Industrial Pharmaceutical Technology Master Program.

MSc. Thesis.

Preparation of Topical diclofenac sodium spray and investigation of the effect of different penetration enhancers on the drug permeation rate.

تحضير رذاذ صوديوم الديكلوفيناك الموضعي والتحقق من تأثير معززات الاختراق على معدل نفاذية الدواء.

This thesis is submitted in partial fulfillment of the requirements for the degree of master in Industrial Pharmaceutical Technology from the faculty if Graduated studies at Birzeit University, Palestine By:

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Supervisor: Dr. Moammal Salah E-deen Qurt.

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Palestine, June 2019

Bayan Farraj.

DECLARATION.

I certify that this thesis submitted for the degree of master is the result of my own research, except where otherwise acknowledged, and this thesis has not been submitted for the higher degree to any other university or institute.

Signed: ……

Bayan Dakhil Farraj.

Date: / / 2019.

TABLE OF CONTENTS.

Contents

LIST OF ABBREVIATIONS.

API Active pharmaceutical ingredient.

AR analytical reagent.

C Concentration.

C_1 concentration in the membrane in the donor compartment.

C_2 concentration in the membrane in the receiver compartment.

C_d concentration in the donor compartment.

CE Cellulose esters.

COX Cyclooxygenase.

C_r concentration in the receiver compartment.

C_{sa} concentration of sample.

D Diffusion coefficient.

DMAC Dimethyl acetamide.

DMF Dimethyl formamide.

DMSO Dimthylsulphoxide.

ER enhancement ratio.

FDC Franz diffusion cell.

h membrane thickness.

HPLC High performance liquid chromatography.

IPA Isopropyl alcohol.

M the amount of material.

M-Pyrol N-Methyl-2-pyrrolidone.

NMF Natural moisturizing factor.

NSAIDs Non-steroidal anti-inflammatory drugs.

P Permeability coefficient.

PA Polyamide.

PAN Poly acrylonitrile.

PC Polycarbonate.

PDMS Polydimethylsiloxane.

PE Penetration enhancer.

PG Propylele glucol.

PO Polyolefin.

PP Polyproylene.

PVP Poly vinyl pyrrolidone.

Q cumulative amount of drug penetrated.

RC Regenerated cellulose.

RES Polysulfone.

RPM round per minute.

SC Stratum Corneum.

SCOP Synergetic Combination of Penetration Enhancers.

SLS Sodium Lauryl Sulphate.

T Time.

TDDS Topical drug delivery system.

T_L Lag time.

USP United state pharmacopeia.

X the distance in cm of movement perpendicular to the surface of the barrier.

LIST OF FIGURES.

LIST OF TABLES.

ABSTRACT.

Diclofenac sodium is an example of NSAIDs that has excellent analgesic, antipyretic and anti-inflammatory activity. It inhibits prostaglandin synthesis through the inhibition of cyclooxygenase (COX) that contributes in pain and inflammation. The main route of administration of Diclofenac sodium is the oral route, it has also been administered topically, intravenously, intramuscularly, intracolonically and rectally. Topically, it is used for local symptomatic relief of mild to moderate pain and inflammation of small and medium sized skin areas.

The aim of this Thesis was to develop a topical diclofenac spray and to investigate the effect of different penetration enhancers on its permeation rate.

The solubility of diclofenac sodium was determined in different solutions and pHs. The highest solubility was found in PG with a value of 29.97 gm/ 50ml.

In the first phase of the experiment the diffusion parameters of (4% w/w) diclofenac sodium spray solution was determined and compared with the marketed RLD which was used as a references control. In the second phase of the experiment a synthetic advanced membrane that mimics the properties of human skin (Start-M® membrane) was used to test the permeation of diclofenac sodium from the optimized spray solution.

In-vitro study using Franz diffusion cell was performed, in the first part the membrane was composed of one layer of polyamide filter membrane soaked in octanol and

sandwiched in between two layers of dialysis membrane that was previously soaked in phosphate buffer pH= 7.4, The receiver compartment was filled with phosphate buffer pH= 7.4. The donor compartment contained 2 g of the prepared solution. In the second part, a novel membrane (Start-M® membrane) was used to separate the donor and receiver compartments.

Samples of 1ml volume were taken from the phosphate buffer at the receiver compartment after half an hour and every half an hour later on up to six hours for each experimental sample. Diclofenac sodium was quantified by using HPLC at $\lambda = 276$ nm.

The penetration enhancer under investigation were: Limonene (0.25%), L-Menthol (1%), Eucalyptol (0.25%), M-pyrol (1%), Tween 80 (0.1%) and Oleic acid (0.5%). They were added in different concentrations to 4% of diclofenac sodium solution in the donor compartment. Diffusion parameters that was determined were cumulative, T_L, D, P, and K. The enhancement Ratio ER was used as criteria for selecting the best penetration enhancer. The ER was found to increase in the order of: Limonene > L-Menthol > Eucalyptol > Limonene+L-Menthol > M-pyrol > Tween > Oleic acid.

Different trials for development of spray solution were formulated using different quantities of PG, Purified water and IPA, including 4% of diclofenac sodium and 0.5% to 1.5% of PVP to increase its viscosity.

Diclofenac spray solution containing 4% of diclofenac sodium and the selected penetration enhancer (Limonene) showed significant higher enhancement ratio than other tested formulae. The final formula with the selected penetration enhancer was tested for permeation through Start-M® membrane. The API showed good penetration through the advanced membrane with permeability coefficient of 0.1610 cm/hr.

PART ONE: INTRODUCTION.

1.1 TRANSDERMAL AND TOPICAL DRUG DELIVERY SYSTEM

Topical drug delivery system (TDDS) are intended for the skin application in order to deliver drugs by topical/transdermal route for both local and systemic action. Advantages of TDDS over conventional oral administration as it avoids first pass metabolism, avoid stomach degradation, provides steady plasma levels, improves bioavailability, being convenient and easy to apply , avoidance of gastro intestinal incompatibility, and improve patient compliance[1–3]. Stratum corneum (SC), the top layer of the skin is the first limiting step for penetration process and transdermal drug delivery. It consists mainly of two components that look like a brick and mortar structure in which dead flattened cells, corneocytes, form the bricks that is surrounded by a lipid extracellular matrix that forms the mortar [4,5].

As the stratum corneum is the main barrier for drug diffusion and delivery, there are several ways used to disturb it and increase the transdermal drug delivery, such as physical enhancers (microneedle, electroporation, ultrasound, magnetophoresis, and iontophoresis), particulate systems and vesicles (solid lipid nanoparticle, micro emulsion, transfersome, noisome, liposome) and chemical enhancers (Azone, Fatty acids, Sulphoxides, pyrolidones, Essential oils, Terpenes etc.)[6–8]. Chemical penetration enhancers must be compatible with the active ingredient and other excipients, it should be pharmacologically inert, non-toxic, non-allergic, and non-irritating. The duration of action of these penetration enhancers must be predictable and reproducible and fast on action [9].

1.2 THE STRUCTURE OF THE SKIN.

Human skin i**s** considered to be the largest body part, it holds the body contents together. The main role of the skin is maintaining body hydration; it also helps in regulation of body temperature, and permits sensation of pain, touch, hot, and cold. Human skin as shown in (Fig.1.1) below is composed of three main layers[10,11]:

1. *The epidermis*, which consist of the nonviable layer of epiderms and viable layers of epidems, the outermost layer of the skin (Non viable layer: Stratum Corneum), also termed as horny layer which is approximately 10-20 μm thick. It has barrier property against macromolecules and hydrophilic drugs due to presence of 79–90% of proteins and 5–15% of lipids, the multilayered viable epidermis located beneath the stratum corneum is considered to be the main reason for generation of stratum corneum.
2. *The dermis*, directly beneath the epidermis with approximately 3-5μm in thickness, consisting of connective tissues and appendages (hair follicles, sweat glands).
3. *Subcutaneous*, hypodermis tissues consisting of some connective and fat tissues those act as a supportive membrane for both dermal and epidermal layer of the skin [12].

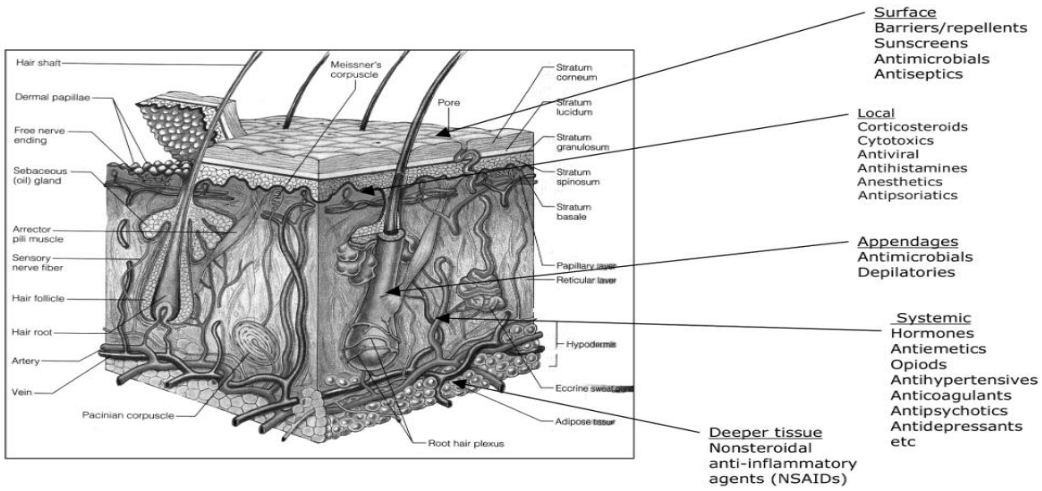

Figure 1. 1*: Anatomy and physiology of the skin shows the potential targets or site of action for cosmetics and drugs* [10].

1.3 DRUG PENETRATION PATHWAY:

1.3.1 Stratum corneum:

Horney layer, the outer most layer of the skin, or the stratum corneum, is considered as the rate limiting step of permeation of the drug across skin. It is approximately 10-20 μm thick[13,14]. At light microscopic level, stratum corneum has two compartments structural organization, corneocytes embedded in a lipid matrix to form such a system

similar to (brick and mortar system) corneocytes as bricks, and mortar is the intercellular lipids (Fig. 1.2)[15].

Figure 1. 2*: Figure 1.2: Bricks and mortar structure of the stratum corneum corneocytes as bricks, and intercellular lipids (arrowheads) as mortar* [15].

Origination of stratum corneum begins from the lowest viable epidermis (stratum basale, stratum granulosum, stratum lucidum) that undergoes several morphological changes before desquamation (Fig. 1.3):

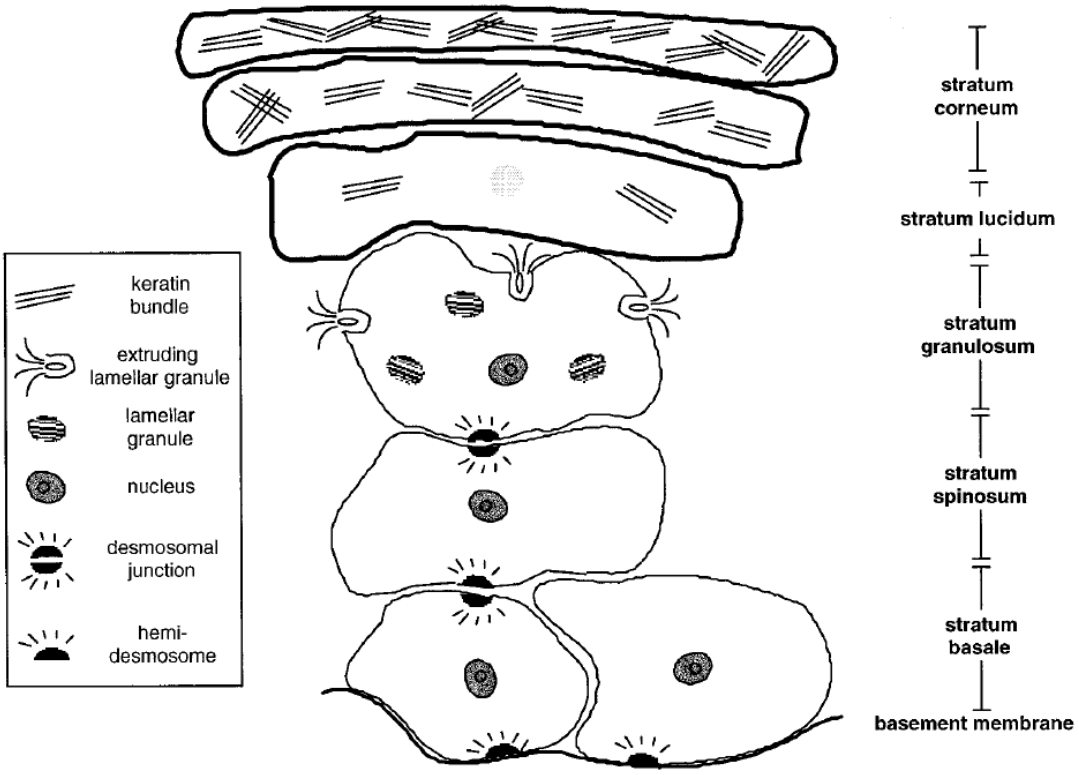

Figure 1. 3*: Epidermal differentiation: extrusion of lamellar bodies, loss of nucleus, increasing amount of keratin in the stratum corneum* [16].

Cells in the basal layer (stratum basale) start to proliferate, leaving, differentiating, and migrating towards the skin surface. At the interface between both staratum granulosum cells and stratum corneum cells, the viable cells elongated and converted to corneocytes (dead keratin filled cells). Stratum Corneum is very important in providing chemical and physical barrier that protects underlying organs from foreign agents such as toxin, and pathogens. It is slightly permeable to water [14].

1.3.2 The Intercellular Lipids

The intercellular lipids approximately occupy 20% of stratum corneum existing as continuous lipid phase of about 20% volume, arranged in multiple lamellar structures. It consists of ceramides 41%, cholesterol 27%, and cholesteryl esters 10%, fatty acids 9% and cholesteryl sulfate 2%. During the epidermal differentiation, the basal layer which is rich in phospholipids, is then converts to glucosylceramide, then to ceramides and free fatty acids, and finally disappear in the outer layer of the stratum corneum. In the final stage of differentiation the levels of free fatty acids and sphingolipids increase. (Ceramide -1) it is a type of eight classes of ceramides that has been identified in human stratum corneum considered to be a stabilizer of intercellular lipid lamella [14,17].

Discharging of the lamellar content in to intercellular space from the keratinocytes in the mid to upper part of the stratum granulosum cell generates the intercellular lipid matrix. The generation of the extrusion material then rearranged forming broad intercellular lamella , that associated in to lipid bilayers with hydrocarbon chains aligned, and polar head groups that dissolves in aqueous layer (Fig.1.4)[14]:

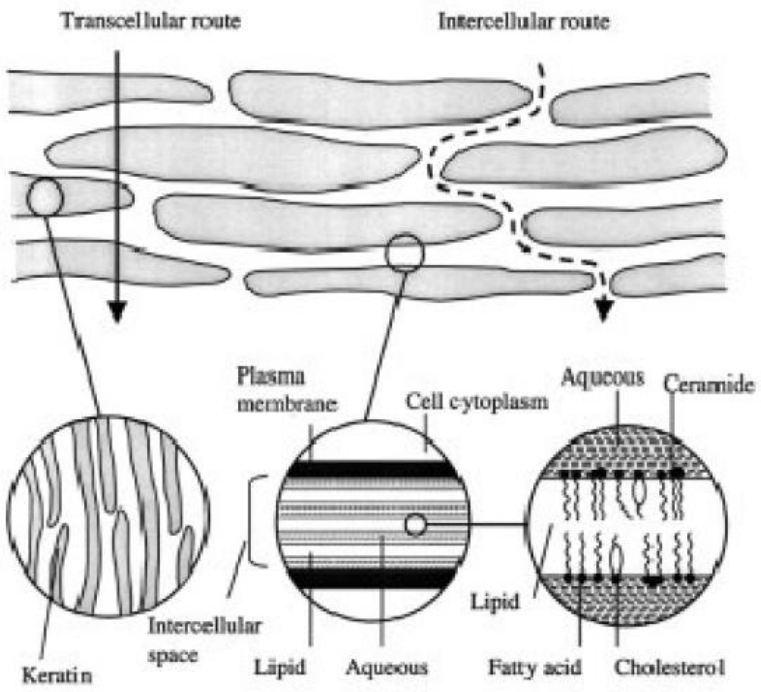

Figure 1. 4: *Figure 1.4: Intercellular lipids* [14].

1.4 TRANSDERMAL PERMEATION:

The transdermal permeation is the passage of substances (e.g.: drug) from outside the skin through its various layers in to the blood circulation at a specific rate. After application of the dosage form to the skin, concentration difference which is created lead to partition of the active ingredient then followed by diffusion of the compound from the external strata to the dermis [18,19]. During the diffusion process of drug

permeation across the skin, Stratum corneum is the major rate limiting step [14].

Simple diffusion Models explains that the flux of compounds through the lipid domains of stratum corneum are adopted by scientists in order to explain the permeation through skin, this model assuming that the interaction between compounds and skin is physiochemical with the multilayer structures of corneocytes within the stratum corneum that are horizontally organized [20].

1.5 FACTORS THAT MAY AFFECT PENETRATION:

Several factors can affect transdermal absorption rate and amount which must be considered when preparing topical preparations. The physiochemical factors that controls the passive diffusion of a substance from a vehicle across the skin are: the skin, the vehicle and the molecular properties of the substance. The affinity of the molecule depends on the skin surface and its molecular size which must be small enough ($<500/mol^{-1}$) to pass easily through stratum corneum, also its compatibility with the intercellular lipids [21–23]. The pharmacology of the vehicle used, the solubility, molecular mass, depth of penetration, and toxicology of its components must be well known. Sprays, gels and micro emulsions are adsorbed more effectively through the skin than creams, and the use of penetration enhancers in the formulation greatly increase the penetration effect through the stratum corneum. Site and method

of application affecting the ability of drug penetration and permeation through skin, barrier properties of the SC may vary at different body sites, in the thickness, number of cells, or the density of skin appendages, studies shows that diclofenac is more likely to reach superficial joints (e.g. knee, finger) more than deeper structure (e.g. hip joint), rubbing and gentle massage after topical application is recommended in order to increase the flux through skin. Bioavailability of the drug can increase by repetitive administration, also penetration can be facilitated by occlusion that hydrates the stratum corneum. Other physiological factors that may affect also are: skin age, the skin metabolism especially in the viable epidermis that contains many enzyme system leading to catalyze processes such as : reduction, hydrolysis, oxidation, or conjugation [20,24,25].

1.6 PATHWAYS OF DRUG PENETRATION:

Drug penetration in the skin could be achieved through one of the following pathways[26,27] (Fig.1.5):

1. *Transcellular route:* straight path to the dermis through both keratinocytes and lipids. This transport route involves repeated partitioning of the molecule between hydrophilic and lipophilic compartments.

2. *Para cellular route:* considered to be the main pathway for penetration of drug molecules (suitable for lipid soluble drugs rather than proteins) drugs remains in lipid moiety and stay around keratin. To achieve penetration of the drugs across the skin, molecules must be in solution in the vehicle to partition in to the lipid from vehicle in the stratum corneum, then diffused through it, then through viable epidermis and dermis, high percentage of molecules at the capillary plexus are transferred in to bloodstream and lower percentage may diffuses in to deeper tissue.
3. *Transappendgeal route:* Which include of sweat ducts and hair follicles it makes continues channel for drug permeation. Polar and large molecules and ions that have difficulty diffusing through the stratum corneum can permeate better through this route [9,14,20].

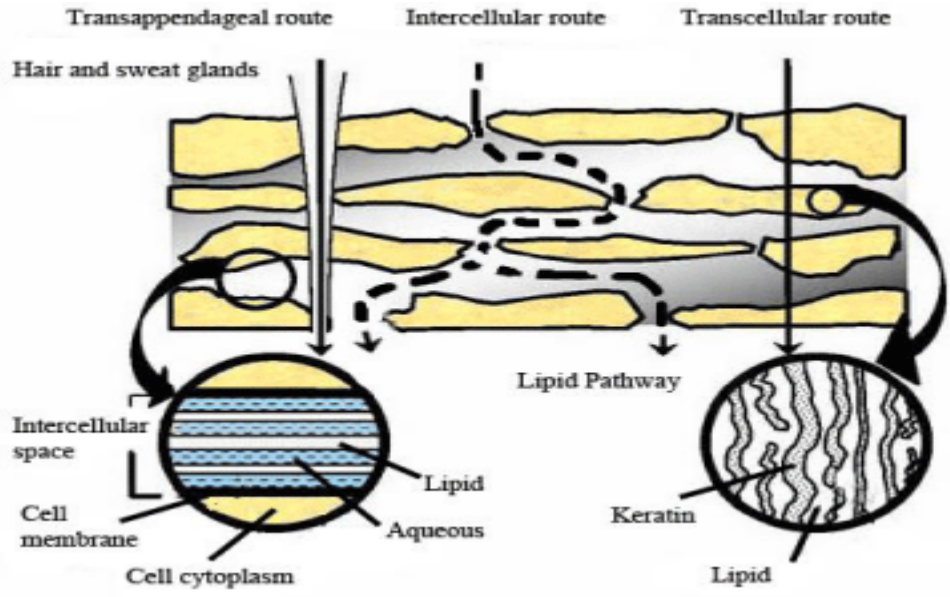

Figure 1. 5*: Drug permeation Pathway through skin*[12].

1.7 PERMEATION ENHANCEMENT.

By acting as a protective barrier ; Stratum corneum provides resistance on drug penetration. Thus various strategies and approaches were employed to penetrate this barrier.

1.7.1 Biochemical penetration enhancers.

Several approaches and strategies used in biochemical penetration enhancement field in order to achieve the transdermal drug delivery such as using prodrug molecules, enzyme inhibition, chemical modification, usage of the colloidal particles or vesicular systems [28].

1.7.2 Physical penetration enhancers.

Iontophoresis, electro-poration, sono-poration, and microneedle are examples of physical methods of penetration enhancement, they produce holes which are wide enough to facilitate permeation of a molecule [28,29].

1.7.3 Chemical penetration enhancers.

The main idea of penetration enhancers is to make the drug more soluble in the stratum corneum, this will lead to diffuse them into the skin surface more easily [30]. Some of penetration enhancers most used:

1.7.3.1 Water:

Water was used widely to facilitate and improve topical drug permeation. Approximately 15-20% of tissue dry weight is the content of water on human Stratum corneum. By soaking the skin in water the humidity of stratum corneum tissues will increase, also an occlusion of the tissues helps to prevent water loss and this will lead to an equilibrium state between the underlying epidermal layer and the stratum corneum layer, increasing the water content up to 400% of tissue dry weight. Occlusive preparations such as patches and ointments alter the water content of stratum corneum leading to an enhancement in the drug delivery rate[31]. Due to the heterogeneous nature of our stratum corneum, water within this layer exists in different ways, about 25-35% of it presents as (bound) i.e. bounded with other structural elements within the stratum corneum, other water particles remains (free) acting as solvent for permeants which are polar. Natural Moisturizing factor (NMF) are substances such as salts, amino acids, derivatives of amino acids aims to preserve the elasticity of the tissues, (-OH, C-OOH) are rich in corneocytes and are existed to bind molecules of water within the stratum corneum. The mechanism of action by which water may enhance the delivery

of transdermal drug is not well known yet. It is supposed that the solubility and partitioning of the permeant through stratum corneum can be modified by the presence of free water molecules. Soaking or occlusion may cause increase in the water content leading to swelling of the skin, thus disruption of the structure of lipids bilayer by distention of polar regions of the lipids bilayer [32].

