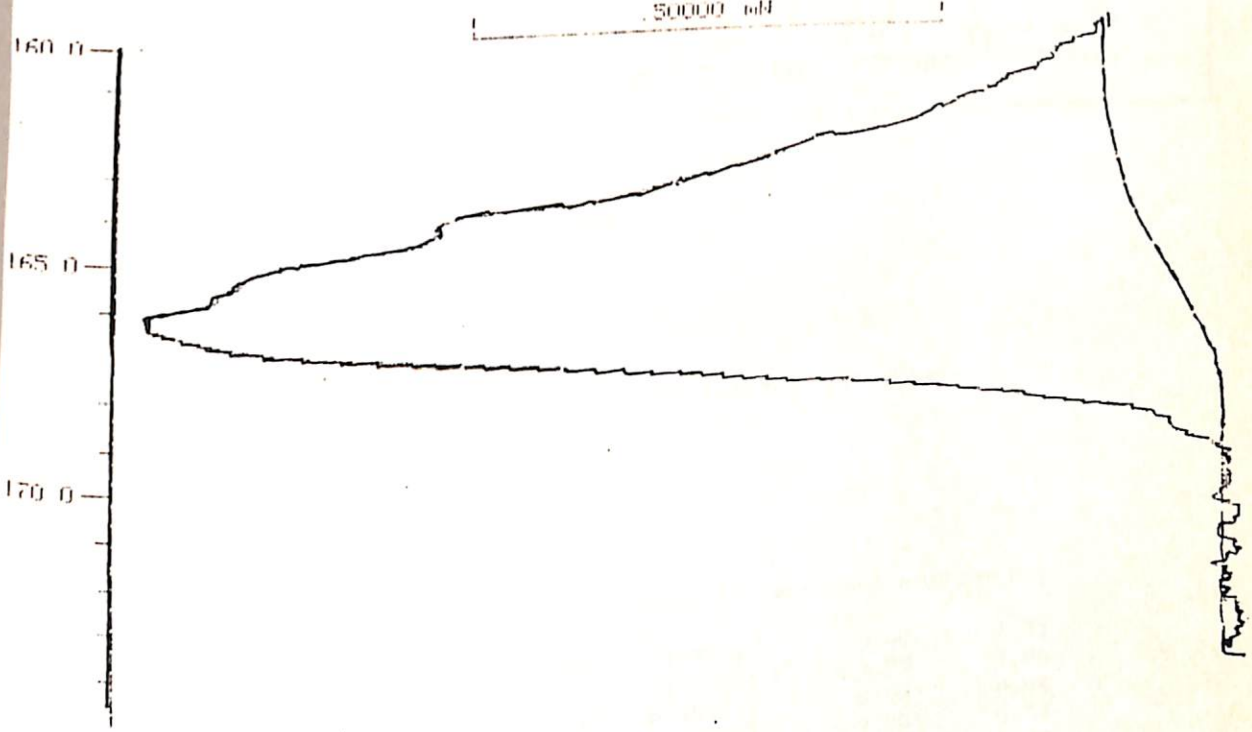

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                          146.55
ΔH ENDO     mJ             61.065
ΔH          J/G           166.2
PEAK TEMP.  °C
        ******** METTLER TA4000 SYSTEM *********
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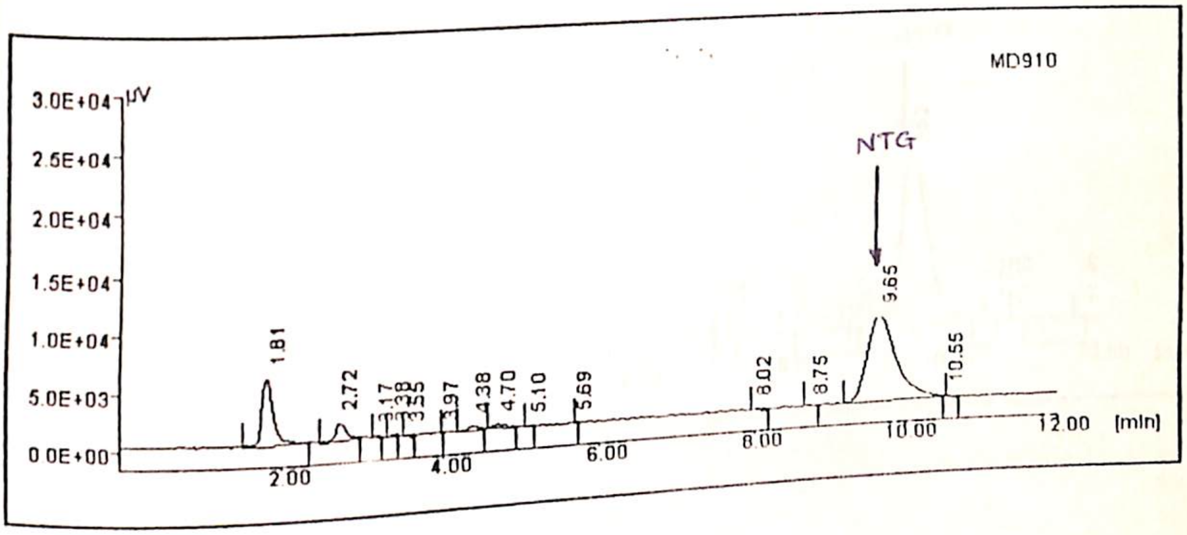
Fig. 5.37: DSC of Nicardipine HCl.

Chromatogram and Report



File name : NTG__042.CH1 User : VINAYAK

Run Length : 12.01 [min]

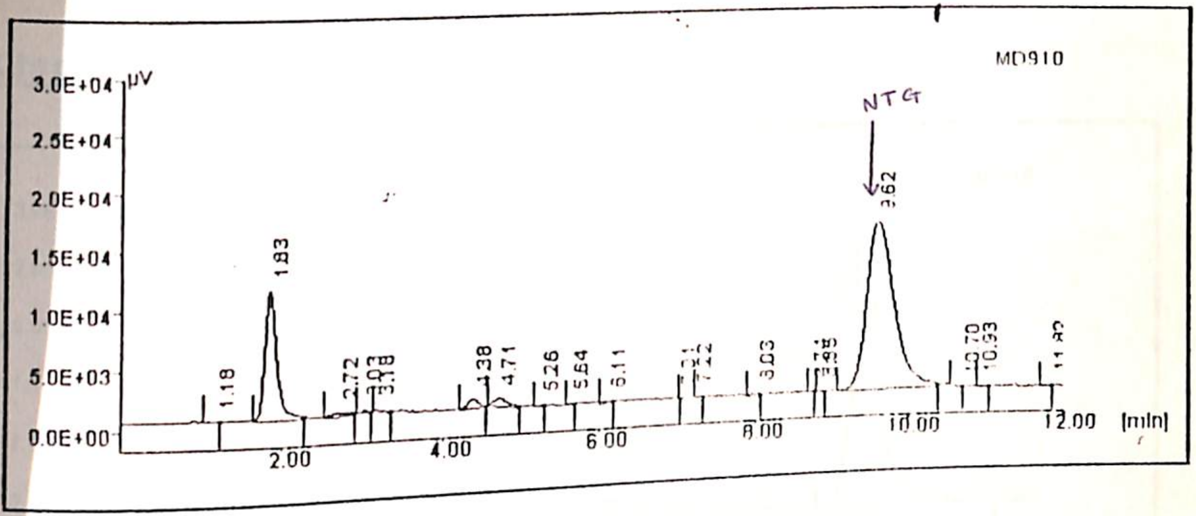
Info :
Standard (20 mcg/ml)
In-vitro Dissolution Studies of Nitrglycerine Patches on Pig Skin.
Column : Hypersil 10 µ, 4.6 X 250 mm,
Mobile Phase : MeOH : H2O (1:1)
Flow rate : 1 ml/min., Detection : 220 nm.

#	Rt	Area	%Area	Height	NPlates	Resolution	Asymmetry
1	1.81	51075.50	18.625	5833	1543.63	0.00	1.73
2	2.72	13943.50	5.085	1545	3505.25	4.99	2.00
3	3.17	828.50	0.302	215	89354.66	3.98	0.86
4	3.38	972.00	0.354	225	25366.43	3.28	0.56
5	3.55	641.75	0.234	148	8308.90	1.39	10.00
6	3.97	-889.50	-0.324	394	0.00	5.47	0.00
7	4.38	4181.00	1.525	436	3831.96	2.89	1.07
8	4.70	4367.25	1.593	412	6883.80	1.25	1.92
9	5.10	552.50	0.201	189	42346.40	2.46	1.20
10	5.69	-942.50	-0.344	431	0.00	11.97	0.00
11	8.02	1255.00	0.458	166	22786.49	21.95	2.57
12	8.75	613.00	0.224	167	381740.63	5.47	1.00
13	9.65	196864.00	71.788	7771	4017.82	2.71	1.77
14	10.55	766.00	0.279	106	52540.05	2.28	6.00

Total Area of Peak = 274228.00

Fig. 5.38: Chromatogram of Nitroglycerine in Validation of Method - Precision-used for in-vitro dissolution studies using pig skin.

Chromatogram and Report



File name : NTG__047.CH1 User : VINAYAK

Run Length : 12.01 [min]

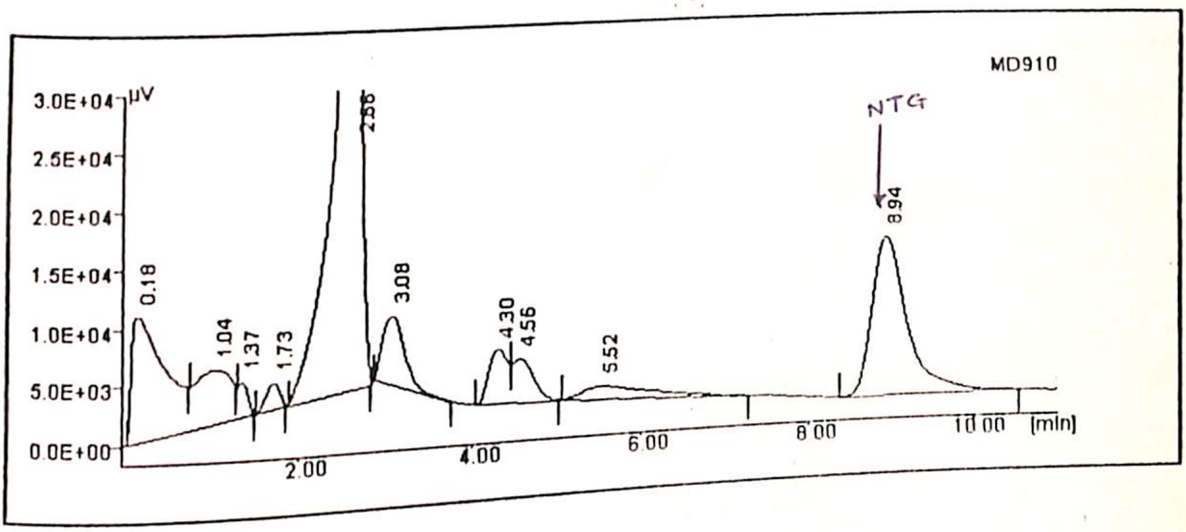
Info :
Standard (30 mcg/ml)
In-vitro Dissolution Studies of Nitroglycerine Patches on Pig Skin.
Column : Hypersil 10 µ, 4.6 X 250 mm,
Mobile Phase : MeOH : H2O (1:1)
Flow rate : 1 ml/min., Detection : 220 nm.

#	Rt	Area	%Area	Height	NPlates	Resolution	Asymmetry
1	1.18	873.50	0.190	149	4468.34	0.00	0.42
2	1.83	90724.50	19.754	10757	1586.60	5.11	1.46
3	2.72	3935.00	0.857	333	947.82	3.32	1.58
4	3.03	1254.50	0.273	278	20277.78	1.37	0.67
5	3.18	979.75	0.213	185	202104.74	2.80	0.63
6	4.38	7053.00	1.536	772	7820.33	10.62	1.00
7	4.71	8032.25	1.749	762	6119.40	1.48	1.13
8	5.26	700.75	0.153	175	88232.40	3.54	4.00
9	5.64	500.50	0.109	182	634785.66	7.75	0.22
10	6.11	1151.00	0.251	212	330731.23	13.22	1.33
11	7.01	-1406.50	-0.306	671	0.00	42.48	0.00
12	7.22	701.00	0.153	147	51411.82	3.41	6.00
13	8.03	1050.75	0.229	200	321768.74	8.80	0.19
14	8.74	543.00	0.118	198	677357.95	14.33	0.25
15	8.85	565.50	0.123	197	390515.99	2.19	1.40
16	9.62	340154.50	74.065	14726	4611.07	2.47	1.30
17	10.70	599.00	0.130	155	9133554.24	3.74	0.00
18	10.93	974.00	0.212	171	56341.50	2.28	3.25
19	11.82	879.00	0.191	177	1237712.33	7.89	0.25

Total Area of Peak = 459265.00

Fig. 5.39: Chromatogram of Nitroglycerine in Validation of Method - Linearity-used for in-vitro dissolution studies using pig skin.

Chromatogram and Report



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File name : NTGP_020.CH1 User :   VINAYAK

Run Length : 11.01 [min]

Info :
Sample : S3/24 hrs
In-vitro Dissolution studies of Nitroglycerine TDDS
using pig skin -1
Column : Hypersil 10 u, Shandon; M.P.: MeOH : H2O (1:1)
Flow rate: 1 ml/min. ; Detection 220 nm.

#  Rt        Area       %Area   Height   NPlates  Resolution Asymmetry

1  0.18   269540.68    12.564   10708       2.79     0.00      5.67
2  1.04   128308.27     5.981    4743      59.95     1.76      0.93
3  1.37    17803.80     0.830    3064    5960.15     1.07      0.40
4  1.73    23066.75     1.075    2300     821.40     2.31      0.81
5  2.66  1011707.50    47.157   53754     626.40     2.81      0.21
6  3.08    98063.50     4.571    6004     967.38     1.04      0.96
7  4.30    69157.76     3.224    4738    5761.95     3.92      1.56
8  4.56    57119.03     2.662    3778   13699.32     1.35      0.89
9  5.52    63562.75     2.963    1093     379.35     1.49      1.98
10 8.94   407059.00    18.974   14278    2887.44     3.82      1.79

Total Area of Peak = 2145389.05
```

Fig. 5.40: A typical chromatogram of sample of Nitroglycerin in the in vitro dissolution studies using pig skin.

