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**Preparation of topical Orphenadrine citrate and
investigating the Effect of different penetration enhancers on
drug permeation rate**

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investigating the Effect of different penetration enhancers on
drug permeation rate**

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Finally I thank everyone who helped me and encouraged me through this research.

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:

Moammal Salah E-deen Qurt

Date: 08/2/2009

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List of Abbreviations

API	Active pharmaceutical ingredient
AR	Analytical reagent
BP	British pharmacopeia
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
CMC	Carboxymethyl cellulose
C_r	Concentration in the receiver compartment
C_{sa}	Concentration of sample
D	Diffusion coefficient
ER	Enhancement ratio
h	Membrane thickness
HPLC	High performance liquid chromatograph
HPMC	Hydroxylpropyl methyl cellulose
K	Partition coefficient
m	The amount of material
MFDC	Modified Franz diffusion cell
MP	Methyl paraben

O.C	Orphenadrine citrate
P	Permeability coefficient
PE	Penetration enhancers
PG	Propylene glycol
RPM	Rounds per minute
r_{sa}	HPLC reading of sample
r_{st}	HPLC reading of standard
S	Area
SLS	Sodium lauryl sulfate
SM	synthetic membrane
T	Time
T_L	Lag Time
U.V.	Ultraviolet
USP	United states pharmacopeia
x	The distance in cm of movement perpendicular to the surface of the barrier

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Abstract

Orphenadrine citrate is a muscle relaxant that is marketed as tablets and ampoules, topical preparations are promising due to the ease of application and acceptability by the patients, but they are not effective because they do not penetrate the skin well.

The aim of this thesis is to investigate the effects of some penetration enhancers on permeation of newly developed orphenadrine citrate gel, as a model of semisolid topical muscle relaxant preparation.

In the first phase the experiment diffusion parameters of (1% w/v) orphenadrine citrate (OC) alone in aqueous solution was specified and used as a reference control to measure the effects of different penetration enhancers on its permeability. In the second phase, gel formulations with 1% OC were used as a reference to measure the effects of the best PE on its permeability in gel preparation, also the effect of viscosity, and type of gelling agent on drug permeation rate were studied. In the third phase a human skin was used to test the permeation of OC from the selected gel-penetration enhancer system.

Modified Franz diffusion cell was used in this in-vitro study. In the first and second phases the membrane was composed of octanol soaked filter membrane sandwiched between two layers of dialysis membrane. The receiver is filled with phosphate buffer pH 7.4. The donor compartment contained 5ml solution of (OC) in aqueous phase and 5 g in gel phase. In the third phase, the stratum corneum of fresh and healthy skin sample obtained from a 42-years old female was used to separate the receiver and donor compartments.

Samples of 1 ml volume were taken from the phosphate buffer at the receiver compartment after the first hour, and every half an hour later on up to (6) hrs for each experimental sample. OC was quantified by using HPLC at $\lambda = 220$ nm.

The solubility of orphenadrine citrate was determined in water and in phosphate buffers with different pHs. The highest solubility was found in phosphate buffer pH 7.4 with a value of 2.43 g/100ml.

The compatibility of orphenadrine citrate was tested with different excipients (HPMC, CMC Na, PG, MP, Tween 80, Cremophore, SLS, Urea, carbapol, Oleic acid, glycerin) for three months at room temperature and 40 °C. It was found that OC is compatible with all excipients except with carbapol and SLS, while Urea was compatible at room temperature only. Also O.C showed stability over a wide range of pH values.

The penetration enhancers (PE) under investigation were Cremophor RH40, Tween 80, Sodium Lauryl Sulphate, Propylene Glycol, β -Cyclodextrin, and Urea. They were added in 1% concentration to 1% OC in the donor compartment. Diffusion parameters determined were cumulative amount, slope and intercept of cumulative amount which is plotted versus time, T_L , D, P, and K. The (ER) was used as criteria for selecting the best penetration enhancer. The enhancement ratio of the penetration enhancers studied in aqueous phase has been found to increase in the order of: PG < Tween80 < Cyclodextrin < Urea < Cremophore RH40.

Different trials of gel were formulated using HPMC and CMC Na as gelling agent including 1% OC and 0.2% to 1.0% of the selected penetration enhancer. The study was conducted using the same MFDC and analytical method.

The HPMC gel containing 1% API and the selected penetration enhancer (Cremophor RH40) showed significant higher enhancement ratio than the CMC Na. gel, and as the PE concentration increased the ER increased for both types of gelling agents formulas.

The final gel formula with the optimal concentration of penetration enhancer was then tested for permeation through healthy human skin. The API showed good penetration through the skin with a permeability coefficient of 0.0009 cm/hr.

Part one

Introduction

Introduction:

Drug delivery through the skin to the systemic circulation provides a convenient and effective route of administration of drugs. However the skin and particularly the stratum corneum resist the transfer of drug from the surface of skin towards the systemic circulation. The Stratum corneum is a horny layer composed of dead cells with a thickness of about 10 μm . It is a multi-layered “brick and mortar” like structure of keratin rich corneocytes(bricks) in an intercellular lipid matrix(mortar). It is markedly hygroscopic; it swells to about three times its original thickness when immersed in water. It functions as a protective chemical and physical barrier and it is only slightly permeable to water, also it retards water loss from underlying tissues (Benson, 2005), (Abate, 2005).

One of the major challenges that faces the scientists today is formulating drugs that penetrate the skin rapidly without inducing significant irreversible alterations to the skin barrier. An approach to do that is by using penetration enhancers, chemicals that interact with the skin constituents to promote drug penetration (Kreilgaard, 2002), (Williams, Barry, 2004)

The mechanism of action of these penetration enhancers on the stratum corneum is either to disrupt the stratum corneum intercellular lipid matrix or by increasing the partitioning of drug into the stratum corneum. In a case where the drug has a high affinity to a solvent and the solvent has a high penetrability to the skin then the portioning into and permeation through the skin can be increased by a solvent drug mechanism in which the drug and the solvent permeate together. (Abate, 2005), (Kamil 2006).

The intercellular lipid matrix exists as continuous lipid phase; occupying about 20% of the stratum corneum and arranged in multiple lamellar liquid crystal structures, with hydrocarbon chains aligned, and polar head groups dissolved in aqueous layer. It resembles the major pathway for drug penetration through the skin (Walters, 2002), (Bouwstra, J, Honeywell-Nguyen,).

That is the hydrophilic substances diffuse through the hydrophilic region, while the hydrophobic substance diffuses through the hydrophobic region. (Benson, 2005)

1.1. Human Skin:

The skin is the largest and heaviest organ of the body accounting for more than 10% of the body mass, with an average area of 2m^2 , it consists of four layers (figure1 .1), the stratum corneum(non viable epidermis), the remaining layers of the epidermis(viable epidermis), dermis, and subcutaneous tissue. There are also several associated appendages: hair follicles, sweat ducts, and nails.(Walters 2002),((Abate, 2005))

The protective homeostatic role of the skin allows the survival of humans in the environment:

- 1- It protects the skin from chemicals, bacteria, allergins, fungi and radiations(Walters, 2002)
- 2- It is responsible for heat regulation, and blood pressure control Bouwstra, J, Honeywell-Nguyen, P. (2002)
- 3- It protects the body against loss of endogenous material (Bouwstra, Honeywell-Nguyen,2002)
- 4- It forms a barrier to the external environment maintaining body fluids and excluding harmful substances(Yamashita, Hashida, 2003)
- 5- It is considered a site of administration of drugs for topical and systemic therapy(Yamashita, Hashida,2003)

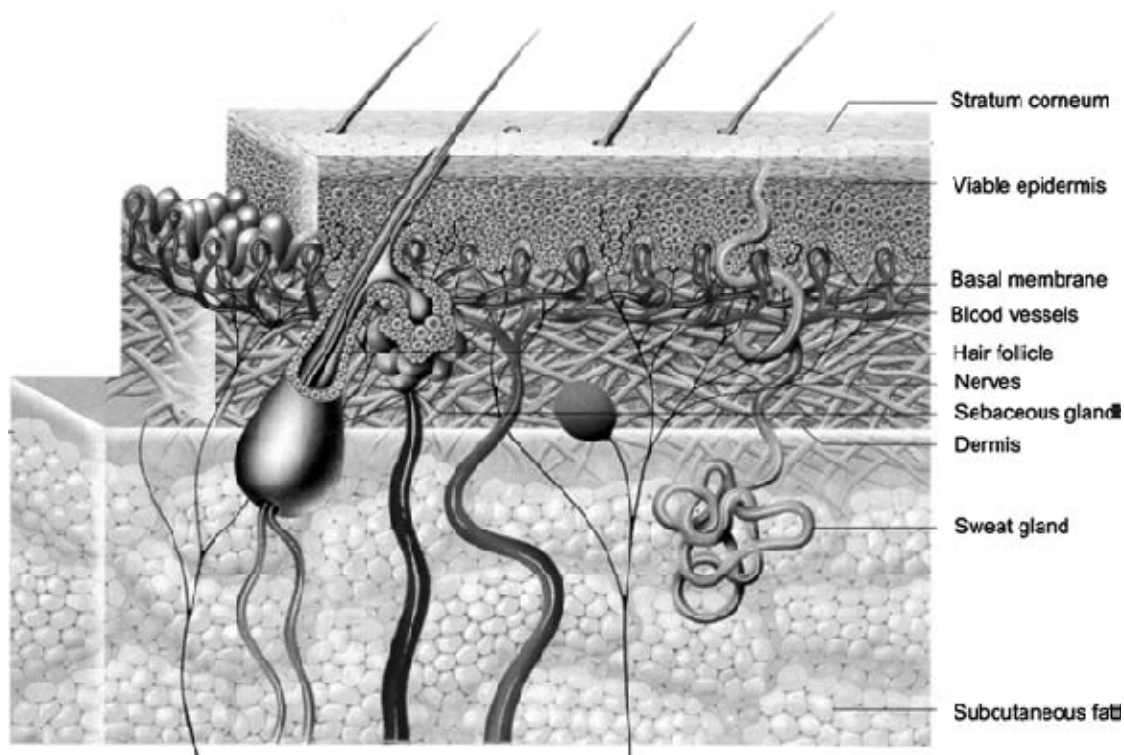


Figure 1.1: Skin structure(Bronaugh,, Maibach, 2005)

1.2. Stratum corneum

The stratum corneum, the outermost layer of epidermis(figure 1.2) , is the primary barrier against permeation of topically applied drugs((Yamashita, Hashida,2003)). It is approximately 10-20 μ m thick(Walter, 2002)

It consists of keratin filled dead cells(the corneocytes) which are entirely surrounded by crystalline lamellar lipid region. The cell boundary , the cornified envelope is, a very densely cross linked protein structure which reduces absorption of drugs into the cell. For This reason most of the active substances applied onto the skin are diffusing along through the lipid lamellae in the intercellular region (Bouwstra, Honeywell-Nguyen, 2002). Its now well recognized that most solutes enter the body through the less than 0.1 μ m –wide intercellular region of the stratum corneum(Walter, 2002): The cells of stratum corneum originate in the

viable epidermis and undergo many morphological changes before desquamation. (Walter, 2002)

The basal layer is composed of (1-2) cell layers, the stratum spinosum (2-7) cell layers, and the stratum granulosum 2-3 cell layers, while the stratum corneum have more than 25 cell layers (Forslind, Lindberg, 2004)

In the basal layer cells proliferate, upon leaving the basal layer cells starts to differentiate and migrate in the direction of the skin surface. At the interface between stratum granulosum and stratum corneum (figure 1.3) final differentiation occurs, during which the viable cell is transformed into dead keratin filled cells (corneocytes).