1.7.3.2 Sulphoxides and similar Chemicals:

Dimethylsulphoxide (DMSO) one of the oldest penetration enhancers has been discovered and used widely, other examples of sulphoxides are Dimethyl acetamide (DMAC), and Dimethyl formamide (DMF), (Fig:1.6). They considered to be a very strong aprotic solvents, they act as penetration enhancers by denaturing the intercellular structure and changing the confirmation of the intercellular keratin from α helical to β sheet[32–34].

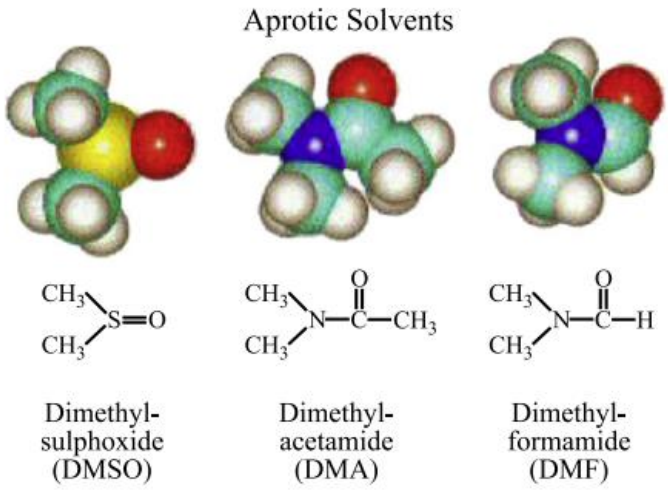

Figure 1. 6: *Aprotic solvents that act as potent penetration enhancers*[32].

1.7.3.3 Azone:

Azone (Laurocapran or 1-dodecylazacycloheptan-2-one) was specifically intended as the first penetration enhancer for skin. Azone is a very lipophilic compound, it has the ability to increase the transdermal drug delivery of many drugs such as antibiotics, antivirals, and steroid agents. Azone is a potent compound, it is very powerful at low concentrations (0.1-5%) [35]. As shown in (Fig:1.7) the unique chemical structure of Azone that has a big polar head is connected with a hydrophobic alkyl chain which explain the mechanism of action and how it disrupt the packing arrangement of the lipids bilayer[32,36].

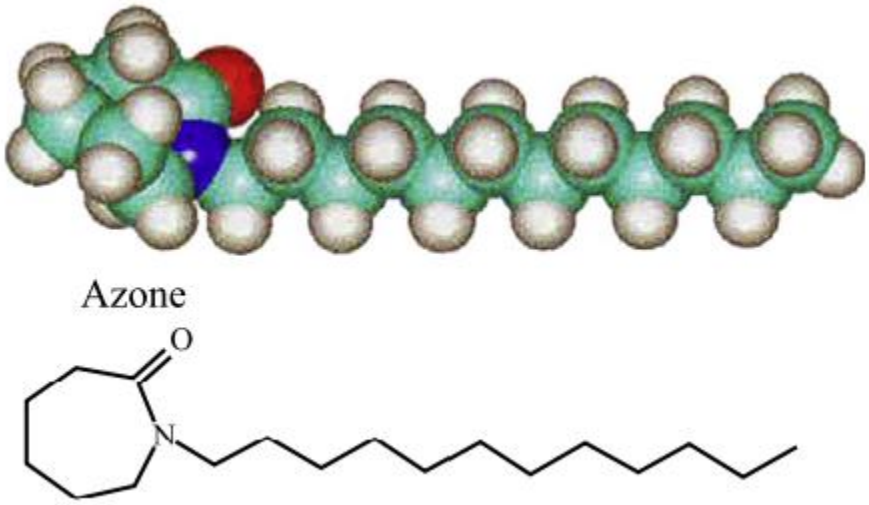

Figure 1. 7: *Figure1.7: Azone, (Laurocapran or 1-dodecylazacycloheptan-2-one) first skin penetration enhancer* [32].

1.7.3.4 Pyrrolidones:

Pyrrolidones is very powerful chemical penetration enhancers. (N-methyl-2-pyrrolidone) is the most famous used one [30]. They partition very well through the stratum corneum, leading to convert the nature of the membrane solvent, thus enhance the drug delivery through skin[35,37].

1.7.3.5 Fatty acids:

Concerning the structure of fatty acids concerning the length of alkyl chain, and the degree of saturation effects of the permeation activity of fatty acids, it is shown that using unsaturated fatty acids indicates more percutaneous drug absorption through stratum corneum than the saturated fatty acids of the same alkyl chain length. Disruption of the lipids packing is the main mode of action of fatty acids [33]. Oleic acid is one example of fatty acid which is widely used as a chemical penetration enhancer [38].

1.7.3.6 Fatty alcohol, Alcohol, and glycols:

Using alcohol in transdermal formulation such as ethanol in high concentrations may lead to lipids extraction from the stratum corneum, also it may affect and cause some modification to the lipid bilayers. 1-propranolol, 1-butanol and 1-octanol are examples of fatty alcohols (Alkanols) applied to the skin usually as co-solvent, especially with propylene glycol (1-10%). Propylene glycol (PG) cause solvation of keratin within stratum corneum leading to disruption of the structure of stratum corneum, thus enhance the delivery of permeants. PG when used as a vehicle for

penetration enhancers such as ethanol and oleic acid shows synergistic effect[32,33]

1.7.3.7 Surfactants:

Depending upon the nature of the polar head groups of surfactants, they are classified to four groups: anionic surfactant, e.g. sodium lauryl sulphate SLS, nonionic surfactant e.g. nonoxynol, cationic surfactant e.g. cetyltrimethyl ammonium bromide and zwitterion surfactant such as dodecyl betaine. Anionic & cationic surfactant shown to be irritant to our skin. Upon contact with skin, surfactants alter the function of the barrier after causing a huge disruption and modification in the properties of the barrier [39].

1.7.3.8 Urea:

Usually it is used in treatment of some skin disorders such as: psoriasis. Urea with salicylic acid used also for keratolysis. Because it is known to be an hydrating agent, urea improves hydration and build a hydrophilic channel within stratum corneum barrier, leading to enhance drug permeability and drug delivery [32].

1.7.3.9 Essential oils, terpenes, and terpenoids:

Terpenes are compounds that naturally occurring they consist of only hydrogen, oxygen and carbon atoms, and they are found in essential oils. They are safe and medically acceptable enhancers for hydrophobic and hydrophilic permeants. In combination with PG these compounds can cause synergistic action. Examples: d-limonene, Nerolidol, 1-8-cineole and carvone [32,35].

1.7.4 Mechanism of action of penetration enhancers:

Penetration enhancers facilitate the passage of an active ingredient (Fig.1.8) through the stratum corneum by several mechanisms, such as disruption of the structure of stratum corneum lipid matrix, increase permeability by improving partition of the drug into stratum corneum, or by direct action with intercellular protein, thus promot flux of the desired penetrant. Major mechanisim of action of PE:

1. **Action at intercellular lipids .**

   This can be through: fluidization, polarity alteration, extraction of lipids, or phase seperation . By fluidization or disordering of the lipid bilayers of the stratum corneum ,so it will be less rigid, several enhancers can improve permeability such as: DMSO, Azone, terpenes, and fatty acids. By forming microcavitis within the lipid bilayers matrix which is done by the aid of these enhancers, the diffusion coefficient of the permeant will increase. Alteration of polarity can be done by using enhancers such as oleic acid and terpenes at high concentration in order to pool with lipids domains and create permeable pores providing less resistance for polar molecules.

2. **Action within corneocytes.**

   Urea, DMSO and surfactants interacts with corneocytes, especially keratin. Penetration of surfactants into the intercellular matrix is followed by binding and interaction with keratin filaments, resulting in corneocyte disruption, thus increase the diffusion coefficient and increase permeability.

   Hydration.

   The safest method in order to increase the permeation rate of the drug (lipophilic & hydrophilic compounds) is using water. The water content of dry weight of stratum corneum is around 15-20 By increasing the skin hydration, there will be swelling of the stratum corneum structure leading to increase of the permeation [40,41].

The dermal penetration enhancers must be non toxic, non irritant to skin, and safe if it is used at high concentrations, unidirectional and predictable, compatible with both excipients and drugs, and must be excellent promoters of percateneous absorption. Famous penetration enhancers which are mainly used are surfactants, terpenes, alcohol, terpenoids or essential oils, azone, pyrrolidones, fatty acids, esters, glycols, phospholipids and glycerides, and phospholipids. Azone, oleic acid, and terpenes with transcutanol and PG are often used to create synergy of action. Recently, there is developing of a new and high- throughput screening approach called SCOPE formulation (Synergetic Combination Of Penetration Enhancers) [40].

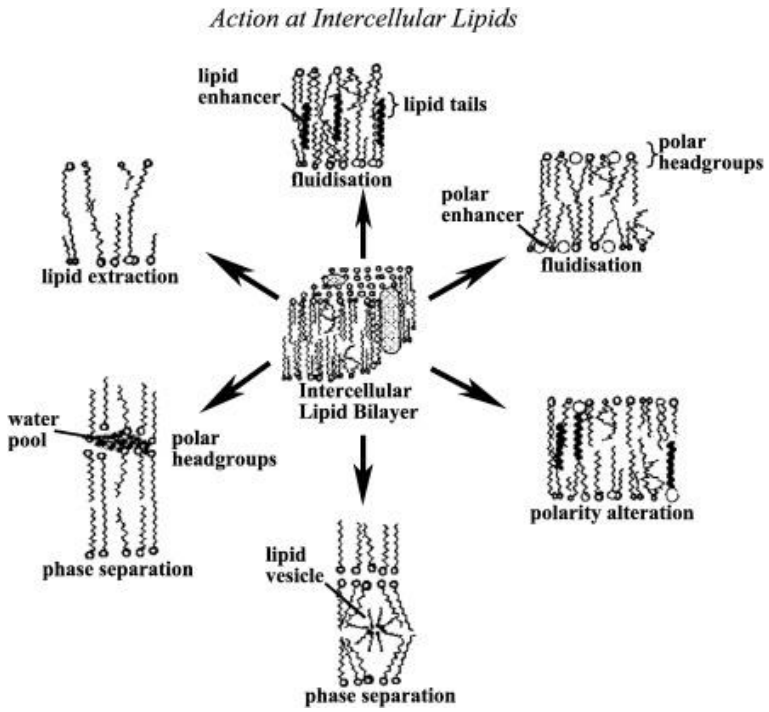

Figure 1. 8: *Figure 1.8: Mode of action of penetration enhancers* [32].

Ideal chemical penetration enhancers:[32]

- They must be cosmetically acceptable.
- They must be compatible with the active pharmaceutical ingredients and other excipients in the formula.
- They must not cause any irritation, toxicity or allergic reaction to the skin tissues.
- These penetration enhancers must work fast, also must predict and reproduce the duration of their activity.

- Pharmacologically must be inert.
- They must work unidirectional.
- Immediately after topical application of any chemical enhancers, the stratum corneum tissue must return to its normal properties.

1.8 DICLOFENAC SODIUM IN TOPICAL PREPARATIONS:

Nonsteroidal Anti-inflammatory Drugs are drugs category having excellent analgesic and anti-inflammatory activity but they produce serious adverse drug reaction such as: gastro-intestinal tract ulceration, liver and kidney problems, and increased with oral administration. Nowadays, due to these adverse drug reaction caused by oral formulations, diclofenac sodium is increasingly administered by topical route [41].

Diclofenac sodium has analgesic and antipyretic properties it works by inhibition of prostaglandin synthesis through the inhibition of cyclooxygenase (COX) that contributes in pain and inflammation. Topically, it is used for local symptomatic relief of mild to moderate pain and inflammation of small and medium sized skin areas [41].

The dosage form of the proposed pharmaceutical product in this project is a cutaneous spray, solution that turns to a gel-like consistency upon spraying the solution on to the skin, avoiding fast flowing of the formulation away from the applied site, the effect is

caused by evaporation of alcoholic components, PVP would also facilitate the formation of gel-like structure during application.

1.8.1Diclofenac sodium physicochemical properties:

The chemical name for diclofenac sodium is Benzeneacetic acid, 2[(2, 6-dichlorophenyl) amino]-, monosodium salt. Its molecular formula is $C_{14}H_{10}Cl_2NNaO_2$, molecular weight is 318.14. And it has the following chemical structure.

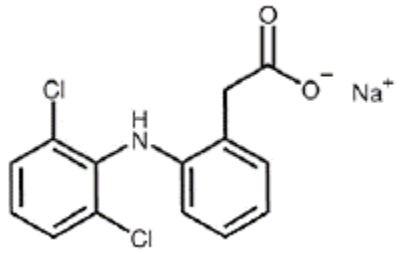

Figure 1. 9*: chemical structure of diclofenac sodium.*

Diclofenac Sodium appear as a hygroscopic crystalline powder white to off-white in color. .Sparingly soluble in water; soluble in alcohol; practically insoluble in chloroform and in ether; freely soluble in methyl alcohol. pH of a 1% solution in water is between 7.0 and 8.5. (USP 31) (Diclofenac Sodium).

1.8.2 Solubility of diclofenac sodium in other solvent

Solubility of a solid in a liquid, one of thermodynamic behavior leading to know the appropriate drug design and appropriate production optimization process. The substance Benzeneacetic acid, 2[(2, 6-dichlorophenyl) amino]-, monosodium salt. Considered to be a salt of weak acid with a pKa of 4 and the partition coefficient (n-octanol/aqueous buffer, pH 7.4) of 13.4 [42]. There is some interaction which may occur with solvents due to the presence of heteroatoms N, O, CL, and Na in diclofenac sodium structure, leading to high polarizability of the molecule affecting the solubility of the drug in different solvent [43].

1.8.3 Pharmacokinetic properties:

Absorption

The main route of administration of Diclofenac sodium is by oral route, it has also been administered topically, intravenously, intramuscularly, intracolonically and rectally. After administration it undergoes first-pass metabolism; 50–60% only of a dose reaches bloodstream as unchanged drug, it is (about 99 %) plasma proteins binding

After application of topical gel, cream, or solution drug absorbed in to bloodstream, the level of plasma concentration is mainly less than level of plasma concentration with oral administration, as after application of 1.5g of cutaneous spray (Voltarol Active 4%), observation of rapid onset of action lead to measurable plasma concentration of

about 1 ng/ml as early as 30 minutes and to maximum levels of about 3 ng/ml at about 24 hours after application [10,44].

The finally systemic concentrations of diclofenac are about 50 times less than oral administration of the same amounts of diclofenac. Systemic plasma levels are not supposed to contribute to the efficacy of Voltarol Active 4% cutaneous spray [10,44].

1.9 DIFFUSION THEORY

1.9.1 Franz- type diffusion cell

The most effective and common method for evaluation of in vitro skin penetration and formulation development is using Franz cell chamber. The only variables are the testing material and the skin, so it can be controlled well. We should make sure that depletion of the donor phase must be negligible, membrane must be a homogeneous slab, and finally receptor phase must be a perfect sink[45].

Mainly the construction materials of the diffusion cell are glass, but also stainless steel and Teflon are used. The membrane mounted as a barrier between a donor compartment and a receptor compartment, the amount of compound of testing material permeating from the donor to the receptor compartment is determined as a function of time. Agitation of the receptor phase must be continues and sampling taken from the

sampling port and accurate replenishment after sampling should be done. We must be sure that there is no air bubbles introduced below the membrane during sampling[14].

The permeation experiment:

- Duration: 24 h mostly to 48 h also 120 h can be used.
- Sampling Intervals: samples should ideally be taken every 30 minutes of the duration of the experiment.
- Temperature: Because the in vivo value of the skin is 32ºC, experiments of in vitro skin diffusion experiments are normally conducted at this value [1].

As shown in (Fig. 1.11) Franz diffusion cell is composed of:

- Donor Compartment.
- Receiver compartment.
- A sampling port.
- Water Jacket.

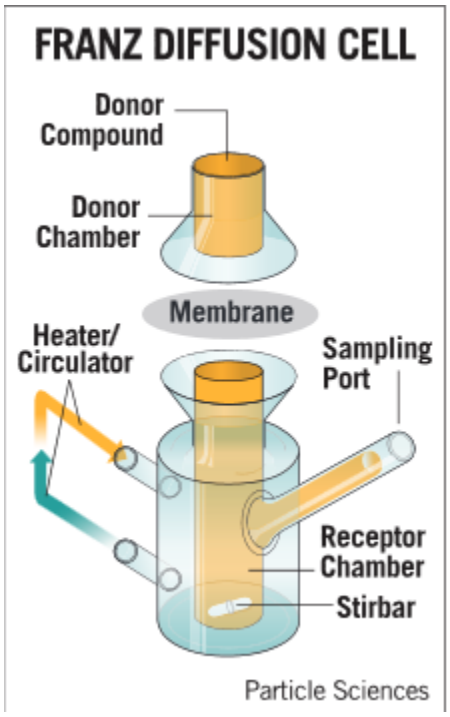

Figure 1. 10: *Franz Diffusion Cell* [1].

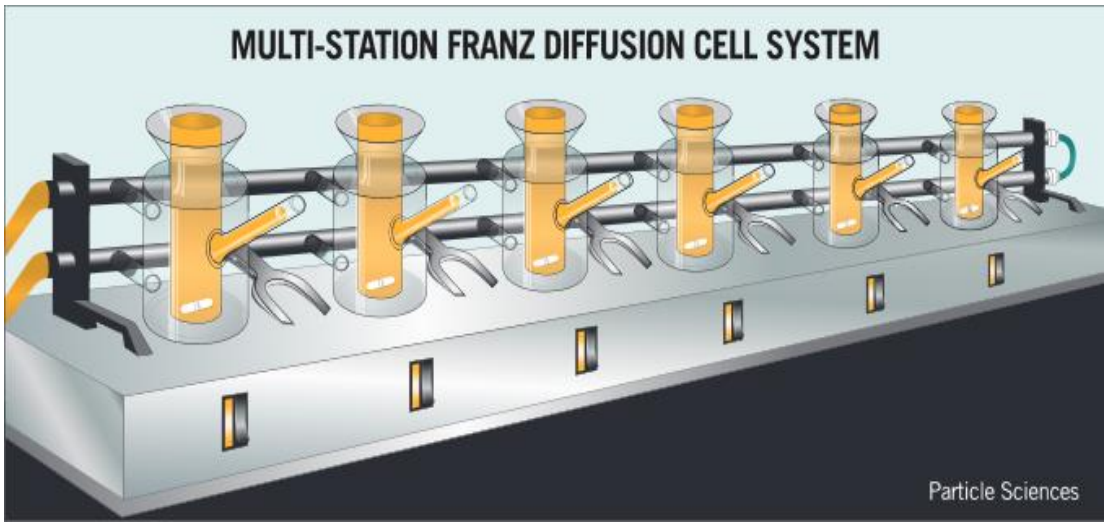

Figure 1. 11: *Multi-station Franz Diffusion cell System* [1].

The donor and receiver compartments are separated by a specific membrane. Several synthetic membrane can be used in Franz Diffusion Cell such as: RC-Regenerated cellulose, CE-Cellulose esters, CN-Cellulose nitrate, PAN-Polycarylonitrile, PA-Polyamide (nylon), PES-Polyethersulfone, PS-Polysulfone, PC-Polycarbonate, PP-Ppolypropylene and PDMS-Polydimethylsiloxane. Synthetic membranes plays two important roles: quality control, as they reduce the diffusion resistance to drug and control the separation of the product from the receptor compartment, and to simulate the skin with providing a limiting barrier properties same as in skin. Synthetic membranes must be a continuous medium of the receptor compartment [46].

1.9.2 Principles of diffusion through membrane:

Simple diffusion laws can be used to describe the percutaneous absorption process. Diffusion can be defined as transfer of individual molecules of a substance, which brought by random molecular motion and associated with concentration difference; the flow of a molecule through a membrane from the higher concentration to the lower one[47].

Fick's first law:

Flux, J is the flowing of the amount M of material through S a unit cross section, of a barrier in t unit time,

$$J = \frac{dM}{S.dt} \quad \text{.....} \quad \textbf{(1)}$$

In turn, the flux is proportional to concentration difference, dc/dx:

$$\boldsymbol{J = -D\,\frac{dC}{dx}} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (2)$$

D: diffusion coefficient of a penetrant in cm^2 /sec.

C: Concentration g/cm^3.

x : Distance in cm.

t: in seconds.

S in cm^2, diffusion is in the direction of decreasing the concentration, and this can indicates the negative sign[25].

Fick's second law represents the change of diffusion concentration with time at specific point in the system. Equation no.3 explains the mass transportation, i.e. the alteration of concentration with time at specific site. Instead of the mass diffusing across unit area of barrier in unit time.

$$\boldsymbol{\frac{\partial c}{\partial t} = -\frac{\partial J}{\partial x}} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (3)$$

Due to the concentration changes that are caused by variances in the output and input, alteration of penetrant concentration in the volume element occurs with time as the flux or amount diffusing differ with distance X.

Differentiating the 1st law expression from equation (2), with respect to X we get:

$$-\frac{\partial J}{\partial x} = D\ \frac{\partial^2 c}{\partial x^2} \quad \text{…………………………………………………………..} \quad (4)$$

Substituting from equation (3) in to equation (4) gives in Fick's 2nd law.

$$\frac{\partial C}{\partial t} = D\frac{\partial^2 C}{\partial x^2} \quad \text{………………………………………………………......} \quad (5)$$

In diffusion process, steady state is an important condition, equation (2) of Fick's 1st law gives the flux/ area in steady state conditions of the flow. The second low explains the change in concentration of diffusion with time at any distance, X. Fick's then may be written as follows [48] :

$$J = \frac{dM}{S.dt} = D\ \left(\frac{C1 - C2}{h}\right) \quad \text{……………………………………………...........} \quad (6)$$

Where, S is the area of the membrane, h is the membrane thickness, C1and C2 represents the concentrations within the membrane boundaries, they are not recognized but they can be substituted by the partition coefficient K multiplied by the concentration of permeant in the donor phase Cd, or in the receiver Cr as follows,

$$K = \frac{C1}{Cd} = \frac{C2}{Cr} \quad \text{…………………………………………………………….} \quad (7)$$

So, from equation (6):

$$\frac{dM}{dt} = \frac{DSK(Cd - Cr)}{h} \quad \text{…………………………………………………………} \quad (8)$$

Cr=0, if sink conditions hold in the receptor phase. Resulting in the following equation:

$$\frac{dM}{dt} = \frac{DSK\,Cd}{h} = PSCd \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (9)$$

Where P is the permeability coefficient given by the next equation:

$$P = \frac{DK}{h}\ cm/hr. \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (10)$$

We measure the cumulative amount of diffusant, m, that passes per unit area through the membrane as a function of time and we obtain the plot shown in (Fig. 1.12).