```
HIREJ   ARREJ   PKWD   ATTN   CHTSPD   ZERO   SLFSTV   PSSTV   SENSTV
  5      12      4      9      0.5      10     255      255      32
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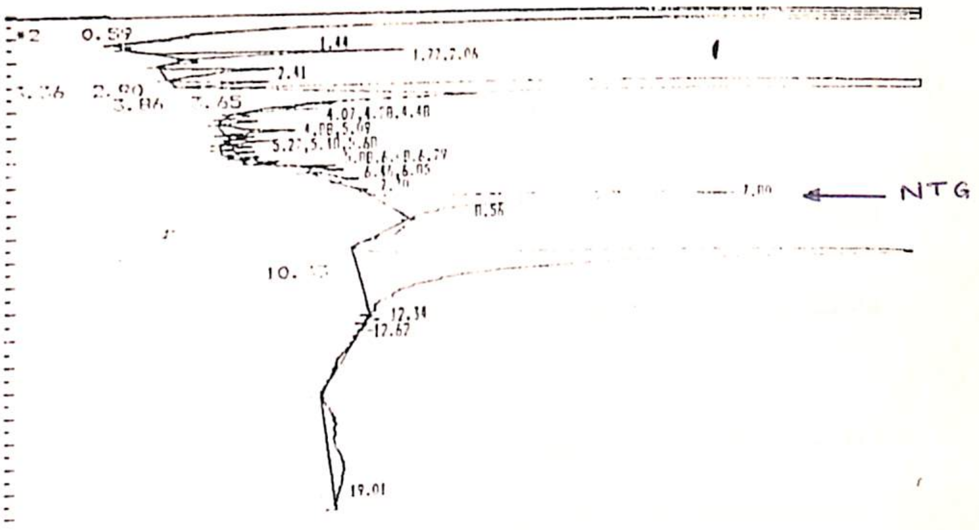

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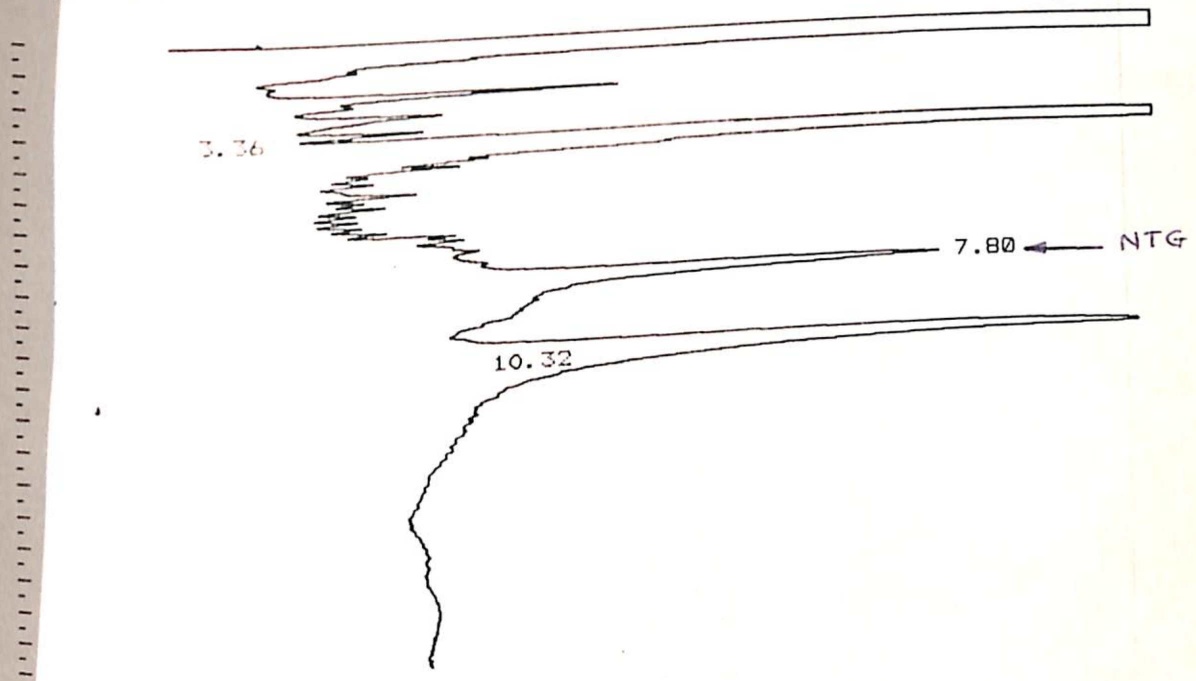
------------------------------------------------------------------------------
SRN  R.TIME   HEIGHT        AREA          HTZ        AREA%   TYPE   AR/HT
------------------------------------------------------------------------------
 1    0.12    17348       1047371       1.5073      0.2349   PB    0.041
 2    0.28   188002      57137024      16.3317     11.0762   BB    0.173
 3    0.59   202771      50907144      17.7048     11.3969   PP    0.171
 4    0.78     4403                     0.3826               PB
 5    1.05     3330       1010250       0.7873      0.2767   PB    0.207
 6    1.44     2482        372924       0.2137      0.0835   PB    0.103
 7    1.77    50612       9366783       5.0925      2.0970   BB    0.109
 8    2.06     5572        921781       0.4841      0.2061   PB    0.113
 9    2.41    26250       7391085       7.2814      1.6345   PB    0.170
10    2.91    22347       4022671       1.9418      0.9006   BP    0.123
11    3.36   265386     124710088      23.0583     27.9197   SB    0.321
12    3.65     8157        456755       0.7087      0.1023   BP    0.038
13    3.86     7857        793654       0.6827      0.1777   PB    0.057
14    4.07    15132       1968259       1.3148      0.4405   PP    0.087
15    4.28     5372        721048       0.4668      0.1411   PP    [illegible]
16    4.48     8585       1194960       0.7459      0.2425   PP    0.035
17    4.88    17362       3630479       1.5085      0.0129   PP    0.113
18    5.10     6223        787130       0.5407      0.1767   PB    0.085
19    5.27    10373       1277407       0.9013      0.2705   PB    0.085
20    5.48     7548        975053       0.6558      0.2778   PB    0.070
71    5.68     7593       1041558       0.6577      0.2332   PB    0.074
22    5.88     6926        885761       0.6010      0.1203   PP    0.087
23    6.08     8412       1171295       0.7309      0.2622   PB    0.075
24    6.27    10576       1152771       0.9276      0.2581   PB    0.071
25    6.46     7736       1041068       0.6771      0.2331   PB    0.097
76    6.85     7408       2420555       0.6436      0.5547   PP    0.220
27    7.30     2601        367823       0.2260      0.0823   PB    0.077
20    7.80    81745      40202492       7.1025      9.0001   BB    0.336
29    8.56     1954       3024105       0.1698      0.6770   BB    1.056
30   10.33   127965     118242568      11.1163     26.4718   PB    0.631
31   12.34     1030                     0.0875               PB    0.069
32   12.63      811                     0.0705               DB
33   19.01     1958      17421068       0.1701      2.7810   PB    4.330
------------------------------------------------------------------------------

TOTAL PEAKS  =       33
TOTAL HEIGHT =  1.15021E+06
TOTAL AREA   =  4.45571E+08
MUL. FACTOR  =       1.0000
```

Fig. 5.41: Chromatogram of Nitroglycerine in Validation of Method - Precision- used for in-vivo studies using rabbits.

```
HTREJ  ARREJ  PKWD  ATTN  CHTSPD  ZERO  SLPSTV  BSSTV  SKMSTV
  5     20     4     9     0.5     20    32      16      8
```

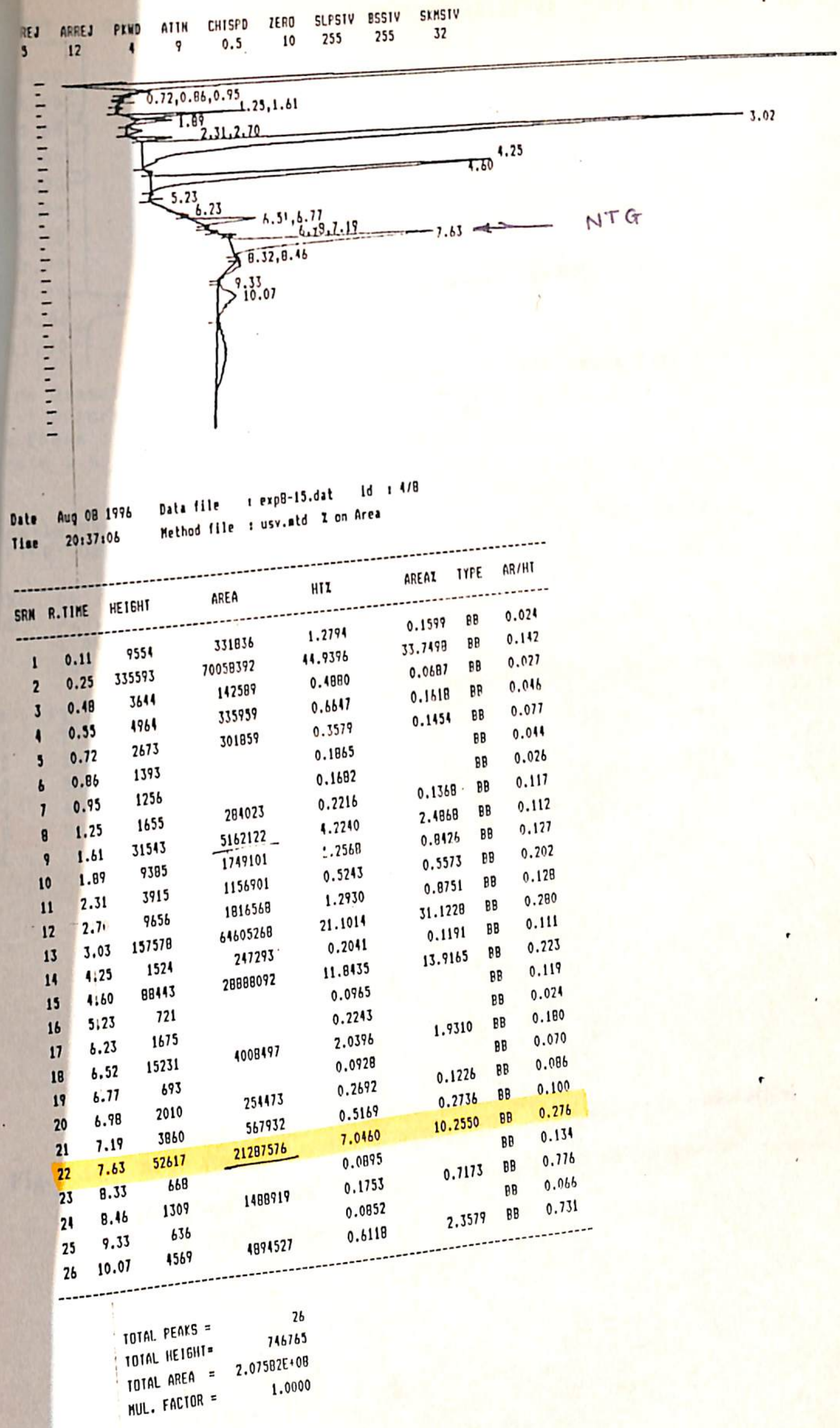


```
Date   Aug 08 1996    Data file    : exp8-8.dat     id   : BLOOD50PICO+IS
Time   17:05:18       Method file  : method1.mtd    % on Area
--------------------------------------------------------------------------
SRN  R.TIME   HEIGHT        AREA        HT%      AREA%    TYPE   AR/HT
--------------------------------------------------------------------------
 1    3.36    235982                  52.3279             BP
 2    7.80     86458    63005324      19.1716   36.2638   PP     0.497
 3   10.32    128528   110736384      28.5005   63.7362   PB     0.588
--------------------------------------------------------------------------

     TOTAL PEAKS  =            3
     TOTAL HEIGHT=        450968
     TOTAL AREA   =  1.73742E+08
     MUL. FACTOR  =       1.0000

Printed on :  8:08:1996    at : 17:09:45
```

Fig. 5.42: Chromatogram of Nitroglycerine in Validation of Method - Linearity-used for in-vivo studies using rabbits.



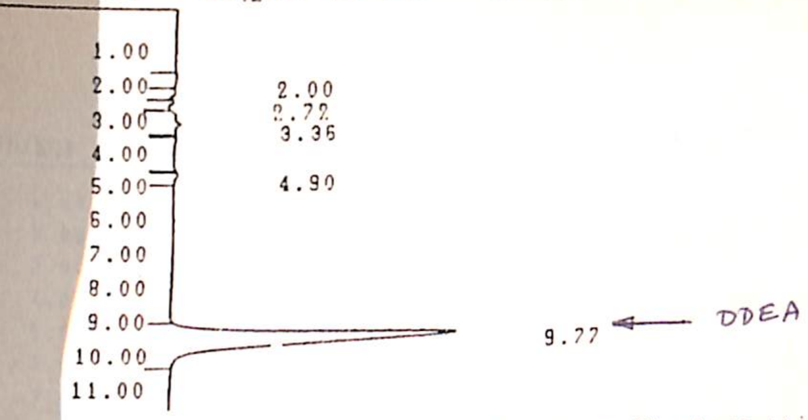
```
REJ  ARREJ  PKWD  ATTN  CHISPD  ZERO  SLPSTV  BSSTV  SKMSTV
5     12      4     9    0.5     10    255     255     32

Date   Aug 08 1996     Data file     : exp8-15.dat      Id  : 4/8
Time      20:37:06     Method file   : usv.mtd   % on Area
--------------------------------------------------------------------------------
SRN  R.TIME   HEIGHT       AREA          HT%         AREA%    TYPE    AR/HT
--------------------------------------------------------------------------------
 1     0.11     9554       331836       1.2794       0.1599    BB     0.024
 2     0.25   335593     70058392      44.9396      33.7498    BB     0.142
 3     0.48     3644       142589       0.4880       0.0687    BB     0.027
 4     0.55     4964       335959       0.6647       0.1618    BB     0.046
 5     0.72     2673       301859       0.3579       0.1454    BB     0.077
 6     0.86     1393                    0.1865                 BB     0.044
 7     0.95     1256                    0.1682                 BB     0.026
 8     1.25     1655       284023       0.2216       0.1368    BB     0.117
 9     1.61    31543      5162122       4.2240       2.4868    BB     0.112
10     1.89     9385      1749101       1.2568       0.8426    BB     0.127
11     2.31     3915      1156901       0.5243       0.5573    BB     0.202
12     2.70     9656      1816568       1.2930       0.8751    BB     0.128
13     3.03   157578     64605268      21.1014      31.1228    BB     0.280
14     4.25     1524       247293       0.2041       0.1191    BB     0.111
15     4.60    88443     28888092      11.8435      13.9165    BB     0.223
16     5.23      721                    0.0965                 BB     0.119
17     6.23     1675                    0.2243                 BB     0.024
18     6.52    15231      4008497       2.0396       1.9310    BB     0.180
19     6.77      693                    0.0928                 BB     0.070
20     6.98     2010       254473       0.2692       0.1226    BB     0.086
21     7.19     3860       567932       0.5169       0.2736    BB     0.100
22     7.63    52617     21287576       7.0460      10.2550    BB     0.276
23     8.33      668                    0.0895                 BB     0.134
24     8.46     1309      1488919       0.1753       0.7173    BB     0.776
25     9.33      636                    0.0852                 BB     0.066
26    10.07     4569      4894527       0.6118       2.3579    BB     0.731
--------------------------------------------------------------------------------

          TOTAL PEAKS  =             26
          TOTAL HEIGHT =         746765
          TOTAL AREA   =    2.07582E+08
          MUL. FACTOR  =         1.0000
```

Fig. 5.43: A typical chromatogram of Nitroglycerin in the blood sample of rabbits.