Epidermal differentiation is a major events that includes extrusion of lamellar bodies, loss of nucleus, and increasing amount of keratin in the stratum corneum (Riviere, 2006).

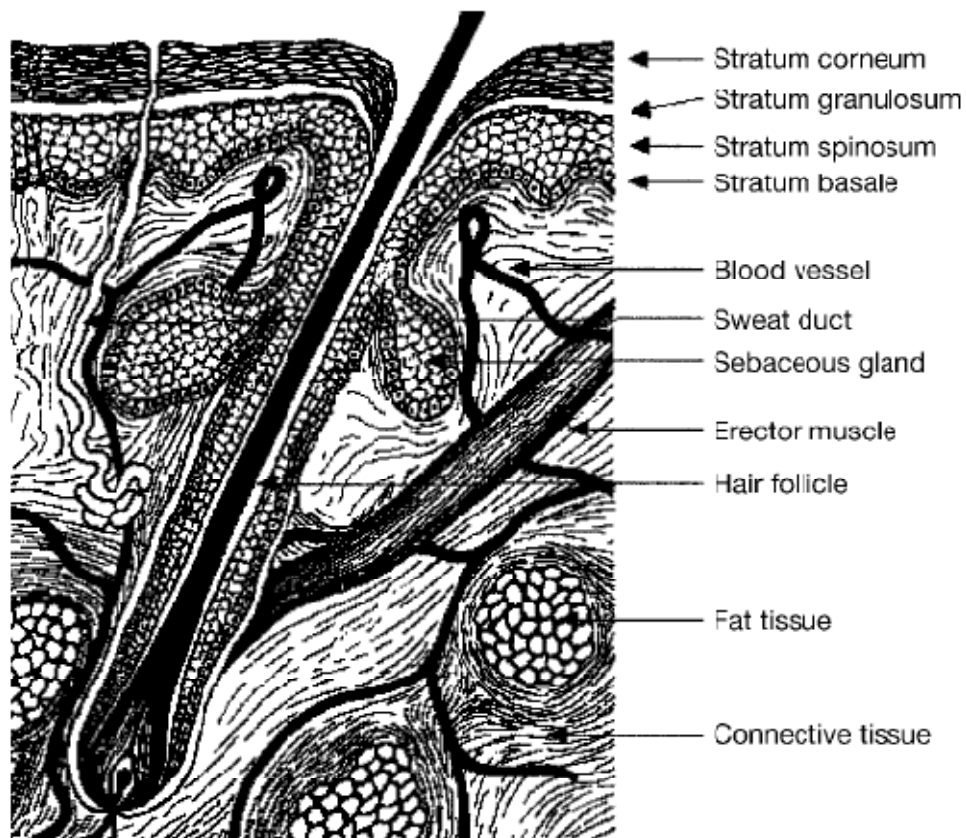


Figure 1.2: Stratum corneum (Walter, 2002)

The Top layer of the skin , The stratum corneum is important for the appearance and function of the skin, it serves as a protective physical and chemical barrier and it is only slightly permeable to water(Abate, *et al* , 2005). It has an average turnover of approximately three weeks. (Walter, 2002)

Hydration of the stratum corneum can lead to profound changes in its barrier properties, it induces swelling of the corneocytes and the expansion of intercellular lipid lamella. Stratum corneum water content can be increased from 15-20% up to 400% of dry weight. The structure of the stratum corneum has been likened to “bricks-and-mortar”, where the bricks are the component cells, or corneocytes, and the mortar is the intercellular lipids (Walter, 2002)

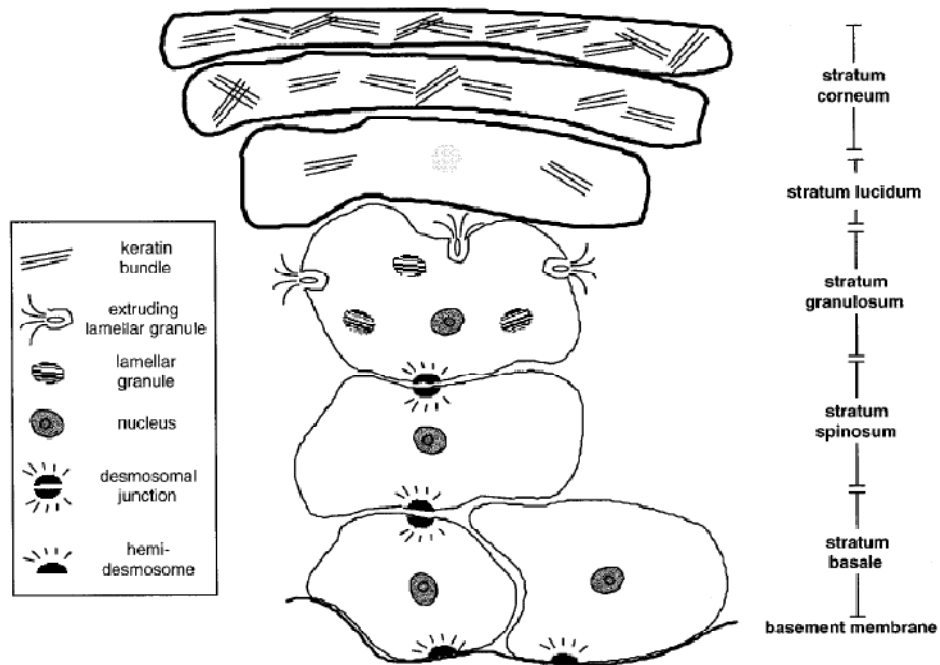


Figure 1.3: Morphological changes of stratum corneum cells (Benson, 2005)

1.3. Dermis

The dermis is a connective tissue (Ranade, Hollinger, 2004) which is a critical component of the body that provides nutritive, immune and support for the epidermis, and play a role in temperature pressure and pain regulation. It is about 0.1-0.5 cm thick consisting of about 70% of collagenous fibers providing Cushioning and elasticity. The blood flow rate to the skin is about $0.05 \text{ ml min}^{-1} \text{ cc}^{-3}$ (Walter, 2002)

The dermis apparently is a gel structure, a fibrous protein(collage, elastin) matrix embeded in an amorphous colloidal ground substance. Blood vessels , lymphatics are within dermis. (Abate, *et al* 2005)

1.4. Subctaneous

The sub cutaneous fat layer serves as a cushion for the dermis and epidermis, and it is important for insulation. Figure 1.4. summarizes the function of the skin(Walter, K. 2002)

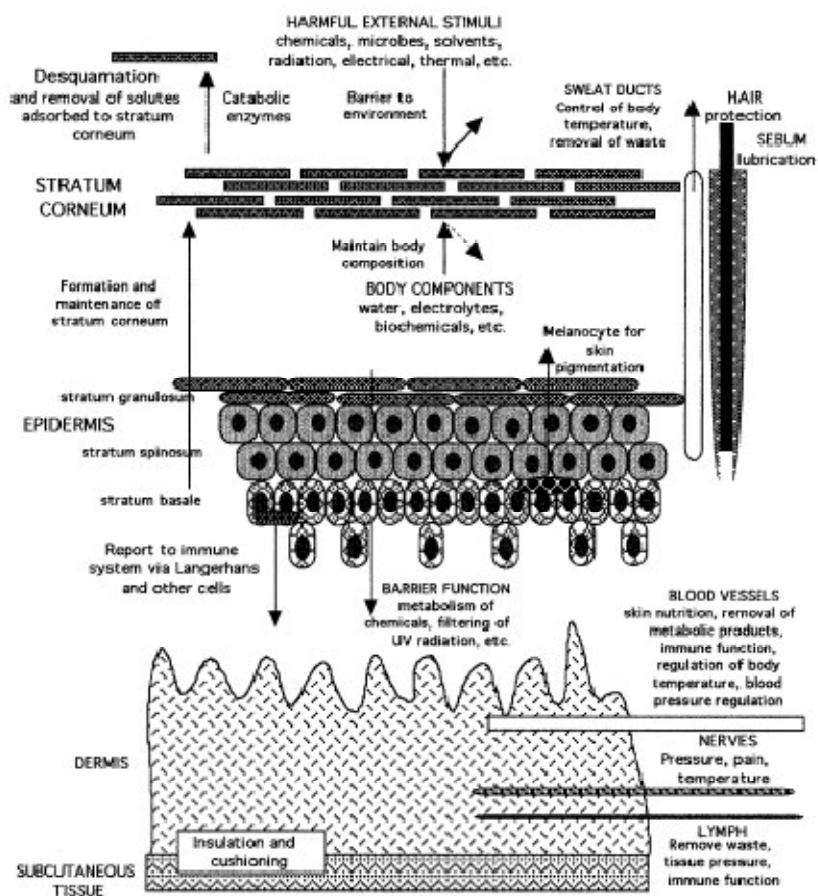


Figure 1.4: Summary of Skin structure and Function(Walter, K. 2002)

1.5. Intercellular lipids

The intercellular region consists mainly of lipids and desmosomes for corneocyte cohesion(Walter, K. 2002). The composition of the stratum corneum lipids strongly differs from that of cell membranes of living cells. Major lipids are Ceramides(CER), Cholesterol(CHOL), and free fatty acids (See figure 1.5). The length of acyl chain of CER varies between 16-33 carbons. While fatty acids chain are mostly C22 and C24 (Bouwstra, Honeywell-Nguyen, 2002).

The intercellular lipids occupy 20% of the Stratum corneum. It is composed of Cholesterol(27%), ceramides(41%), free fatty acids(9%), cholesteryl esters (10%) and cholesteryl sulfate 2%. Ceramide 1-8 with 1 is the least polar (Stabilizer of the intercellular lipids). (Walter, K. 2002).