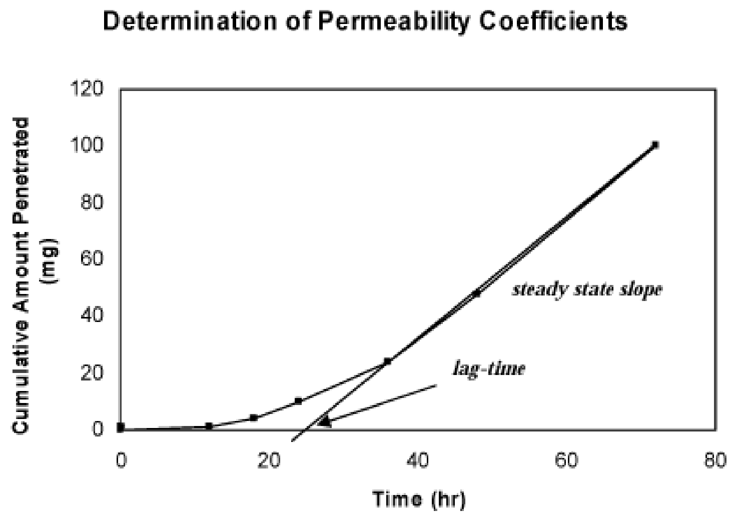

Figure 1. 12: *Determination of steady state flux and lag-time.*

After prolonged times the plot has a straight line and a steady state flow is obtained. Intercept with x axis gives the lag time, TL which can be expressed by the following equation:

$$TL = h^2/6D \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (11)$$

11 PART 2: OBJECTIVE AND SIGNIFICANCE OF THE STUDY

2.1 SIGNIFICANCE OF THE STUDY

Nonsteroidal Anti-inflammatory Drugs are drugs category having excellent analgesic and anti-inflammatory activity but they produce serious adverse drug reaction such as: gastro-intestinal tract ulceration, liver and kidney problems which increased with oral administration. Nowadays, due to these adverse drug reaction caused by oral formulations, diclofenac sodium is increasingly administered by topical route.

Permeation of drugs across skin still faces serious difficulties due to the nature of the skin tissues. As the stratum corneum (SC) is the outermost layer it is considered to be the rate limiting step for absorption and facilitating the drug flux via the skin, it is comprises keratin-rich cell embedded in multiple lipid layer.

Drug permeation through SC is limited due to the hydrophobicity and densely packed structure of this layer. The usage of selected penetration enhancers have been proposed to increase and facilitate the flux of drug through the skin tissues [20].

In this study a topical diclofenac sodium spray was prepared and the effects of different penetration enhancers on drug permeation rate was investigated.

2.2 OBJECTIVE:

1. Preparation of topical diclofenac sodium spray.
2. Investigation of the effects of different penetration enhancers on drug permeation rate through a synthetic membrane using a modified Franz- type diffusion cell.
3. Development and evaluation of analytical method to determine the content of diclofenac sodium in topical dosage form and solutions.
4. Comparison of these formulation with the RLD

To complete the project, the following steps were carry out:

1. Developing and evaluation of the spray solution analysis method.
2. Solubilization of diclofenac sodium in different solvents, solvent mixtures and buffers to select the appropriate ones in the preparation of spray solution/ or in the diffusion studies.
3. Preparation of different spray solutions containing different penetration enhancers in different concentrations.
4. Select the most acceptable formula.
5. Determination of permeability of diclofenac through a synthetic membrane using Franz- type diffusion cell.
6. Investigation of the effect of the penetration enhancers on drug penetration from the spray solution and comparing it to control formulation.

7. Collection of samples and data analysis to determine the amount of the drug that penetrate the synthetic membrane.

PART 3: RESEARCH METHODOLOGY

3.1MATERIALS AND REAGENTS.

All reagents used in this study were of analytical grade, and all materials were of pharmaceutical grade. These materials and reagents were supplied from Birzeit University laboratories, Diclofenac Sodium gifted by Jerusalem Pharmaceutical Co, Ltd. Ramallah Al Bireh- Palestine (table 3.1).

Table 3. 1: *The reagents and materials used in the study of diclofenac sodium spray.*

Purpose	No.	Item	Description	Function
Formulation	1.	Diclofenac Sodium	USP	API
	2.	Isopropyl Alcohol.	USP	Solvent
	3.	Propylene Glycol.	USP	Solvent.
	4.	Purified water.	USP	Vehicle.
	5.	Sodium metabisulfite.	USP	Antioxidant.
	6.	Polyvinyl pyrrolidone PVP k 30.	USP	Wetting agent.
	7.	Tween 80.	USP	PE

	8.	Oleic Acid.	USP	PE
	9.	Limonene.	USP	PE
	10.	L-Menthol.	USP	PE
	11.	Eucalyptol.	USP	PE
Packaging Material.	1.	Amber glass Bottle		Primary packaging material.
Analytical	1.	Potassium Phosphate monobasic.	USP	Buffering agent.
	2.	Sodium Hydroxide pellets.	USP	Buffering agent.
	3.	Ortho-phosphoric acid 85%.	Merk.	Mobile phase.
	4.	Acetonitrile	USP	Mobile phase.
	5.	PARAFILM.		
	6.	1-Octanol 99%.	USP	Lipid layer simulator

3.2 EQUIPMENT AND TOOLS

Vials, pipettes, glassware, syringes, tubes and stands were supplied by Birzeit University laboratories (table 3.2).

Table 3. 2: *The equipment and tools used in the study of diclofenac sodium spray*.

Equipment.	Source/ Model.
HPLC.	Agilent Technologies.
U.V. Spectrophotometer.	PerkinElmer, Lambda 25.
Diffusion Cell Apparatus.	Orchid Scintific, Model No. : FDC-06.
pH meter.	HANNA instruments. pH/ ORP meter.
Hot Plate and magnetic stirrer.	Thermo scientific
Magnetic stirrer bar.	Large, Small, mini.
Bath Sonicator.	Elma, S 300 H, Elmasonic.
Centrifuge.	Centurion Scientific, Model: K2015R.
Water Bath shaker.	Mrc.
Brookfield Digital Viscometer.	Brookfield Ametek, Model: DV1MLVTJ0.
Refrigerator	
Vacuum filter	KNF lab, Laboport.

Cellulose Nitrate Filter.	Sartorius Stedim Biotech, Pore size (µm): 0.45.
Polyamide Membrane Filters	Whatman, Pore size (µm): 0.45.
Dialysis tubing cellulose membrane.	SIGMA-ALDRICH.
Precision Balance	

3.3 SOLUBILITY DETERMINATION:

To determine the diclofenac sodium solubility in different pH conditions and different solvents, diclofenac sodium was added in excess amount in separated flasks, containing:

- Water.
- Phosphate buffer pH=7.4.
- Propylene glycol.
- Isopropyl Alcohol.

The flask was sealed and shaken for 24 hours at 25 C°, speed: 75 rpm. After 24 hours, a quantity of 10 ml were transferred from the content of each flask of diclofenac sodium with different solvents to plastic tubes. Each tube was covered by Para film, by using the centrifuge machine at 3000 rpm/ 15 min, then the

supernatant of each solvent was taken, about 0.5g was measured from each solvent to be diluted and to find the absorbance using the U.V.

2.4 PREPARATION OF DICLOFENAC SODIUM SPRAY SOLUTION.

3.4.1 Composition of formulation trials:

Table (3.3) Illustrate the general formula for Diclofenac sodium spray and the function of each component of it.

Table 3. 3: *General formula for Diclofenac Sodium spray.*

No.	Component	%	Function.
1	Diclofenac Sodium	4	Active ingredient.
2	Propylene Glycol	50	Solvent
3	Isopropyl Alcohol.	10	Solvent.
4	Polyvinylpyrrolidone.	1.5	Wetting agent.
5	Sodium mitabisulfite.	0.5	Antioxidant.
6	Penetration enhancers.	X	Penetration enhancer.
7	Water.	40	Solvent
8	Hydrochloric acid, diluted	*	pH adjustment

Table (3.3) illustrate type and percentage of each formula which has been used.

3.4.2 Method for preparation:

1. Place PG in a suitable beaker.
2. Add isopropyl alcohol and water to PG and stir well.
3. Add diclofenac sodium, sodium mitabisulfite, and mix well until complete dissolving.
4. Add PVP with continuous stirring.
5. Adjustment of the pH value to 6.4, as the pH plays an important role in evaluation of drug penetration from the formulation through our used membrane.

Table 3. 4: *several formulas of diclofenac sodium, with different penetration enhancers.*

Formula no.	Composition											
	Diclofenac Sodium	PG	Purified Water	IPA	Sodium Metabisulfite	PVP	Tween 80	Oleic acid	Limonene	L-Menthol	M-Pyrol	Eucalyptol
F0	4 %	50%	39.5%	10%	0.5%	-	-	-	-	-	-	-
F1	4 %	50%	38%	10%	0.5%	1.5%	-	-	-	-	-	-
F2	4 %	50%	37.9%	10%	0.5%	1.5%	0.1%	-	-	-	-	-
F3	4 %	50%	37.5%	10%	0.5%	1.5%	-	0.5%	-	-	-	-
F4	4 %	50%	37.75%	10%	0.5%	1.5%	-	-	0.25%	-	-	-
F5	4 %	50%	37%	10%	0.5%	1.5%	-	-	-	1%	-	-
F6	4 %	50%	37%	10%	0.5%	1.5%	-	-	-	-	1%	-
F7	4 %	50%	37.75%	10%	0.5%	1.5%	-	-	-	-	-	0.25%
F8	4 %	50%	36.75%	10%	0.5%	1.5%	-	-	0.25%	1%	-	-

2.5 PERMEABILITY BEHAVIOR OF DICLOFENAC SODIUM SPRAY SOLUTION WITH DIFFERENT PENETRATION ENHANCERS UNDER TEST USING FRANZ DIFFUSION CELL (FDC) THROUGH SYNTHETIC MEMBRANE.

3.5.1 Description of diffusion apparatus.

ORCHD scientific diffusion cell apparatus was used in this study. Diffusion cells are made of two separated glass compartments. Donor compartment is the upper part, receiver compartment is the lower one. The innermost of each cell is jacketed. Stainless steel clips, rubber rings are used to adjust the apparatus. The apparatus equipped with temperature controller in the range of 0 °C – 60 °C with controller accuracy ± 0.1 °C, and with circulating pump. Receptor volume of each cell is 20 ml, with 22 mm orifice/ mouth diameter.

Figure 3. 1*: Franz Diffusion Cell used in the study of diclofenac Sodium skin permeation.*

3.5.2 The receiver phase.

Phosphate buffer with pH= 7.4 was used as receiver phase in the receiver chamber to dissolve the Diclofenac Sodium that diffuse through the used membrane. In order to mimic the pH of Human blood pH= 7.4, we prepared the phosphate buffer by dissolving 13.67g of potassium phosphate monobasic and 3.249g of sodium hydroxide pellets in 2000 ml of distilled water. Before placing the receptor phase in each receiver compartment, a very important step must be kept in mind which is degassing of the receptor phase. Degassing is done by heating it up to 45°C and degas it using the sonicator. This step will help to exclude any bubbles in the receiver phase. Appearing of bubbles during the process, due to stirring and not well degassing lead them to be sticked under the separating membrane and reduce the permeable area for the permeants, thus reduce permeability, and faulty results.

3.5.3 Preparation of synthetic membrane.

Two different synthetic membranes were used in this study:

First membrane composed of two layers of dialysis membrane and one layer of Polyamide membrane filters with pore size 0.45µm. The dialysis membrane was soaked in phosphate buffer pH=7.4 for half an hour, polyamide membrane filters also soaked for half an hour before the beginning of the experiment in octanol, in order to resemble the lipophilicity nature of the intercellular lipid domains of the stratum corneum. Then the polyamide membrane filter was sandwiched in between the

dialysis membrane layers, in order to prevent the leaving of the octanol from the filter membrane and mimic the aqueous layer of Human skin [14].

This innovative use of a membrane may mimic the superficial layer of the human skin SC. It was done by Dr. Muammal Qurt in previous studies which proved the effectiveness of the membrane and the possibility of comparing the results of the permeability of the drug through this membrane with real human skin.

Second used membrane was start M-membrane (Fig.3.2) This membrane is composed of Polyethersulfone (PES) and Polyolefin (PO) and top synthetic lipid layer [49]. This membrane was soaked in phosphate buffer before the beginning of experimental trial by half an hour.

Also this membrane has been studied by several researchers and proved effective in the penetration of drugs through it and give results very close to those studied on real human skin [32,47,49].

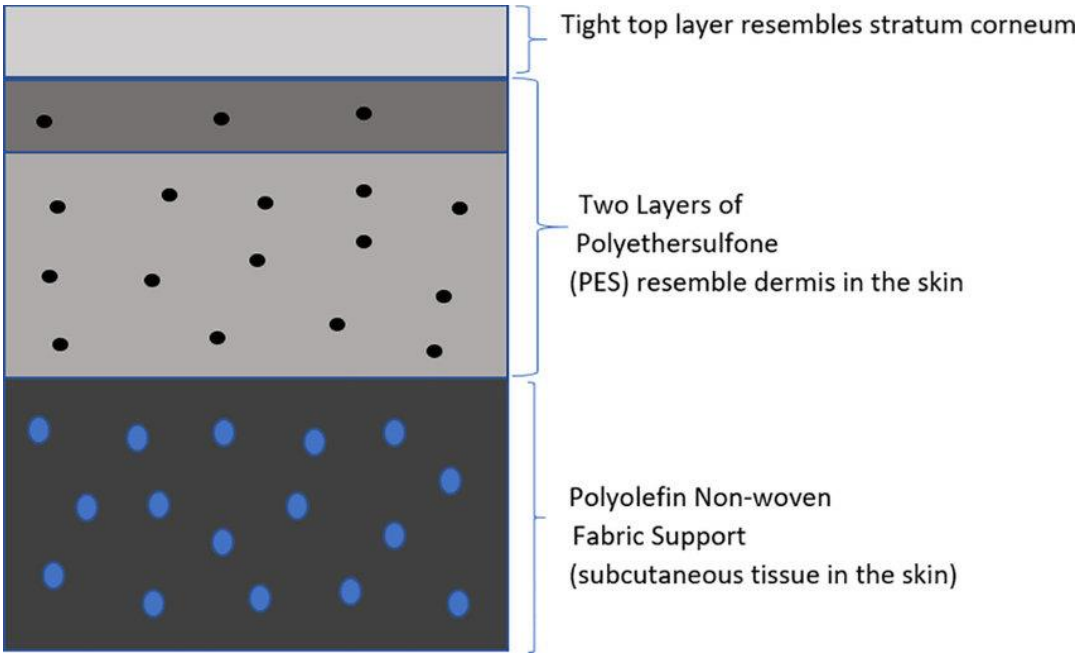

Figure 3. 2*: structure of Start M®-membrane27.*

3.5.4 Diffusion procedure:

Each cell of FDC apparatus is supported by a water jacket with inlet and outlet orifice that is connected with rubber tubes, and the receiver chamber is stable in place using a holder and a stainless steel clips. The water bath consist of water pump which helps to pump and circulate the water from the water bath through the rubber tubes reaching the water jacket of FDC and circulate back to the water bath remaining a stable temperature of 32 ±1 °C.

A magnetic bar is introduced to each receiver chamber of FDCs and the receiver chamber filled to top with phosphate buffer pH= 7.4. Previously prepared membrane is mounted on the top of receiver chamber of FDC, making sure that no air bubbles stick under it. In order to prevent any leakage a rubber ring is mounted above the placed membrane, then the donor chamber is mounted over the rubber ring covered by a layer of parafilm and closed tightly using stainless steel clips.

Now the donor and receiver chambers are totally separated by the membrane, adequate amount (2ml) of solution to be tested is introduced in the donor chamber. Tightly the orifice of each of donor chambers and sampling ports are covered using parafilm to prevent any evaporation of contents with time. Magnetic induction is activated to maximum, speed of stirrer reaches 750 rpm and the water pump is on (zero time of experiment) (Fig. 3.3). From sampling port and before taking samples from the receiver phase about 1 ml is pulled from the receiver chamber and return it, this process is done three time in order to be sure that the concentration of the taken sample in the ports

area is the same as in the receiver chamber. After every sample is taken from the cell an equal amount (1 ml) of phosphate buffer pH= 7.4 is introduced to receiver chamber of FDC to be sure that the volume is not affected using HPLC. And every experiment was done in triplicate. The cumulative amount of diclofenac sodium is calculated according to the following equation:

Cumulative amount of penetrant at time

$$(t) = Ct \times V + \sum_{t=0}^{t-0.5} Ct \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (12)$$

Ct: is the measured concentration of the penetrant at time t in the receiver chamber in mg/ml.

V: is the volume of the solution in the receiver chamber.

The thickness of the membranes polyamide filter membrane with dialysis membrane and Start M-membrane (h) equals 0.032, 4.7 cm respectively, area of membrane (S) equals 4 cm^2 and the volume of the receiver is 20 ml.

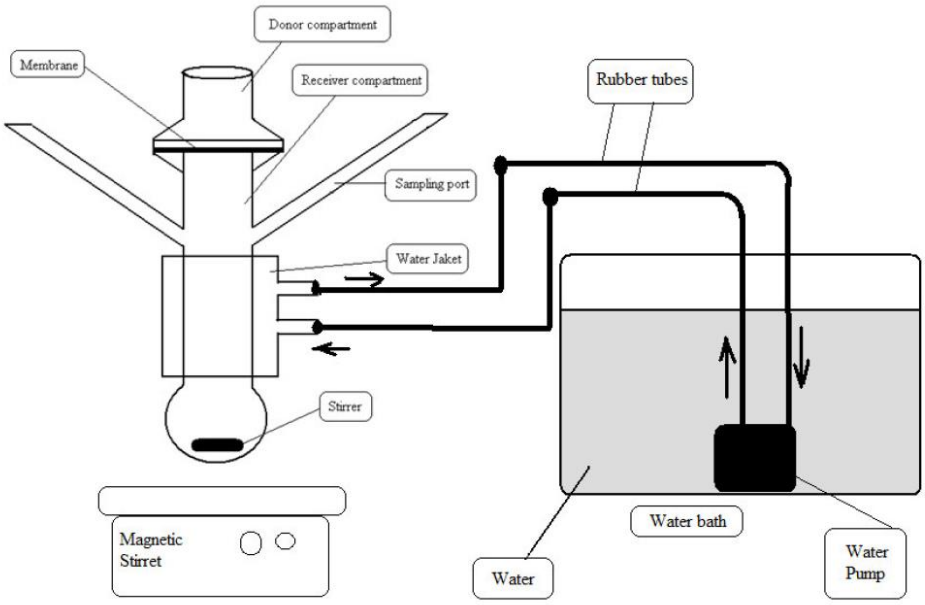

Figure 3. 3: *Franz Diffusion cell attached to water bath and water pump and including a magnetic stirrer* [14].

3.5.5 Calculation of diffusion parameters.

At every sampling time a sample was withdrawn and the amount of diclofenac sodium was determined by HPLC analysis. A cumulative amount of diclofenac sodium through time is then drawn as flux per time, and the diffusion parameters will be calculated. The curve was then extrapolated using Excel 2007 to find the steady state line. The x intercept of the line will be the lag time.

According to equation (8):

$$\frac{dM}{dt} = \frac{DSK(Cd - Cr)}{h} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (13)$$

The slope = PSCd.

Where S is the area, P is the permeability coefficient; Cd is the concentration in the donor compartment. The permeability coefficient can be calculated as the slope. The area of membrane and concentration in donor compartment are known.

According to equation (11):

$$TL = h^2/6D \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (14)$$

Where h is thickness of membrane that was measured during the experiment, TL was calculated from the plot so D the diffusion coefficient is calculated.

According to equation no. (9):

The permeability coefficient

$$P = \frac{DK}{h}$$

Where h is thickness of membrane that was measured during the experiment, P is the permeability coefficient that was calculated previously, and thus the partition coefficient K is calculated [14,44,45].

Table (3.5) illustrate the diffusion parameters and their method of calculation.

Table 3. 5: *Diffusion Parameter and their method of calculation.*

Slop	Lag time TL	Diffusion coefficient	Permeability coefficient	Partition coefficient.	Enhancement Ratio.
Calculated from the plot	Intercept with x axes.	$\frac{h^2}{6\,Tl}$	$slop/_{Cd}$	$\frac{P.h}{D}$	Permeability with enhancer/ permeability without enhancer, or Brand.

3.5.6 Selecting the best penetration enhancer.

In order to study the effect of penetration enhancers on the permeability of diclofenac sodium through a synthetic membrane, different PE were mixed with solution of the API and investigated for permeability using different synthetic membranes and FDC. The following penetration enhancers were used:

1. Tween 80.
2. Oleic acid.
3. Limonene.
4. L-menthol.
5. N-Methyl-2-pyrrolidone (M-pyrol).
6. Eucalyptol.

The diffusion parameters are calculated and the permeability coefficient of diclofenac sodium with PE is compared to the permeability coefficient of the diclofenac sodium without PE in order to calculate the enhancement ratio. Selection of the best penetration enhancer was according to the best permeability coefficient value.

3.5.7 Formulation of Diclofenac Sodium spray solution with the best penetration enhancer:

Table 3. 6: *The composition of Diclofenac sodium with the selected penetration enhancer.*

No.	Component	%	Function.
1	Diclofenac Sodium	4	Active ingredient.
2	Propylene Glycol	50	Solvent
3	Isopropyl Alcohol.	10	Solvent.
4	Polyvinylpyrrolidone.	1.5	Wetting agent.
5	Sodium mitabisulfite.	0.5	Antioxidant.
6	Limonene	0.25	Penetration enhancer.
7	Water.	37.75	Solvent
8	Hydrochloric acid, diluted	*	pH adjustment

PART 4: RESULTS AND DISCUSSION.

4.1 SOLUBILITY DETERMINING RESULTS.

Saturated solutions of diclofenac sodium were prepared at different pHs, their concentrations were determined by HPLC and measuring the responses at 25°C. Results are shown in table (4.1).

Table 4. 1: *The solubility results of diclofenac Sodium in different solutions at 25 °C.*

Diclofenac Sodium in	**Solubility mg / ml**	**SD**
Distilled water	21.44	0.1
Phosphate buffer pH 7.4	12.96	0.0
Isopropyl Alcohol	7.32	0.1
Propylene Glycol	599.32	0.9

The solubility of diclofenac Sodium depends on the pH, being practically insoluble under acidic conditions, but the solubility increases with pH increase, beginning from a pH of 6.

4.2 DETERMINATION OF DICLOFENAC SODIUM IN SPRAY SOLUTION.

***Table 4. 2**: List of Diclofenac sodium formulation in spray solution.*

Formula no.	Composition					
	Diclofenac sodium	PG	Purified water	IPA	Sodium Metabisulfite	PVP
Fa	4%	50%	39.5%	10%	0.5%	-
Fb	4%	60%	29.5%	10%	0.5%	-
Fc	4%	40%	49.5%	10%	0.5%	-
Fd	4%	50%	39%	10%	0.5%	0.5%
Fe	4%	50%	38.5%	10%	0.5%	1%
F1	4%	50%	38%	10%	0.5%	1.5%

As shown in table (4.2) several formulations were developed using different quantities of PG, purified water, and IPA in order to develop a stable, colorless, transparent and clear solution of diclofenac sodium. Later the best formula which is formula F1 according to the results in table (4.3), will be used as a control formulation for comparison.