```
INJECT     SEQ_NO. 001-008     10/23/1995 18:12:44     CH 1  AT 32     SF 0.50
```



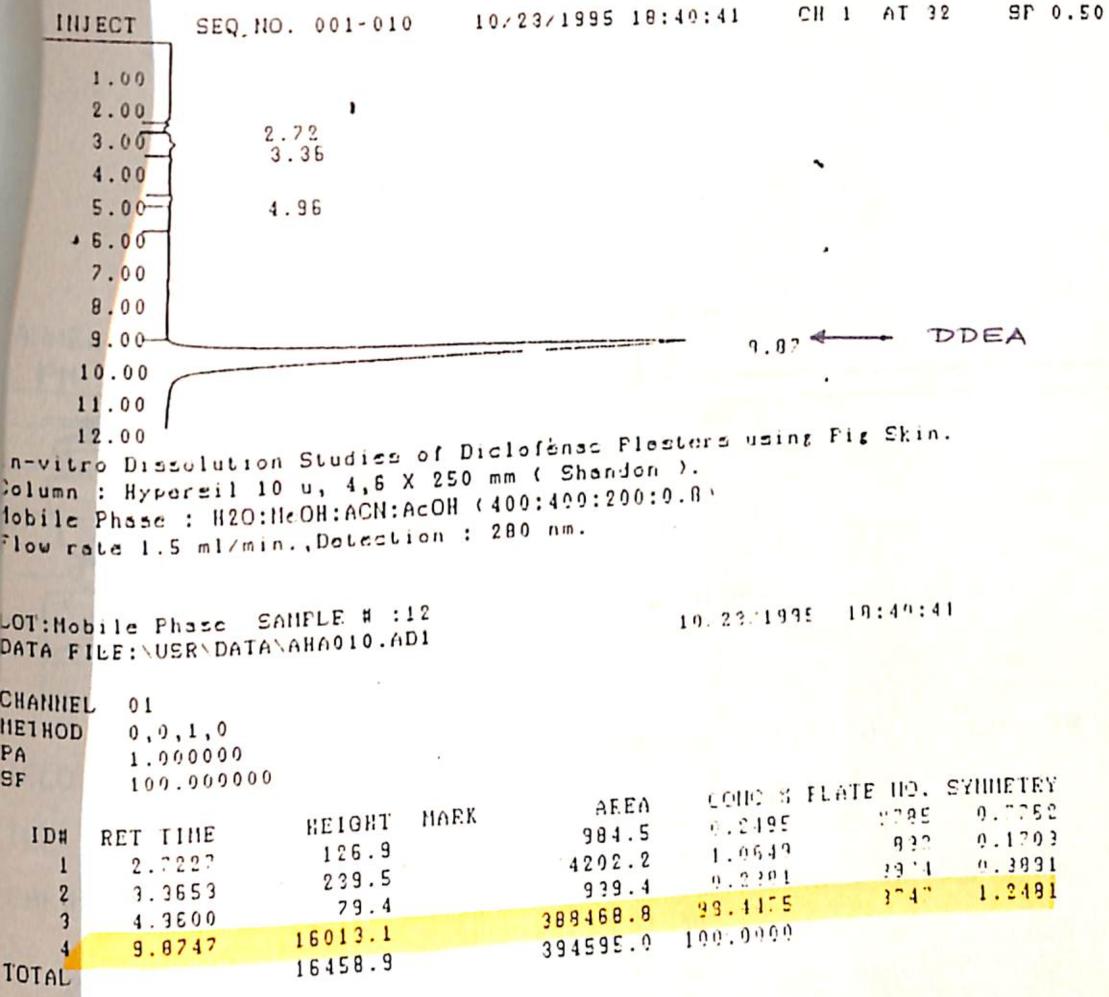
```
n-vitro Dissolution Studies of Diclofenac Plasters using Pig Skin.
olumn : Hypersil 10 u, 4,6 X 250 mm ( Shandon ).
obile Phase : H2O:MeOH:ACN:AcOH (400:400:200:0.8)
low rate 1.5 ml/min.,Detection : 280 nm.


OT:Mobile Phase   SAMPLE # :10                        10/23/1995   18:12:44
ATA FILE:\USR\DATA\AHA008.AD1

HANNEL   01
ETHOD    0,0,1,0
A        1.000000
F        100.000000

                                                    CONC %  PLATE NO. SYMMETRY
 ID#  RET TIME      HEIGHT  MARK         AREA
   1    2.0080        53.3               882.1     0.4292        332    1.1225
   2    2.7280       120.8               728.3     0.3544       4527    0.3530
   3    3.3627       232.1              4204.3     2.0456        779    0.1439
   4    4.9067       115.3              1282.2     0.6233       4101    2.7088
   5    9.7787      8409.7            198432.3    96.5470       3884    1.2295
OTAL                8931.0            205529.2   100.0000
```

Fig. 5.44: Chromatogram of Diclofenac diethyl ammonium in Validation of Method- Precision- used for in-vitro dissolution studies.



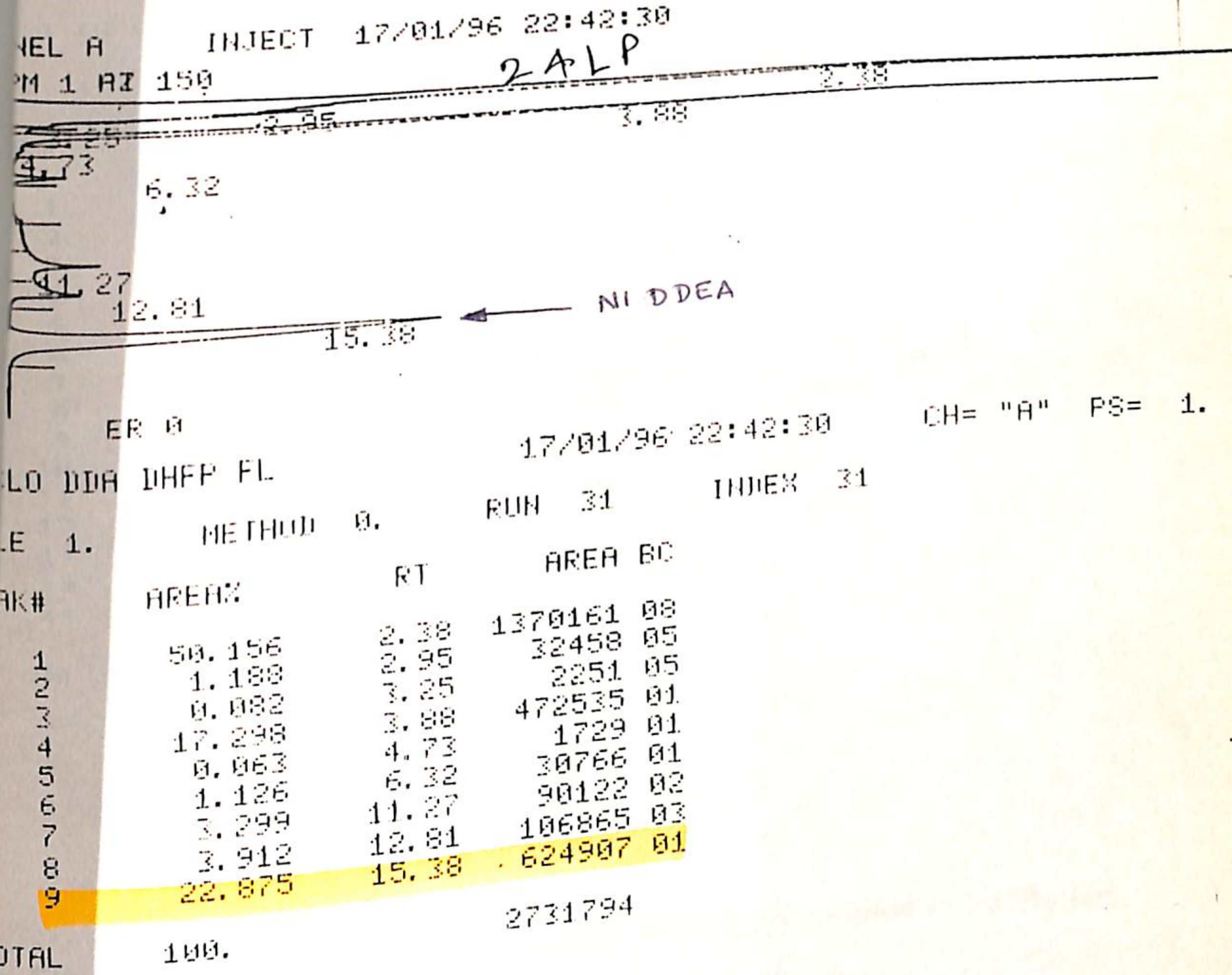
```
INJECT      SEQ.NO. 001-010     10/23/1995 18:40:41     CH 1   AT 32     SP 0.50

n-vitro Dissolution Studies of Diclofenac Plasters using Pig Skin.
;olumn : Hypersil 10 u, 4,6 X 250 mm ( Shandon ).
lobile Phase : H2O:MeOH:ACN:AcOH (400:400:200:0.8)
low rate 1.5 ml/min.,Detection : 280 nm.


LOT:Mobile Phase  SAMPLE # :12                        10.23.1995  18:40:41
DATA FILE:\USR\DATA\AHA010.AD1

CHANNEL   01
METHOD    0,0,1,0
PA        1.000000
SF        100.000000
                                                    CONC % FLATE NO. SYMMETRY
  ID#  RET TIME        HEIGHT  MARK          AREA
   1     2.7227         126.9                984.5    0.2495     1785   0.7752
   2     3.3653         239.5               4202.2    1.0649      832   0.1703
   3     4.3600          79.4                939.4    0.2381     2974   0.3891
   4     9.8747       16013.1             388468.8   98.4475     3747   1.2481
TOTAL                 16458.9             394595.0  100.0000
```

ig. 5.45: **Chromatogram of Diclofenac diethyl ammonium in Validation of Method-Linearity - used for in-vitro dissolution studies.**



```
NEL A       INJECT   17/01/96 22:42:30
PM 1 AI 150

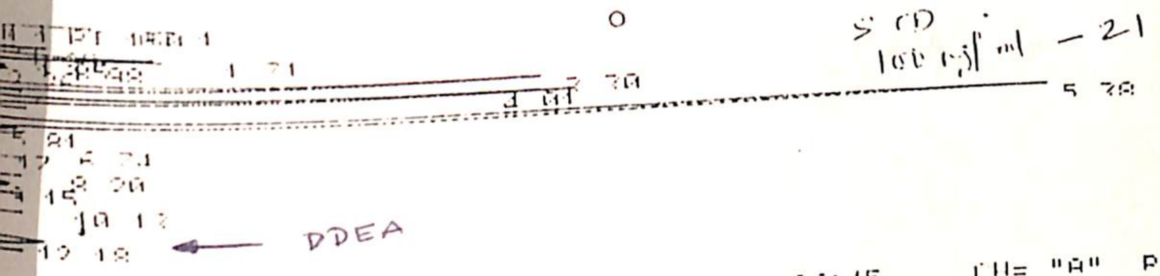
       ER 0                                  17/01/96 22:42:30      CH= "A"   PS=  1.
LO DDA DHFP FL
                                                                    INDEX  31
                                               RUN   31
E   1.          METHOD   0.

AK#       AREA%             RT         AREA BC

  1        50.156           2.38     1370161 08
  2         1.188           2.95       32458 05
  3         0.082           3.25        2251 05
  4        17.298           3.88      472535 01
  5         0.063           4.73        1729 01
  6         1.126           6.32       30766 01
  7         3.299          11.27       90122 02
  8         3.912          12.81      106865 03
  9        22.875          15.38    . 624907 01

                                     2731794
OTAL      100.
```

Fig. 5.46: A typical chromatogram of Diclofenac diethyl ammonium in the samples using pig skin.



S (D)
100 ng/ml – 21

```
ICLO IN VIVO II                       14/06/96 15:26:45     CH= "A"   PS=  1

ILE  1          METHOD  0          RUN  13        INDEX  13              0

EAK#          AREA%            RT           AREA BC

  1             1 418        1 25           13676 01
  2             6 316        1 71           60903 02
  3             3 722        1 85           35889 02
  4             0 804        1 98            7757 02
  5            17 468        2 26          168435 02
  6            14 738        3 3           142108 03
  7            15 218        4 01          146738 01
  8            32 706        5 38          315362 02
  9             1 098        5 81           10583 03
 10             0 844        6 74            8137 02
 11             0 184        7 17            1773 03
 12             0 675        8 2             6507 01
 13             1 238        9 15           11935 01
 14             0 606       10 13            5845 01
 15             2 965       12 18           28589 01

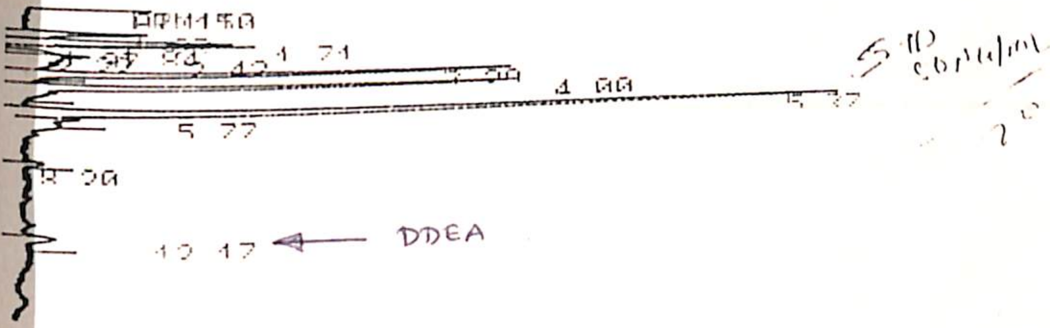
TOTAL         100                          964237
```

0.0906

Fig. 5.47: Chromatogram of Diclofenac diethyl ammonium in Validation of Method-Precision- used for in-vivo studies

```
PIA7I RT 150

ANNEL A        INJECT  14/06/96 15:06:21
```



```
                                    14/06/96 15:06:21      CH= "A"   PS=  1
CLO IN VIVO II
                                              RUN  12        INDEX  12
LE  1          METHOD  0

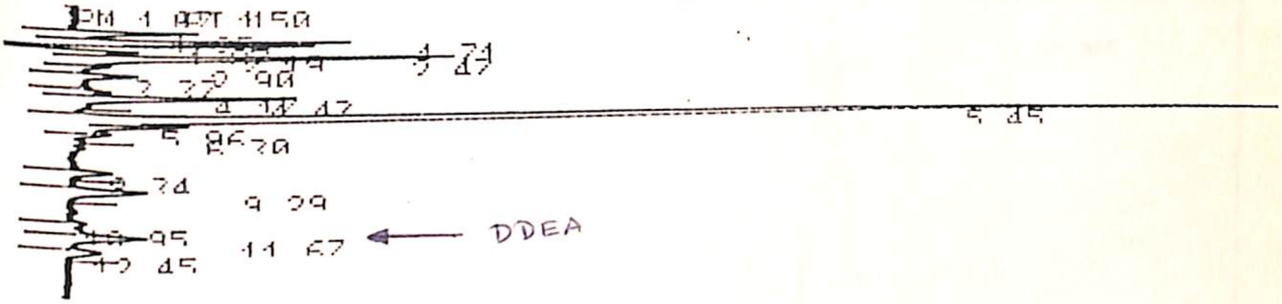
                                   RT       AREA BC
AK#          AREA%
                                            48370 02
 1             5.366            1.23        95537 02
 2            10.598            1.71        45084 02
 3             5.001            1.84        10593 02
 4             1.175            1.97        64808 02
 5             7.199            2.22       156269 02
 6            17.334            2.42       123146 03
 7            13.66             3.29       113362 01
 8            12.575            4          222973 03
 9            24.734            5.37        10806 03
10             1.199            5.77         2278 04
11             0.253            8.2          8269 04
12             0.917           12.17
                                           901495

TAL          100
```

0.03/03

Fig. 5.48: Chromatogram of Diclofenac diethyl ammonium in Validation of Method- Linearity- used for in-vivo studies.