Creation of free space (free volume) in the intercellular lipid lamellae of the stratum corneum allows a greater mobility of the hydrocarbon chains that may result in enhanced diffusivity. This could be induced by enhancer shape for example, oleic acid and laurocapram (Azone)] or by electrostatic head group interactions. (Walter, K. 2002).

The combination of the long chain soaps and carboxylic acids spontaneously form a layered structure when combined with water in certain portions. Water soluble compounds dissolve in the aqueous layer while lipid compounds dissolve in the hydrocarbon region of the structure (Friberg, *et al*, 1987).

The intercellular lipids matrix is generated by keratinocytes in the mid to the upper part of the stratum granulosum discharging their lamellar content into the intercellular space. (Benson, 2005) In the initial layers of the stratum corneum this extruded material rearranges to form broad intercellular lipid lamella which then associates into lipid bilayers, with the hydrocarbon chains aligned and polar head groups dissolved in aqueous layer. (figure 1.5).

The hydrocarbon chains are arranged in region of crystalline, lamellar gel and lamellar liquid crystal phases by creating various domains within the lipid bilayers. Water acts as a plasticizer to prevent cracking of the stratum corneum. (Benson, 2005)

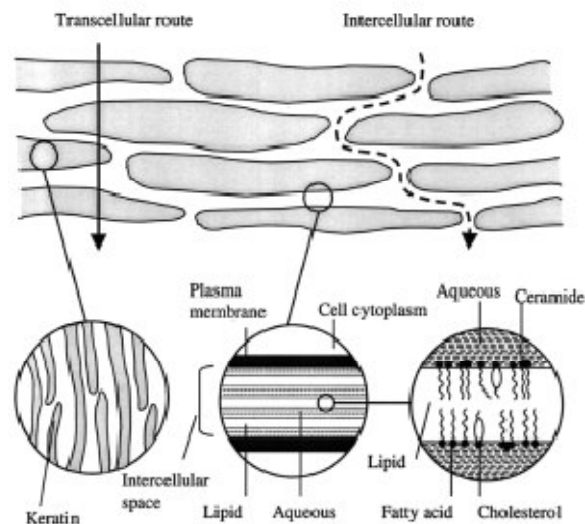


Figure 1.5: Intercellular lipids. (Moser, *et al*, 2001)

1.6. Transdermal Permeation

Transdermal permeation, or percutaneous absorption, can be defined as the passage of a substance, such as a drug, from the outside of the skin through its various layers into the bloodstream, preferably at a specific rate (Ranade, Hollinger, 2004), (Walker, Smith, 1996). The exposure of the skin to a dosage form creates a concentration difference which the active ingredient must partition, followed by diffusion of the compound through the external strata to the dermis. (Walker, Smith, 1996), (Cevec, *et al*, 1996)

The barrier properties of the Stratum corneum are now recognized the major rate-limiting step in the diffusion process of a drug permeating across skin (Walter, 2002).

Despite the small number of drugs delivered through the transdermal route, it is estimated that the worldwide market revenues of this route are about three billion dollars (Benson, 2005)

Transport across the stratum corneum is largely a passive process, and thus the physicochemical properties of a permeant are an important determinant of its ability to penetrate and diffuse across the membrane. Once it has penetrated through the epidermis, a compound may be carried away by the dermal blood supply or be transported to deeper tissues (Walter, 2002).

Transdermal permeation can be attained by the combination of appropriate solute, properties for skin with appropriate dosage form design (e.g., patches, gels, creams, ointments). (Walter, 2002)

Advantages of transdermal absorption:

- 1- Avoid the problem of stomach emptying, pH effects, enzymes(drug deactivation).
(Walter, 2002)
- 2- Avoid hepatic first pass effect (Walter, 2002)
- 3- Ability to discontinue administration by removal of the system (Kanikkannan, *et al*, 1999)

Difficulties of transdermal permeation (Walter, 2002):

- 1- Variability of transdermal absorption regarding to site of application

- 2- Skin metabolic effect
- 3- Irritation and toxicity to the skin

Disadvantages of transdermal delivery (Walter, 2002):

- 1- The main drawback being that not all compounds are suitable candidates for transdermal absorption
- 2- diffusion rate can differ between individuals(races, ages, skin conditions and diseases)
- 3- Diminished skin absorption may start to be observed with compounds of molecular weight above 500 Da.(Bronaugh, Maibach, 2005)

There are generally considered to be three routes by which compounds can diffuse across the SC (Walter, 2002):

- 1- Transcellular,
- 2- Intercellular
- 3- Transappendageal

1.6.1. Transcellular route

It was originally believed in the past that transcellular diffusion mechanisms dominated over the intercellular and transappendageal routes during the passage of solutes through the stratum corneum. However, the transport by the transcellular route would involve the repeated partitioning of the molecule between lipophilic and hydrophilic compartments, including the almost impenetrable corneocyte intracellular matrix of keratin. Most experimental evidence now suggests that transport through the stratum corneum is by the intercellular route (Walter, 2002)

1.6.2. Intercellular route

The intercellular stratum corneum spaces were initially dismissed as a potentially significant diffusion pathway because of the small volume they occupy. However, the physical structure

of the intercellular lipids was thought to be a significant factor in the barrier properties of the skin. Tracer studies provided evidence that the intercellular lipids are the main epidermal permeability barrier

Two key determinants for a solute crossing a membrane are solubility and diffusivity. The relative solubility of a solute in two phases determines its partition coefficient and therefore, the likelihood of the solute being taken up into the Stratum corneum from a vehicle. Also, solubility will determine whether a solute is likely to be desorbed from the Stratum corneum into deeper layers. The diffusivity is a measure of the speed at which a solute crosses.

In the first step of the transport process, molecules must be in solution in the vehicle to partition from the vehicle into the lipids in the outermost part of the SC; they must then diffuse through it; partition back out of the Stratum corneum and into the viable epidermis. Next, molecules diffuse through the viable epidermis and papillary dermis.

At the capillary plexus a high percentage of molecules are transferred into the circulating blood and a lower percentage diffuses into deeper tissues. (Walter, 2002)

The physicochemical properties of the diffusing drug and the vehicle (e.g., molecular size, stability, binding affinity, solubility, and partition coefficient),(Ranade, Hollinger, 2004), (Benson, 2005) influence permeation across the stratum corneum and thereby optimize the delivery.

The maximum skin penetration rate is obtained when a drug is in a supersaturated solution ((Benson, 2005). Also hydration of the skin cause swelling of the stratum corneum and decreases protein network density and diffusional pathway length.(can be done by occlusion) (Abate, 2005).

1.6.3 Appendageal route.

Appendageal route is limited to about 0.1% of total surface area of the skin (Barry, 2002) and include sweat ducts and hair follicles (Walter, 2002). Penetration through hair follicles is responsible for the presteady state permeation of polar molecules and the flux of large polar molecules and ions that have difficulty diffusing through the stratum corneum.

Majority of penetration enhancement techniques focus on increasing the transport of drugs through the stratum corneum due to its large surface area(Benson,2005)

1.7. Penetration enhancers

There are physical and chemical transdermal enhancement techniques. Physical penetration enhancement involves an externally applied force to augment the delivery of the target agent across the skin. Examples are iontophoresis (the use of a small electric current), sonophoresis (the use of ultrasound), and electroporation (the use of short-duration, high-voltage electric pulses). (Potts, Guy, 1997)

Another approach for improving transdermal drug delivery uses chemical penetration enhancers (also called sorption promoters or accelerants) which penetrate into skin to reversibly decrease the barrier resistance. (Williams, Barry, 2004). Penetration enhancers may disrupt the packing of the intercellular lipid matrix or may increase the drug partitioning into the tissue by acting as a solvent for the permeant within the membrane (Williams, Barry, 2004). See figure (1.6).

Desirable properties of penetration enhancers are (Williams, Barry, 2004).

- 1- They should be non-toxic, non-irritating and nonallergenic.
- 2- They would ideally work rapidly, and the activity and duration of effect should be both predictable and reproducible.
- 3- They should have no pharmacological activity within the body—i.e. should not bind to receptor sites.
- 4- The penetration enhancers should work unidirectionally, i.e. should allow therapeutic agents into the body whilst preventing the loss of endogenous material from the body.
- 5- When removed from the skin, barrier properties should return both rapidly and fully.
- 6- . The penetration enhancers should be appropriate for formulation into diverse topical preparations, thus should be compatible with both excipients and drugs.
- 7- . They should be cosmetically acceptable with an appropriate skin 'feel'.

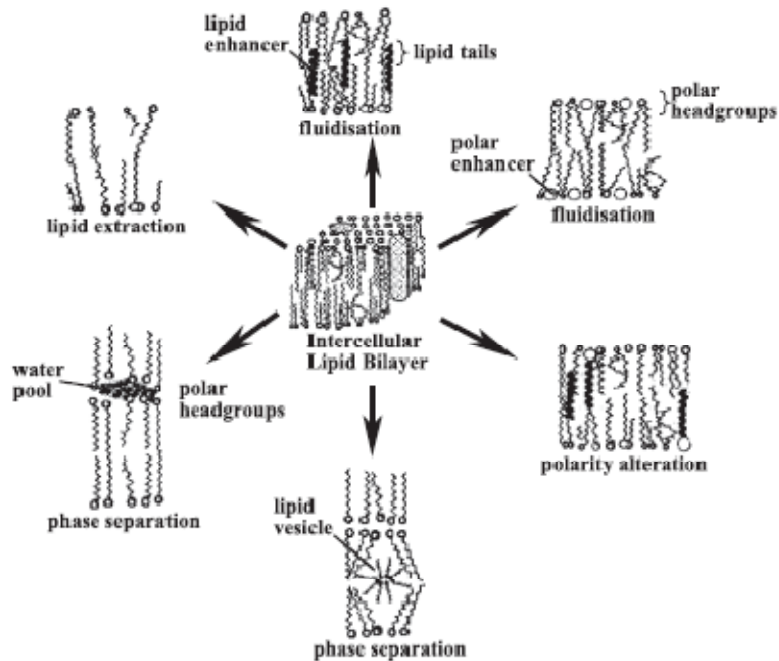


Figure 1.6: Disruption of intercellular lipids by penetration enhancers(Williams, Barry, 2004).

Many Penetration enhancers such as Azone, DMSO, Alcohols,, fatty acids and terpenes have been shown to increase permeability by disordering (fluidizing) the lipid structure of the stratum corneum.