Formula of 4% diclofenac sodium containing 49.5% of purified water (Fc) was immediately excluded from the study, because it became turbid and exhibited physical instability. Formulas Fa, Fc were tested at zero time and after 1 week, and 1 month Fa represented more stable results so it was chosen for further study.

Table 4. 3: *the assay content of 4% diclofenac sodium of Fa and Fc formulas.*

	Fa (zero time)	Fa (1 week)	Fa (1 month)
Area 1	8464.5	8136.6	8454.9
Area 2	8451.5	8128.9	8404.6
Area 3	8470.9	8126.4	8318.1
Avg.	8462.3	8130.6	8392.5
STDEV	9.9	5.3	69.2
% RSD	0.1	0.1	0.8
Assay %	**100.6 %**	**98.7%**	**98.1%**
	Fc (zero time)	Fc (1 week)	Fc (1 week)
Area 1	8136.6	8464.5	8254.9
Area 2	8128.9	8451.5	8204.6
Area 3	8126.4	8470.9	8218.1

Avg.	8130.6	8462.3	8225.9
STDEV	5.3	9.9	26.0
% RSD	0.1	0.1	0.3
Assay %	**99.7%**	**98.4%**	**96.2%**

Polyvinylpyrrolidone (PVP) is a synthetic polymer, used widely in different pharmaceutical formulations, in this study addition of PVP was for viscosity increasing of the solution in order to develop a gel-like consistency after being sprayed onto the skin. Table (4.4) shows that with the increase in the percentage of PVP the viscosity of the solution increases. Formula (F1) achieves the effect on the solution that it does not spill after spraying on the skin.

Table 4. 4: *viscosity of 4% diclofenac Sodium solution with different quantities of PVP using viscometer at 100 rpm.*

composition	Viscosity (cp)			Avg.	STDEV	Temperature (°C)
Without PVP	13.02	13.98	13.86	13.62	0.5	21.3
PVP 0.5%	13.99	13.62	13.82	13.62	0.1	20.9
PVP 1%	15.84	16.08	16.20	16.04	0.1	20.5
PVP 1.5%	16.68	17.28	17.52	17.16	0.4	20.6

4.3 DETERMINATION OF BEST DICLOFENAC SODIUM FORMULA.

The lag time (TL) reflects the time required by the Active Pharmaceutical Ingredient API to pass through the intact membrane and reach the receiver compartment. Diffusion coefficient (D) measures the membrane resistance encountered by the diffusant. Permeability coefficient (P) gives an indication about the distance passed by the substance within specific period. The partition coefficient (K) gives an indication about the ability of API to partition between the oily phase and the aqueous phase, this parameter includes other diffusion parameters as previously shown in the calculation of diffusion parameter (part 3). Later on in this thesis we will made some attempts to compare the enhancement ratio (ER) of various penetration enhancers (P after / P before) another comparison will be done with the permeability coefficient of the Brand (P after/ P Brand). The greater the ER the greater the penetration enhancement ability of penetration enhancer used.

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Table 4. 5: *a list of Diclofenac sodium spray formulation to be studied for permeability*.

Formula no.	Composition											
	Diclofenac Sodium	PG	Purified Water	IPA	Sodium Metabisulfite	PVP	Tween 80	Oleic acid	Limonene	L-Menthol	M-Pyrol	Eucalyptol
F1	4 %	50%	38%	10%	0.5%	1.5%	-	-	-	-	-	-
F2	4 %	50%	37.9%	10%	0.5%	1.5%	0.1%	-	-	-	-	-
F3	4 %	50%	37.5%	10%	0.5%	1.5%	-	0.5%	-	-	-	-
F4	4 %	50%	37.75%	10%	0.5%	1.5%	-	-	0.25%	-	-	-
F5	4 %	50%	37%	10%	0.5%	1.5%	-	-	-	1%	-	-
F6	4 %	50%	37%	10%	0.5%	1.5%	-	-	-	-	1%	-
F7	4 %	50%	37.75%	10%	0.5%	1.5%	-	-	-	-	-	0.25%
F8	4 %	50%	36.75%	10%	0.5%	1.5%	-	-	0.25%	1%	-	-

4.3.1 Formula no. F1, 4% diclofenac sodium solution without penetration enhancers.

The basic diclofenac sodium was prepared without using penetration enhancer as a control formulation to constitute a base for comparison. Samples were taken every half hour from the sampling port of the receiver compartment and analyzed by HPLC for the amount of diclofenac sodium.

Tables (4.6), (4.7) illustrate the assay results of API penetrated to the receiving compartment by time. Area under the peak (Area1, Area2 Area3) was presented in triplicates, and the cumulative amount of drug penetrated (Q) per unit of the membrane area was determined and plotted as a function of time (Fig. 4.1). The linear part of the curve was plotted in (Fig. 4.2) and the diffusion parameter were calculated for API alone and tabulated in table (4.7)

The TL was calculated by dividing the intercept of the equation of flux profile on the slop from the same equation.

The diffusion parameter (D) was calculating using equation 11:

$$\boldsymbol{TL = h^2/6D} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (15)$$

The permeability coefficient (P) was calculated by dividing the value of the slop of the flux profile by the concentration of API in the donor compartment (4mg/ml). The permeability coefficient (P) was used here as the main value in the comparison between

the activity of different penetration enhancers, since its value was obtained from all diffusion parameters as shown in equation 12.

$$\boldsymbol{K = (P.h)/D} \quad \text{.....} \quad (16)$$

Table 4. 6: *Data obtained from the diffusion of formula no.F1, through poly amide membrane using Franz diffusion cell, without addition of penetration enhancers (part 1).*

Time (HR)	Avg for 3 SA Area 1	Avg for 3 SA Area 2	Avg for 3 SA Area 3	Concentration of sample 1 (mg/ml)	Concentration of sample 2 (mg/ml)	Concentration of sample 3 (mg/ml)	Q: cumulative amount released 1 [mg]	Q: cumulative amount released 2 [mg]	Q: cumulative amount released 3 [mg]
0.5	789.2	788.8	789.7	0.03267	0.03265	0.03269	0.6534	0.6530	0.6539
1.0	1507.5	1504.2	1505.2	0.06687	0.06672	0.06677	1.3375	1.3344	1.3354
1.5	2245.0	2245.6	2245.0	0.10200	0.10202	0.10200	2.0400	2.0405	2.0400
2.0	2986.8	2985.3	2984.8	0.13732	0.13725	0.13723	2.7465	2.7450	2.7446
2.5	3700.6	3704.7	3703.8	0.17131	0.17151	0.17147	3.4263	3.4302	3.4294
3.0	4412.2	4408.6	4422.6	0.20520	0.20503	0.20570	4.1041	4.1006	4.1140
3.5	5202.2	5197.5	5194.2	0.24282	0.24260	0.24244	4.8565	4.8520	4.8489
4.0	5676.6	5677.2	5672.3	0.26541	0.2654	0.2652	5.3083	5.3089	5.3042
4.5	6343.1	6345.1	6339.5	0.29715	0.29725	0.2969	5.9431	5.9450	5.9397
5.0	7056.4	7045.2	7038.6	0.33112	0.33059	0.3302	6.6225	6.6118	6.6055
5.5	7578.3	7567.5	7568.9	0.35597	0.35546	0.3555	7.1195	7.1093	7.1106
6.0	8281.1	8284.9	8284.0	0.38944	0.38962	0.3895	7.7889	7.7925	7.7917

Table 4. 7: *Data obtained from the diffusion of formula no.F1, through poly amide membrane using Franz diffusion cell, without addition of penetration enhancers (part 2).*

Time (HR)	Q: cumulative amount released 1 [mg]	Q: cumulative amount released 2 [mg]	Q: cumulative amount released 3 [mg]	m: cumulative amount released 1 cm^2[mg/cm^2]	m: cumulative amount released 2 cm^2[mg/cm^2]	m: cumulative amount released 3 cm^2[mg/cm^2]	Mean	SD	%RSD
0.5	0.653469213	0.653088242	0.653945426	0.208044958	0.207923668	0.20819657	0.2081	0.0001	0.07
1.0	1.370270489	1.367108434	1.368103719	0.436252941	0.435246238	0.435563107	0.4357	0.001	0.12
1.5	2.139564741	2.139959998	2.139479023	0.681173111	0.681298949	0.681145821	0.6812	0.000	0.01
2.0	2.948075146	2.946498881	2.946084575	0.938578525	0.938076689	0.937944787	0.9382	0.000	0.04
2.5	3.765243107	3.768928997	3.76810991	1.198740244	1.199913721	1.199652948	1.1994	0.001	0.05
3.0	4.614307824	4.610855279	4.624184485	1.469056932	1.467957746	1.472201364	1.4697	0.002	0.15
3.5	5.571930092	5.567258441	5.56477737	1.773935082	1.772447769	1.77165787	1.7727	0.001	0.07
4.0	6.266587457	6.266739845	6.262577742	1.995093109	1.995141625	1.993816537	1.9947	0.001	0.04
4.5	7.166797467	7.168311824	7.163249679	2.281692922	2.282175048	2.280563412	2.2815	0.001	0.04
5.0	8.143320634	8.132358208	8.126076956	2.59258855	2.589098442	2.587098681	2.5896	0.003	0.11
5.5	8.971517691	8.960402876	8.961426735	2.856261602	2.852722979	2.853048944	2.8540	0.002	0.07
6.0	9.996862232	9.99913853	9.998038478	3.182700488	3.183425193	3.183074969	3.1831	0.000	0.01

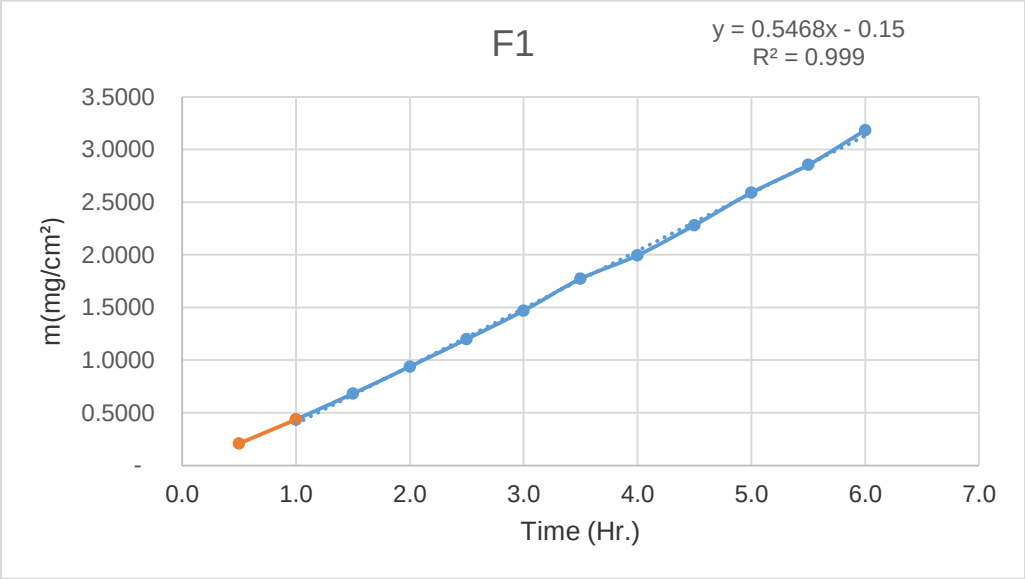


Figure 4. 1*: In vitro permeation profile for the cumulative amount of DS penetrated per unit area of polyamide membrane (mg/cm²) for formula no.F1, without addition of penetration enhancers.*

The best linear line is determined of (Fig.4.1) by Excel 2007, from which the linear line equation is determined. The equation helps in determining the slop and the x intercept, these are used for further calculation of diffusion parameters. The diffusion parameters are calculated according to table (3.3). The diffusion parameter shown in table (4.8).

Table 4. 8: *Diffusion parameters for formula no. F1, without addition of PE.*

Formulation	Slop	Intercept	T_L	D	P	K	ER
F1	0.5468	0.15	0.2743233	0.06221	0.1367	0.703124	1

The permeability parameter of this basic diclofenac sodium solution formula F1 was used as a reference for penetration enhancement comparison.

4.3.2 Formula no.F2, 4% diclofenac sodium with 0.1% of tween 80 as penetration enhancer.

Tween 80, or polysorbate 80 is a nonionic surfactant, as previously discussed in section (1.7.3.7) several surfactants may be used as penetration enhancers in topical pharmaceutical formulation.

Basic formulation of 4% diclofenac sodium and 0.1% of tween 80 was prepared according to general method described in section (3.4.2). The permeability results of drug through the polyamide membrane are shown in tables (4.9), (4.10) and (Fig.4.2).

The cumulative amount of API permeated through unit area of membrane was then calculated as mentioned before, the linear section, i.e. the steady state flux was plotted versus time (Fig.4.2).

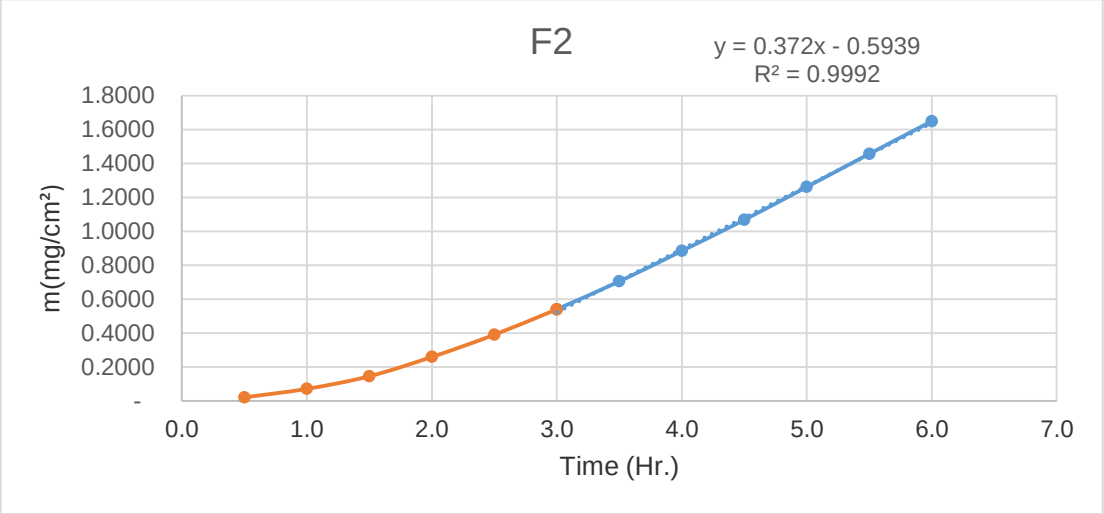
Table 4. 9: *Data obtained from the diffusion of formula no. F2, through poly amide membrane using Franz diffusion cell, with 0.1% of tween 80 as PE (part 1).*

Time (HR)	Avg for 3 SA Area 1	Avg for 3 SA Area 2	Avg for 3 SA Area 3	Concentration of sample 1 (mg/ml)	Concentration of sample 2 (mg/ml)	Concentration of sample 3 (mg/ml)	Q: cumulative amount released 1 [mg]	Q: cumulative amount released 2 [mg]	Q: cumulative amount released 3 [mg]
0.5	171.2	171.2	171.9	0.003243488	0.003243488	0.003276823	0.064869756	0.064869756	0.065536454
1.0	333.4	333.2	333.6	0.010967665	0.010958141	0.010977189	0.219353303	0.219162817	0.219543788
1.5	565.8	567.0	567.9	0.022034859	0.022092004	0.022134864	0.440697176	0.441840088	0.442697271
2.0	918.7	921.2	918.2	0.038840421	0.038959474	0.03881661	0.776808419	0.779189485	0.776332206
2.5	1308.4	1310.0	1311.8	0.057398448	0.057474642	0.05756036	1.147968951	1.149492833	1.1512072
3.0	1739.8	1747.8	1745.3	0.077942283	0.078323253	0.0782042	1.558845659	1.56646507	1.564084004
3.5	2206.3	2204.1	2204.0	0.100157627	0.10005286	0.100048098	2.003152531	2.001057193	2.000961951
4.0	2691.8	2693.5	2692.1	0.123277775	0.123358731	0.123292062	2.465555503	2.467174627	2.465841231
4.5	3160.0	3170.7	3165.5	0.145574075	0.146083623	0.145835992	2.911481499	2.921672461	2.916719844
5.0	3654.7	3651.5	3655.8	0.16913234	0.168979951	0.169184723	3.382646793	3.379599029	3.383694462
5.5	4116.1	4126.0	4116.2	0.191104815	0.191576266	0.191109577	3.82209629	3.831525311	3.822191533
6.0	4550.4	4554.2	4557.7	0.211786752	0.211967713	0.212134387	4.235735035	4.239354255	4.242687747

Table 4. 10: *Data obtained from the diffusion of formula no. F2, through poly amide membrane using Franz diffusion cell, with 0.1% of tween 80 as PE (part 2).*

Time (HR)	Q: cumulative amount released 1 [mg]	Q: cumulative amount released 2 [mg]	Q: cumulative amount released 3 [mg]	m: cumulative amount released 1 cm^2[mg/cm^2]	m: cumulative amount released 2 cm^2[mg/cm^2]	m: cumulative amount released 3 cm^2[mg/cm^2]	Mean	SD	%RSD
0.5	0.064869756	0.064869756	0.065536454	0.020652581	0.020652581	0.020864837	0.0207	0.000	0.59
1.0	0.22259679	0.222406305	0.222820611	0.070868128	0.070807483	0.070939386	0.0709	0.000	0.09
1.5	0.454908329	0.456041716	0.456951283	0.14482914	0.145189977	0.145479555	0.1452	0.000	0.22
2.0	0.813054431	0.815483118	0.812721082	0.258852095	0.259625316	0.258745967	0.2591	0.000	0.19
2.5	1.223055384	1.22474594	1.226412686	0.389384076	0.389922299	0.390452941	0.3899	0.001	0.14
3.0	1.69133054	1.699192819	1.69684985	0.538468812	0.540971926	0.540225995	0.5399	0.001	0.24
3.5	2.213579694	2.212108196	2.211931997	0.704737247	0.704268767	0.70421267	0.7044	0.000	0.04
4.0	2.776140292	2.778278489	2.776859374	0.883839635	0.884520372	0.884068569	0.8841	0.000	0.04
4.5	3.345344064	3.356135054	3.351030049	1.065057009	1.068492536	1.066867255	1.0668	0.002	0.16
5.0	3.962083433	3.960145245	3.963840659	1.261408288	1.260791227	1.261967736	1.2614	0.001	0.05
5.5	4.57066527	4.581051479	4.571522453	1.455162455	1.458469111	1.455435356	1.4564	0.002	0.13
6.0	5.175408829	5.180456688	5.183128244	1.647694629	1.649301716	1.650152259	1.6490	0.001	0.08

Figure 4. 2: *in vitro permeation profile for the cumulative amount of diclofenac sodium penetrated per unit area of poly amide membrane (mg/cm²) for formula no. F2, with 0.1% of tween 80 as penetration enhancer.*



The diffusion parameters are calculated according to table (4.8) and the enhancement ratio is determined (see table 4.11).

Table 4. 11: *Diffusion parameters for formula no.F2, with 0.1% of tween 80 as penetration enhancer.*

Formulation	Slop	Intercept	TL	D	P	K	ER
F2	0.372	0.5939	1.5965	0.01069	0.093	2.78391	0.6803

The rate of diffusion of API in the presence of tween 80 is slower than when it was alone, this is indicated by the value of high permeability coefficient (P) from which enhancement ratio (ER) is found to be (0.6803).

4.3.3 Formula no. F3, 4% of diclofenac sodium with 0.5% of Oleic acid as penetration enhancer.

Oleic acid is used widely as a penetration enhancer in topical and transdermal formulation [50].Oleic acid is an example of fatty acid acts by disruption of the lipids packing and increase the permeation rate[38]. Using 0.5% of oleic acid as penetration enhancer and following the same general procedure, the data measured are shown in tables (4.12), (4.13), (Fig. 4.3).

Figure 4. 2: *In vitro permeation profile for the cumulative amount of diclofenac sodium penetrated per unit area of poly amide membrane (mg/cm²) for formula no. F3, with 0.5% of oleic acid as penetration enhancer.*

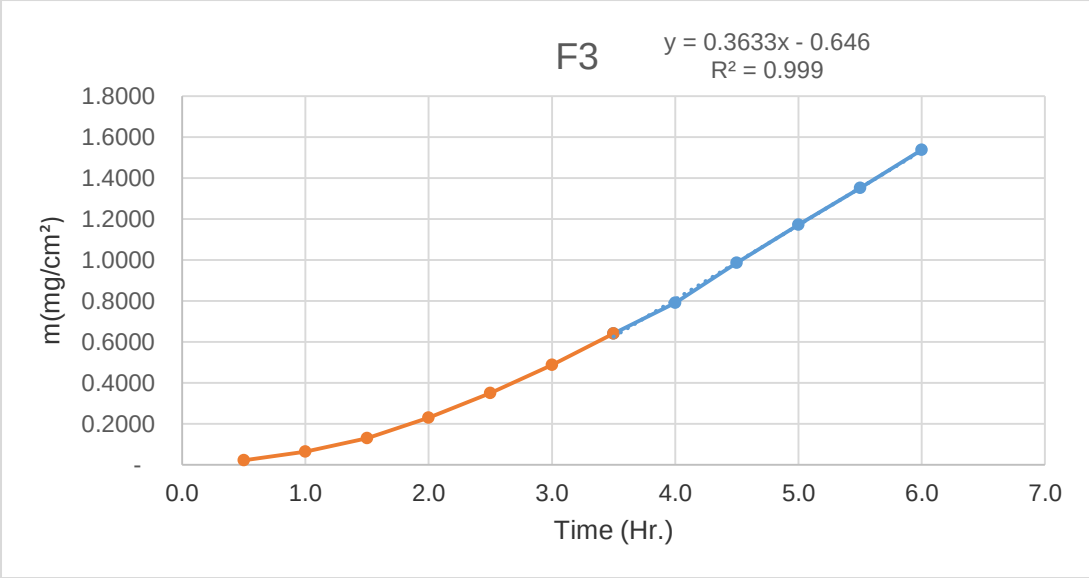


Table 4. 12: *Data obtained from the diffusion of Formula no.F3, through poly amide membrane using Franz diffusion cell, with 0.5% of Oleic acid as penetration enhancer (part 1).*

Time (HR)	Avg for 3 SA Area 1	Avg for 3 SA Area 2	Avg for 3 SA Area 3	Concentration of sample 1 (mg/ml)	Concentration of sample 2 (mg/ml)	Concentration of sample 3 (mg/ml)	Q: cumulative amount released 1 [mg]	Q: cumulative amount released 2 [mg]	Q: cumulative amount released 3 [mg]
0.5	173.0	172.8	181.0	0.003329206	0.003319682	0.003710177	0.066584123	0.066393638	0.074203534
1.0	311.5	310.9	310.7	0.009924758	0.009896186	0.009886661	0.198495166	0.197923711	0.197733225
1.5	515.7	515.3	516.8	0.019649031	0.019629982	0.019701414	0.392980618	0.392599648	0.394028287
2.0	823.6	825.8	826.2	0.034311634	0.034416401	0.034435449	0.686232678	0.688328016	0.688708986
2.5	1182.7	1187.7	1185.4	0.051412448	0.051650555	0.051541026	1.028248964	1.033011096	1.030820515
3.0	1585.6	1584.5	1584.1	0.070599076	0.070546693	0.070527644	1.411981523	1.410933854	1.410552883
3.5	2020.1	2014.1	2016.9	0.091290538	0.09100481	0.091138149	1.825810753	1.820096195	1.822762989
4.0	2410.7	2417.8	2415.7	0.109891423	0.110229535	0.11012953	2.197828468	2.204590695	2.2025906
4.5	2946.8	2941.8	2942.8	0.135421211	0.135183104	0.135230725	2.708424211	2.703662079	2.704614505
5.0	3424.9	3417.7	3415.1	0.158188961	0.157846088	0.157722272	3.163779228	3.156921758	3.15444545
5.5	3845.6	3839.1	3840.7	0.178223249	0.17791371	0.177989904	3.564464975	3.558274204	3.559798086
6.0	4275.9	4270.9	4266.7	0.198714701	0.198476594	0.198276585	3.974294014	3.969531882	3.965531692

Table 4. 13: *Data obtained from the diffusion of Formula no.F3, through poly amide membrane using Franz diffusion cell, with 0.5% of Oleic acid as penetration enhancer (part 2).*

Time (HR)	Q: cumulative amount released 1 [mg]	Q: cumulative amount released 2 [mg]	Q: cumulative amount released 3 [mg]	m: cumulative amount released 1 cm^2[mg/cm^2]	m: cumulative amount released 2 cm^2[mg/cm^2]	m: cumulative amount released 3 cm^2[mg/cm^2]	Mean	SD	%RSD
0.5	0.066584123	0.066393638	0.074203534	0.021198384	0.021137739	0.023624175	0.0220	0.001	6.45
1.0	0.201824373	0.201243393	0.201443402	0.064254815	0.064069848	0.064133525	0.0642	0.000	0.15
1.5	0.406234583	0.405815515	0.407625125	0.129332882	0.129199464	0.129775589	0.1294	0.000	0.23
2.0	0.719135673	0.721173865	0.722007238	0.228951185	0.229600085	0.229865405	0.2295	0.000	0.20
2.5	1.095463594	1.100273346	1.098554217	0.348762685	0.350293966	0.349746647	0.3496	0.001	0.22
3.0	1.5306086	1.529846659	1.529827611	0.487299777	0.487057198	0.487051134	0.4871	0.000	0.03
3.5	2.015036907	2.009555693	2.01256536	0.641527191	0.639782137	0.640740325	0.6407	0.001	0.14
4.0	2.478345159	2.485055003	2.483531121	0.789030614	0.791166827	0.790681668	0.7903	0.001	0.14
4.5	3.098832325	3.094355922	3.095684556	0.98657508	0.985149927	0.985572925	0.9858	0.001	0.07
5.0	3.689608553	3.682798705	3.680746226	1.174660475	1.172492424	1.171838977	1.1730	0.001	0.13
5.5	4.248483261	4.241997238	4.243821134	1.352589386	1.350524431	1.351105105	1.3514	0.001	0.08
6.0	4.836535549	4.831168627	4.827544645	1.539807561	1.538098894	1.536945127	1.5383	0.001	0.09

According to table (4.14) the diffusion parameter are calculated and the enhancement ratio is determined (see table 4.11).