```
CHANNEL A        INJECT
```



```
DICLO IN VIVO II

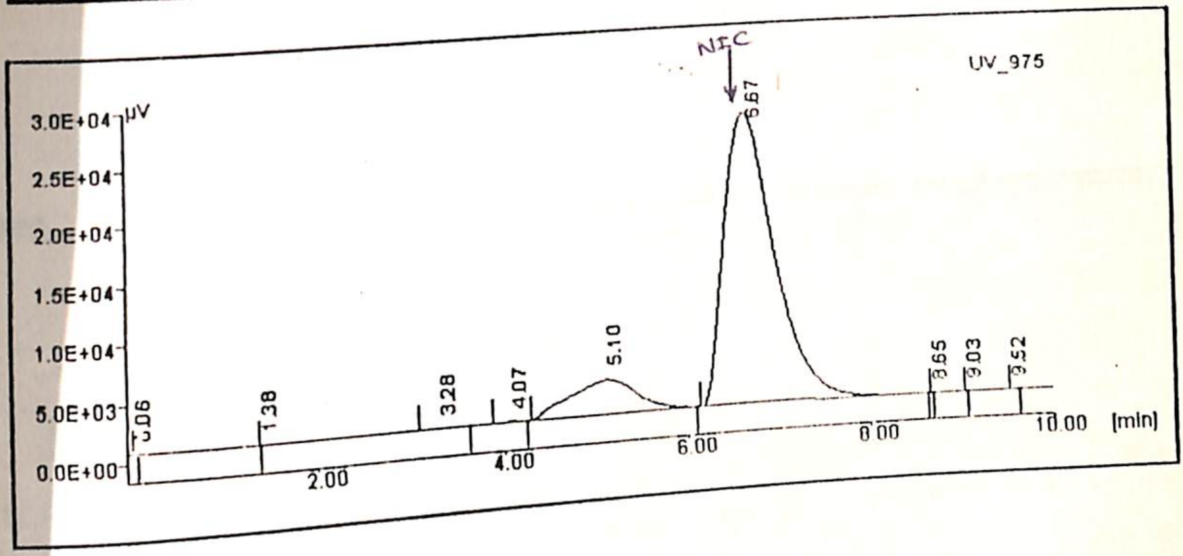
FILE  1          METHOD  0          RUN  31          INDEX  31

                                       AREA BC
PEAK#          AREA%          RT

                                1 25    48198 02
  1            4 13             1 71    93507 02
  2            8 013            1 87    48586 02
  3            4 164            2 19    76548 02
  4            6 56             2 47   187243 02
  5           16 046            2 9     66428 02
  6            5 693            3 37    93084 02
  7            7 977            4 17    42117 02
  8            3 609            4 47    74183 03
  9            6 357            5 45   313620 02
 10           26 875            5 86    29780 02
 11            2 552            6 3      7829 03
 12            0 671            8 74    10050 01
 13            0 861            9 29    27781 01
 14            2 381           10 95     7091 02
 15            0 608           11 67    29478 02
 16            2 526           12 45    11418 03
 17            0 978
                                      1166937
TOTAL         100
```

Fig. 5.49: A typical chromatogram of Diclofenac diethyl ammonium in the blood samples of rabbits.

Chromatogram and Report



```
File name : NIC__007.CH3 User :  VINAYAK

Run Length : 10.01 [min]

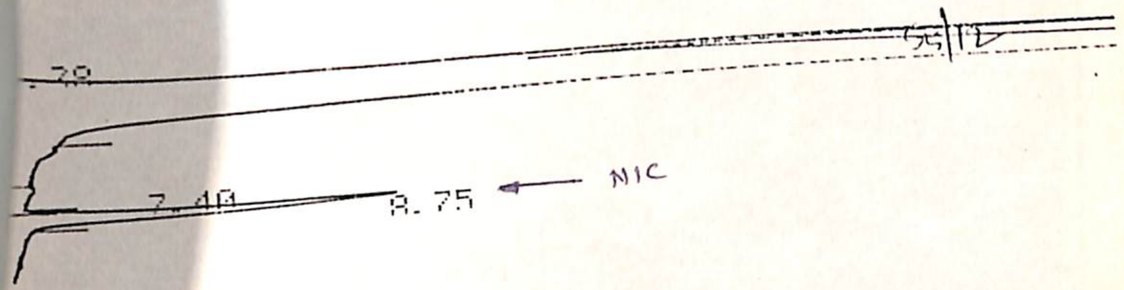
Info :
Nicardipine HCl (14 mcg)
Column : µ-Bondapak ODS, 3.9 X 300 mm
Mobile Phase : MeOH : H2O ( 8: 2 ).
Flow 1.2 ml/min., Detection UV- 240 nm.

 #  Rt      Area       %Area    Height  NPlates   Resolution Asymmetry

 1 0.06    3106.50    0.281     896       0.00      0.00      0.00
 2 1.38    1325.00    0.120     889       0.00      0.00      0.00
 3 3.28    1265.00    0.114      63     424.70      6.00      2.28
 4 4.07    1472.25    0.133     103    1568.75      1.50      0.90
 5 5.10  154308.50   13.947    3051     211.71      1.14      0.91
 6 6.67  952369.50   86.079   25921     789.84      1.34      1.58
 7 8.65   -1500.50   -0.136     748       0.00      4.19      0.00
 8 9.03   -1480.00   -0.134     739       0.00      0.00      0.00
 9 9.52   -4473.50   -0.404     742       0.00      0.00      0.00

 Total Area of Peak = 1106392.75
```

Fig. 5.51: Chromatogram of Nicardipine HCl in Validation of Method - Linearity -used for in-vitro dissolution studies using pig skin.



```
ARTD  T                                                INDEX   10
                                         RUN   10
F   1.          METHOD   0.
                                  RT         AREA  BC
AK#        AREA%
                                  1.38        17330  01
              0.32                2.7        691852  02
 1           12.787               3.66      4424830  03
 2           81.783               7.4          6047  01
 3            0.112               8.75       270382  01
 4            4.997
 5                                          5410441