Other penetration enhancers such as oleic acid , at high concentration, pool within the lipid domains to create permeable pores. In addition to their effect on stratum corneum lipids, urea and surfactants also react with keratin and may result in a disruption of order within the corneocyte (Smith, Maibach, 2006)

Urea promotes transdermal permeation by facilitating hydration of the stratum corneum and by the formation of hydrophilic diffusion channels within the barrier (Walker, Smith,1996).

A number of solvents increase permeant partitioning and solubility within the stratum corneum(Propylene glycol, ethanol) (Smith, Maibach, 2006).

Soaking the skin in water, exposing the membrane to high humidities or, as is more usual under clinical conditions, occluding the tissue so preventing transepidermal water loss can allow the stratum corneum to reach water contents in equilibrium with that of the underlying

epidermal skin cells. Thus, on occlusion, the water content of this outer membrane can approach 400% of the tissue dry weight. ((Williams, Barry, 2004).)

Freeze Fracture electron microscopy showed that fully hydrated stratum corneum shows that the intercellular lipid bilayers contain water pools with occasional vesicle-like structures (Touitou, Barry, 2007)

It is difficult to select rationally a penetration enhancer for a given permeant. Penetration enhancer potencies appear to be drug specific, or at best may be predictive for a series of permeants with similar physico-chemical properties (such as similar partition coefficients, molecular weights and solubilities). Some broad generic trends are apparent, such as the use of hydrocarbon monoterpenes for lipophilic permeants, but the level of enhancement expected for these agents is unpredictable. ((Williams, Barry, 2004).

1.8. Orphenadrine citrate

Orphenadrine citrate is the citrate salt of orphenadrine (2-dimethylaminoethyl 2-methylbenzhydryl ether citrate)(figure 1.7). It is a congener of diphenhydramine and a tertiary amine (Sweetman, 2007)

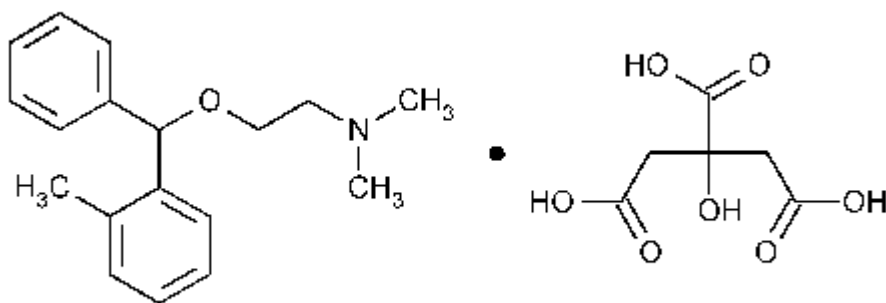


Figure 1.7: Chemical structure of Orphenadrine citrate

It occurs as a white, crystalline powder having a bitter taste. It is practically odorless; sparingly soluble in water, slightly soluble in alcohol. It melts at 136 °C (The Stationary office, 2005),Molecular weight of orphenadrine citrate is 461.5. The mode of therapeutic

action has not been clearly identified, but may be related to its analgesic properties. Orphenadrine citrate also possesses anti-cholinergic actions. (Abate, *et al* 2005)

It relieves the discomfort associated with acute painful musculoskeletal conditions by a mode of action not quite defined. It also has weak antihistaminic and local anaesthetic properties.

It is given by mouth in a dose of 100 mg twice daily or by intramuscular or slow intravenous injection in a dose of 60 mg which could be repeated every 12 hours (Thomson PDR, 2006)

Orphenadrine citrate has been used in some countries for the treatment of intractable hiccup (Sweetman, 2007)

The half-life of orphenadrine has been reported to be 14 hours (Sweetman, 2007)

Orphenadrine citrate should be used cautiously in patients with gastrointestinal obstruction.

Orphenadrine citrate is used as hydrochloride and citrate salts (Sweetman, 2007)

In one study orphenadrine citrate showed an anticholinergic effect directly on muscarinic receptors in chicken heart, rabbit lung, rabbit heart (Lazareno, *et al*, 1990). Orphenadrine citrate is a non competitive antagonist at NMDA receptor complexes. In addition the drug is an antagonist at histamine receptors H1 also it may act as antagonist at M1, M2,M3, M4 muscarinic acetylcholine receptors (Neurotransmitters.net, 2008)

1.9. Gels

Gels are semi solid systems in which a liquid phase is trapped within an interlocking three dimensional polymeric matrix of a natural or synthetic gum. A high degree of physical or chemical cross linking of the polymer is involved. Gelling agents include Carboxymethyl cellulose(Banker, Rhodes, 2002), HPMC gelling agent (Mohamed, 2004). Gels have favorable properties such as being greaseless, easily spreadable, easily removable, emollient, non staining, compatible with several excipients and water soluble or miscible, thixotropic (Mohamed, 2004). The preparation of gels can involve high temperature processing. It is easier to disperse methyl cellulose in hot than in cold water. The polymer then goes into solution and thickens or sets up as the temperature is lowered. (Banker, Rhodes, 2002). Gels can be investigated visually such as color , homogeneity, consistency, spreadability, and phase separation. (Mohamed, 2004).

In vitro dissolution method for topical dosage forms is based on an open chamber diffusion cell system such as Franz cell, fitted usually with a synthetic membrane. The test product is placed in the donor compartment of the diffusion cell and a sampling fluid is placed on the other side of the membrane in a receptor compartment. Diffusion of the product through the membrane is monitored by assay. (According to FDA) This guideline is the most commonly used method (Abate, et al, 2005).

1.10. In vitro diffusion cells and skin isolation

Most common methods for evaluation of in vitro skin penetration is using diffusion cells, The major advantage of in vitro investigations is that the experimental conditions can be controlled precisely, such that the only variables are the skin and the test material.

Diffusion equations that are applied to the in vitro situation make the following assumptions(Walter, 2002):

1. The receptor phase is a perfect sink.
2. Depletion of the donor phase is negligible.
3. The membrane is a homogeneous slab.

A well-designed skin diffusion cell should (Walter, 2002)

1. Be inert
2. Be robust and easy to handle
3. Allow the use of membranes of different thicknesses
4. Provide thorough mixing of the receptor chamber contents
5. Ensure intimate contact between membrane and receptor phase
6. Be maintainable at constant temperature
7. Have precisely calibrated volumes and diffusional areas
8. Maintain membrane integrity
9. Provide easy sampling and replenishment of receptor phase
10. be available at reasonable cost

Franz diffusion cell (figure 1.8) is composed of (Kamil, 2006):

- 1- Donor compartment
- 2- Receiver compartment
- 3- Two sampling ports
- 4- Water jacket

To isolate the Stratum corneum from epidermal membranes, the latter is placed in trypsin solution (0.0001%), incubated at 37°C for 12 h, rubbed (with a cotton bud) to remove the epidermal cells, rinsed in distilled water, and air-dried on a surface from which they can be easily removed.

In vitro skin diffusion experiments are normally conducted with a skin temperature of 32°C (the in vivo value of the human skin) (Walter, 2002)

As human skin is difficult to obtain. Several membranes were used to simulate skin, including cellulose acetate, silicon, egg shell membrane but all these membranes are not as complex as human skin. (Aulton, 1996).

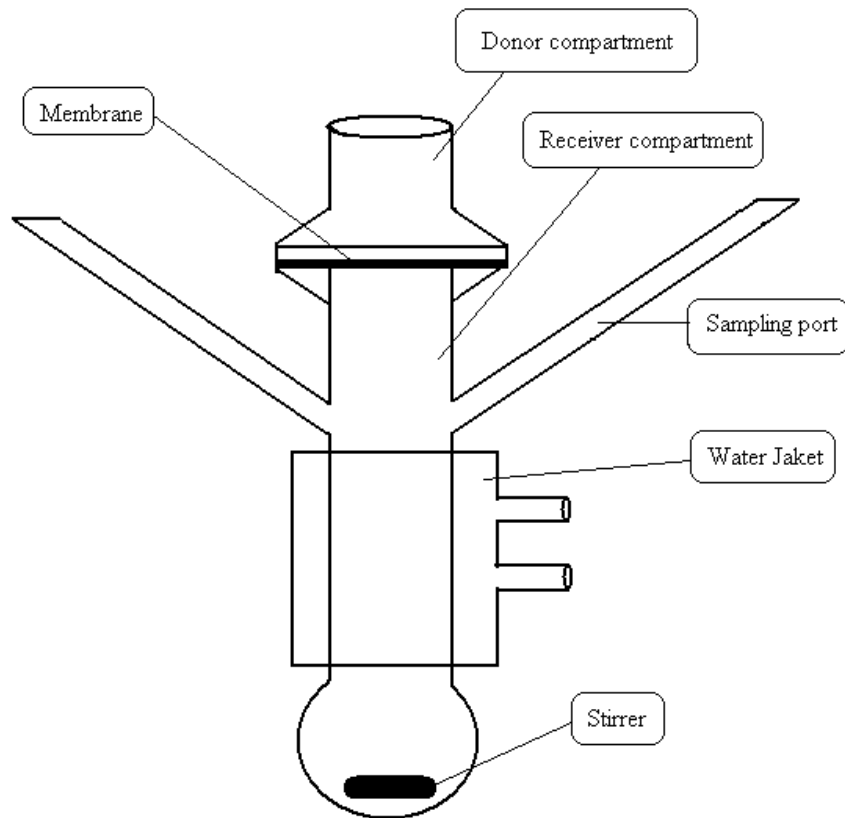


Figure 1.8: Modified Franz diffusion cell

1.11. Diffusion Theory

Simple diffusion laws can be used to describe the percutaneous absorption process (Guy, Hadgraft, 2003)

Diffusion is defined as a process of mass transfer of individual molecules of a substance, brought about by random molecular motion and associated with concentration gradient; the flow of a molecule through a membrane is a convenient way to study diffusion (Martin, *et al*, 1983).

Fick's first law :

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

$$J = \frac{dM}{S \cdot dt} \dots\dots\dots (1)$$

The flux in turn is proportional to concentration gradient, dC/dx:

$$J = -D \frac{dC}{dx} \dots\dots\dots (2)$$

In which D is the diffusion coefficient of a penetrant in cm²/sec, C its concentration g/cm³ and x is the distance in cm of movement perpendicular to the surface of the barrier, t usually in seconds and S in cm², the negative sign indicates that the diffusion is in the direction of decreasing concentration.