Table 4. 14: *Diffusion parameters for formula no. F3, with 0.5% of Oleic acid as penetration enhancer.*

Formulation	Slop	Intercept	TL	D	P	K	ER
F3	0.3633	0.646	1.77814	0.00959	0.090825	3.030656	0.6644

The rate of diffusion of diclofenac sodium in the presence of 0.5% of oleic acid is slower than it was alone also, and this is indicated by the value of high permeability coefficient (P) from which enhancement ratio (ER) is found to be (0.6644), and consequently works as penetration retardant.

4.3.4 Formula no.F4, 4% of diclofenac sodium with 0.25% of Limonene as penetration enhancer.

Limonene is one example of terpenes. Terpenes shows their effectiveness to be used as topical penetration enhancers in pharmaceutical formulations as they act by interaction with the stratum corneum keratin and lipids content, and helps to increase the solubility of the permeant in to lipids domains, hence increase the skin permeability.

Using 0.25% of Limonene as potential penetration enhancer and following the same general procedure, the data measured are shown in tables (4.15),(4.16) the cumulative amount of diclofenac sodium penetrated per unit area is shown in (Fig.4.4), and the calculated diffusion parameters are illustered in table (4.17).

Table 4. 15: *Data obtained from the diffusion of formula no.F4, through poly amide membrane using Franz diffusion cell, with o.25%of limonene as penetration enhancer (part 1).*

Time (HR)	Avg for 3 SA Area 1	Avg for 3 SA Area 2	Avg for 3 SA Area 3	Concentration of sample (mg/ml) 1	Concentration of sample (mg/ml) 2	Concentration of sample (mg/ml) 3	Q: cumulative amount released [mg] 1	Q: cumulative amount released [mg] 2	Q: cumulative amount released [mg] 3
0.5	1095.0	1097.8	1093.4	0.047236059	0.047369399	0.047159865	0.944721177	0.947387971	0.943197295
1.0	1728.0	1730.8	1725.6	0.077380351	0.077513691	0.07726606	1.547607029	1.550273823	1.545321206
1.5	2515.0	2512.7	2514.3	0.114858327	0.114748798	0.114824992	2.297166532	2.294975951	2.296499833
2.0	3330.8	3333.1	3333.6	0.153707796	0.153817325	0.153841135	3.074155912	3.076346493	3.076822706
2.5	4172.9	4172.0	4171.5	0.193809705	0.193766846	0.193743035	3.876194104	3.875336921	3.874860708
3.0	5078.7	5082.5	5078.7	0.236945093	0.237126054	0.236945093	4.738901852	4.742521072	4.738901852
3.5	5654.8	5648.3	5644.0	0.264379732	0.264070194	0.263865422	5.287594647	5.281403876	5.277308443
4.0	6382.9	6389.9	6379.5	0.299052812	0.299386161	0.2988909	5.981056241	5.987723225	5.977817991
4.5	6920.4	6913.9	6913.7	0.324649269	0.32433973	0.324330206	6.49298538	6.486794609	6.486604124
5.0	7471.4	7473.2	7476.6	0.350888614	0.350974332	0.351136245	7.017772275	7.019486642	7.022724892
5.5	8069.3	8059.8	8065.2	0.379361398	0.378908996	0.379166151	7.587227963	7.578179913	7.583323015
6.0	8377.3	8372.8	8379.5	0.394028763	0.393814467	0.39413353	7.880575265	7.876289347	7.882670603

Table 4. 16: *Data obtained from the diffusion of formula no.F4, through poly amide membrane using Franz diffusion cell, with o.25%of limonene as penetration enhancer (part 2).*

Time (HR)	Q: cumulative amount released [mg] 1	Q: cumulative amount released [mg] 2	Q: cumulative amount released [mg] 3	m: cumulative amount released cm^2[mg/cm^2] 1	m: cumulative amount released cm^2[mg/cm^2] 2	m: cumulative amount released cm^2[mg/cm^2] 3	Mean	SD	%RSD
0.5	0.944721177	0.947387971	0.943197295	0.30077083	0.301619857	0.300285672	0.3009	0.001	0.22
1.0	1.594843088	1.597643221	1.592481071	0.507750108	0.508641586	0.506998112	0.5078	0.001	0.16
1.5	2.421782942	2.419859041	2.420925758	0.771022904	0.770410392	0.770750003	0.7707	0.000	0.04
2.0	3.313630649	3.31597838	3.316073623	1.05496041	1.055707857	1.05573818	1.0555	0.000	0.04
2.5	4.269376637	4.268786133	4.26795276	1.359241209	1.35905321	1.358787889	1.3590	0.000	0.02
3.0	5.32589409	5.32973713	5.32573694	1.695604613	1.696828122	1.695554581	1.6960	0.001	0.04
3.5	6.111531978	6.105745988	6.101088623	1.945728105	1.94388602	1.942403255	1.9440	0.002	0.09
4.0	7.069373303	7.07613553	7.065463594	2.250675996	2.252828886	2.249431262	2.2510	0.002	0.08
4.5	7.880355255	7.874593076	7.873140626	2.508868276	2.507033771	2.506571355	2.5075	0.001	0.05
5.0	8.729791419	8.731624839	8.7335916	2.779303221	2.779886928	2.780513085	2.7799	0.001	0.02
5.5	9.650135721	9.641292442	9.645325968	3.072313187	3.069497753	3.070781906	3.0709	0.001	0.05
6.0	10.32284442	10.31831087	10.32383971	3.28648342	3.285040074	3.286800289	3.2861	0.001	0.03

Figure 4. 3: *In vitro permeation profile for the cumulative amount of diclofenac sodium penetrated per unit area of poly amide membrane (mg/cm²) for formula no. F4, with 0.25% of Limonene as penetration enhancer.*

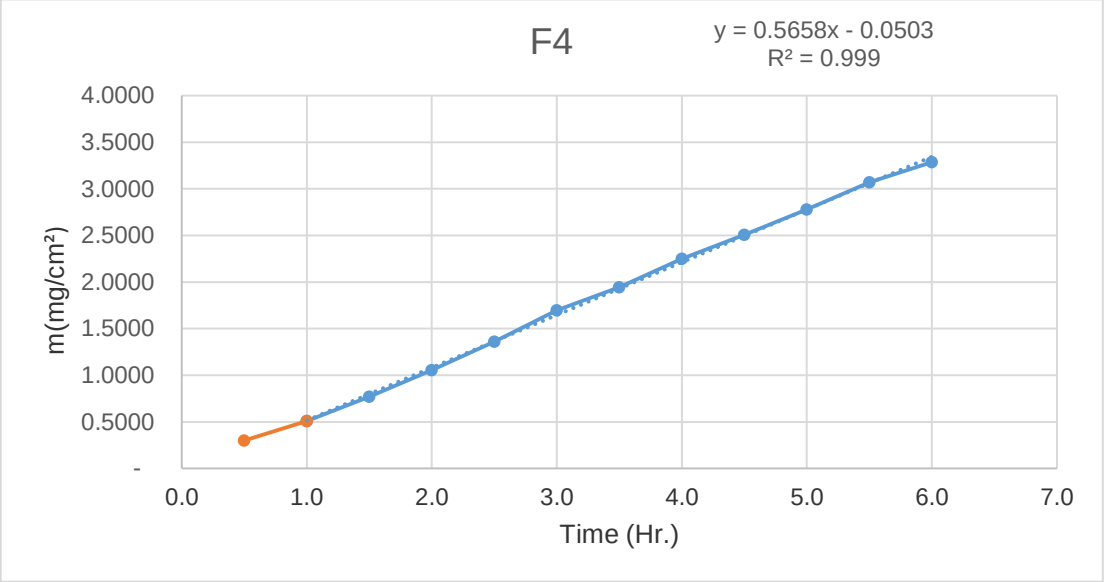


Table 4. 17: *Diffusion parameters for formula no. F4, with 0.25% of limonene as penetration enhancer.*

Formulation	Slop	Intercept	TL	D	P	K	ER
F4	0.5658	0.0503	0.088900	0.1919745	0.14145	0.23578125	1.03474

The rate of diffusion of diclofenac sodium in the presence of limonene is faster than when it is alone, this is indicated by the value of high permeability coefficient (P) from which the enhancement ratio (ER) is found to be (1.03474).

4.3.5 Formula no. F5, 4 % of diclofenac sodium with 1% of L-Menthol as penetration enhancer**.**

Another example of terpenes is L-Menthol used in transdermal pharmaceutical formulation in order to enhance the delivery of drugs through skin. The rate of diffusion of diclofenac sodium for this experiment in the presence of L- Menthol was slower than when it is alone, this is indicated by the value of high permeability coefficient (P) from which the enhancement ratio found to be (0.6280) as shown in table (4.15). And following the same general procedure, the data measured are shown in table (4.18), (4.19) and (Fig. 4.5).

Table 4. 18: *Data obtained from the diffusion of formula no. F5, through poly amide membrane using Franz diffusion cell, with 1% of L-Menthol as penetration enhancer (part 1).*

Time (HR)	Avg for 3 SA Area 1	Avg for 3 SA Area 2	Avg for 3 SA Area 3	Concentration of sample 1 (mg/ml)	Concentration of sample 2 (mg/ml)	Concentration of sample 3 (mg/ml)	Q: cumulative amount released 1 [mg]	Q: cumulative amount released 2 [mg]	Q: cumulative amount released 3 [mg]
0.5	1187.5	1187.4	1186.0	0.051641031	0.051636268	0.051569599	1.032820611	1.032725368	1.031391971
1.0	2443.0	2440.8	2441.8	0.111429592	0.111324825	0.111372446	2.228591838	2.2264965	2.227448926
1.5	3195.6	3191.0	3193.9	0.147269394	0.147050336	0.147188438	2.945387876	2.941006715	2.943768751
2.0	3837.5	3840.1	3834.8	0.177837516	0.177961331	0.177708939	3.556750321	3.55922663	3.55417877
2.5	4453.2	4462.3	4453.5	0.20715796	0.207591314	0.207172246	4.143159198	4.151826277	4.143444926
3.0	5122.1	5110.8	5114.2	0.239011858	0.238473737	0.238635649	4.780237154	4.769474737	4.772712986
3.5	5520.9	5526.8	5519.2	0.258003238	0.258284204	0.257922282	5.160064765	5.16568408	5.15844564
4.0	5932.4	5935.5	5934.3	0.277599409	0.277747036	0.27768989	5.55198819	5.554940711	5.5537978
4.5	6242.3	6239.9	6239.6	0.292357255	0.292242964	0.292228678	5.847145102	5.844859279	5.844573551
5.0	6533.6	6534.1	6537.4	0.306229344	0.306253155	0.306410305	6.124586885	6.125063098	6.128206105
5.5	6800.9	6810.8	6803.3	0.318958522	0.319429973	0.319072813	6.379170437	6.388599457	6.38145626
6.0	6730.0	6714.4	6736.7	0.315582171	0.314839278	0.315901233	6.311643412	6.296785561	6.318024668

Table 4. 19: *Data obtained from the diffusion of formula no. F5, through poly amide membrane using Franz diffusion cell, with 1% of L-Menthol as penetration enhancer (part 2).*

Time (HR)	Q: cumulative amount released 1 [mg]	Q: cumulative amount released 2 [mg]	Q: cumulative amount released 3 [mg]	m: cumulative amount released 1 cm^2[mg/cm^2]	m: cumulative amount released 2 cm^2[mg/cm^2]	m: cumulative amount released 3 cm^2[mg/cm^2]	Mean	SD	%RSD
0.5	1.032820611	1.032725368	1.031391971	0.328819042	0.328788719	0.328364206	0.3287	0.000	0.08
1.0	2.280232868	2.278132768	2.279018525	0.725957615	0.725289006	0.725571004	0.7256	0.000	0.05
1.5	3.108458498	3.103967808	3.106710796	0.989639764	0.988210063	0.989083348	0.9890	0.001	0.07
2.0	3.867090338	3.869238059	3.864309253	1.231165341	1.231849111	1.230279928	1.2311	0.001	0.06
2.5	4.63133673	4.639799038	4.631284347	1.474478424	1.477172569	1.474461747	1.4754	0.002	0.11
3.0	5.475572646	5.465038811	5.467724654	1.743257767	1.739904111	1.740759202	1.7413	0.002	0.10
3.5	6.094412115	6.099721892	6.092092957	1.940277655	1.941968128	1.939539305	1.9406	0.001	0.06
4.0	6.744338778	6.747262727	6.745367398	2.147194772	2.148125669	2.147522254	2.1476	0.000	0.02
4.5	7.3170951	7.31492833	7.31383304	2.329543171	2.328853336	2.328504629	2.3290	0.001	0.02
5.0	7.886894138	7.887375113	7.889694271	2.51095006	2.511103188	2.511841538	2.5113	0.000	0.02
5.5	8.447707034	8.457164627	8.449354731	2.689496031	2.692507045	2.690020608	2.6907	0.002	0.06
6.0	8.876976046	8.862742035	8.882704891	2.826162383	2.821630702	2.827986275	2.8253	0.003	0.12

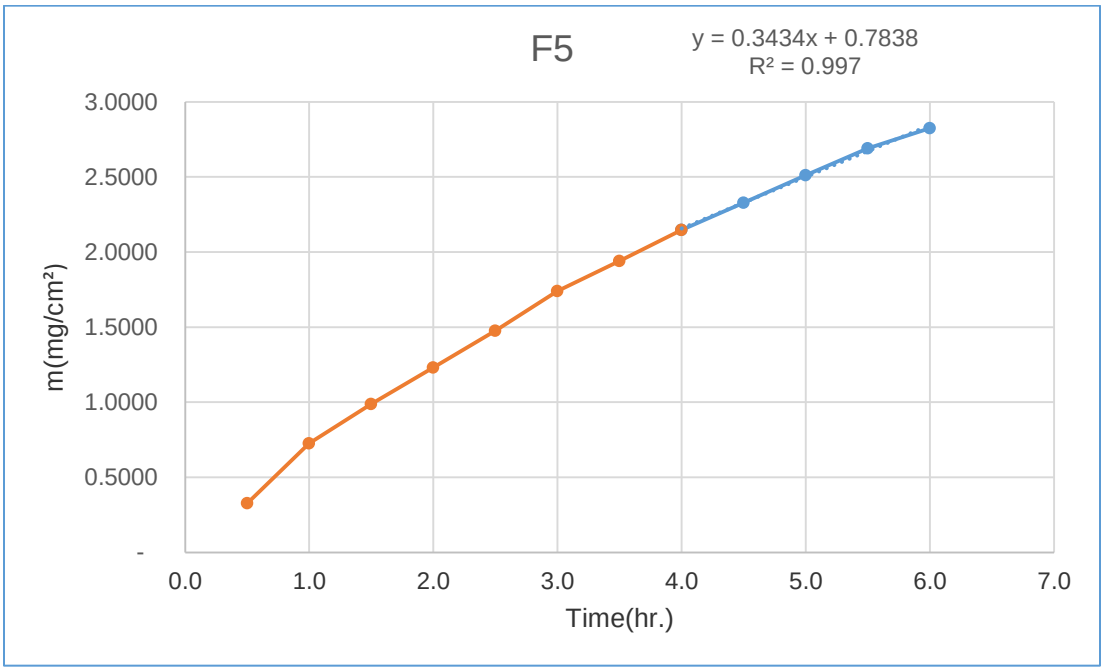


***Figure 4. 4**: In vitro permeation profile for the cumulative amount of diclofenac sodium penetrated per unit area of poly amide membrane (mg/cm²) for formula no. F5, with 1% of L-Menthol as penetration enhancer.*

***Table 4. 20:** Diffusion parameters for formula no. F5, with 1% of L-Menthol as penetration enhancer*

Formulation	Slop	Intercept	TL	D	P	K	ER
F5	0.3434	0.7838	2.282469	0.007477	0.08585	3.674200	0.6280

4.3.6 Formula no. F6, 4% of diclofenac sodium with 0.25% of Eucalyptol as penetration enhancer.

Eucalyptol is a cyclic ether used in different pharmaceutical formulation such as: in cosmetic, flavoring, and fragrance industries due to its unique spicy aroma and taste. Also it is used to enhance the transdermal absorption of lipophilic permeants through the skin.

Using 0.25% of eucalyptol as penetration enhancer in the formulation and following the same general procedure, the data measured are shown in table (4.21), (4.22) and (Fig. 4.6).

Table 4. 21: *Data obtained from the diffusion of formula F6, through poly amide membrane using Franz diffusion cell, with 0.25% of eucalyptol as penetration enhancer (part 1).*

Time (HR)	Avg for 3 SA Area 1	Avg for 3 SA Area 2	Avg for 3 SA Area 3	Concentration of sample 1 (mg/ml)	Concentration of sample 2 (mg/ml)	Concentration of sample 3 (mg/ml)	Q: cumulative amount released 1 [mg]	Q: cumulative amount released 2 [mg]	Q: cumulative amount released 3 [mg]
0.5	168.8	168.3	168.4	0.003129197	0.003105386	0.003110148	0.062583933	0.062107719	0.062202962
1.0	566.9	565.5	567.6	0.022087242	0.022020572	0.022120577	0.441744845	0.440411448	0.442411543
1.5	1129.7	1126.6	1130.0	0.048888519	0.048740892	0.048902805	0.97777037	0.974817848	0.978056098
2.0	1708.7	1710.9	1710.2	0.07646126	0.076566027	0.076532692	1.529225201	1.531320539	1.530653841
2.5	2361.2	2367.5	2369.9	0.107534168	0.107834183	0.107948474	2.150683366	2.156683652	2.158969475
3.0	2979.6	2979.8	2979.7	0.13698319	0.136992714	0.136987952	2.739663794	2.739854279	2.739759036
3.5	3526.7	3518.7	3518.0	0.163036811	0.162655841	0.162622506	3.260736226	3.253116815	3.252450117
4.0	4189.4	4179.7	4179.5	0.194595457	0.19413353	0.194124006	3.891909139	3.882670603	3.882480118
4.5	4717.4	4717.4	4713.9	0.219739511	0.219739511	0.219572837	4.394790228	4.394790228	4.391456736
5.0	5250.6	5247.7	5249.8	0.245131197	0.244993095	0.2450931	4.902623934	4.899861898	4.901861993
5.5	5676.8	5670.4	5670.8	0.265427401	0.265122625	0.265141673	5.308548026	5.302452498	5.302833468
6.0	6179.8	6172.8	6176.4	0.289380923	0.289047574	0.28921901	5.787618458	5.780951474	5.784380209

Table 4. 22: *Data obtained from the diffusion of formula F6, through poly amide membrane using Franz diffusion cell, with 0.25% of eucalyptol as penetration enhancer (part 2).*

Time (HR)	Q: cumulative amount released 1 [mg]	Q: cumulative amount released 2 [mg]	Q: cumulative amount released 3 [mg]	m: cumulative amount released cm^2 1 [mg/cm^2]	m: cumulative amount released cm^2 2 [mg/cm^2]	m: cumulative amount released cm^2 3 [mg/cm^2]	Mean	SD	%RSD
0.5	0.062583933	0.062107719	0.062202962	0.019924843	0.019773231	0.019803554	0.020	0.000	0.40
1.0	0.444874042	0.443516834	0.445521692	0.141634525	0.14120243	0.141840717	0.142	0.000	0.23
1.5	1.002986809	0.999943807	1.003286823	0.319320856	0.318352056	0.319416372	0.319	0.001	0.18
2.0	1.603330159	1.60518739	1.604787371	0.510452136	0.511043422	0.510916068	0.511	0.000	0.06
2.5	2.301249583	2.307116529	2.309635697	0.732648705	0.734516565	0.735318592	0.734	0.001	0.19
3.0	2.997764179	2.998121339	2.998373732	0.954398019	0.954511728	0.954592083	0.955	0.000	0.01
3.5	3.655819801	3.648376589	3.648052764	1.163903152	1.161533457	1.161430361	1.162	0.001	0.12
4.0	4.450029525	4.440586218	4.440705272	1.416755659	1.413749194	1.413787097	1.415	0.002	0.12
4.5	5.147506072	5.146839373	5.143805896	1.638811229	1.638598973	1.637633205	1.638	0.001	0.04
5.0	5.875079289	5.871650555	5.87378399	1.870448675	1.869357069	1.870036291	1.870	0.001	0.03
5.5	6.526134578	6.519234249	6.519848564	2.077725112	2.075528255	2.075723835	2.076	0.001	0.06
6.0	7.270632411	7.26285585	7.266536978	2.314750847	2.312275024	2.313446984	2.313	0.001	0.05

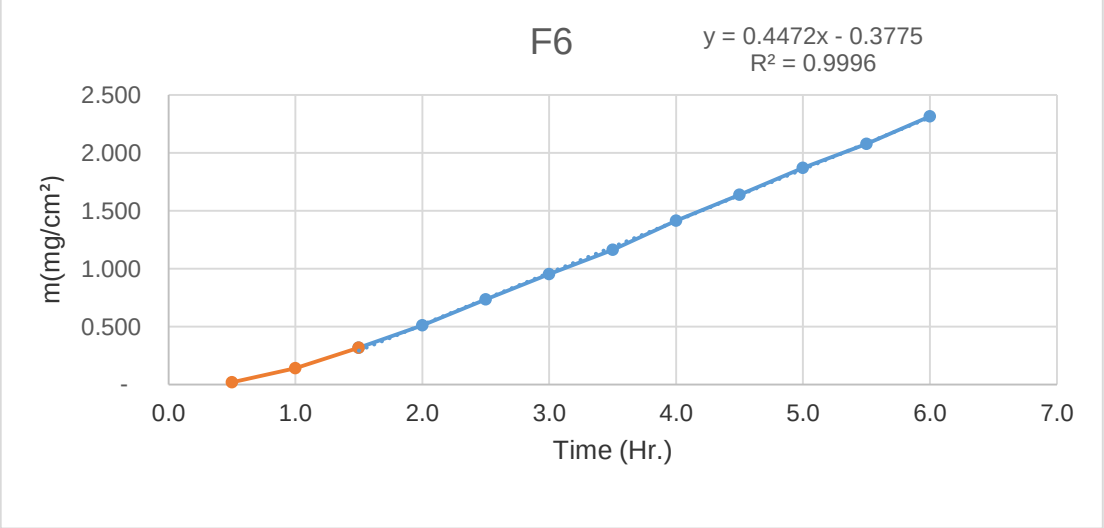


Figure 4. 5*: In vitro permeation profile for the cumulative amount of diclofenac sodium penetrated per unit area of poly amide membrane (mg/cm²) for formula F6, with 0.25% of Eucalyptol as penetration enhancer.*

The diffusion parameters are calculated according to tables (4.23), (4.22) and the enhancement ration is obtained (see table 4.23).