OTAL        100.
```

ig. 5.52: A typical chromatogram of Nicardipine HCl in the samples using pig skin.

Fig. 5.53a: Application machine for matrix-dispersion system.

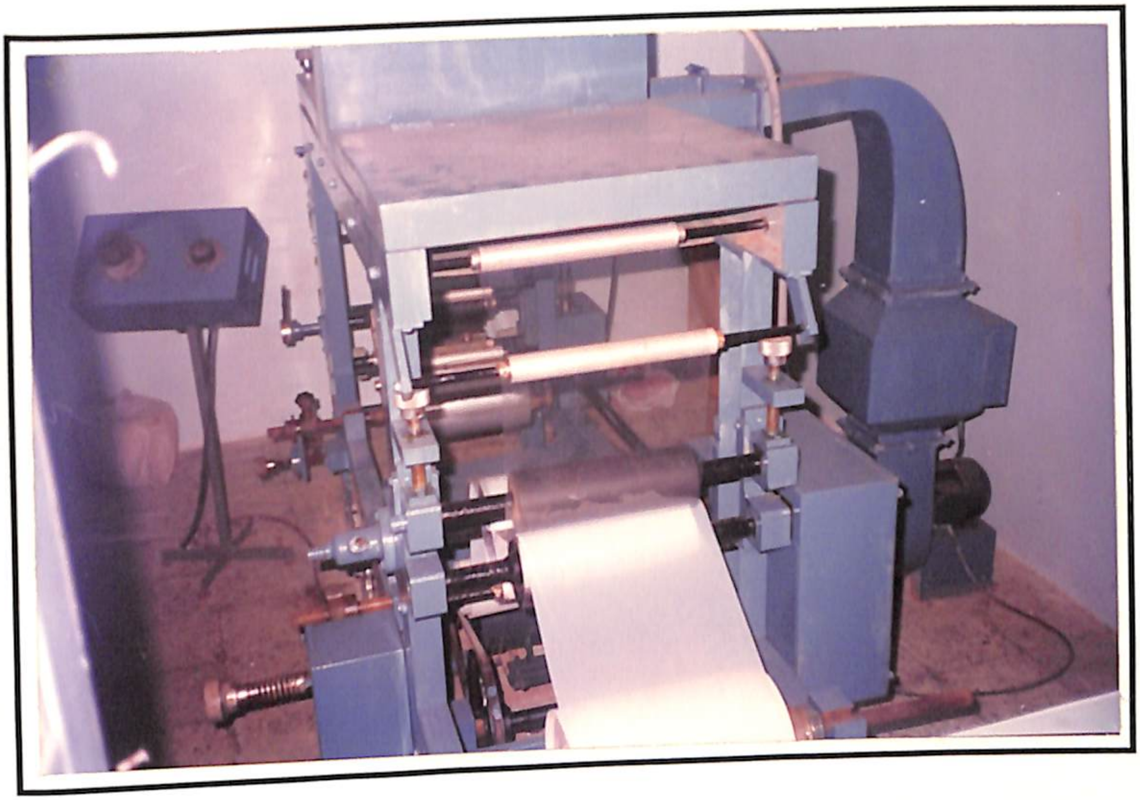


Fig. 5.53b: Close view of application roller of application machine.

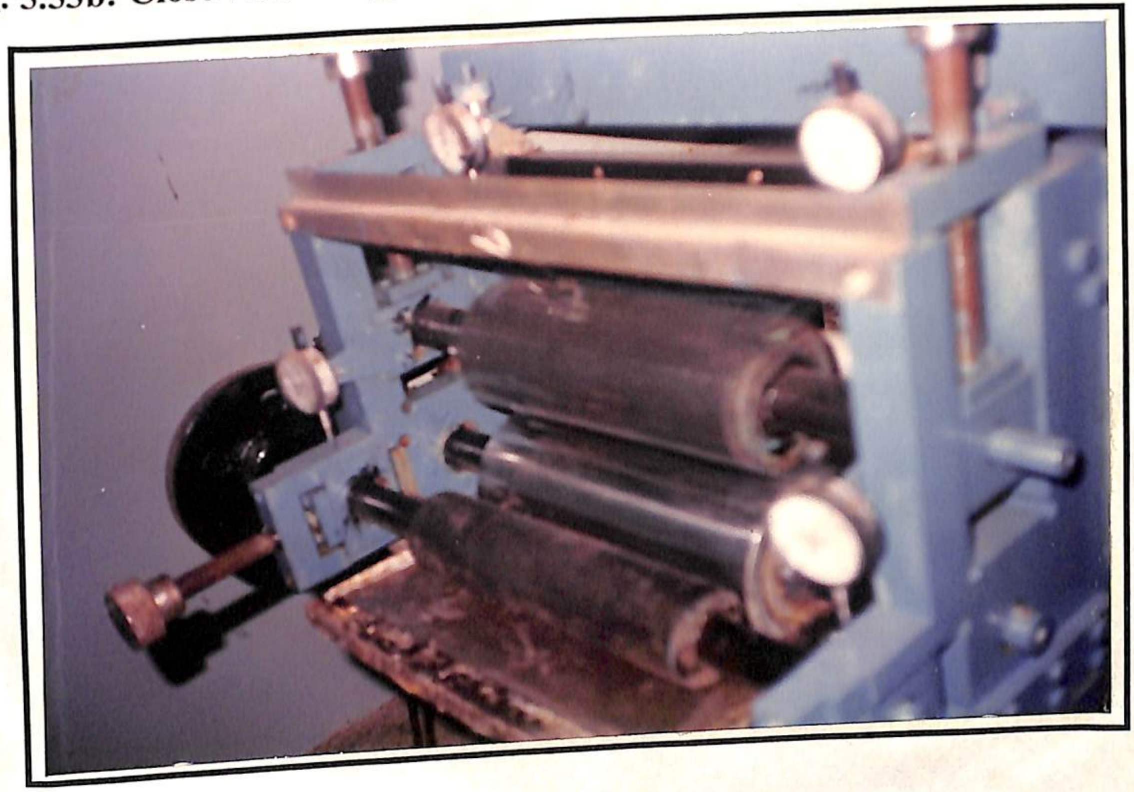


Fig. 5.55: Modified Franz cell (60 ml).

Fig. 5.56: Experimental assembly of modified Franz cell (60 ml)

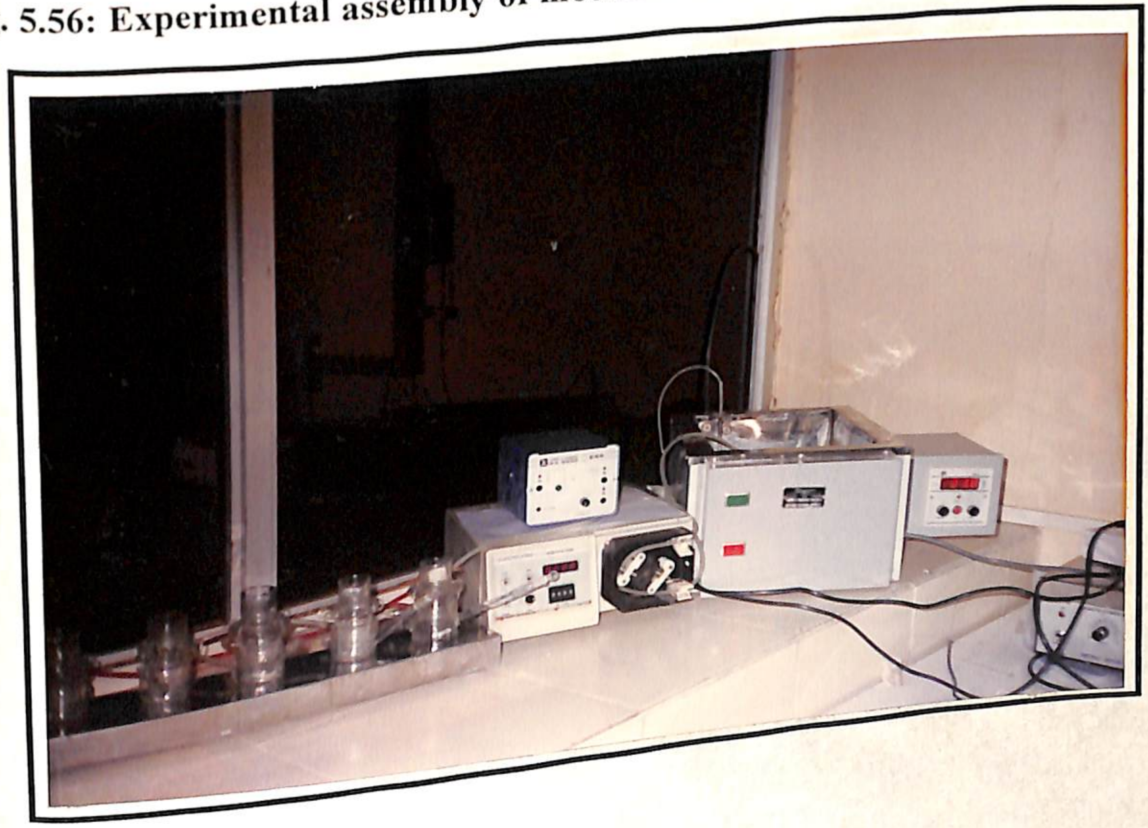


Fig. 5.57: Modified Franz cell (460 ml).

Fig. 5.58: Experimental assembly of modified Franz (460 ml)

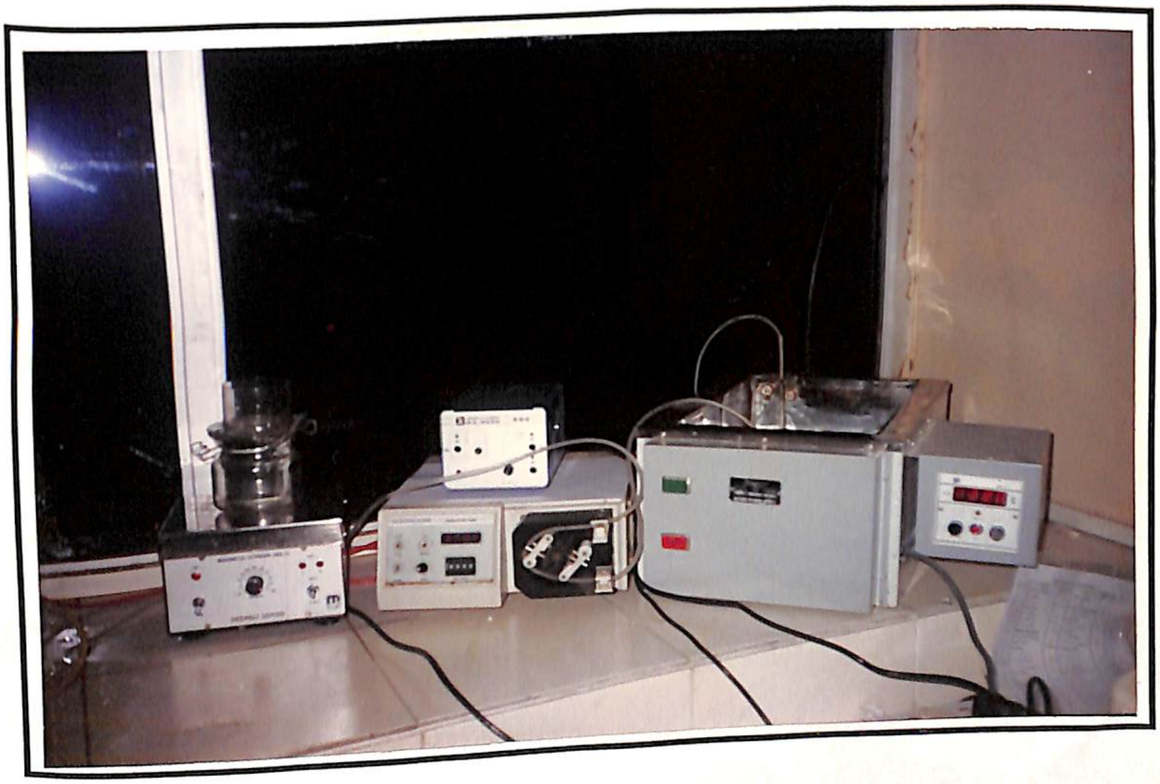


Table 5.37: Certificate of analysis of sodium benzoate.

Sr. No.	Test	Specification	Results
1.	Description	White, amorphous, Granular or crystalline powder, odorless or with a faint odor, taste-unpleasant	Complies
2.	Solubility	To pass test as per I.P.1985	Complies
3.	Identification	To pass test as per I.P. 1985	Complies
4.	Acidity or Alkalinity	To pass test as per I.P. 1985	Complies
5.	Arsenic	NMT 2 PPM	Complies
6.	Heavy metals	NMT 2 PPM	Complies
7.	Chloride	To pass test as per I.P. 1985	Complies
8.	Sulfate	To pass test as per I.P. 1985	Complies
9.	Chlorinated compounds	To pass test as per I.P. 1985	Complies
10.	Loss on drying	NMT 1.5%	Complies 0.7733%
11.	Assay (on dried basis)	NLT 99%	Complies 99.0%

Table 5.38: Certificate of analysis of Gelatin I.P.

Sr. No.	Test	Specification	Results
1.	Description	Colorless or pale yellowish translucent sheets, Flakes, Shreds or a coarse to fine powder	Complies
2.	Solubility	To pass test as per I.P.1985	Complies
3.	Identification	To pass test as per I.P. 1985	Complies
4.	Odor and Water Insol. Subs.	To pass test as per I.P. 1985	Complies
5.	Arsenic	NMT 2 PPM	Complies
6.	Heavy metals	NMT 50 PPM	Complies
7.	Copper	NMT 30 PPM	Complies
8.	Zinc	NMT 100 PPM	Complies
9.	Microbial limits	To pass test as per I.P. 1985 To pass test as per I.P. 1985	Complies
10.	Loss on drying	NMT 16.0%	Complies 4.55%
11.	Ash	NMT 3.25%	Complies 1.625%

Table 5.39: Certificate of analysis of C. M. C. Sodium.

Sr. No.	Test	Specification	Results
1.	Description	White to cream colored powder or granules, odorless, hygroscopic	Complies
2.	Solubility	To pass test as per I.P.1985	Complies
3.	Identification	To pass test as per I.P. 1985	Complies
4.	pH	Between 6.0 to 8.0 (1% solution)	Complies 6.78
5.	Viscosity	NLT 80% AND NMT 110%	Complies 2250 cp.
6.	Arsenic	NMT 1.0 PPM	Complies
7.	Heavy metals	NMT 10 PPM	Complies
8.	Loss on drying	NMT 10.0%	Complies 9.646%
9.	Assay (on dried basis)	NLT 6.5% and NMT 9.5% of Sodium	Complies 6.811%

Table 5.40: Certificate of analysis Of Propylene Glycol I.P.

Sr. No.	Test	Specification	Results
1.	Description	Clear colorless, Viscous liquid, practically odorless, taste-slightly sweet, hygroscopic	Complies
2.	Solubility	To pass test as per I.P.1985	Complies
3.	Identification	To pass test as per I.P. 1985	Complies
4.	Boiling range	Between 184 & 189 °C	Complies 184 - 186 °C
5.	Specific gravity	Between 1.035 to 1.037	Complies 1.03686
6.	Acidity / Alkalinity	NMT 0.2 ml of 0.1N NaOH required	Complies 0.06 ml of 0.1 N NaOH
7.	Chloride	To pass test as per I.P. 1985	Complies
8.	Sulfate	To pass test as per I.P. 1985	Complies
9.	Arsenic	NMT 5 PPM	Complies
10.	Heavy metals	NMT 10 PPM	Complies
11.	Sulfated ash	NMT 0.2%	Complies 0.0019%
12.	Water	NMT 0.2%	Complies 0.10903%

Table 5.43: Certificate of analysis of Titanium dioxide I. P.

Sr. No.	Test	Specification	Results
1.	Description	White or almost white infusible powder, odorless, tasteless	Complies
2.	Solubility	To pass test as per I.P.1985	Complies
3.	Identification	To pass test as per I.P. 1985	Complies
4.	Clarity and color of solution	To pass test as per I.P. 1985	Complies
5.	Acidity / Alkalinity	To pass test as per I.P.	Complies
6.	Water soluble matter	NMT 0.5%	Complies 0.1669%
7.	Arsenic	NMT 8 PPM	Complies
8.	Barium	NMT 5 PPM	Complies
9.	Heavy metals	NMT 20 PPM	Complies
10.	Iron	NMT 200 PPM	Complies
11.	Loss on drying	NMT 0.5%	Complies 0.1634%
12.	Loss on ignition	NMT 0.5%	Complies 0.0595%
13.	Assay (on dried basis)	NLT 98.0%	Complies 99.68%

Table 5.44: Certificate of analysis of Glycerin I.P.

Sr. No.	Test	Specification	Results
1.	Description	Clear colorless liquid of syrupy consistency, odorless, taste-wet	Complies
2.	Solubility	To pass test as per I.P.1985	Complies
3.	Identification	To pass test as per I.P. 1985	Complies
4.	Acidity	NMT 0.2 ml of 0.1N NaOH	Complies, 0.07 ml
5.	Weight per ml	1.252 to 1.257 gm/ml	Complies 1.2563 gm/ml
6.	Refractive Index	1.470 to 1.475	Complies,1.471
7.	Arsenic / copper	To pass test as per I.P. 1985	Complies
8.	Iron / Heavy metal	To pass test as per I.P. 1985	Complies
9.	Sulfate	To pass test as peer I.P. 1985	Complies
10.	Chloride	To pass test as per I.P. 1985	Complies
11.	Acraldehyde and Glucose	To pass test as per I.P. 1985	Complies
12.	Sugar	To pass test as per I.P. 1985	Complies
13.	Aldehyde and related substance	To pass test as per I.P. 1985	Complies
14.	Fatty acids and esters	NMT 1.0 ml of 0.5N NaOH	Complies
15.	Sulfated ash	NMT 0.01%	Complies,0.00097%
16.	Assay	NLT 98.0%	Complies, 99.7%

Table 5.45: Certificate of analysis of Ethyl acetate (MERCK).

Sr. No.	Test	Specification	Results
1.	Assay	99.50%	Complies 99.465%
2.	Residue after evaporation	≤ 0.003%	Complies 0.00198%
3.	Water	≤ 0.2%	Complies
4.	Titrable acid	≤ 0.0009 meq/gm	Complies
5.	Substances darkened by sulfuric acid	Passes test	Complies

Table 5.46: Certificate of analysis of Butyl acrylate(FLUKA).

Sr. No.	Test	Specification	Results
1.	Description	Polymer, elastic tacky substance	Complies
2.	Boiling point	145 to 146 °C	Complies 146 °C
3.	Density at 20 °C	0.898	Complies 0.8975
4.	Assay (GC)	NLT 99.0%	Complies 98.95%

Table 5.47: Certificate of analysis of 2-Ethyl hexyl acrylate (FLUKA).

Sr. No.	Test	Specification	Results
1.	Boiling point	213 TO 215 °C	Complies 213 to 214 °C
2.	Density at 20 °C	0.884	Complies 0.8832
3.	Assay (GC)	NLT 98.0%	Complies 98.12%

Table 5.48: Certificate of analysis of Vinyl acetate (FLUKA).

Sr. No.	Test	Specification	Results
1.	Boiling point	71 TO 73 °C	Complies 72 to 73 °C
2.	Density at 20 °C	0.932	Complies 0.9321
3.	Assay (GC)	NLT 99.0%	Complies 98.7%
4.	Description	Liquid, polymerizes in light to a colorless transparent mass	Complies

Table 5.49: Certificate of analysis of Ammonium persulfate (SIGMA).

Sr. No.	Test	Specification	Results
1.	Insoluble matter	≤ 0.005%	Complies, 0.004%
2.	Residue after ignition	≤ 0.05%	Complies, 0.045%
3.	Assay	< 98.0%	Complies, 98.2%
4.	Tritable free acid	≤ 0.04 meq/gm	Complies, 0.038%
5.	Chlorides (as Cl)	≤ 0.001%	Complies
6.	Heavy metals (as Pb)	≤ 0.005%	Complies
7.	Iron	≤ 0.001 %	Complies

Table 5.50: Certificate of analysis of Potassium persulfate (SIGMA).

Sr. No.	Test	Specification	Results
1.	Description	Colorless or white, odorless crystals	Complies
2.	Assay	99.0 %	Complies, 99.%
3.	Insoluble matter	≤ 0.005%	Complies,0.004%
4.	Chloride	≤ 0.001%	Complies
5.	Iron	≤ 5 PPM	Complies

Table 5.51: Certificate of analysis of Dibutyl phthalate (FLUKA).

Sr. No.	Test	Specification	Results
1.	Assay	98.0%	Complies
2.	Density	1.046 gm/ml	Complies
3.	Refractive index	1.493	Complies

Table 5.52: Certificate of analysis of Formalin I.P.

Sr. No.	Test	Specification	Results
1.	Description	Colorless aqueous solution with a lacrymatory vapor	Complies
2.	Weight per ml	1.08 gm/ml	Complies
3.	Assay	NLT 34.0% and NMT 37.0%w/v	Complies

Table 5.53: Certificate of analysis of Ethanol I.P.

Sr. No.	Test	Specification	Results
1.	Description	Clear, colorless, mobile and volatile liquid, characteristic odor	Complies
2.	Solubility	To pass test as per I.P. 1996	Complies
3.	Assay	NLT 92% and NMT 92.7%w/w	Complies, 92.021%
4.	Acidity / Alkalinity	NMT 0.2 ml of 0.1M NaOH	Complies
5.	Relative density	Between 0.8084 and 0.8104	Complies, 0.8097
6.	Clarity of solution	To test pass as per I.P. 1996	Complies
7.	Aldehydes	NMT 10 PPM	Complies

Table 5.54: Certificate of analysis of Methanol I.P.

Sr. No.	Test	Specification	Results
1.	Description	Clear, colorless, mobile and volatile liquid, characteristic odor	*Complies*
2.	Boiling point	65 °C	Complies 64 to 65°C
3.	Weight per ml	0.791 mg/ml	Complies, 0.7906 mg/ml

Table 5.55: Certificate of analysis of Purified water I.P.

Sr. No.	Test	Specification	Results
1.	Description	Clear, colorless liquid, odorless and tasteless	Complies
2.	Acidity / Alkalinity	To pass test as per I.P. 1996	Complies
3.	Ammonium	To Pass test as per I.P. 1996	Complies
4.	Calcium and Magnesium	To pass test as per I.P. 1996	Complies
5.	Heavy metals	NMT 0.1 PPM	Complies
6.	Chloride	To pass test as per I.P. 1996	Complies
7.	Nitrate	To pass test as per I.P. 1996	Complies
8.	Sulfate	To pass test as per I.P. 1996	Complies
9.	Oxidisable substance	To pass test as per I. P. 1996	Complies
10.	Residue on evaporation	NMT 1 mg / 100 ml	Complies

Table 5.56: Certificate of analysis of Pectin I.P.

Sr. No.	Test	Specification	Results
1.	Description	Coarse or fine, yellowish white powder, almost odorless	Complies
2.	Solubility	To pass test as per I.P. 1996	Complies
3.	Identification	To Pass test as per I.P. 1996	Complies
4.	Acidity	To pass test as per I.P. 1996	Complies
5.	Starch	To pass test as per I.P. 1996	Complies
6.	Sugars and organic acids	To pass test as per I.P. 1996	Complies
7.	Arsenic	NMT 3 PPM	Complies
8.	Lead	NMT 5 PPM	Complies
9.	Ash	NMT 4 %	Complies
10.	Acid insoluble ash	NMT 0.4%	Complies
11.	Loss on drying	NMT 10%	Complies
12.	Microbial limit	to pass test as per I.P. 1996	Complies

Table 5.57: Certificate of analysis of Nicardipine Hydrochloride J.P.

Sr. No.	Test	Specification	Results
1.	Description	Pale greenish yellow powder	Complies
2.	Solubility	To pass test as per J. P. 1996	Complies
3.	Identification	To Pass test as per JP 1996	Complies
4.	Melting point	167 to 171 °C	Complies
5.	Heavy metals	NMT 20 PPM	Complies
6.	Related substances	To pass test as per JP 1996	Complies
7.	Loss on drying	NMT 1.0 %	Complies
8.	Residue on ignition	NMT 0.1%	Complies