An equation for mass transport that emphasizes the change in concentration with time at a definite location rather than the mass diffusing across a unit area of a barrier in unit time is known as Fick's second law

The concentration of diffusant in volume element changes with time, ΔC/Δt as the flux or amount diffusing changes with distance ΔJ/Δx:

$$\frac{\partial C}{\partial t} = - \frac{\partial J}{\partial x} \dots\dots\dots (3)$$

Differentiating the first law expression, equation (2) with respect to x one obtains :

$$- \frac{\partial J}{\partial x} = D \frac{\partial^2 C}{\partial x^2} \dots\dots\dots (4)$$

Substituting ∂C/∂t from equation (3) into equation (4) results in Fick's second law:

$$\frac{\partial C}{\partial t} = D \frac{\partial^2 C}{\partial x^2} \dots\dots\dots (5)$$

Fick's second law states that the change in concentration with time in a particular region is proportional to the change in concentration gradient at that point in the system.

An important condition in diffusion is the steady state. Fick's first law, equation (2), gives the flux or rate of diffusion through unit area in the steady state of flow while the second law refers in general to a change in concentration of diffusant with time at any distance (equation (5)).

Originally the diffusate concentration will fall in the donor compartment and will increase in the receiver one until the system come to equilibrium.

If a membrane separates the two compartments of a diffusion cell of a cross sectional area S and thickness h , and if the concentrations in the membrane on the donor C_1 and on the receiver C_2 respectively (Figure 1.9, Fick's first law could be written :

$$J = \frac{dM}{S \cdot dt} = D \left(\frac{C_1 - C_2}{h} \right) \dots\dots\dots (6)$$

The concentrations C_1 and C_2 within the membrane ordinarily are not known but can be replaced by the partition coefficient multiplied by the concentration C_d on the donor side of the membrane or C_r on the receiver side as follows:

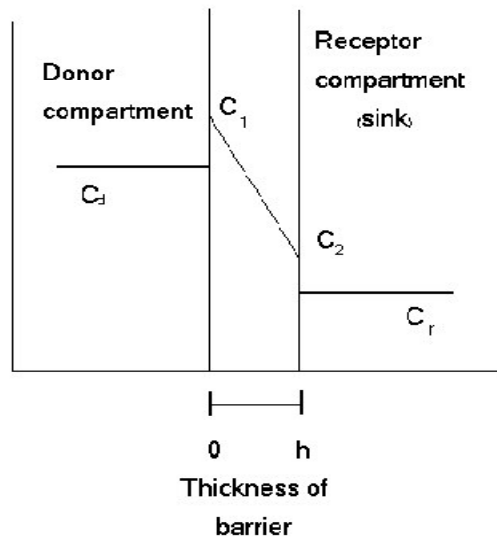


Figure 1.9: Donor and receptor compartments separated by a barrier.

$$K = \frac{C_1}{C_d} = \frac{C_2}{C_r} \dots\dots\dots (7)$$

Hence according to equation(6)

$$\frac{dM}{dt} = \frac{DSK (C_d - C_r)}{h} \dots\dots\dots (8)$$

And if sink conditions is maintained in the receptor then $C_r \approx 0$ then equation (8) becomes :

$$\frac{dM}{dt} = \frac{DSK C_d}{h} = PSC_d \dots\dots\dots (9)$$

In which

$$P = \frac{DK}{h} \text{ cm/sec} \dots\dots\dots (10)$$

Where P is the permeability coefficient.

To calculate diffusion parameters for a specific drug we use a diffusion cell and we put the drug solution in the donor phase separated by a membrane from the receptor phase ensuring that sink conditions is available, we take samples from the receiver phase at time intervals and we plot the curve(see figure 1.10).

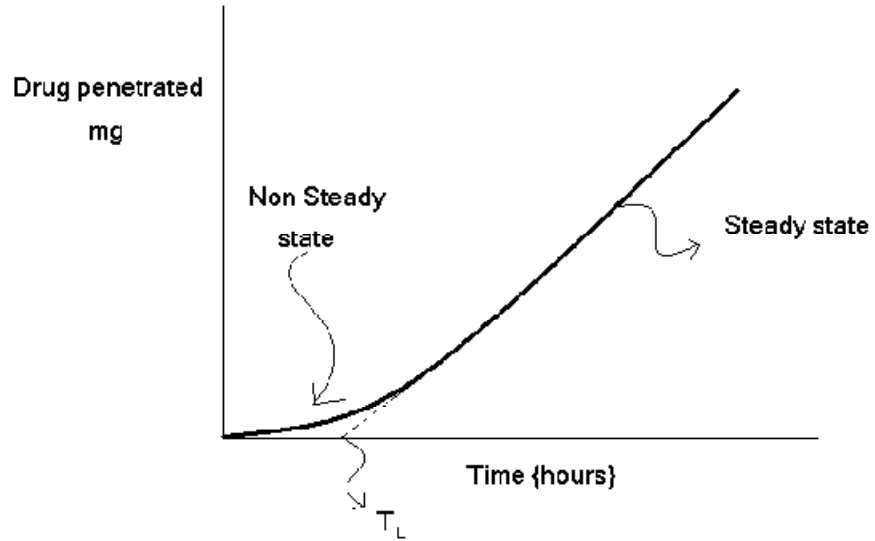


Figure 1.10: Cumulative amount of drug permeated through a barrier over a time period.

The slope of the steady state plot is dM/dt , the X intercept of the steady state line is T_L .

Going back to equation(9)

$$\frac{dM}{dt} = \frac{DSK C_d}{h} = PSC_d \dots\dots\dots (9)$$

One concludes that the slope of the plot = PSC_d

The lag time is given by the equation:

$$T_L = \frac{h^2}{6D} \dots\dots\dots (12)$$

Part two
Experimental

2.1. Introduction:

Orphenadrine citrate is an anti-cholinergic drug, It relieves the discomfort associated with acute painful musculoskeletal conditions by a mode of action not quite defined. It also has weak antihistaminic and local anaesthetic , and analgesic properties.(Sweetman, 2007).

It is given by mouth in a dose of 100 mg twice daily or by intramuscular or slow intravenous injection in a dose of 60 mg which could be repeated every 12 hours(PDR). Orphenadrine citrate has been used in some countries for the treatment of intractable hiccup (Sweetman, 2007).

In this research a new dosage form of orphenadrine citrate is formulated (gel), and mixed with several penetration enhancers in order to increase its permeation through the skin.

The addition of penetration enhancers may change the physicochemical properties such as solubility and partition coefficient which may alter its delivery.

Gel dosage forms are more acceptable by the patients and can be investigated visually such as color, homogeneity, consistency, spreadability, and phase separation. (Mohamed, 2004).

2.2. The aim of this work

In this research a new topical gel formula of orphenadrine citrate is prepared and investigated for the effect of several chemical penetration enhancers on the drug permeation rate through a synthetic membrane and through human skin using a MFDC.

2.2.1. The specific objectives of this work

- 1- Determining the solubility of orphenadrine citrate in water and in different pH buffer solutions in order to determine the concentration of orphenadrine citrate to be used in the formula and the effect of the pH on its solubility.
- 2- Studying the compatibility of orphenadrine citrate with the excipients and the penetration enhancers in order to determine the materials that can be used for further formulation and study.
- 3- Selecting the best penetration enhancer in solution depending on diffusion study using MFDC and a synthetic membrane.

- 4- Preparation of several gel formulas using different gelling agents, compatible excipients and the best penetration enhancer, while keeping the concentration of the orphenadrine citrate constant through all.
- 5- Studying the permeability of the best gel formula through a synthetic membrane using a MFDC
- 6- Determining the effect of gel viscosity on its permeation through a synthetic membrane using a MFDC.
- 7- Studying the effect of the concentration of the penetration enhancer on the drug permeation through a synthetic membrane using MFDC.
- 8- Studying the permeation of the best orphenadrine citrate gel formula that penetrated the synthetic membrane through human skin using MFDC.

2.3. Materials and Reagents

In this research a synthetic membrane was used in the permeation study. It is obtained by sandwiching octanol soaked membrane filter between two layers of water soaked dialysis membranes.

The materials and reagents used in this research are listed in table (2.1).

Table 2.1: Materials and reagents used in the study

Items	Source	Grade
Orphenadine citrate	Nortec Quimica	USP
Potassium dihydrogen phosphate	Merck	AR
Picric acid	Merck	AR
Double distilled water	Jerusalem Pharmaceuticals	BP
Hydrochloric Acid	Merck	AR
Toluene	Merck	AR
Carpabon	Lubrizol	BP
Triethanolamine	Garlot Chemical Terminals	BP
Methyl paraben	Sharon Laboratories	USP
Hydroxypropyl methylcellulose	FMC	USP
Urea	Zhejiang Zhongbuo	BP
Propylene Glycol	Dow Chemical company	BP
Oleic Acid	Woodland Food Industries	USP
Tween 80	KOLB	USP
Sodium laurylsulphate	Merck	USP
Cremophore RH 40	Carmel	USP
Sodium carboxy methyl cellulose	CPK Elcco	USP
1-Octanol	Sigma-Aldrich	AR
Glycerin	Oleo chemicals	BP
Methanol	J.T Baker	AR
Acetonitril	Mallinckrodt Chemicals	AR
Ammonium phosphate	Merck	AR
Trypsin	Merck	AR
Sodium Hydroxide	Merck	AR
Ammonia solution	Merck	AR
Cyclodextrin	Zhejiang	BP

2.4. Tools and equipments

Syringes, vials, pipettes, glassware, stands and tubes was supplied by Jerusalem pharmaceuticals Tools and equipments used in the study are listed in table (2.2).

Table 2.2: Equipments and tools used in the study.

Equipment	Source/Model
HPLC	Merck- Hitachi.
U.V. Spectrophotometer	Hitachi U2900
Modifed Franz diffusion cell	Jordan University of science and technology work shop
pH meter	Metrohm
Percision Balance	Percisia
Magnetic Stirrer	Fried Electronic
Incubator 25C°	Advantec CL-310
Incubator 40C°	Advantec CL-310
Incubator 37C°	WTB binder
Water Pump	Atman At-101
Synthetic membrane filter HVLP 04700 0.45µm	Millipore
Dialysis membrane Cellu Sep H1	Membrane Filtration Products, Inc.
Water bath	Tuttnauer Co. LTD
Vaccum filter	Sartorius
Sonicator	Elmasonic
Refrigerator	L.G.
Vaccum pump	Millipore
HPLC	Dionex Ult.mate 3000
Brookfield Digital Viscometer	Brookfield engineering laboratories, Inc.