Table 4. 23: *Diffusion parameters for formula no. F6, with 0.25% of eucalyptol as penetration enhancer.*

Formulation	Slop	Intercept	TL	D	P	K	ER
F6	0.4472	0.3775	0.84414	0.02021	0.1118	1.77021	0.81784

4.3.7 Formula no.F7, 4%of diclofenac sodium with 1% of M-pyrol as penetration enhancer.

M-pyrol (N-methyl 2 pyrrolidone) is a powerful and versatile aprotic solvent, in topical administration it appears to be effective as penetration enhancer.

Using 1%M-pyrol as penetration enhancer and following the same general procedure, the data measured are shown in tables (4.24), (4.25).

The cumulative amount of diclofenac sodium penetrated per unit area is shown in (Fig 4.7).

The calculated diffusion parameters are presented in table (4.26).

Table 4. 24: *Data obtained from the diffusion of formula no. F7, through poly amide membrane using Franz diffusion cell, with 1% of M-pyrol as penetration enhancer (part 1).*

Time (HR)	Avg for 3 SA Area 1	Avg for 3 SA Area 2	Avg for 3 SA Area 3	Concentration of sample 1 (mg/ml)	Concentration of sample 2 (mg/ml)	Concentration of sample 3 (mg/ml)	Q: cumulative amount released 1 [mg]	Q: cumulative amount released 2 [mg]	Q: cumulative amount released 3 [mg]
0.5	1056.4	1055.3	1054.6	0.045397876	0.045345493	0.045312158	0.907957522	0.906909853	0.906243154
1.0	1396.8	1394.1	1396.3	0.061608172	0.061479594	0.061584361	1.232163436	1.229591885	1.231687223
1.5	1841.1	1845.2	1844.4	0.082766322	0.08296157	0.082923473	1.655326444	1.659231392	1.658469451
2.0	2353.6	2352.5	2354.0	0.107172246	0.107119863	0.107191295	2.143444926	2.142397257	2.143825896
2.5	2741.4	2739.7	2742.0	0.125639792	0.125558836	0.125668365	2.512795847	2.511176723	2.513367303
3.0	2992.5	2990.6	2995.2	0.137597505	0.137507024	0.137726082	2.751950093	2.750140483	2.754521644
3.5	3460.7	3461.8	3462.7	0.159893804	0.159946188	0.159989047	3.197876089	3.198923758	3.199780942
4.0	3924.9	3921.0	3917.8	0.181999619	0.181813896	0.181661508	3.639992381	3.636277918	3.633230154
4.5	4316.2	4314.3	4314.9	0.20063384	0.200543359	0.200571932	4.012676794	4.010867184	4.01143864
5.0	4552.6	4549.0	4552.5	0.211891519	0.211720082	0.211886757	4.237830373	4.234401638	4.23773513
5.5	4920.3	4917.9	4916.7	0.229401876	0.229287585	0.22923044	4.588037526	4.585751702	4.584608791
6.0	5192.0	5189.2	5188.8	0.242340588	0.242207248	0.242188199	4.846811753	4.844144959	4.843763989

Table 4. 25: *Data obtained from the diffusion of formula no. F7, through poly amide membrane using Franz diffusion cell, with 1% of M-pyrol as penetration enhancer (part 2).*

Time (HR)	Q: cumulative amount released 1 [mg]	Q: cumulative amount released 2 [mg]	Q: cumulative amount released 3 [mg]	m: cumulative amount released cm^2 1 [mg/cm^2]	m: cumulative amount released cm^2 2 [mg/cm^2]	m: cumulative amount released cm^2 3 [mg/cm^2]	Mean	SD	%RSD
0.5	0.907957522	0.906909853	0.906243154	0.289066387	0.288732841	0.288520584	0.2888	0.000	0.10
1.0	1.277561312	1.274937378	1.276999381	0.406737126	0.405901744	0.406558224	0.4064	0.000	0.11
1.5	1.762332492	1.766056479	1.76536597	0.5610737	0.562259306	0.562039468	0.5618	0.001	0.11
2.0	2.333217296	2.332183914	2.333645888	0.742826264	0.742497266	0.742962715	0.7428	0.000	0.03
2.5	2.809740464	2.808083242	2.810378589	0.894536919	0.89400931	0.894740079	0.8944	0.000	0.04
3.0	3.174534502	3.172605838	3.177201295	1.010676377	1.010062349	1.011525404	1.0108	0.001	0.07
3.5	3.758058003	3.758896138	3.760186676	1.196452723	1.19671956	1.197130428	1.1968	0.000	0.03
4.0	4.360068098	4.356196486	4.353624935	1.388114645	1.386882039	1.386063335	1.3870	0.001	0.07
4.5	4.914752131	4.912599648	4.913494928	1.56470937	1.564024084	1.564309114	1.5643	0.000	0.02
5.0	5.34053955	5.336677461	5.340363351	1.700267287	1.699037714	1.700211191	1.6998	0.001	0.04
5.5	5.902638221	5.899747607	5.899123768	1.879222611	1.878302326	1.878103715	1.8785	0.001	0.03
6.0	6.390814324	6.387428449	6.387509405	2.034643211	2.03356525	2.033591024	2.0339	0.001	0.03

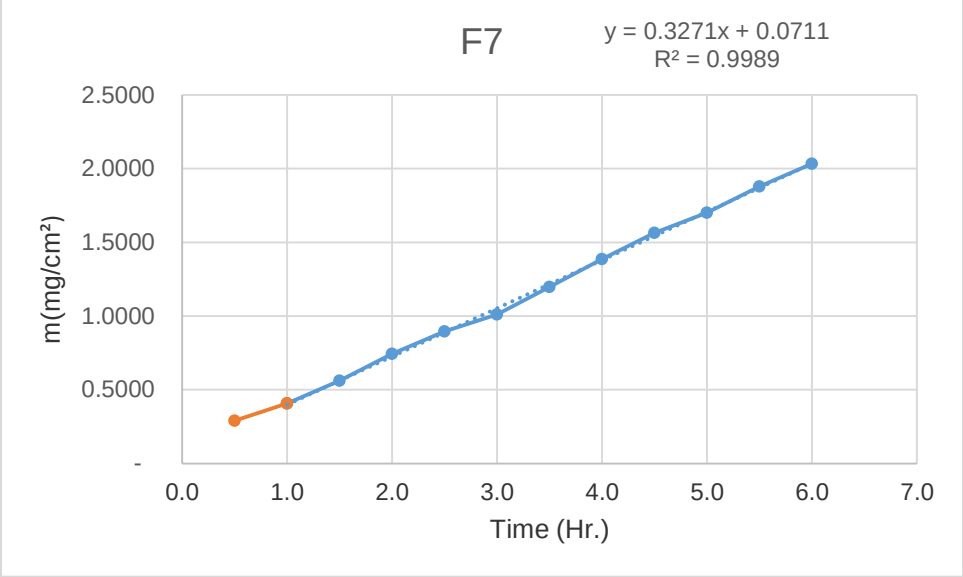


***Figure 4. 6**: In vitro permeation profile for the cumulative amount per unit area of poly amide membrane (mg/cm²) for formula no. F7, with 1% of M-pyrol as penetration enhancer.*

***Table 4. 26:** Diffusion parameters for formula no. F7, with 1% of M-pyrol as penetration enhancer.*

Formulation	Slop	Intercept	TL	D	P	K	ER
F7	0.3271	0.0711	0.21736	0.078516	0.081775	0.3332823	0.5982

The rate of diffusion of API in the presence of M-pyrol is slower than when it was alone, this is indicated by the value of high permeability coefficient (P) from which the enhancement ratio (ER) is found to be approximately half the enhancement ratio of the basic formula (0.5982).

4.3.8 Brand permeation results.

Swiss Relief – Spray Gel 4%, is a pharmaceutical product in the market contains 4% of diclofenac sodium and we have adopted it as a reference to compare our trials with it and to select the best penetration enhancer in the study.

The permeability results of drug through the poly amide membrane are shown in table (4.27),(4.28) and (Fig. 4.8).

The cumulative amount of API permeated through unit area of membrane was then calculated as mentioned before the linear section (the steady state flux) was plotted versus time (Fig. 4.29).

Table 4. 27: *Data obtained from the diffusion of the Brand formula, through poly amide membrane using Franz diffusion cell (part 1).*

Time (HR)	Avg for 3 SA Area 1	Avg for 3 SA Area 2	Avg for 3 SA Area 3	Concentration of sample 1 (mg/ml)	Concentration of sample 2 (mg/ml)	Concentration of sample 3 (mg/ml)	Q: cumulative amount released 1 [mg]	Q: cumulative amount released 2 [mg]	Q: cumulative amount released 3 [mg]
0.5	303.5	302.7	303.1	0.009543788	0.009505691	0.009524739	0.190875756	0.190113815	0.190494785
1.0	507.7	508.7	522.4	0.01926806	0.019315682	0.019968094	0.385361208	0.386313634	0.399361874
1.5	680.3	682.1	680.2	0.027487499	0.027573218	0.027482737	0.549749988	0.551464355	0.549654745
2.0	856.1	856.4	855.6	0.035859327	0.035873613	0.035835516	0.717186533	0.717472261	0.71671032
2.5	1057.2	1056.9	1056.2	0.045435973	0.045421687	0.045388352	0.908719463	0.908433735	0.907767037
3.0	1125.8	1125.2	1124.9	0.048702795	0.048674223	0.048659936	0.974055907	0.973484452	0.973198724
3.5	1256.4	1256.3	1254.8	0.054922139	0.054917377	0.054845945	1.098442783	1.09834754	1.096918901
4.0	1344.9	1344.5	1344.7	0.059136626	0.059117577	0.059127101	1.182732511	1.182351541	1.182542026
4.5	1469.2	1467.3	1434.2	0.065055955	0.064965475	0.063389209	1.301119101	1.299309491	1.26778418
5.0	1541.9	1539.2	1538.5	0.068518025	0.068389447	0.068356112	1.370360493	1.367788942	1.367122244
5.5	1629.0	1627.5	1628.2	0.072665841	0.072594409	0.072627744	1.453316825	1.451888185	1.452554884
6.0	1724.7	1723.5	1724.0	0.077223201	0.077166056	0.077189866	1.544464022	1.543321111	1.543797324

Table 4. 28: *Data obtained from the diffusion of the Brand formula, through poly amide membrane using Franz diffusion cell (part 2).*

Time (HR)	Q: cumulative amount released 1 [mg]	Q: cumulative amount released 2 [mg]	Q: cumulative amount released 3 [mg]	m: cumulative amount released cm^2 1 [mg/cm^2]	m: cumulative amount released cm^2 2 [mg/cm^2]	m: cumulative amount released cm^2 3 [mg/cm^2]	Mean	SD	%RSD
0.5	0.190875756	0.190113815	0.190494785	0.060769104	0.060526525	0.060647815	0.0606	0.000	0.20
1.0	0.394904995	0.395819325	0.408886614	0.125725882	0.126016977	0.130177209	0.1273	0.002	1.96
1.5	0.578561836	0.580285728	0.579147578	0.184196701	0.184745536	0.184383183	0.1844	0.000	0.15
2.0	0.77348588	0.773866851	0.77368589	0.246254658	0.246375947	0.246318335	0.2463	0.000	0.02
2.5	1.000878137	1.000701938	1.000578123	0.318649518	0.318593422	0.318554003	0.3186	0.000	0.02
3.0	1.111650555	1.111174342	1.111398162	0.353916127	0.353764515	0.353835773	0.3538	0.000	0.02
3.5	1.284740226	1.284711653	1.283778275	0.409022676	0.409013579	0.40871642	0.4089	0.000	0.04
4.0	1.423952093	1.42363303	1.424247345	0.453343551	0.453241971	0.45343755	0.4533	0.000	0.02
4.5	1.601475308	1.599708558	1.568616601	0.509861607	0.509299127	0.499400382	0.5062	0.006	1.16
5.0	1.735772656	1.733153483	1.731343874	0.552617846	0.551783981	0.551207855	0.5519	0.001	0.13
5.5	1.887247012	1.885642173	1.885132625	0.600842729	0.600331797	0.600169572	0.6004	0.000	0.06
6.0	2.05106005	2.049669508	2.04900281	0.652995877	0.65255317	0.652340914	0.6526	0.000	0.05

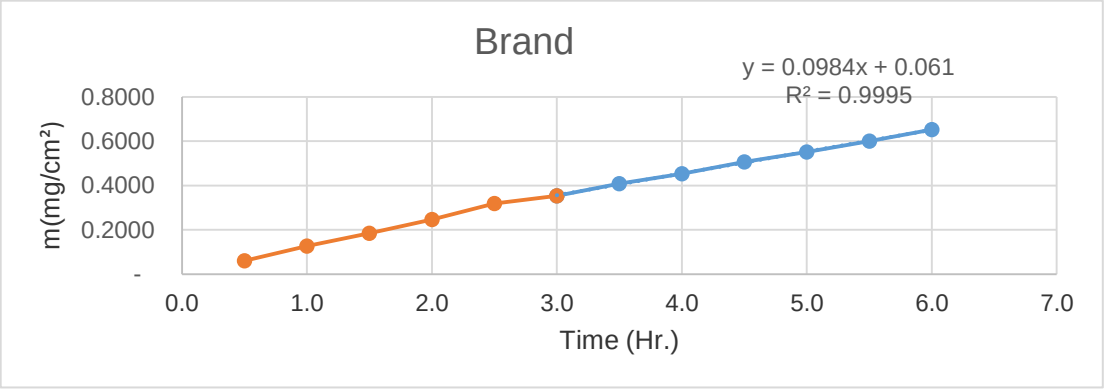


Figure 4. 7: *Figure 4.8: In vitro permeation profile for the cumulative amount of diclofenac sodium penetrated per unit area of poly amid membrane (mg/cm²) for the brand formula.*

Table 4. 29: *Diffusion parameters for the Brand formula.*

Formulation	Slop	Intercept	TL	D	P	K	ER
Brand	0.0984	0.061	0.619918	0.027530	0.0246	0.2859375	0.1799

The permeability parameter of the Brand formula table (4.29) was used as a reference for penetration enhancement comparison.

4.3 SELECTING THE BEST PENETRATION ENHANCER.

Table (4.30) summarize the diffusion parameters of all formulations tested.

Table 4. 30: *summary of diffusion parameters for different formulations of diclofenac sodium solution and comparison between all the enhancement ratio of formulas with the basic formula and the Brand one.*

Formulation	Slop	Intercept	TL	D	P	K	ER of basic formula	ER of the brand
Brand	0.0984	0.061	0.619918	0.027530	0.0246	0.2859375	0.1799	1
F1	0.5468	0.15	0.2743233	0.06221	0.1367	0.703124	1	5.5569
F2	0.372	0.5939	1.5965	0.01069	0.093	2.78391	0.6803	3.7804
F3	0.3633	0.646	1.77814	0.00959	0.090825	3.030656	0.6644	3.6920
F4	0.5658	0.0503	0.088900	0.1919745	0.14145	0.23578125	1.03474	5.75
F5	0.3434	0.7838	2.282469	0.007477	0.08585	3.674200	0.6280	3.4898
F6	0.4472	0.3775	0.84414	0.02021	0.1118	1.77021	0.81784	4.5447
F7	0.3271	0.0711	0.21736	0.078516	0.081775	0.3332823	0.5982	3.3241

It is obvious from the above table that all formulas tested with different types of penetration enhancers exhibited enhancement ratios better than the enhancement ratio of the Brand formulation. On the other hand and with comparison of our formulation tested with the basic formula, formula no. F1, it's obvious that the best penetration enhancement was found for formulation no. F4.

Formulation no. F4, exhibited ER about (1.035) better than the basic formulation formula no. F1, and about (5.75) times relative to the Brand one.

Formulation no.F5 which contains L-Menthol as penetration enhancer was the second formula that exhibited good enhancement ratio after formulation no. F4, so it was chosen for further study.

Formula no. F8 is a combination of 0.25% of Limonene and 1% of L-Menthol in 4% of diclofenac sodium solution which was developed in order to have synergistic effect and better enhancement ratio than the previous results. Using 0.25% of Limonene and 1% of L-Menthol as combination of penetration enhancers in order to get synergistic effect and following the same general procedure the data measured are shown in tables (4.31),(4.32) the cumulative amount of diclofenac sodium penetrated per unit area is shown in (Fig 4.9), and the calculated diffusion parameters are illustered in table (4.33).

Table 4. 31: *Data obtained from the diffusion of formula no. F8, through poly amide membrane using Franz diffusion cell, with combination of 0.25% of Limonene and 1% of L- Menthol as synergistic effect on the formulation (part 1).*

Time (HR)	Avg for 3 SA Area 1	Avg for 3 SA Area 2	Avg for 3 SA Area 3	Concentration of sample 1 (mg/ml)	Concentration of sample 2 (mg/ml)	Concentration of sample 3 (mg/ml)	Q: cumulative amount released 1 [mg]	Q: cumulative amount released 2 [mg]	Q: cumulative amount released 3 [mg]
0.5	140.5	140.6	140.4	0.001781513	0.001786276	0.001776751	0.035630268	0.035725511	0.035535025
1.0	498.5	497.2	496.8	0.018829944	0.018768037	0.018748988	0.376598886	0.375360731	0.374979761
1.5	1041.8	1042.8	1041.5	0.044702605	0.044750226	0.044688318	0.894052098	0.895004524	0.89376637
2.0	1624.5	1622.7	1617.9	0.072451545	0.072365827	0.072137245	1.449030906	1.447316539	1.442744893
2.5	2226.5	2226.1	2218.6	0.101119577	0.101100529	0.100743369	2.022391542	2.022010572	2.014867375
3.0	2755.3	2750.1	2753.1	0.126301729	0.126054098	0.126196962	2.526034573	2.521081956	2.523939235
3.5	3368.1	3369.0	3367.2	0.155484071	0.15552693	0.155441211	3.109681413	3.110538597	3.10882423
4.0	3926.7	3922.7	3921.7	0.182085337	0.181894852	0.181847231	3.641706748	3.637897043	3.636944616
4.5	4440.3	4437.9	4432.6	0.206543645	0.206429354	0.206176961	4.130872899	4.128587076	4.123539216
5.0	4957.4	4945.5	4944.6	0.231168627	0.230601933	0.230559074	4.623372542	4.612038669	4.611181485
5.5	5339.2	5346.9	5337.2	0.249350445	0.249717129	0.249255203	4.987008905	4.994342588	4.985104053
6.0	5794.7	5791.7	5788.0	0.271041954	0.27089909	0.270722892	5.420839088	5.417981809	5.414457831

Table 4. 32: *Data obtained from the diffusion of formula no. F8, through poly amide membrane using Franz diffusion cell, with combination of 0.25% of Limonene and 1% of L- Menthol to check synergistic effect on the formulation (part 2).*

Time (HR)	Q: cumulative amount released 1 [mg]	Q: cumulative amount released 2 [mg]	Q: cumulative amount released 3 [mg]	m: cumulative amount released cm^2 1 [mg/cm^2]	m: cumulative amount released cm^2 2 [mg/cm^2]	m: cumulative amount released cm^2 3 [mg/cm^2]	Mean	SD	%RSD
0.5	0.035630268	0.035725511	0.035535025	0.011343607	0.011373929	0.011313284	0.0113	0.000	0.27
1.0	0.378380399	0.377147007	0.376756512	0.120464947	0.120072272	0.11994795	0.1202	0.000	0.22
1.5	0.914663555	0.915558836	0.914292109	0.291201387	0.291486417	0.291083129	0.2913	0.000	0.07
2.0	1.514344969	1.512621077	1.50795895	0.482121926	0.48157309	0.480088809	0.4813	0.001	0.22
2.5	2.16015715	2.159680937	2.152218677	0.687729115	0.687577503	0.685201744	0.6868	0.001	0.21
3.0	2.764919758	2.75985285	2.762033906	0.880267354	0.878654203	0.879348585	0.8794	0.001	0.09
3.5	3.474868327	3.475363589	3.473115863	1.106293641	1.106451318	1.105735709	1.1062	0.000	0.03
4.0	4.162377732	4.158248964	4.156677461	1.325175973	1.323861498	1.323361178	1.3241	0.001	0.07
4.5	4.83362922	4.830833849	4.825119291	1.538882273	1.537992311	1.536172968	1.5377	0.001	0.09
5.0	5.532672508	5.520714796	5.518938521	1.761436647	1.757629671	1.757064158	1.7587	0.002	0.14
5.5	6.127477499	6.133620649	6.123420163	1.95080468	1.952760474	1.949512946	1.9510	0.002	0.08
6.0	6.810658127	6.806976999	6.802029144	2.168308859	2.167136899	2.165561651	2.1670	0.001	0.06

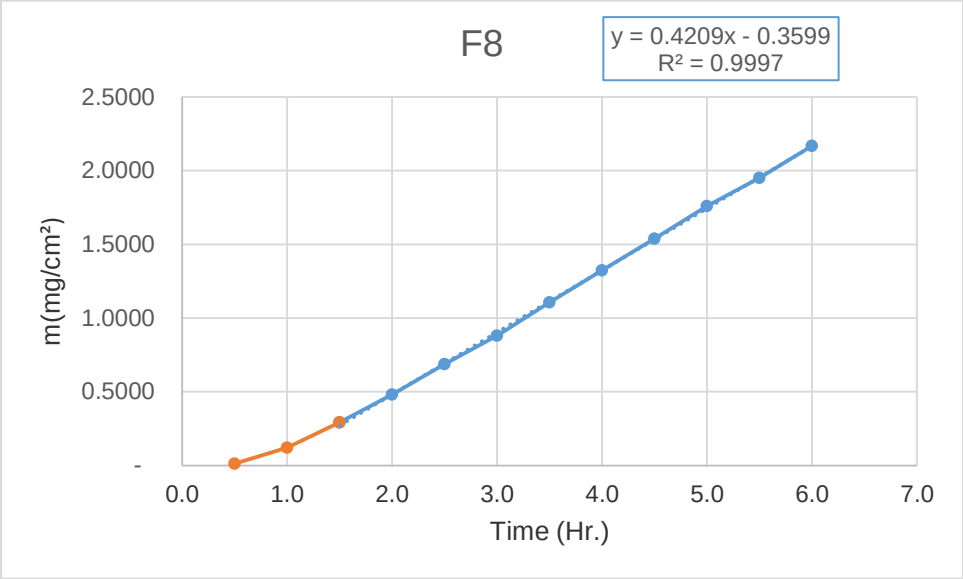


Figure 4. 8: *In vitro permeation profile for the cumulative amount of diclofenac sodium penetrated per unit area of poly amide membrane (mg/cm²) for formula no. F8 as synergistic effect of penetration enhancer.*

Table 4. 33: *Diffusion parameters for formula no. F8 with 0.25% of limonene + 1% of L-Menthol as penetration enhancers.*

Formulation	Slop	Intercept	TL	D	P	K	ER
F8	0.4209	0.3599	0.85507	0.019959	0.105225	1.68703125	0.76975

The calculated enhancement ratio ER of formula no. F8 was reduced relative to basic formulation F1 and in relative to formulation with Limonene (F4). The expected synergistic effect of combination of limonene and L-Menthol did unfortunately not occur and consequently works as penetration retardant.