9.	Assay	NLT 98.5%	Complies

Table 5.58: Certificate of analysis of Azoisobutarylnitrile (FLUKA).

Sr. No.	Test	Specification	Results
1.	Assay (GC)	> 98%	Complies
2.	Water	< 2%	Complies
3.	Ash	< 0.1 %	Complies
4.	Melting point	102 to 104 °C	Complies

Table 5.59: Certificate of analysis of Nitroglycerin 1% solution in ethanol USP.

Sr. No.	Test	Specification	Results
1.	Assay	NLT 90% AND NMT 110%	Complies
2.	Identification	To pass test as per USP 1990	Complies
3.	Chromatographic purity	To pass test as per USP 1990	Complies

Table 5.60: Certificate of analysis of Diclofenac diethyl ammonium.

Sr. No.	Test	Specification	Results
1.	Description	White or white crystalline taste-bitter, hygroscopic	Complies
2.	Identification	To pass test as per Amoli Organic Pvt. Ltd.	Complies
3.	Melting range	149 to 153 °C	Complies
4.	pH	Between 6.8 to 8.5 (1% w/v suspension)	Complies
5.	Moisture	NMT 0.5 %	Complies
6.	Heavy metals	NMT 10 PPM	Complies
7.	Residue on ignition	NMT 10 PPM`	Complies
8.	Assay (on dry basis)	NLT 98 %	Complies

Table 5.61: Certificate of analysis of Dimethyl isosorbide (ICI).

Sr. No.	Test	Specification	Results
1.	Description	Colorless liquid	Complies
2.	Viscosity at 25 °C	5 mps.	Complies
3.	Boiling point	234 °C	Complies
4.	Density at 25 °C	1.1635	Complies
5.	Refractive index	1.4623	Complies
6.	Flash point	120 °C	Complies
7.	Solubility	Miscible with all polar solvents	Complies
8.	Acid number	Max. 1.5 mg KOH/gm	Complies
9.	Hydroxyl number	Max. 10 mg KOH/gm	Complies
10.	Water content	Max. 0.5%	Complies
11.	Assay	Min. 97%	Complies

Table No. 5.62: Certificate of analysis of Hydroxy ethyl cellulose USP.

Sr. No.	Test	Specification	Results
1.	Description	Light tan or cream to white colored, odorless and tasteless, hygroscopic powder	Complies
2.	Identification	To pass test as per USP	Complies
3.	pH (1 in 100)	6 to 8.5	Complies 7.25
4.	Loss on drying	≤ 10%	Complies 4.6%
5.	Lead	NMT 0.001%	Complies
6.	Residue on ignition	NMT 5.0%	Complies
7.	Arsenic	NMT 3 ppm	Complies
8.	Heavy metals	NMT 0.04%	Complies
9.	Chloride	NMT 0.07%	Complies
10.	Free pthalic acid	≤ 11.0%	Complies
11.	Viscosity	1500-2500 mps (1% w/v aqueous solution)	Complies

Table No. 5.63: Certificate of analysis of Hydroxy propyl cellulose USP.

Sr. No.	Test	Specification	Results
1.	Identification	To pass test as per USP	Complies
2.	Apparent viscosity		
3.	pH	5.0 to 8.0 (1 in 100)	Complies 6.56
4.	Loss on drying	≤ 5.0 %	Complies 1.32%
5.	Residue on ignition	≤ 0.2%	Complies 0.025%
6.	Arsenic	NMT 3 ppm	Complies
7.	Lead	NMT 0.001%	Complies
8.	Heavy metals	NMT 0.004%	Complies
9.	Silica	≤ 0.6%	Complies 0.432%
10.	Assay of hydroxypropyl group	≤ 80.5%	Complies 80.78%
11.	Viscosity	1500-3500 mps (1% aqueous solution)	Complies

Table No. 5.64: Certificate of analysis of Dioctyl maleate.

Sr. No.	Test	Specification	Results
1.	Assay (GC method)	98 to 99 %	Complies 98.65%

Table No. 5.65: Certificate of analysis of Nonyl Phenyl Ethylene Oxalate Condensate.

Sr. No.	Test	Specification	Results
01.	Assay (GC)	97 to 98%	Complies 97.23%

Chapter VI.

DISCUSSION

CHAPTER VI. DISCUSSION :

PART I. NITROGLYCERIN PATCHES :

Nitroglycerin (NTG) is a well known and established drug for management of angina pectoris. Abroad, it is marketed in almost all type of transdermal delivery systems i.e. adhesive-matrix dispersion, adhesive-diffusion, membrane-controlled and micro-reservoir type drug delivery systems. These different types of systems are currently marketed in western countries by as many as six to seven companies but none of them have manufactured these products in India. This became the motivating factor for developing TDDS containing NTG. The objective of the study was to develop drug-matrix dispersion as well as membrane-controlled drug delivery system in order to understand the effects of formulation on the release profile of NTG as well *as suitability of the system for desired release profiles* and subsequently, commercialize the finally developed product.

Through out the development, for matrix-dispersion system, Nitro Dur® 40 mg/10 cm^2 was used as a reference standard while for membrane-controlled system, Nitroderm TTS® was used.

Methodology involved during the development of both the systems is categorized in the following steps:

1. Extensive literature survey.
2. Analysis of raw materials and packaging materials used in the work.
3. Preformulation and formulation development.
4. Development of machine for both laboratory as well as scaling processes.
5. Development and validation of analytical methods for the evaluation of patches which includes in vitro and in vivo NTG permeation studies.
6. NTG content of the finally developed product kept for stability.

Preformulation studies were mainly conducted by making combinations of adhesives and NTG in various ratios which were subjected to different storage temperatures to study the effect of temperature. The temperatures employed were RT(30±2 °C), 37 °C and refrigeration over a period of six months. Such of the combinations, which were found to be showing at least 95% of the recovery levels, were selected further in the formulation development work.

The further work done is divided into two sections, Matrix dispersion system and membrane controlled drug delivery system.

A. Matrix-Adhesive Dispersion System :

Formulation of the matrix-adhesive dispersion system involved selection of vehicle for NTG, suitable adhesive, primary and secondary packaging material as well as development of machines for laboratory scale and scale up process development.

It was a difficult exercise to select an adhesive for TDDS from the currently marketed adhesives in India, as none of the manufacturers was able to provide detailed account of adhesives which include toxicity, skin irritation profile and compatibility of the adhesive with the other components of TDDS. Finally, it was decided to synthesize adhesives based on the work done by Dr. Lalla et al. After some changes in ingredients and process variables, solvent based acrylic adhesives were synthesized to suit the requirements (refer Chapter 3) and evaluated.

After the preformulation studies, successful formulations were subjected to laboratory scale process development for TDDS. Initial efforts with the Mayor's rod were unsuccessful as there was no uniformity in application process. Later in the work, a special laboratory scale applicator was developed with which desired uniformity in application was achieved which had shown negligible weight variation in patches (Av. Wt. of patch 0.292 ± 0.0058 gm).

The laboratory scale developed batches were repeated to get reproducibility on few occasions and the final batch developed (NTG-A40-01) was subjected to in vitro drug permeation through synthetic membrane (Cellulose acetate, 0.45µm pore size) and pig skin using modified Franz cell. The cumulative release through synthetic membrane (11.465 ± 0.487 mg/cell) was found to compare with that obtained with NitroDur® 40 mg (14.86±1.535 mg), whereas using pig skin the cumulative release was found to be less (6.382±1.1 mg/cell) and (5.853±1.979 mg/cell) for NTG patches and NitroDur® 40 mg, respectively. In the series of initial experiments, much variation in the in vitro cumulative release using pig skin (std. dev. for NTG 1.034 and for NitroDur® 1.749) was observed, which was subsequently eliminated by equilibrating the pig skin with dissolution medium for longer time.

Also, the patches were studied for primary skin irritation in rabbits. The results were extremely encouraging and the patches were found to be non-irritant.

The scale up process development was accomplished by using the machine available at KMK College of Pharmacy, Mumbai with little modification in design to reduce the minimum requirement of the raw material for processing. The final formulation developed on laboratory scale was modified to suit the application process. After validating the application process with few repetitive experiments, the final batch (NTG-A4-02) was subjected to in vitro as well in vivo evaluation.

In vitro Study:

The in vitro cumulative release of NTG patches (6.387±0.39 mg/cell) was comparable with that of Nitro Dur (6.87±0.06 mg/cell) using pig skin and human cadaver skin (1.460 versus 1.875 mg/cell). The in vitro release using cadaver skin was carried out on only one occasion using one sample because of the scarcity of cadaver skin and complex legal formalities involved which were difficult to overcome. The release profile of NTG from the NTG patches B. No. NTG-A40-02 and Nitro Dur are observed to follow linear

Q/√t pattern in a biphasic manner as expected from matrix-diffusion drug release kinetics as shown in fig 6.1 & 6.2.

Fig. 6.1: In vitro release of NTG from NTG patches B. No. NTG-A40-02 showing biphasic pattern.

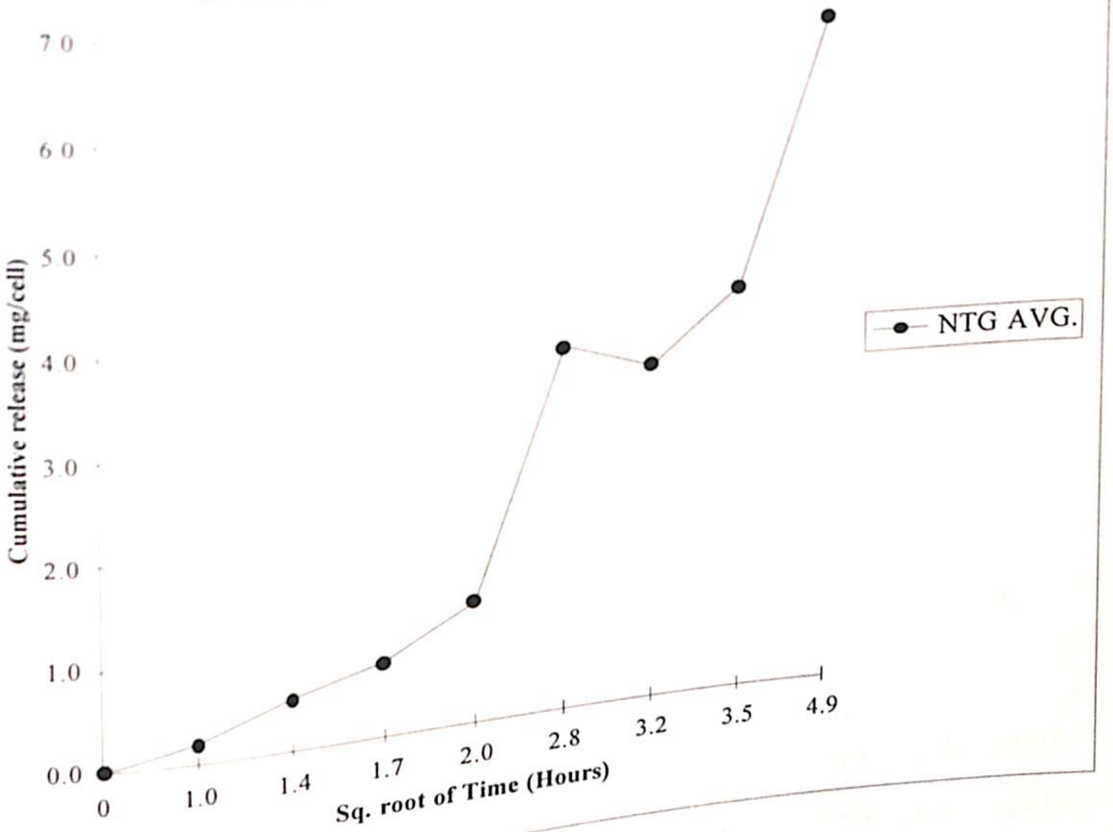
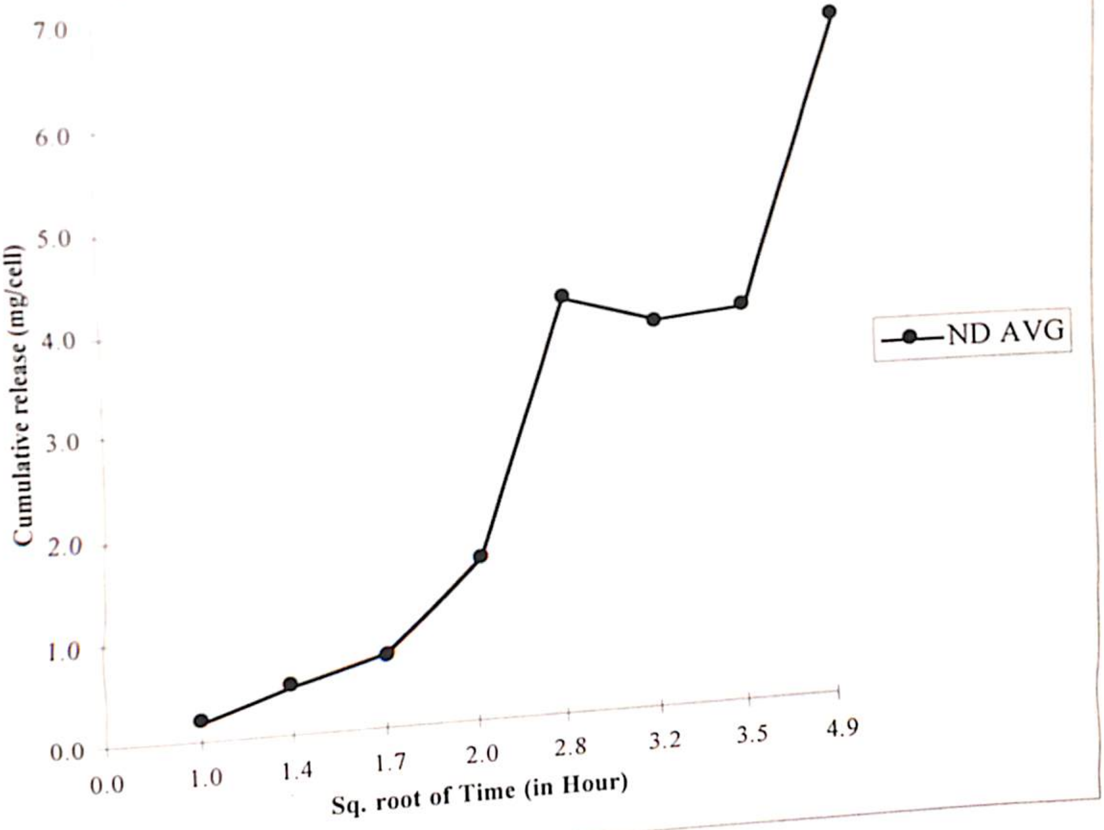


Fig. 6.2: In vitro release of NTG from NitroDur 40 mg showing bipasic release pattern.



In vivo Study:

Extensive literature survey revealed only two methods of testing the patches in picogram quantities **in vivo**, viz. GC-ECD and GC-MS. Unfortunately both the equipments are quite expensive although sensitive and were not available either in our laboratory or with outside agencies who could lend us for doing our work. Due to close contacts with industry personnel, and after learning the background of the total analysis involved, all reported methods were screened and with the help of M/s Shimadzu Japan, reported methods by A. Gerardin et al in **Biomedical Mass Spectrometry**[*] without the Nitroglycerin synthesized using ^{15}N nitroglycerin as internal standard were tried and though linearity could be achieved in the electron ionization (EI) mode in PPM quantities but due to the lower capacity vacuum pump, the desired linearity, in picogram levels, could not be achieved; hence we had to abandon the work on GC-MS.