2.5. Methods:

2.5.1. Solubility determination

To determine the solubility of Orphenadine citrate in water and in different pH conditions, Orphenadrine citrate was added in excess to separate vials containing:

- 1- Water
- 2- Potassium dihydrogen phosphate Buffer with pH=5
- 3- Potassium dihydrogen phosphate Buffer with pH=6
- 4- Potassium dihydrogen phosphate Buffer with pH=7.4

Each vial was shaken, sonicated for 10 minutes and filtered from excess orphenadrine through 0.45 μ m filter membrane, The clear solution containing soluble orphenadrine citrate was then analyzed spectrophotometrically to determine the amount of soluble orphenadrine citrate (United States Pharmacopeial Convention, 1990)

2.5.2. Spectrophotometrical analysis of Orphenadrine citrate

200 mg of Trinitrophenol was dissolved in 1000ml toluene, a standard solution of orphenadrine citrate in 0.1M HCL was prepared with concentration of 0.00061g/ml. 2ml of each sample was taken and diluted with 25ml 0.1M HCL. 1 ml of each standard, assay preparation and 0.1 M HCL (to provide blank) was transferred into separate test tubes, to each tube 10ml of toluene and 1ml of 0.1M NaOH were added and shaken for 10 minutes.

5ml of each clear toluene layer was transferred to 3 three separate test tubes containing 5ml of Trinitrophenol reagent and mixed; the absorbance of solution at wave length 410nm was then recorded.

The concentration of sample is calculated according to the following equation:

$$\text{Sample concentration in mg/ml} = \left(\frac{0.61 \times 12.5 \times \text{Sample Absorption}}{\text{Standard Absorption}} \right)$$

2.5.3. Compatibility and stability studies

In order to investigate the compatibility of orphenadrine citrate with the expected excipients and penetration enhancers under study, and to determine the stability of orphenadrine citrate in different pH medias the following solutions were prepared:

- 1- Orphenadrine citrate 1% in water
- 2- Orphenadrine citrate 1% and carboxypol 2% and triethanolamine 2%
- 3- Orphenadrine citrate 1% in phosphate buffer of pH= 7.4
- 4- Orphenadrine citrate 1% in phosphate buffer of pH= 6
- 5- Orphenadrine citrate 1% in phosphate buffer of pH= 5
- 6- Orphenadrine citrate 1% in phosphate buffer of pH= 5.5
- 7- Orphenadrine citrate 1% in water and methyl paraben 0.1%
- 8- Orphenadrine citrate 1% in water and HPMC 2%
- 9- Orphenadrine citrate 1% in water and Urea 10%
- 10- Orphenadrine citrate 1% in water and propylene glycol 10%
- 11- Orphenadrine citrate 1% in water and oleic acid 0.2 % and tween 80 0.5%
- 12- Orphenadrine citrate 1% in water and tween 80 2%
- 13- Orphenadrine citrate 1% in water and sodium laurylsulphate 1%
- 14- Orphenadrine citrate 1% in water and cremophore RH 40 1%
- 15- Orphenadrine citrate 1% in water and cyclodextrin 2%
- 16- Orphenadrine citrate 1% in water and Na CMC 2% and glycerin 6%

Each solution was prepared by adding 1 g of orphenadrine citrate to 80 ml water or buffer and sonicated for 10 minutes to dissolve the orphenadrine citrate. The excipients are then added in the desired concentration and mixed for 10 minutes, then the volume of solution is completed to 100ml.

Each solution is poured into separate vials, labeled and closed tightly. Vials from each solution are placed in an incubator at 40 C° (Accelerated stability study) and at 25 C° for three months. During this period samples are withdrawn from each solution by a syringe every month and analyzed by HPLC according to USP30. (United States Pharmacopeial Convention, 2006)

2.5.4. Analysis of Orphenadrine citrate by HPLC.

Buffer Preparation:

0.05 M Ammonium phosphate buffer was prepared by dissolving 5.8g of monobasic ammonium phosphate in 1000ml water and the pH adjusted to 7.9 using ammonium hydroxide or phosphoric acid

Mobile Phase Preparation:

Mobile phase was prepared by mixing the methanol, Ammonium phosphate buffer, and acetonitrile in the ratios (9:8:3) respectively.

Standard Preparation:

A standard was prepared by adding 90mg orphenadrine citrate to 80ml water and sonicating for 10 minutes to dissolve the Orphenadrine citrate, the volume is made up with water to 100ml to obtain a concentration of 0.9mg/ml.

System Sensitivity Solution Preparation:

A system sensitivity solution is prepared by adding 1ml of the standard solution to 1000ml beaker and completing the volume with water, then 500ml of the diluted solution is added to 1000ml beaker and the volume is made up with water to 1000ml to obtain a concentration of 0.00045mg/ml

Chromatographic Conditions

The liquid chromatography is equipped with a 220nm detector and a 4.6mm x 15cm column that contains 5 μ m packing L1, the flow rate is about 1.5ml/min and the temperature of the column is maintained at 40C°

Assay preparation for Compatability and Stability Study:

The sample is prepared by taking a 2 ml of filtered sample (1% orphenadrine citrate) and diluting it to 25ml with bi-distilled water to obtain a theoretical concentration of 0.8mg/ml.

Procedure:

Separately inject equal volumes of both Standard and Assay preparation into the HPLC maintaining the specified chromatographic conditions and record the readings.

Calculations:

The concentration of orphenadrin citrate in sample is calculated as follows

$$C_{sa} = \frac{0.9 r_{sa}}{r_{st}}$$

Where C_{sa} is the concentration of sample and r_{sa} , r_{st} are the HPLC readings of sample and standard respectively.

The percentage of Orphenadrine citrate is calculated by dividing the concentration of sample on 0.8 and multiplying by 100

2.6. Diffusion Study

To study the diffusion of Orphenadrine citrate a MFDC was used that have two compartments a donor and a receiver one. The sample to be studied for diffusion is placed in the donor phase and separated from the receiver phase by a synthetic membrane that resembles the skin barrier by its hydrophilic and lipophilic regions. The synthetic membrane was used in the study to select the best gel formula with the best penetration, after that the synthetic membrane was replaced by fresh Human skin obtained from Ramallah Hospital.

The receiver phase is formulated to dissolve the diffused drug through the membrane or the skin.

2.6.1. Preparation of synthetic membrane

The Synthetic membrane is composed from three layers, a HVLP Filter membrane layer with pore size $0.45\mu\text{m}$, the filter membrane is soaked in octanol for 24 hours to resemble the lipophilic layer of intercellular lipids in the stratum corneum.

Two dialysis membranes are soaked in water for half an hour before the diffusion experiment begins. The HVLP filter membrane is sandwiched between the two dialysis membranes to prevent the octanol from leaving the filter membrane and to resemble the aqueous layer of the skin (Mohammad, 2004). The dialysis membrane used had a molecular weight cutoff of 10000.

2.6.2. Preparation of Human Skin

A fresh and healthy skin sample was obtained from Ramallah Hospital, it was obtained from a female patient in the 40s who had breast surgery. The stratum corneum, the main barrier

against permeation of drugs into the human body, was used to separate the receiver and donor compartments of the MFDC. The adipose tissue from the skin sample is removed as much as possible and the skin is immersed in trypsin solution for 12 hours at 37°C (Walter, 2002).

After that the stratum corneum is removed carefully from the rest of the skin by a tweezers, rubbed with a cotton bud to remove any depresses, washed with distilled water and dried (Walter, 2002)

The dried Stratum corneum is then mounted on a Franz diffusion cell and diffusion study is performed.

2.6.3. The Receiver Phase.

A phosphate buffer with pH 7.4 was used in the receiver compartment to dissolve the orphenadrine citrate that penetrated through synthetic membrane or through the human skin. The pH of the receiver compartment is the same as the pH of Human blood (7.4). The phosphate buffer was prepared by dissolving 6.8g of KH_2PO_4 in 1000ml of bi-distilled water. The pH is then adjusted by a solution of 5M NaOH to the desired pH.

The receptor phase was then degassed by heating the buffer to 45°C and vacuum filtration through a HVLP filter membrane with pore size of 0.45µm.

Degassing is very important, in order to keep the receiver phase bubble free when stirring to maintain sink conditions. If not degassed bubbles will appear due to stirring and will stick under the separating membrane in the MFDC. This will lead to a decrease in the permeable area of the separating membrane resulting in decreased permeability and faulty results.

2.6.4. Diffusion Procedure

An in and out rubber tubes are attached to the inlet and outlet of the water jacket of the MFDC and the receiver compartment of the MFDC is fixed in place using a stand and a clamp.

A water pump is introduced in the water bath to pump the water from the water bath through the water jacket of the MFDC and back to the water bath to maintain a stable temperature of 32 ± 2 °C. The water bath is switched on and its temperature is set to 34 ± 2 °C to maintain a temperature of 32 ± 2 °C in the MFDC. At this point the water pump is still off.

The receiver compartment is filled to top with the phosphate buffer of pH=7 and a magnetic bar is introduced in the compartment. The octanol soaked membrane is sandwiched between two water soaked dialysis membranes and mounted on the receiver compartment of the MFDC (Habes, 2005) (see figure(1.8)), making sure that no bubbles appear. A rubber ring is mounted over the membrane to prevent leakage and the donor compartment is mounted over the rubber ring.

The receiver and donor compartments are now separated by the membrane and a clamp is used to maintain them in their position while the rubber ring prevents leakage due to pressure made by the clamp. 5grams solution or gel to be tested is introduced in the donor compartment. The receiver compartment, the sampling ports and the receiver-donor junction were covered with parafilm to assure that no part of the donor or receiver phase is lost.

A magnetic stirrer is introduced below the MFDC, The water pump and the stirrer is activated (zero time for the experiment) (See figure (2.1)). 1ml samples are collected from sampling port using a syringe every half an hour for six hours. Before every sample is taken about 1.5ml from the receiver phase is taken from one port and introduced into the other. This process was repeated three times before every sample to ensure that the concentration in the ports is the same as in the receiver compartment.

After every sample is taken from the cell an equal amount of phosphate buffer (1ml) is introduced to the receiver compartment of the MFDC to ensure that its volume is not affected. Samples taken were analyzed by HPLC according to USP30 (see section 2.5.4) and every experiment was done in triplicates. The cumulative amount of orphenadrine citrate is calculated and plotted versus time, (see section (1.10)), and the diffusion parameter is calculated.

The cumulative amount of the penetrant is calculated according to the following equation:

$$\text{Cumulative amount of penetrant at time (t)} = C_t \times V + \sum_{t=0}^{t-0.5} C_t$$

C_t : is the measured concentration of the penetrant at time t in the receptor compartment in mg/ml.

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

The thickness of the membrane (h) equals 0.019cm, area of membrane (S) equals 3.14cm² and the volume of the receiver compartment is 25.8ml.