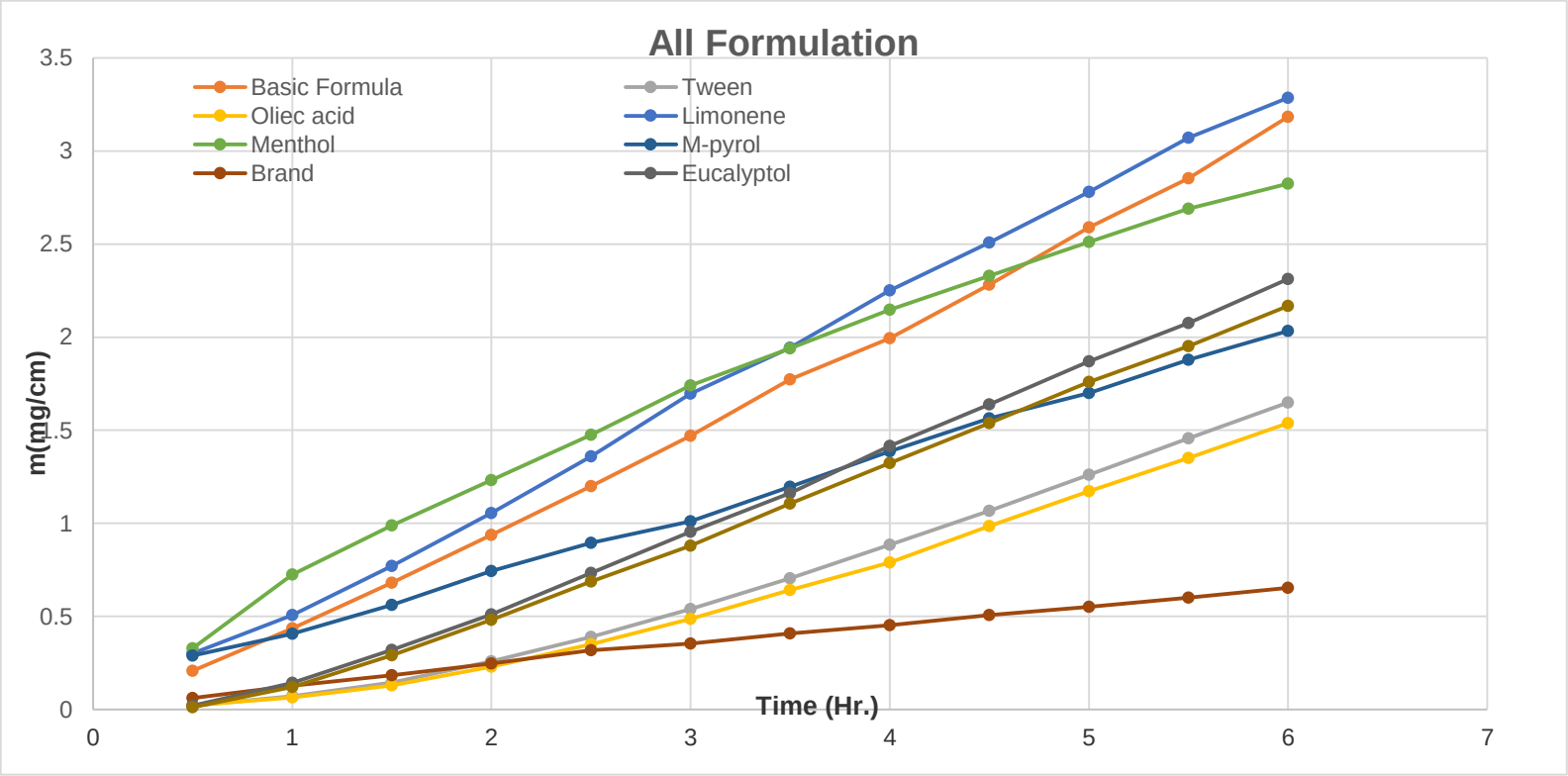


Figure 4. 9: *In vitro permeation profile for the cumulative amount of diclofenac sodium penetrated per unit area of polyamid membrane (mg/cm²) for all formulas tested.*

(Fig. 4.10) clarify summary of in vitro permeation profile for cumulative amount of diclofenac sodium for all formulas tested through the poly amid membrane.

4.3 START-M MEMBRANE PERMEATION RESULTS.

As mentioned in section 4.4, the best penetration enhancement was found for formulation no. F4. This formulation was scaled up and tested for permeation through advanced membrane (Start-M® membrane) along with the basic formulation F1, and the Brand formulation, using the same configuration of Franz diffusion cell FDC apparatus.

Start-M® membrane is a novel synthetic membrane, it was designed to imitative the nature of human skin layers (epidermis, dermis, and subcutaneous layer). These multiple layers of start-M® membrane was found to be 47 mm in thickness consist of top layer confirming by double layers of porous polyether sulfone (PES) followed by one layer of polyolefin Non-wover fabric support (see Fig. 4.2). Start-M® membrane composed of some lipids similar to lipids which found in normal human skin such as (cholesterol, free fatty acids, ceramides and other compounds) [49]. The samples were collected each half an hour for six hours. Permeation data for Brand, F1, and F4 obtained are shown in table (4.34) (4.35), (4.36) (4.37), (4.38) and (Fig. 4.11), (Fig.4.12), (Fig4.13)

respectively. From the steady state flux diagram of each formulations, the diffusion parameters were calculated and summaries in table (4.37).

***Table 4. 34**: Data obtained from the diffusion of the Brand, through Start-M membrane using Franz diffusion cell (part 1).*

Time (HR)	Avg for 3 SA Area 1	Avg for 3 SA Area 2	Avg for 3 SA Area 3	Concentration of sample 1 (mg/ml)	Concentration of sample2 (mg/ml)	Concentration of sample 3 (mg/ml)	Q: cumulative amount released 1 [mg]	Q: cumulative amount released 2 [mg]	Q: cumulative amount released 3 [mg]
0.5	349.8	349.1	349.2	0.011748655	0.01171532	0.011720082	0.234973094	0.234306396	0.234401638
1.0	420.7	420.6	420.5	0.015125006	0.015120244	0.015115482	0.302500119	0.302404876	0.302309634
1.5	589.1	590.2	591.0	0.023144435	0.023196819	0.023234916	0.462888709	0.463936378	0.464698319
2.0	790.5	790.1	790.0	0.032735368	0.03271632	0.032711558	0.654707367	0.654326396	0.654231154
2.5	959.8	960.7	960.1	0.040797657	0.040840516	0.040811943	0.815953141	0.816810324	0.816238869
3.0	1180.4	1170.4	1179.7	0.051302919	0.050826706	0.051269584	1.026058384	1.016534121	1.025391685
3.5	1389.8	1389.4	1386.7	0.061274823	0.061255774	0.061127197	1.225496452	1.225115482	1.222543931
4.0	1617.4	1616.0	1615.7	0.072113434	0.072046764	0.072032478	1.442268679	1.440935283	1.440649555
4.5	1836.7	1836.1	1835.0	0.082556788	0.082528216	0.082475832	1.651135768	1.650564313	1.649516644
5.0	2056.4	2056.0	2054.6	0.093019191	0.093000143	0.092933473	1.860383828	1.860002857	1.85866946
5.5	2163.0	2163.7	2164.1	0.098095624	0.098128959	0.098148007	1.961912472	1.96257917	1.962960141
6.0	2488.1	2490.3	2492.9	0.113577313	0.11368208	0.113805896	2.271546264	2.273641602	2.27611791

Table 4. 35: *Data obtained from the diffusion of the Brand, through Start-M membrane using Franz diffusion cell (part 2).*

Time (HR)	Q: cumulative amount released 1 [mg]	Q: cumulative amount released 2 [mg]	Q: cumulative amount released 3 [mg]	m: cumulative amount released cm^2 1 [mg/cm^2]	m: cumulative amount released cm^2 2 [mg/cm^2]	m: cumulative amount released cm^2 3 [mg/cm^2]	Mean	SD	%RSD
0.5	0.234973094	0.234306396	0.234401638	0.074808371	0.074596114	0.074626437	0.07468	0.000	0.15
1.0	0.314248774	0.314120196	0.314029716	0.100047365	0.10000643	0.099977624	0.10001	0.000	0.04
1.5	0.48976237	0.490771942	0.491533883	0.155925619	0.156247036	0.156489616	0.15622	0.000	0.18
2.0	0.704725463	0.704358779	0.704301633	0.224363408	0.224246666	0.224228473	0.22428	0.000	0.03
2.5	0.898706605	0.899559027	0.899020906	0.286121173	0.286392559	0.286221237	0.28624	0.000	0.05
3.0	1.149609505	1.140123339	1.148985666	0.366001116	0.362981006	0.365802504	0.36493	0.002	0.46
3.5	1.400350493	1.399531406	1.397407496	0.445829511	0.445568738	0.444892549	0.44543	0.000	0.11
4.0	1.678397543	1.676606981	1.676640316	0.534351335	0.533781274	0.533791887	0.53397	0.000	0.06
4.5	1.959378066	1.958282775	1.957539883	0.623807089	0.623458381	0.623221867	0.62350	0.000	0.05
5.0	2.251182913	2.250249536	2.249168532	0.716708982	0.716411823	0.716067664	0.71640	0.000	0.04
5.5	2.445730749	2.445825992	2.446392685	0.778647166	0.778677489	0.778857907	0.77873	0.000	0.01
6.0	2.853460165	2.855017382	2.857698462	0.908455958	0.908951729	0.909805305	0.90907	0.001	0.08

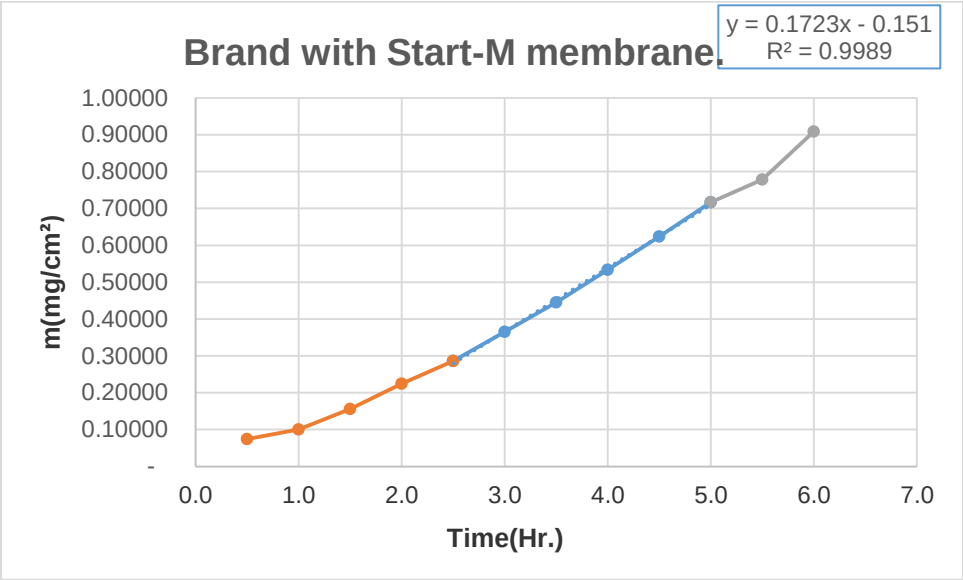


***Figure 4. 10**: In vitro permeation profile for the cumulative amount of diclofenac sodium penetrated per unit area of Start-M membrane (mg/cm²) for the Brand.*

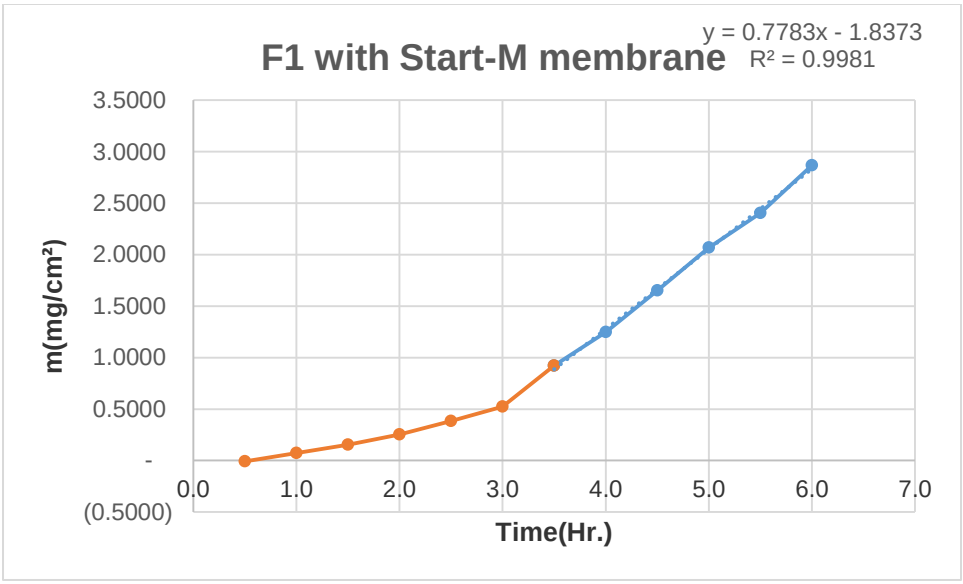


***Figure 4. 11:** In vitro permeation profile for the cumulative amount of diclofenac sodium penetrated per unit area of Start-M membrane (mg/cm²) for the formulation no.F1 without addition of penetration enhancers.*

Table 4. 36: *Data obtained from the diffusion of the formulation no. F1, through Start-M membrane using Franz diffusion cell, without penetration enhancers (part 1).*

Time (HR)	Avg for 3 SA Area 1	Avg for 3 SA Area 2	Avg for 3 SA Area 3	Concentration of sample 1 (mg/ml)	Concentration of sample 2 (mg/ml)	Concentration of sample 3 (mg/ml)	Q: cumulative amount released 1 [mg]	Q: cumulative amount released 2 [mg]	Q: cumulative amount released 3 [mg]
0.5	77.5	76.8	76.7	-0.001218629	-0.001251964	-0.001256727	-0.024372589	-0.025039288	-0.02513453
1.0	345.5	346.3	345.7	0.011543883	0.01158198	0.011553407	0.230877661	0.231639602	0.231068146
1.5	603.1	603.2	602.0	0.023811134	0.023815896	0.02375875	0.476222677	0.47631792	0.475175008
2.0	901.7	902.3	899.6	0.038030859	0.038059431	0.037930854	0.760617172	0.761188628	0.758617077
2.5	1292.0	1292.2	1289.4	0.056617458	0.056626982	0.056493643	1.132349159	1.132539645	1.129872851
3.0	1689.3	1690.7	1692.9	0.075537407	0.075604076	0.075708843	1.510748131	1.512081528	1.514176866
3.5	2917.8	2926.0	2927.0	0.134040192	0.134430687	0.134478308	2.680803848	2.688613744	2.68956617
4.0	3855.2	3856.2	3862.3	0.178680413	0.178728035	0.179018525	3.573608267	3.574560693	3.580370494
4.5	5015.1	4993.0	5011.3	0.233916377	0.232863946	0.233735416	4.678327539	4.657278918	4.674708319
5.0	6141.3	6125.4	6133.3	0.287547502	0.286790323	0.287166532	5.750950045	5.735806467	5.743330635
5.5	6941.3	6949.0	6942.4	0.325644555	0.326011239	0.325696938	6.51289109	6.520224773	6.513938759
6.0	8124.5	8122.8	8120.0	0.381990095	0.381909139	0.381775799	7.639801895	7.638182771	7.635515977

Table 4. 37: *Data obtained from the diffusion of the formulation no. F1, through Start-M membrane using Franz diffusion cell, without penetration enhancers (part 2).*

Time (HR)	Q: cumulative amount released 1 [mg]	Q: cumulative amount released 2 [mg]	Q: cumulative amount released 3 [mg]	m: cumulative amount released $cm^2$1 [mg/cm^2]	m: cumulative amount released cm^2 2 [mg/cm^2]	m: cumulative amount released cm^2 3 [mg/cm^2]	Mean	SD	%RSD
0.5	-0.024372589	-0.025039288	-0.02513453	-0.0077595	-0.007971757	-0.008002079	(0.0079)	0.000	-1.67
1.0	0.229659031	0.230387638	0.22981142	0.073116533	0.0733485	0.073165049	0.0732	0.000	0.17
1.5	0.486547931	0.486647936	0.485471689	0.154902238	0.154934077	0.154559595	0.1548	0.000	0.13
2.0	0.79475356	0.79533454	0.792672508	0.253025648	0.253210614	0.252363104	0.2529	0.000	0.18
2.5	1.204516406	1.204744988	1.201859136	0.383481823	0.383554597	0.382635828	0.3832	0.001	0.13
3.0	1.639532835	1.640913853	1.642656793	0.52197798	0.522417655	0.522972554	0.5225	0.000	0.10
3.5	2.885125958	2.893050145	2.893754941	0.918537395	0.921060218	0.921284604	0.9203	0.002	0.17
4.0	3.91197057	3.913427782	3.919037573	1.245453859	1.245917791	1.24770378	1.2464	0.001	0.10
4.5	5.195370256	5.174874042	5.192393924	1.654049747	1.647524369	1.653102172	1.6516	0.004	0.21
5.0	6.501909139	6.486265536	6.494751655	2.070012461	2.065032008	2.067733733	2.0676	0.002	0.12
5.5	7.551397686	7.557474165	7.552526311	2.404138072	2.406072641	2.404497393	2.4049	0.001	0.04
6.0	9.003953045	9.001443402	8.999800467	2.866588044	2.865789049	2.865265987	2.8659	0.001	0.02

Table 4. 38:*Data obtained from the diffusion of the formulation no. F4, through Start-M membrane using Franz diffusion cell, with 0.25% of Limonene as penetration enhancer (part 1).*

Time (HR)	Avg for 3 SA Area 1	Avg for 3 SA Area 2	Avg for 3 SA Area 3	Concentration of sample 1 (mg/ml)	Concentration of sample 2 (mg/ml)	Concentration of sample 3 (mg/ml)	Q: cumulative amount released 1 [mg]	Q: cumulative amount released 2 [mg]	Q: cumulative amount released 3 [mg]
0.5	278.5	277.9	278.8	0.008353255	0.008324682	0.008367541	0.167065098	0.166493643	0.167350826
1.0	589.2	589.6	587.8	0.023149198	0.023168246	0.023082528	0.462983952	0.463364922	0.461650555
1.5	882.6	881.3	881.9	0.037121291	0.037059384	0.037087957	0.74242583	0.741187676	0.741759131
2.0	1327.9	1326.1	1325.4	0.058327063	0.058241345	0.05820801	1.166541264	1.164826897	1.164160198
2.5	1802.2	1800.3	1798.9	0.080913853	0.080823373	0.080756703	1.618277061	1.616467451	1.615134054
3.0	2289.7	2287.6	2289.3	0.104129244	0.104029239	0.104110196	2.082584885	2.08058479	2.082203914
3.5	2914.6	2910.2	2915.1	0.133887804	0.13367827	0.133911615	2.677756084	2.673565408	2.678232297
4.0	3706.3	3705.9	3712.0	0.1715896	0.171570551	0.171861041	3.43179199	3.43141102	3.43722082
4.5	4659.1	4655.4	4662.4	0.216963189	0.21678699	0.217120339	4.339263774	4.335739797	4.342406781
5.0	5480.7	5486.4	5476.0	0.256088861	0.256360303	0.255865041	5.121777227	5.127206057	5.117300824
5.5	6318.7	6313.6	6314.5	0.295995524	0.295752655	0.295795514	5.919910472	5.915053098	5.915910281
6.0	7617.8	7638.8	7623.7	0.357860374	0.358860422	0.35814134	7.157207486	7.177208438	7.162826801

Table 4. 39: *Data obtained from the diffusion of the formulation no. F4, through Start-M membrane using Franz diffusion cell, with 0.25% of Limonene as penetration enhancer (part 2).*

Time (HR)	Q: cumulative amount released 1 [mg]	Q: cumulative amount released 2 [mg]	Q: cumulative amount released 3 [mg]	m: cumulative amount released cm² 1 [mg/cm²]	m: cumulative amount released cm² 2 [mg/cm²]	m: cumulative amount released cm² 3 [mg/cm²]	Mean	SD	%RSD
0.5	0.167065098	0.166493643	0.167350826	0.053188506	0.053006572	0.053279473	0.05316	0.000	0.26
1.0	0.471337207	0.471689604	0.470018096	0.150059601	0.150171794	0.149639636	0.14996	0.000	0.19
1.5	0.773928282	0.772680604	0.7732092	0.246395505	0.245998282	0.246166571	0.24619	0.000	0.08
2.0	1.235165008	1.233379209	1.232698224	0.393239417	0.392670872	0.392454067	0.39279	0.000	0.10
2.5	1.745227868	1.743261108	1.74188009	0.555628102	0.555001944	0.55456227	0.55506	0.001	0.10
3.0	2.290449545	2.288201819	2.289706653	0.729210298	0.728494689	0.728973783	0.72889	0.000	0.05
3.5	2.989749988	2.985211677	2.989845231	0.951846542	0.95040168	0.951876864	0.95138	0.001	0.09
4.0	3.877673699	3.876735559	3.882745369	1.234534766	1.23423609	1.236149433	1.23497	0.001	0.08
4.5	4.956735083	4.952634887	4.959792371	1.57807548	1.576770101	1.579048829	1.57796	0.001	0.07
5.0	5.956211724	5.960888138	5.951806753	1.896278804	1.897767634	1.894876394	1.89631	0.001	0.08
5.5	7.01043383	7.005095481	7.006281251	2.231911439	2.230211869	2.230589383	2.23090	0.001	0.04
6.0	8.543726368	8.563003476	8.548993285	2.720065701	2.726202953	2.72174253	2.72267	0.003	0.12

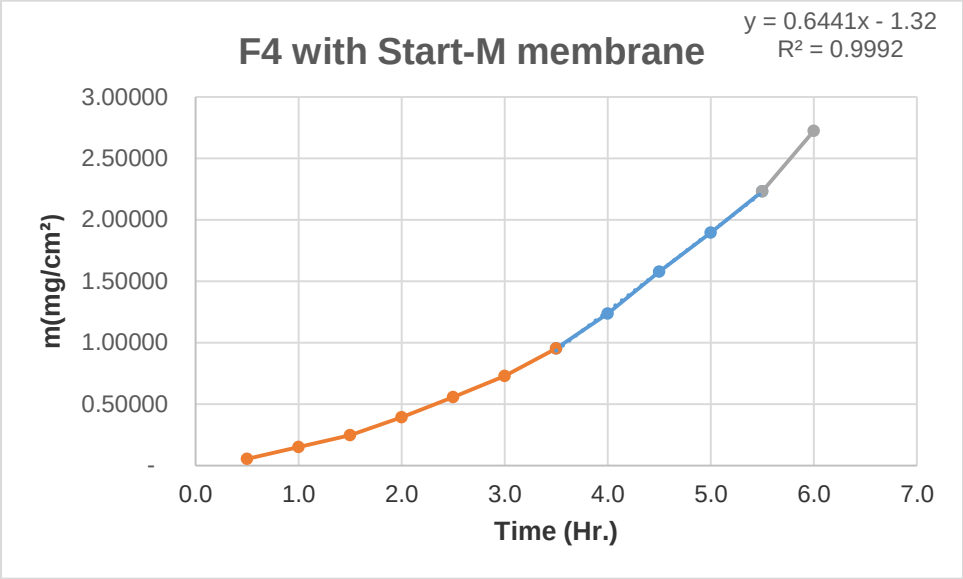


Figure 4. 12: *In vitro permeation profile for the cumulative amount of diclofenac sodium penetrated per unit area of Start-M membrane (mg/cm²) for the formulation no.F4 with 0.25% of Limonene as penetration enhancer.*

Table 4. 40: summary of diffusion parameters for the Brand, F1 and F4 of diclofenac sodium solution.