Due to failure of GC-MS method and GC-ECD not being available in our laboratory, contract analytical laboratories were contacted and one such laboratory, having GC equipped with ECD detector, was located. Several packed columns, as reported in the literature, have been tested so far, but *the method, reported* by Chien Chin Wu et al[†] was selected for the *determination of pg quantities of Nitroglycerin from plasma*. The linearity for the same could not be established, even after trying with different columns and was thought of due to bad ECD detector (due to improper maintenance), the work had to be abandoned with this particular laboratory. Local manufacturer of GC, equipped with ECD detector, M/s Netel Chromatograph Ltd. was then contacted and this problem was discussed with them who confirmed our suspicion. The work was started with their own equipment. Finally, a validated analytical method was developed, using widebore capillary column, as mentioned in the experimental section. After the analysis, AUC of drug calculated for the NTG patch (544.8±85.59 pg/L.hr) was comparable with that of NitroDur® (572.12±45.347 pg/L.hr). However, there was slight difference in T_{max} but that can be attributed to sampling time interval of 4 hrs. and animal size preventing from repetitive bleeding.

B. Membrane-Controlled Release System:

The equipment available for developing patches, employing this principle are quite complex in construction and working and this is perhaps the reason why number of entrepreneurs desirous of introducing patches on commercial scale have been discouraged. An extensive discussion with various technocrats and fabricators led us to believe that this machine has to be a form-fill-seal type machine. However, an intricate look at Nitroderm TTS®

[†] J. Chromatogr., **228**: 333-339 (1982).

reveals a more complex technology as the rate-controlling membrane is impregnated with adhesive followed by release liner. This was the practical difficulty in developing such a machine. However after considering many factors, the development work was initiated and as a result of intense working, totally indigenous machine has been developed in this laboratory (see photograph). The patch developed using the final formula (B. No. NTG-M25-01) showed that major portion of NTG was released as compared to Nitroderm TTS®. Some difficulties experienced with this machine included (I) rupturing of controlled-release membrane (sometimes due to high temperature used for sealing) and (II) the application of adhesive in a controlled manner to deliver uniform quantities which currently is under progress.

Currently, it has been possible to achieve 60-70% of release in vitro using pig skin (6.525±2.76 mg/cell) compared to Nitroderm TTS® 25 mg (11.901±0.804 mg/cell), further work in this direction is in progress.

PART II. DICLOFENAC DIETHYL AMMONIUM (DDEA) PLASTER:

Diclofenac sodium is most widely used salt both in oral and in intramuscular injections for all arthritic and other related pain and inflammatory conditions. Diclofenac diethyl ammonium salt is available in the form of a gel manufactured by several companies in India and abroad for local pain and inflammatory conditions. It was decided to make a plaster formulation of the same hopefully obviating the need to swallow the pills or apply the cream, repeatedly. As mentioned earlier, during literature survey on "Dialog", it was found that ISBA of Switzerland used Diclofenac Hydroxy Ethyl Pyrrolidone (DHEP) salt in the form of a plaster. This prompted us to develop the same here in this country and to take this as a research project. To achieve this objective, an attempt was made to synthesize the salt, Diclofenac Hydroxy Ethyl Pyrrolidone (DHEP). But due to shortage of time and non-availability of synthetic method, the salt could not be synthesized. As an alternative, it was decided to use Diclofenac Diethyl Ammonium (DDEA) salt for plaster. Attempts were made to match the performance, in terms of in vitro release rates, in vivo release rates and performance of the plaster in toto with the imported plaster.

I. Pharmacokinetic Claims And Solutions:

The superiority of DHEP over other salts of diclofenac has been claimed in literature while not mentioning any details or data of comparison with Diclofenac Diethyl Ammonium salt. Three factors concerning DHEP were considered during the development of the plaster;

1. The literature claims that the solubility of DHEP is 1.8 gms./100 ml in water and 8.34 gms/100 ml in octanol , ratio of o/w is 4.4.
2. DHEP has surfactant activity and has critical micelle concentration above 35 mM.
3. It has ability to dissolve hydrophobic compounds such as lecithin.

The studies conducted, in this laboratory, showed that the material procured from local manufacturers has solubility of 1.4 gms/100 ml in water and 14 gms/100 ml in n-octanol, resulting in much higher o/w partition coefficient of 10. Thus DDEA can be a much better salt of diclofenac as compared to DHEP as a candidate for transdermal delivery system . The critical micelle concentration, determined by the method reported in Chapter 3, is higher than DHEP (40 mM). Lastly, the surfactant behavior of DDEA is identical to DHEP i.e. it has ability to dissolve lecithin as demonstrated by the similar experiments. Based on this data, DDEA was selected as a final candidate, for starting the work on plasters.

II. Methodology Followed:

The steps followed for the development of plaster are as follows:

1. Analysis of raw materials involved in the manufacturing of plaster as well as that of packaging materials.

2. Development and validation of the analytical methods for drug content in plaster, in vitro and in vivo analysis.
3. Development of suitable formulation and machines for laboratory scale as well as scale up work.

III. Preformulation Studies:

This was done broadly to study any instability and physical changes in the material at moderately elevated temperature which involved analysis of samples stored at RT and 45 °C.

IV. Formulation Of Plaster:

The purpose of these plasters is not to deliver the drug at predetermined rate but only to get as high a concentration as possible, at the site of application. The entire attempt was to get similar or higher release rates of DDEA as compared to that of Flector. As already discussed in the experimental section, various combinations of aqueous adhesives, hydrocolloids, thickening agents and solubility enhancers such as propylene glycol, glycerin etc. were tried and scale up batches of the size of 10 Kg were made. A considerable amount of time was consumed in selecting a proper cloth as a backing liner. As the composition of backing liner used for Flector, could not be ascertained, most of the work was done using surgical grade cotton cloth, confirming to B.P. specifications. Jersey cloth composed of 65% viscose and 35% cotton of special variety was also employed here. The other major work, included the development of the machine for application in scale up batches, which was developed indigenously where as the other machines required (such as slitting and cutting) were available commercially.

V. Evaluation Of Plaster:

1. In Vitro Study:

This was accomplished by using only pig skin. Considering the size of the patch, three modified Franz cells having neck diameter of 73.4 mm and volume 460 ml were got fabricated and release rates were established for both

Flector tissue gel and indigenously developed DDEA plasters (both at laboratory & scale up level). The release obtained was(3.421±0.3854 mg/cell) for DDEA plaster versus (5.101 ±1.309 mg/cell) for Flector developed in laboratory. And for pilot batches was (5.240±0.729 mg/cell) versus (5.840±0.952 mg/cell) for Flector. Though the absolute release rate was not considered important, the wide variations also could not be accepted while selecting the formulation. Since the bigger (neck diameter of 73.4 mm and volume 460 ml) cells did not offer any potential advantage, small (neck diameter 37.14 mm; volume 60 ml) cells were used for further in vitro studies. The release profile of Diclofenac from DDEA plasters and Flector are observed to follow linear Q /√t pattern in biphasic manner as expected from matrix- diffusion drug release kinetics as shown in fig 6.3 & 6.4.

Fig. 6.3: In vitro release of DDEA platser B. No. DDA-5 showing biphasic pattern.

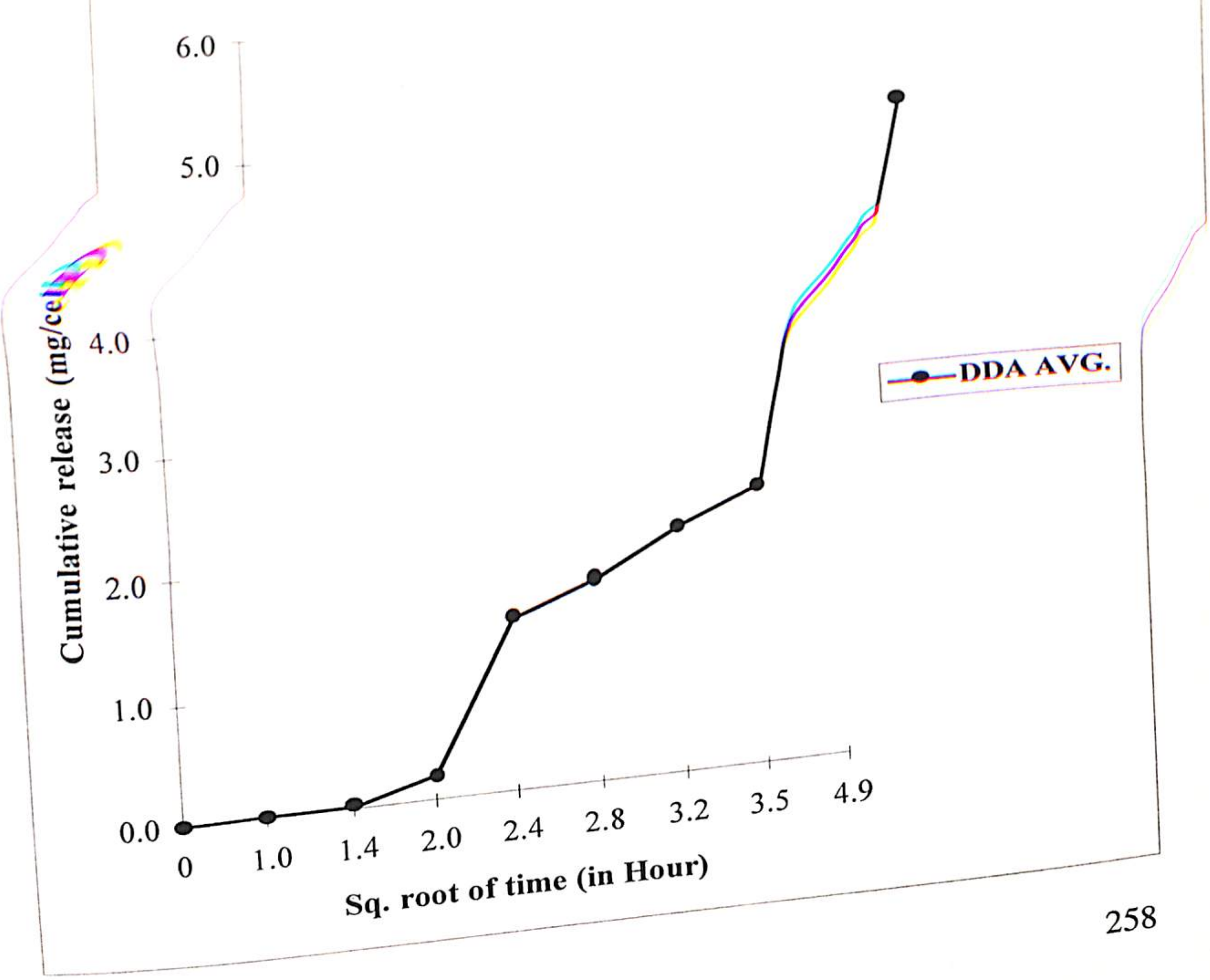
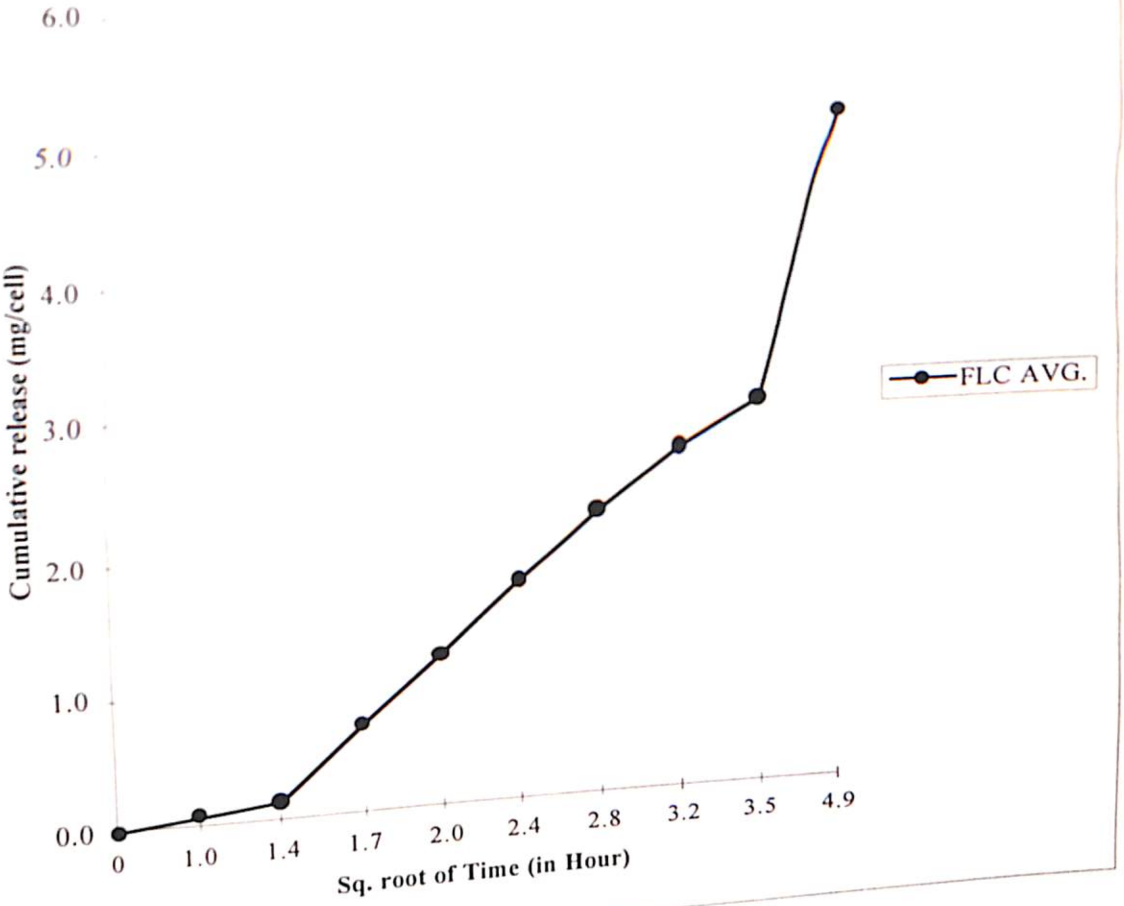


Fig. 6.4: In vitro release of DHEP from Flector showing biphasic release pattern.



2. In Vivo Study:

This study was performed on six rabbits. The blood samples collected at regular interval for 24 hrs. were analyzed by validated HPLC method. The aim of the study was to compare the release pattern of the drug with that obtained with Flector to establish the equivalence of the developed plasters. The AUC calculated, as per the trapezoidal least square method, was (4.61±0.617 µg/L.hr) of Flector versus (3.917±1.04 µg/L.hr) for DDEA plaster while the C_{max} and T_{max} values were nearly identical.

PART III. NICARDIPINE PATCHES :

Nicardipine is a Ca^{++} channel antagonist which inhibits the transmembrane influx of calcium ions into cardiac muscle and smooth muscles without changing the serum calcium concentrations. The effects of Nicardipine HCl are more selective in vascular smooth muscle than in cardiac muscles which produces relaxation of coronary smooth muscles at drug levels and cause little or no ionotropic effect. This is desirable for management of chronic stable angina and hypertension.

Considering the theoretical modeling, partition coefficient and availability of Nicardipine HCl, some indigenous work on this molecule was initiated. After proving the suitability of Nicardipine HCl as a candidate for the transdermal delivery system as discussed in Chapter 3, attempts were made to formulate the adhesive matrix dispersion system as well as the membrane-controlled delivery system.