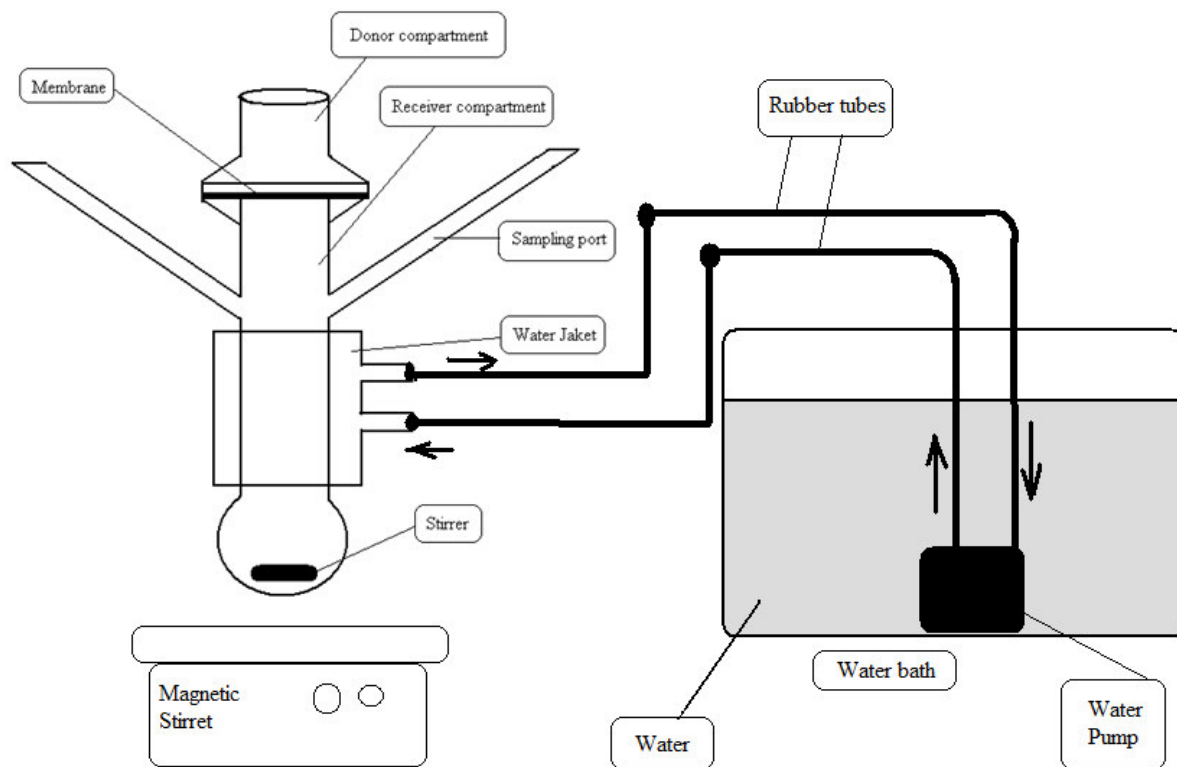


Figure 2.1: Modified Franz diffusion cell mounted on a magnetic stirrer and attached to a water pump and a water bath.

2.6.5. Calculation of diffusion parameters.

At every sampling time a sample is withdrawn and the amount of orphenadrine citrate is determined by HPLC analysis, (see section 2.5.4). A cumulative amount of orphenadrine citrate through time is drawn, (see Figure (1.10)), and the diffusion parameters are calculated. The curve is extrapolated using Excel 2007 to find the steady state line. The x intercept of the line will be the lag time. According to equation (9) in section (1.9)

The slope = PSC_d

Where S is the area, P is the permeability coefficient; C_d 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 (12) in section (1.9).

$$T_L = \frac{h^2}{6D}$$

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

According to equation (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 .

A summary of the diffusion parameters and their method of calculation is seen in table (2.3)

Table 2.3: Summary of diffusion parameters and their method of calculation

Slope	Lag Time(TL)	Diffusion Coefficient	Permability Coefficient	Partition coefficient	Enhancement ratio
Calculated from the plot	Intercept with x axes	$\frac{h^2}{6TL}$	Slope/ C_d	$\frac{P \cdot h}{D}$	Permeability with enhancer/ permeability without enhancer

2.7. Selecting the best penetration enhancer in solution

To study the effect of penetration enhancers on the permeability of orphenadrine citrate through a synthetic membrane, several penetration enhancers were mixed with solution of the API and investigated for permeability using MFDC and a synthetic membrane.

The following PE used are: Propylene glycol, Tween 80, Urea, cremophore RH40, and Cyclodextrin.

An aqueous solution of the API with concentration of 1% is prepared and tested for permeability through the synthetic membrane using MFDC. Its diffusion parameters are determined and the permeability coefficient is used as a reference to calculate the enhancement ratio.

Each penetration enhancer is mixed with orphenadrine citrate aqueous solution to have a solution containing 1% O.C and 1% PE. Then each solution is run through the diffusion procedure using the MFDC and the synthetic membrane. The diffusion parameters are calculated and the permeability coefficient of the solution with the penetration enhancer is compared to the permeability coefficient of solution without penetration enhancer to calculate the enhancement ratio. The best penetration enhancer is selected according to the best permeability coefficient value.

2.8. Formulating of orphenadrine citrate gel with the best penetration enhancer

Different gel formulas are prepared using the best PE (cremophore RH40), compatible excipients and different gelling agents, making sure that the formula is clear, with good viscosity, and good spreadability. The formulas are tested for permeability using MFDC through a synthetic membrane by the same diffusion procedure mentioned in section 2.6.4.

The effect of concentration of gelling agent, penetration enhancer, and the effect of the amount of gelling agent on drug permeation is determined. The O.C. Gel formulations is shown in the next tables.

Table 2.4: Gel Formula A

Material	Percent w/w	Function
Orphenadrine citrate	1%	API
CMC Na	2%	Gelling agent
Cremophore RH 40	0.2%	PE
Glycerin	5%	Enhance Spreadability
Methyl paraben	0.1%	Preservative
Water	To 100g	Solvent

Table 2.5: Gel Formula B.

Material	Percent w/w	Function
Orphenadrine citrate	1%	API
CMC Na	2%	Gelling agent
Cremophore RH 40	1%	PE
Glycerin	5%	Enhance Spreadability
Methyl paraben	0.1%	Preservative
Water	To 100g	Solvent

Formula A and B were formulated with different PE concentrations to determine the effect of the PE concentration on drug permeation while other excipients are the same in concentration in both formulations.

The procedure for preparation of the gel formulas A and B is the following :

Orphenadrine citrate is added to 80 ml water and stirred for 20 minutes until totally dissolved with mild heating. Glycerin is added and stirred for 2 minutes, Cremophore RH 40 is added and stirred for 2 minutes. Methyparaben is added and stirred for 20 minutes. The appropriate amount of CMC Na (2g) is spreaded over the solution and stirred with mild heating for 20 minutes. Water is added to complete the weight to 100g. The sample is left for 12 hours and stirred again for 10 minutes.

Table 2.6: Gel Formula C

Material	Percent w/w	Qt/ grams	Function
Orphenadrine citrate	1%	1	API
HPMC	3%	3	Gelling agent
Cremophore RH 40	1%	1	PE
Glycerin	5%	5	Enhance Spreadability
Methyl paraben	0.1%	0.1	Preservative
Water	To 100 g	X	Solvent

Table 2.7: Formula D

Material	Percent w/w	Qt/ grams	Function
Orphenadrine citrate	1%	1	API
HPMC	2%	2	Gelling agent
Cremophore RH 40	1%	1	PE
Glycerin	5%	5	Enhance Spreadability
Methyl paraben	0.1%	0.1	Preservative
Water	To 100g	X	Solvent

In the Gel Formulas C and D the concentration of O.C, PE and other excipients are the same and the only difference is in the gelling agent concentration, these two formulas are used to evaluate the effect of viscosity on drug permeation.

Table 2.8: Formula E.

Material	Percent w/w	Qt/grams	Function
Orphenadrine citrate	1%	1	API
HPMC	3%	3	Gelling agent
Cremophore RH 40	0.2%	0.2	PE
Glycerin	5%	5	Enhance Spreadability
Methyl paraben	0.1%	0.1	Preservative
Water	To 100g	X	Solvent

In the Gel Formulas C and E the concentration of O.C, gelling agent and other excipients are the same and the only difference is in the PE concentration. These two formulas are used to evaluate the effect of the concentration of PE on the drug permeation.

The procedure used to prepare the HPMC gel formulas(C, D, E) is as follows:

Orphenadrine citrate is added to 80 ml water and stirred for 20 minutes until totally dissolved with mild heating. Glycerin is added and stirred for 2 minutes. Cremophore RH 40 is added and stirred for 2 minutes. Methyparaben is added and stirred for 20 minutes. The appropriate amount of HPMC is spreaded over the solution and stirred under mild heat for 10 minutes Water is added to complete the weight to 100g; The sample is left for 12 hours and stirred again for 10 minutes.

2.9. Determining the effect of different gelling agents on drug permeation

Gel formulas are studied for their permeation through a synthetic membrane using the MFDC and the diffusion parameter is determined. The permeability coefficient is used to determine the best gel in synthetic membrane penetration.

The Permeability coefficient of Formula A (CMC Na as gelling agent) is compared to Formula C (HPMC as a gelling agent) and the permeability coefficient of Formula B (CMC Na as gelling agent) is compared to the formula D(HPMC as gelling agent).

2.10. Determining the effect of viscosity on the drug permeation

The best gel formula is selected according to the best permeability coefficient, the ease of use on human skin, and acceptability.(Formulas C and D in section 2.8.)

The viscosity of the two formulas is determined using a Brookfield viscometer, with spindle number 94 at 6 RPM. The formula D was less viscous than Formula C as will be shown in the results and discussion part later. The two formulas are studied for permeation using MFDC and a synthetic membrane.

2.11. Determining the effect of the concentration of penetration enhancer on drug permeation.

Formulas C and E (from section 2.8) is studied using a MFDC and a synthetic membrane. The diffusion parameters is calculated and compared.

The gel with the more cremophore RH 40 showed better penetration as expected.

2.12. Studying the permeation of drug through human skin.

Formula C from section 2.8 was selected for permeation study using human skin mounted on a MFDC, due to its acceptability, spreadability, and good permeation coefficient.

The diffusion parameter is determined using MFDC and human stratum corneum.

Part 3

Results and Discussion

3.1. Solubility determination results

Saturated solutions of Orphenadrine citrate were prepared at different pHs, their concentrations were determined by complexing with trinitrophenol and measuring the absorbance at 410 nm (see section 2.5.1). Results are shown in table (3.1).