Formulation	Slop	Intercept	TL	D	P	K	ER (compare with basic formula)	ER (compare with Brand)
F1	0.7783	1.8373	2.36065	155.9593	0.194575	0.05863	1	4.5171213
F4	0.6441	1.32	2.049371	179.648598	0.161025	0.0421276	0.8275729	3.7382472
Brand	0.1723	0.151	0.876378	420.1001104	0.043075	0.00481914	0.1580367	1

Previous table (4.28) clarify that formulation no. F1 and F4 exhibited better penetration enhancement than the Brand. Formulation no. F1 exhibited enhancement ratio about (4.5) better than the Brand. And formulation no. F4 presented about (3.7) times relative to the Brand Too.

There was a slight difference between formulation no.F1 and F4. Formulation no. F1, 4% of diclofenac sodium solution without addition any penetration enhancer was better in penetration enhancement activity by (0.1724) relative to each other, country to what happened in the previous comparison discussed in section (4.4) were formulation no. F4, 4% of diclofenac sodium solution with 0.25% of limonene as penetration enhancer which was better in penetration enhancement (1.03474) than F1 (1). This slight difference occur maybe due to the nature of each membrane used.

PART FIVE: CONCLUSION.

CONCLUSION.

In the present work, the solubility of diclofenac sodium was determined in different solutions and was found to increase as the pH increases.

The influence of selected penetration enhancer included in a 4% w/w of diclofenac sodium solution was investigated through synthetic membrane, poly amide filter membrane soaked and saturated in octanol and was sandwiched in between 2 layers of dialysis membrane presoaked with phosphate buffer pH= 7.4. The enhancement ratio was calculated for each penetration enhancer and found to be in the following order (in compare with the Brand):

Limonene > L-Menthol > Eucalyptol > Limonene+L-Menthol > M-pyrol > Tween 80 > Oleic acid.

Topical 4% diclofenac sodium was formulated with the selected penetration enhancer Limonene, and the permeation was investigated through 47 mm thickness of an advanced synthetic membrane (Start- M® membrane). A comparison between the diffusion parameters was made between using the poly amide filter membrane and Start M® membrane.

The lag time was found to be tow folds in case of Start-M® membrane relative to poly amide filter membrane, and the diffusion coefficient was about (179.64) in case of Start-M® membrane and (0.1919) in case of poly amide membrane, while the

permeability coefficient was (0.16) in the case of Start- M® membrane, in relative to (0.14) of poly amide filter membrane.

Poly amide membrane showed better permeation results than Start-M® membrane, suggesting the difference between their thickness, and the characteristics that characterize the Start-M® membrane make it very similar to the characteristics of human skin layers.

PART SIX: APPENDIX

Data File: C:\CHEM32\1\DATA\DICLOFENAC SODIUM 2018-08-14 12-28-27\011-1001>
Sample Name: SA- Solubility in Buffer

==========

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Acq. Instrument	: Instrument 1	Location	: Vial 11
Injection Date	: 8/14/2018	Inj	: 1
Injection Time:	2:48:15 PM	Inj. Volume (Method)	: 10 µl
		Act. Inj. Vol. from Sequence	: 10 µl

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DICLOFENAC SODIUM.M
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Method Info:
Diclofenac Sodium

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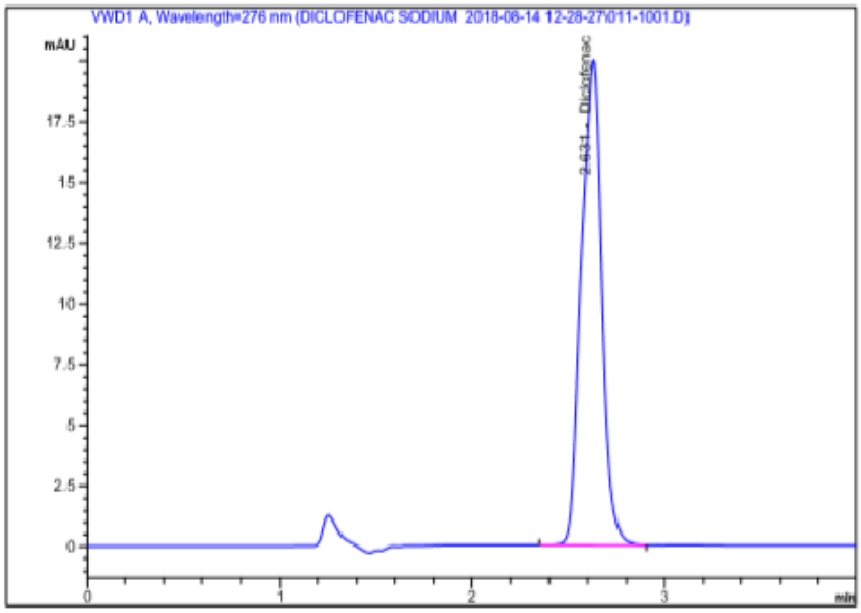

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Customized Report: Performance Report per Signal
This report template has been designed for uncalibrated methods.

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Available Signals:
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Figure 5. 1: *Chromatograph of diclofenac sodium solubility study in phosphate buffer pH= 7.4 using HPLC.*

```
==========================================================================
Acq. Operator    : Ramsi Muqedi                        Seq. Line : 11
Acq. Instrument  : Instrument 1                        Location  : Vial 12
Injection Date   : 8/14/2018                           Inj       : 1
Injection Time:    3:04:36 PM              Inj. Volume  (Method): 10  µl
                                  Act. Inj. Vol. from Sequence: 10  µl

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Method Info:
Diclofenac Sodium

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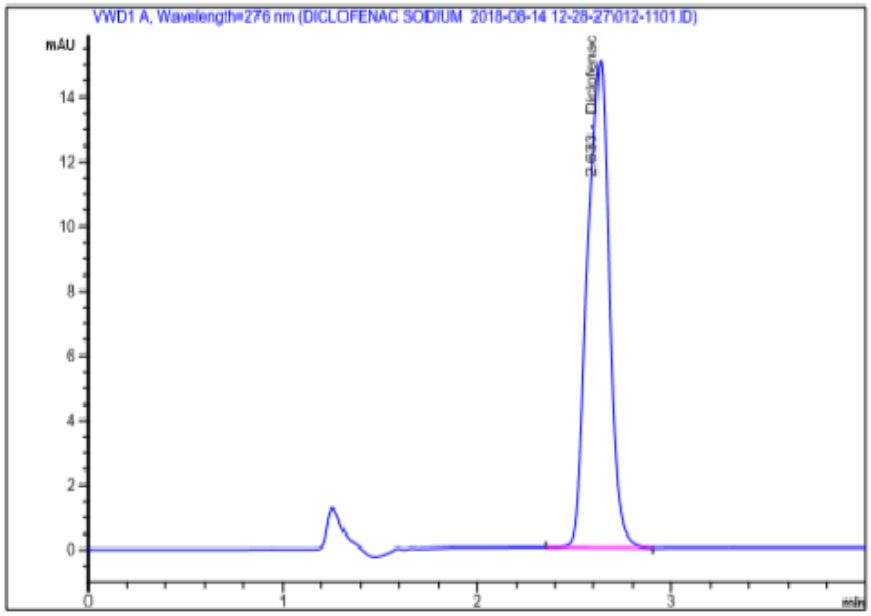


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Available Signals:
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Figure 5. 2: *Chromatograph of diclofenac sodium solubility study in IPA using HPLC.*

```
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                                  Act. Inj. Vol. from Sequence: 10  µl

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Method Info:
Diclofenac Sodium
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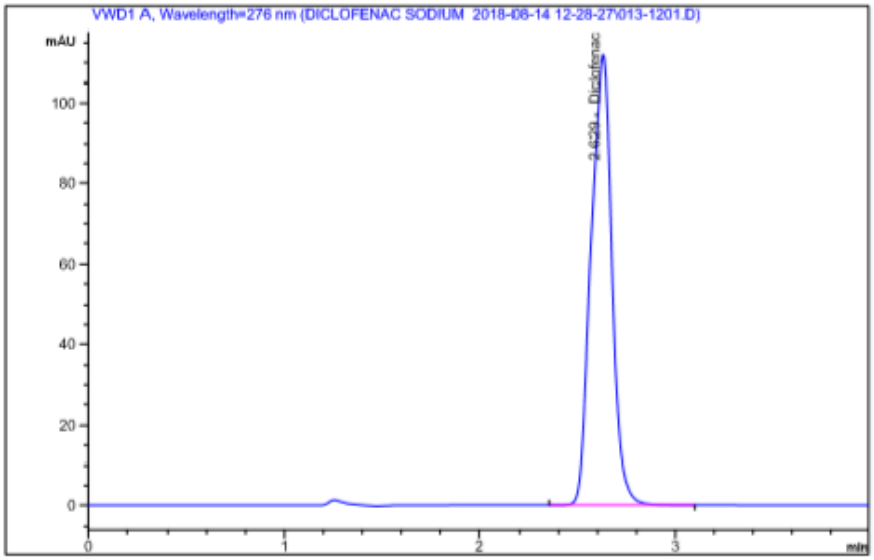


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Available Signals:
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Signal:   VWD1 A, Wavelength=276 nm

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[min]    Name      [mAU*s]   Width [min] k`     Tail.
-------|---------|----------|----------|-----|-------|-------|---------
2.629  Diclofenac     832.940    0.119          0.989  2683
```

Figure 5. 3: *Chromatograph of diclofenac sodium solubility study in PG using HPLC.*

Data File: C:\CHEM32\1\DATA\DICLOFENAC SODIUM 2018-08-14 12-28-27\014-130->
Sample Name: SA- Solubility F1

Acq. Operator : Ramzi Muqedi Seq. Line : 13
Acq. Instrument : Instrument 1 Location : Vial 14
Injection Date : 8/14/2018 Inj : 1
Injection Time: 3:37:45 PM Inj. Volume (Method): 10 µl
Act. Inj. Vol. from Sequence: 10 µl

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DICLOFENAC SODIUM.M
Analysis Method : C:\CHEM32\1\METHODS\DICLOFENAC SODIUM\DICLOFENAC SODIUM.M
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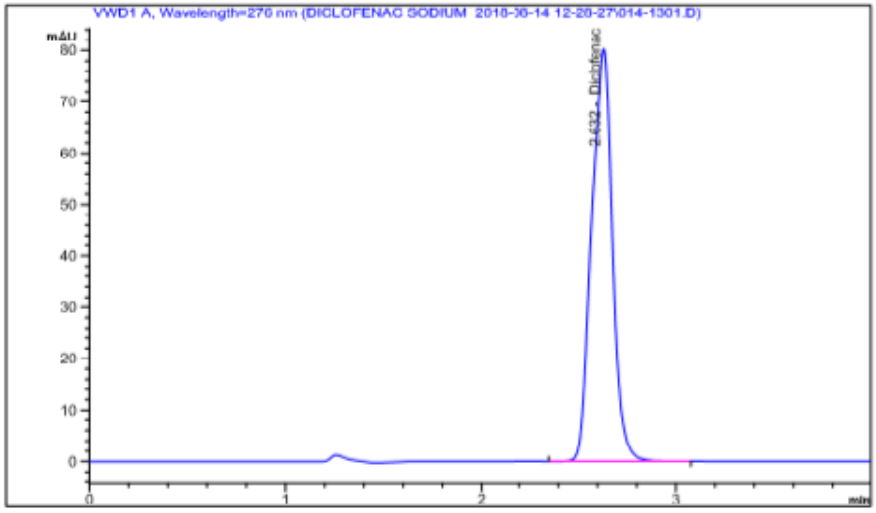
Method Info:
Diclofenac Sodium



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Available Signals:
VWD1 A, Wavelength=276 nm

Signal: VWD1 A, Wavelength=276 nm

RetTime [min]	Name	Area [mAU*s]	Halfh. Width [min]	k'	USP Tail.	Plates	Resolution
2.632	Diclofenac	588.706	0.118		0.969	2774	

Figure 5. 4: *Chromatograph of diclofenac sodium solubility study in Formula no. Fa using HPLC.*

Options

Data File: C:\CHEM32\1\DATA\DICLOFENAC SODIUM 2018-08-14 12-28-27\015-140->
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Injection Time:	8:54:20 PM	Inj. Volume (Method):	10 µl
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Method Info:
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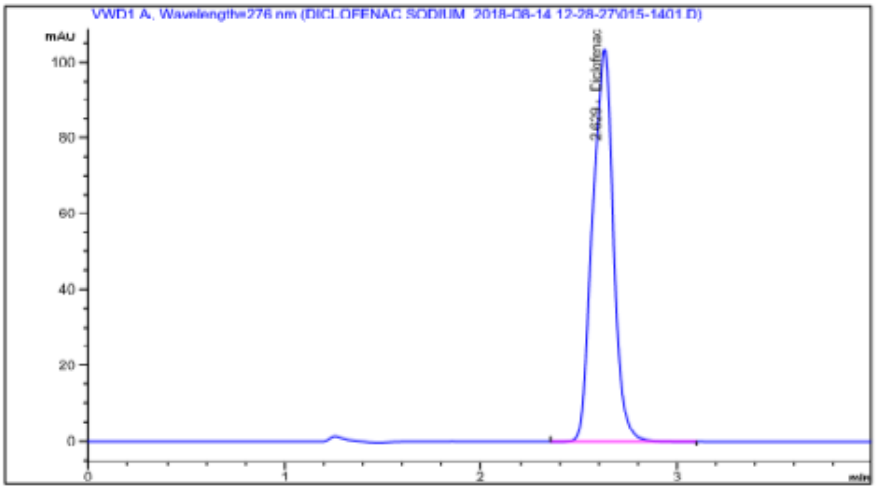

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Available Signals:
VWD1 A, Wavelength=276 nm

Signal: VWD1 A, Wavelength=276 nm

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2.629	Diclofenac	760.475	0.118		0.989	2770	

Figure 5. 5: *Chromatograph of diclofenac sodium solubility study in Formula no. Fb using HPLC.*

Options

Data File: C:\CHEM32\1\DATA\DICLOFENAC SODIUM 2018-08-14 12-28-27\016-150->
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Injection Time: 4:10:58 PM Inj. Volume (Method): 10 µl
Act. Inj. Vol. from Sequence: 10 µl

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Method Info:
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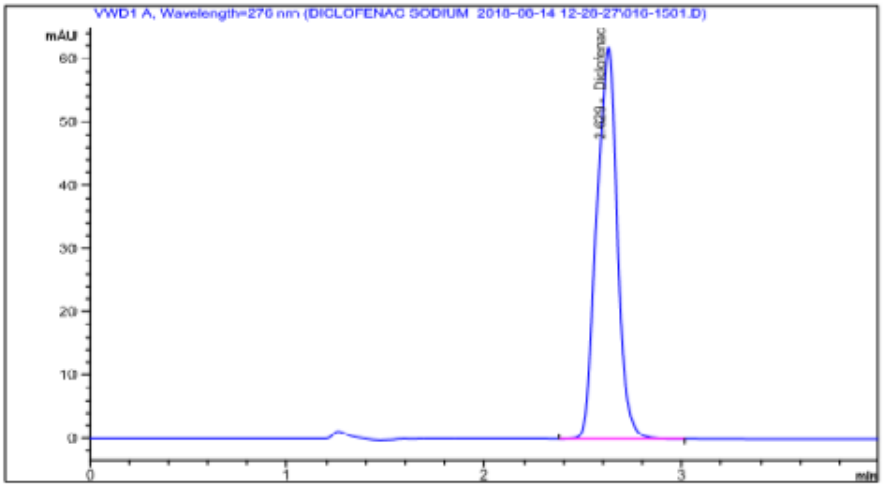

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Customized Report: Performance Report per Signal
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Available Signals:
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Signal: VWD1 A, Wavelength=276 nm

RetTime [min]	Name	Area [mAU*s]	Halfh. Width [min]	k'	USP Tail.	Plates	Resolution
2.629	Diclofenac	451.031	0.116		0.980	2823	

Figure 5. 6: *Chromatograph of diclofenac sodium solubility study in Formula no. Fc using HPLC.*

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تحضير رذاذ صوديوم الديكلوفيناك الموضعي والتحقق من تأثير معززات الاختراق على معدل نفاذية الدواء.

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ملخص

يعتبر ديكلوفيناك الصوديوم مثالاً على مضادات الالتهاب غير الستيروئيدية التي لها نشاط مسكن وخافض التي للحرارة ومضاد للالتهابات ممتاز. يمنع تخليق البروستاجلاندين من خلال تثبيط إنزيمات الأكسدة الحلقية تساهم في الألم والالتهابات. الطريق الرئيسي لتناول الديكلوفيناك صوديوم هو عن طريق الفم ، كما أنه يدار موضعيًا ، عن طريق الوريد ، العضل ، داخل العضل والمستقيم. موضعيا ، يتم استخدامه لتخفيف الأعراض من آلام خفيفة إلى معتدلة والتهاب مناطق الجلد الصغيرة والمتوسطة.

كان الهدف من هذه الرسالة هو تطوير رذاذ موضعي للديكلوفيناك واستقصاء تأثير معززات الاختراق المختلفة على معدل تغلغلها.

تم تحديد قابلية ذوبان ديكلوفيناك الصوديوم في محاليل مختلفة. تم العثور على أعلى قابلية للذوبان في

بقيمة 29.97 جم / 50 مل من البروبايلين جلايكول.

في المرحلة الأولى من التجربة ، تم تحديد معلمات الانتشار لمحلول رذاذ الصوديوم ديكلوفيناك (4٪ وزن / وزن) المسوق الذي تم استخدامه كعنصر تحكم في المراجع. في المرحلة الثانية من التجربة ، تم استخدام RLD ومقارنتها مع لاختبار تخلل الصوديوم ديكلوفيناك من (Start-M® غشاء) غشاء صناعي متقدم يحاكي خصائص جلد الإنسان محلول الرش الأمثل.

تم إجراء دراسة داخل المختبر باستخدام خلية فرانز للنشر ، في الجزء الأول ، كان الغشاء مكونًا من طبقة واحدة من غشاء مرشح مادة البولي أميد المنقوع في الأوكتانول وموجود بين طبقتين من غشاء آخر الذي كان غارقًا في فوسفات درجة الحموضة 7.4 ؛ تمتلئ مقصورة الاستقبال مع درجة الحموضة الفوسفات العازلة = 7.أما المقصورة العليا فكانت تحتوي 2 غرام من المحاليل المراد فحصها تفصل المقصورتين الغشاء المجهز مسبقا. في الجزء الثاني ، تم استخدام لفصل المقصورات الغشاء الآخر.

تم أخذ عينات من حجم 1 مل من مقصورة الاستقبال بعد نصف ساعة وكل نصف ساعة في وقت لاحق لمدة تصل إلى ست ساعات لكل عينة تجريبية. تم تقدير كمية ديكلوفيناك الصوديوم باستخدام

نانومتر 276 = λ عند HPLC

كان محسن الاختراق قيد التحقيق:

بيرول (1 ٪) ، توين 80 (0.1 ٪) -M، (0.25 ٪) المنثول (1 ٪) ، أوكالبتول -L، (0.25 ٪) ليمونين وحمض الأوليك (0.5 ٪). تمت إضافتهم بتركيزات مختلفة إلى 4٪ من محلول ديكلوفيناك الصوديوم في حجرة ER تم استخدام نسبة التعزيز .K ، و P، D، TLالمانحين. كانت معلمات الانتشار التي تم تحديدها تراكمية ، :لزيادة في ترتيب ER كمعيار لاختيار أفضل مُحسِّن للاختراق. تم العثور على

Limonene> L-Menthol> Eucalyptol> Limonene + L-Menthol> M-pyrol> Tween> Oleic acid.

، بما في IPA ، والمياه النقية و PG صيغت تجارب مختلفة لتطوير محلول الرش باستخدام كميات مختلفة من .لزيادة اللزوجة PVP ذلك 4 ٪ من ديكلوفيناك الصوديوم و 0.5 ٪ إلى 1.5 ٪ من

أظهر محلول رش ديكلوفيناك المحتوي على 4٪ من ديكلوفيناك الصوديوم ومحسن الاختراق المحدد (الليمونين) نسبة تعزيز أعلى بكثير من الصيغ الأخرى المختبرة. تم اختبار الصيغة النهائية مع محسن الاختراق المحدد اختراقًا جيدًا من خلال الغشاء المتقدم مع معامل نفاذية تبلغ API أظهر .®Start-M للاختراق من خلال غشاء .0.1610 سم / ساعة