A. Adhesive-Matrix Dispersion System:

1. Formulation Development:

The adhesives and method used was same as for NTG except dissolving Nicardipine HCl in alcohol was initially attempted. However, while studying the in vitro release of the drug even after prolonged lag time the amount released was negligible. As an alternative, formulations based on aqueous adhesives, used for diclofenac plaster, along with Gelatin as a substrate was attempted. This formulation gave good in vitro release rates. PET film, 50 µm thickness, was used as backing membrane while the release liner used was also the PET film of 125 µm thickness. The application method used, was similar to that used for diclofenac plaster employing the applicator used in laboratory scale preparations.

2. Evaluation Of The Patches:

The patches were evaluated for in vitro release using both cellulose acetate membrane (2.260±0.10 mg/cell) as well as pig skin (0.420±0.118 mg/cell). As the release was not very encouraging, it was decided to develop membrane-controlled delivery system parallely.

B. Membrane-Controlled Delivery System:

Development of this type of system was mainly based on the technology used for development of nitroglycerin membrane-controlled delivery system using same machinery used earlier for NTG formulations. Some formulations were first developed to establish release rates which failed to comply with requirements. Inclusion of as high as 50% methyl diisosorbide as a penetration enhancer and 50 % propylene glycol increased release rates substantially (0.273±0.044 mg/cell) with prolonged lag times of 12-15 hrs.

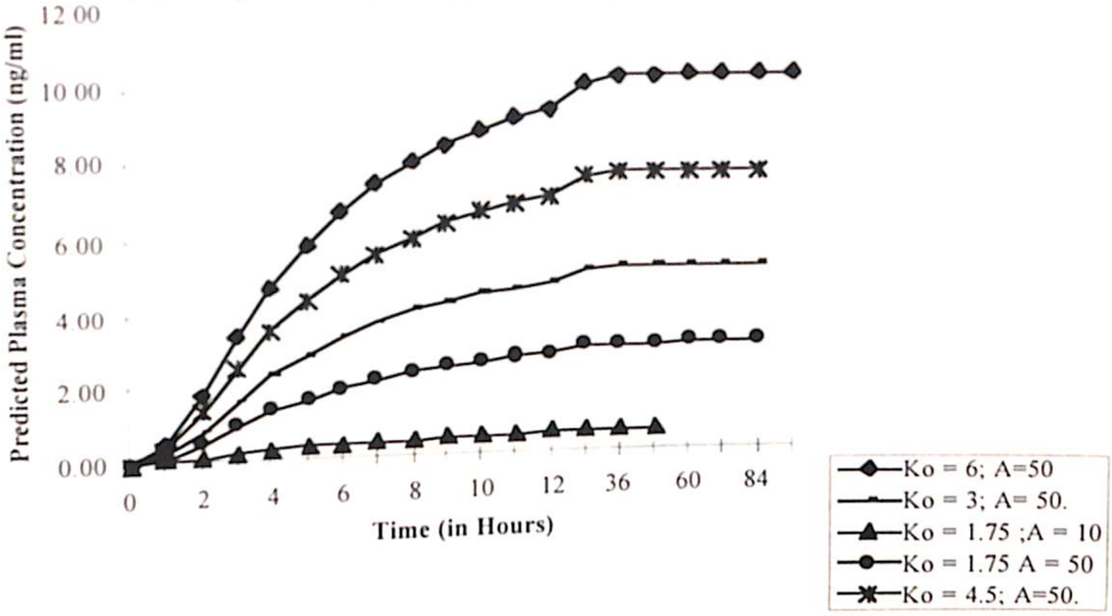
PART IV. *Theoretical Modeling* And Prediction Of Plasma Levels:

As both matrix diffusion *controlled systems and membrane-controlled drug* delivery systems failed to show sufficient levels of drug through pig skin even after 24 hrs. (expected concentration of 12 mg/cell). It was decided to re-examine the theoretical model developed (based on Guy and Hadgraft equations described in chapter 3) in BASIC language written with the help of mathematics dept. of KMK College of Pharmacy, Mumbai. Since it was known that minimum amount of 6-10 ng/ml of blood level is therapeutically effective, an innovative interpretation has been attempted with suitable in vitro concentrations to be achieved for both the type of system for prediction of plasma conc. at different time intervals.

I. Matrix-Diffusion System:

Since the amount of drug released in 24 hrs. from best possible system developed in this laboratory was only 420 mcg/cell from a 10 cm^2 patch. Hence, it can be seen that a release of only 1.75 $\mu g/cm^2/hr$. was achieved. In order to achieve plasma concentration of above 6 ng/ml, only three possibilities are there (I) increase the drug dissolution by inclusion of permeation enhancers and other formulation changes to get higher release rates; (II) increase the area of the patches and (III) use a combination of both (refer Fig 6.5).

Fig 6.5: Effect of Ko and A on the prediction of plasma levels of Nicardipine HCl patch (matrix-dispersion system).



Different values of K_0 (expected in vitro dissolution from the patch in μg/cm^2/hr.) and different areas were fed into the computer while using the same values of k_1, k_2, k_3, k_4 and k_r. A plot of plasma concentration versus time was drawn using the values obtained from the computer for as much as four days. It can be clearly seen from the Fig 6.5 that even if the area is increased to 50 cm^2 without affecting k_0, it is possible to achieve only 2 ng/ml of the plasma levels. However, using an intermediate level of 4.5 μg/cm^2/hr. and area of 50 cm^2 it is possible to achieve levels of above 6 ng/ml in about 8 hrs. of time, using still higher values of k_0 of 6 μg/cm^2/hr and area of 50 cm^2, the time can be further reduced to about 5 hrs.

II. Membrane-Controlled Release System:

As the levels obtained in 24 hrs. are half of the values obtained in the matrix-diffusion system, first order release part of differential equation used by Guy and Hadgraft was included for calculation in the program. In addition to

k_0, area of the patch and amount to be loaded on rate-controlling membrane for first order release rates was considered. Fig 6.6 shows the effect all these factors and it can be clearly seen that only a patch of 50 cm^2, M_{ad} of 20 mg and a system having dissolution of 6 μg/cm^2/hr. can only deliver desirable drug concentration after 10-12 hrs (refer Fig. 6.6).

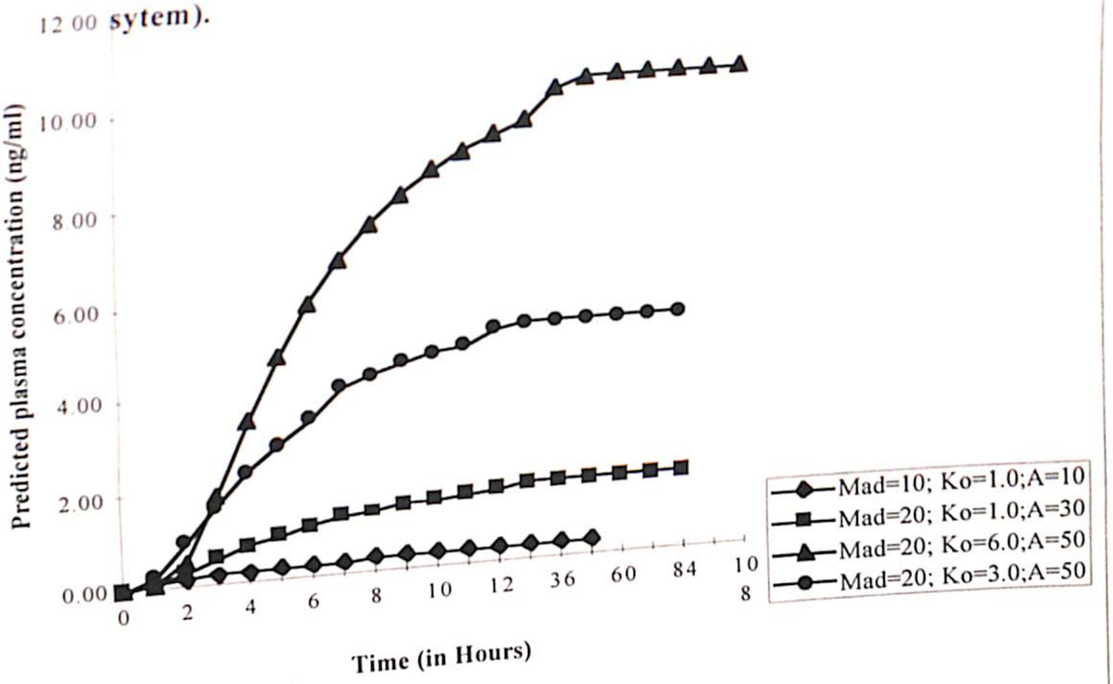


Fig. 6.6: Effect of Mad, Ko and A on the prediction of plasma levels of Nicardipine HCl patch (membrane-controlled release sytem).

In absence of encouraging in vitro release rates, size of the patch, lack of commercial commitment and non-availability of commercial samples it was decided to abandon the work at this juncture. It is apparently clear that Nicardipine shows better release rates (in vitro) using matrix diffusion system than rate controlling membrane system in preliminary experiments carried out so far.

Chapter VII.

SUMMARY & CONCLUSION

Chapter VII. Summary and Conclusion

Summary:

In this section an attempt has been made to summarize the entire work done in seven chapters.

Chapter one includes, general introduction to anatomy and physiology of skin laying stress on stratum corneum, permeation enhancers, thermodynamics of skin permeation, fundamentals in pharmacokinetics interpretation for prediction of steady state plasma levels. This section describes the pharmaceutical aspects of transdermal drug delivery system and succeeding section discusses the factors affecting the same. Finally, in vitro and in vivo evaluation methods for this mode of drug delivery system has been discussed in the last section.

As the study reported here includes three drugs, namely Diclofenac Diethyl Ammonium, Nitroglycerin and Nicardipine HCl , chapters subsequent to 1 have been divided into three sub-parts.

Chapter 2 gives the detailed literature survey for all three drugs mentioned above.

Chapter 3 describes introduction to the experiments i.e. details of adhesives used for such systems, the packing material employed, methodology used for analysis of all components and various techniques which were used for developing the drug delivery system including development of equipment.

Chapter 4 comprises of details of methodology employed for analysis of raw materials including validation of all the analytical methods, process involved in formulation and evaluation of the system.

The results of the experiments conducted are illustrated by table and graphs in chapter 5.

The discussion is included in the Chapter 6. While Chapter 7 includes summary and conclusion.

In addition to these generalized topics, discussions and conclusions about use of DDEA as the choice of plaster versus DHEP based on physicochemical property have been included in Chapter 3. Similarly, for Nicardipine HCl, substantial work done on theoretical modeling for prediction of plasma levels using different mathematical models have been dealt with at length in Chapter 3.

Conclusion:

The transdermal patches are very popular in western countries where people believe in compliance and convenience for treating any disease. With the priority accorded to health and the amount of budget allocated for health and personal care, one can easily afford a plaster or a patch costing 2 dollars for 12 hours relief. But such a proposition is unthinkable by majority of the doctors in India. With this point at the back of mind, the development work was taken up, encompassing synthesis and evaluation of adhesive, procuring right packaging components, developing the machinery to produce the patches, on commercial scale, in a manner to keep the cost of delivery system low at 2 rupees a piece and not 2 dollars.

The same has been achieved to a great extent for all the drug candidates considered.

1. Nitroglycerin Patches:

Ready to use, compatible, stable, elegant and pharmaceutically marketable patches giving demonstrable blood levels, in animals, have been developed awaiting for bioequivalence studies in human beings and for clinical evaluation. Considering DCI permission for marketing and other legal formalities to be completed for the purpose, NTG patch has been completely developed for marketing using totally indigenous system except for importing the drug substance. Development of membrane-controlled drug delivery system is going to take some more time as some modifications in machine for scaling purpose are required.

2. Diclofenac Plaster:

The greatest challenge here was the size and weight of the plaster and non-availability of the any information regarding the formulation (except the preservatives) which made this job challenging. The challenging task of making the machine both for laboratory and commercial scale production has been successfully achieved besides developing the analytical procedures for determination of low levels of DDEA in the blood in this laboratory in vivo. Final commercial samples are ready for commercial exploitation while arrangements are being made to complete the blood level studies in humans using GC-MS which is being procured for this purpose. It is hoped that a large number of arthritis patients and persons incurring sports injuries should be benefited largely with this kind of system manufactured in our homeland at a very reasonable cost.

3. Nicardipine HCl Patches :

As this drug has cardio-selective action and is used both in stable angina and hypertension and found appropriate candidate for TDDS in theoretical models. Thus, it was considered for inclusion in patch. Sufficient work has been done employing both systems. However, unseen difficulties forced the termination of work at this juncture.

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