Table 3.1: The solubility results of orphenadrine citrate in different solutions at 20C.

Orphenadrine citrate in	Solubility g/100ml
Buffer pH 7.4	2.43
Buffer pH 6	1.63l
Buffer pH 5	1.39
Water	1.5

The solubility of orphenadrine citrate increases as the pH increases, the highest solubility was found when the pH of the solution was 7.4.

The solubility of orphenadrine citrate in water was found to be 1 in 70 in accordance with values mentioned in the literature (Abate, *et al* 2005).

3.2. Studying the compatibility of orphenadrine citrate with excipients and penetration enhancers in aqueous solutions.

Different penetration enhancers and excipients were tested for their compatibility with orphenadrine citrate in water at different pHs for a period of three months, the results are shown in Table (3.2).

Table 3.2-A: The results of the compatibility study for three months on 25 C° and 40C°.

Aqueous solution	% API at Zero time	40 °C ± 2 °C (Accelerated Stability Study)			25 °C ± 2 °C
		% API after 1 M	% API after 2 M	% API after 3 M	% API after 3 M
Orphenadrine citrate 1%	100%	99%	96%	94%	98%
Orphenadrine citrate 1% , carpabob, triethanolamine	Non compatible	Non compatible	Non compatible	Non compatible	Non Compatible
Orphenadrine citrate 1%, phosphate buffer of pH= 7.4	100%	95%	X	98%	97.2%
Orphenadrine citrate 1% , phosphate buffer of pH= 6	100%	X	X	99%	100%
Orphenadrine citrate 1% , phosphate buffer of pH= 5	100%	X	98%	X	104%
Orphenadrine citrate 1%, phosphate buffer of pH= 5.5	100%	X	94%	93%	102%
Orphenadrine citrate 1%, methyl paraben	100%	X	97%	93%	102%
Orphenadrine citrate 1% , HPMC	100%	94%	94%	90%	99%

Table 3.2-B: The results of Compatibility for three months on 25 C° and 40C°.

Aqueous solution	% API at Zero time	40 °C ± 2 °C			25 °C ± 2 °C
		% API after 1 M	% API after 2 M	% API after 3 M	% API after 3 M
Orphenadrine citrate 1% in water and Urea	100%	25%	25%	17%	102%
Orphenadrine citrate 1% in water and propylene glycol	100%	X	102%	96.8%	101%
Orphenadrine citrate 1% in water and oleic acid and tween 80	100%	95%	94%	91%	91%
Orphenadrine citrate 1% in water and tween 80	100%	96%	96%	89%	97%
Orphenadrine citrate 1% in water and sodium laurylsulphate	100%	X	100%	69%	165%
Orphenadrine citrate 1% in water and cremophore RH 40	100%	99%	99%	97%	99%
Orphenadrine citrate 1% in water and cyclodextrin	100%	100%	103%	90%	101%
Orphenadrine citrate 1% in water and Na CMC	100%	98%	96.2%	94%	97%

Table 3.2-C: The results of Compatibility study for three months on 25 C° and 40C°.

Aqueous solution	Zero time	40 °C ± 2 °C			25 °C ± 2 °C
		% API after 1 M	% API after 2 M	% API after 3 M	% API after 3 M
Orphenadrine citrate 1% in water and Na CMC+ glycerin	100%	X	X	99%	101%

The (X) letter is used whenever an analysis was not possible.

When carbopol was added to a solution of orphenadrine citrate a white precipitate was formed which indicates that carbopol is incompatible with Orphenadrine citrate and hence cannot be used as a gelling agent for formulation. Carbopol is an anionic gelling agent, and it may have formed a non soluble complex with Orphenadrine.

In the presence of urea, the concentration of orphenadrine citrate falls to 17% after 3 months of incubation at 40±2°C, while at room temperature no significant change was observed in the assay. The presence of heat may result in the hydrolysis of Urea (10% concentration is used) and the production of ammonia which may reacts with orphenadrine causing precipitation.

With Sodium lauryl sulphate the assay of orphenadrine citrate was not stable at 40±2°C. Orphenadrine is suggested to form a complex with lauryl sulphate releasing the citrate. This complex is not stable at 40 C° and degrades with time.

It is clear that orphenadrine citrate is stable over a wide range of pHs (5.0-7.4) which is in accordance with the limits of Orphenadrine Citrate Injections pHs (5.0-6.0) approved in USP-31. The other excipients (MP, HPMC, CMC Na, Tween 80, Oleic acid, glycerin, Cyclodextrin and Propylene glycol), were found to be compatible with OC and may be used in the formulation of OC gel

3.3. Determination of best Penetration enhancer in solution.

In this part the lag time (T_L) reflects the time required by API to pass through the intact membrane and reach to the receiver compartment. Diffusion coefficient (D) measures the

membrane resistance encountered by a 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 aqueous phase, this parameter includes other diffusion parameters as shown in the calculation of diffusion parameters (part two). In the final part of this, thesis we made attempts to compare the enhancement ratio (ER) of various penetration enhancers used. This is a ratio of permeability coefficients constants (P) following the use of penetration enhancer divided by the permeability coefficient before the use of penetration enhancer (P after / P before). The greater the ER, the greater the penetration enhancement ability of penetration enhancer used.

Table 3.3: A list of solutions to be studied for permeability.

Solution no.	Composition
1	Orphenadrine citrate 1%
2	cremophor RH40 1%, Orphenadrine citrate 1%
3	Cyclodextrin 1% and orphenadrine 1%
4	Urea 1% and orphenadrine citrate 1% in water
5	Tween 80 1% and orphenadrine citrate 1%
6	Propylene glycol 1%, orphenadrine citrate 1%

3.3.1. Solution No. 1 Orphenadrine citrate 1% and Water

For the preparation of Orphenadrine citrate 1% in water, 1g of O.C was dissolved in 80ml of water by the aid of sonicator for 10 minutes; the volume was made up to 100ml with water and mixed.

Samples are taken every 30 minutes from the receiver compartment and analyzed by HPLC for the amount of orphenadrine citrate.

Table (3.4) illustrates the assay results of API penetrated to the receiving compartment by time. Area under peaks (Area1, Area2, Area3) was presented in triplicates, and the cumulative amount of drug penetrated (Q) per unit of membrane area was determined and plotted as a function of time Fig. (3-1). The linear part of the curve was plotted in Fig. (3.2) from which the diffusion parameters were calculated for API alone and tabulated in Table (3-4). The T_L was calculated by dividing the intercept of the equation of flux profile on the slope from the same equation. The diffusion parameter (D) was calculated using equation 1:

$$D = h^2/6.T_1 \quad (1)$$

The permeability coefficient (P) was calculated by dividing the value of the slope of the flux profile by the concentration of API in the donor compartment (10mg/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 2.

$$K = (P. h) / D \quad (2)$$

Table 3.4: Data obtained from the diffusion of solution No. 1 through a synthetic membrane using a Modified Franz diffusion cell, with no penetration enhancer introduced.

Hrs	Area 1	Area 2	Area 3	Mean Area	SD	RSD	Amount mg/ml	mg/25.8ml	Q [mg]	m [mg/cm²]
0.0	0	0	0	0	0	0	0	0	0	0
1.0	1294138	1517924	2903885	1905316	871995	45.8	0.0321	0.8286	0.8286	0.2639
1.5	1777708	2152610	4128733	2686350	1263127	47.0	0.0453	1.1682	1.2003	0.3823
2.0	2150727	2881389	5077651	3369922	1523391	45.2	0.0568	1.4655	1.5107	0.4811
2.5	2494094	3429543	5881011	3934883	1749093	44.5	0.0663	1.7112	1.7680	0.5630
3.0	2877533	3950219	6337057	4388270	1770873	40.4	0.0740	1.9083	1.9746	0.6289
3.5	3315750	4707504	7365077	5129444	2057374	40.1	0.0865	2.2306	2.3046	0.7339
4.0	3596938	5327683	7896880	5607167	2163552	38.6	0.0945	2.4384	2.5248	0.8041
4.5	3918197	5833517	8969353	6240356	2550036	40.9	0.1052	2.7137	2.8082	0.8943
5.0	4274966	6477851	9620921	6791246	2686721	39.6	0.1145	2.9533	3.0585	0.9740
5.5	4612057	7136978	10532922	7427319	2971091	40.0	0.1252	3.2299	3.3444	1.0651
6.0	5173753	7932375	11526897	8211008	3185724	38.8	0.1384	3.5707	3.6959	1.1770

Table (3.4) shows the assay results of orphenadrine citrate, this diffusion study was done three times. The cumulative amount of orphenadrine citrate per unit of membrane area was calculated and plotted as a function of time. See figure (3.1).

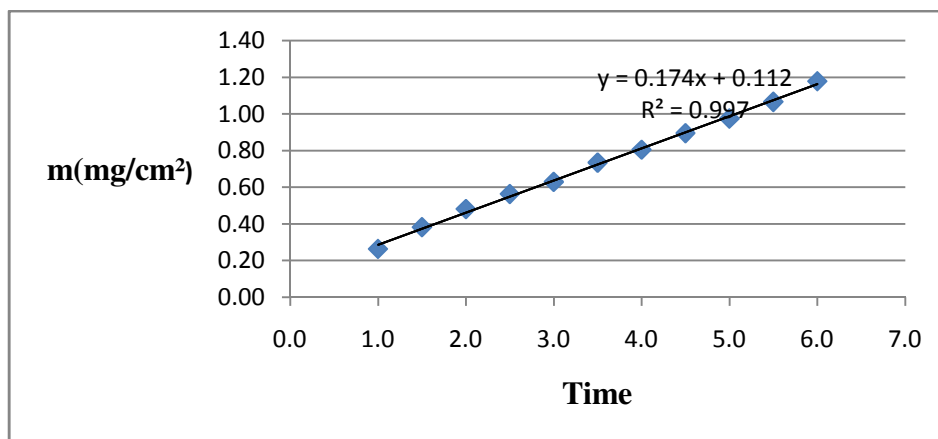


Figure 3.1: In vitro permeation profile for the cumulative amount of O.C penetrated per unit area of synthetic membrane (mg/cm²) for solution no. 1, with no penetration enhancer

The best linear line is determined on figure (3.2) by Excel 2007, from which the linear line equation is determined. This equation helps in determining the slope and the x intercept; these are used for further calculation of diffusion parameters. The diffusion parameters are calculated according to table (2.3).

The steady state Flux diagram and the diffusion parameters are shown in figure (3.2) and table (3.5).

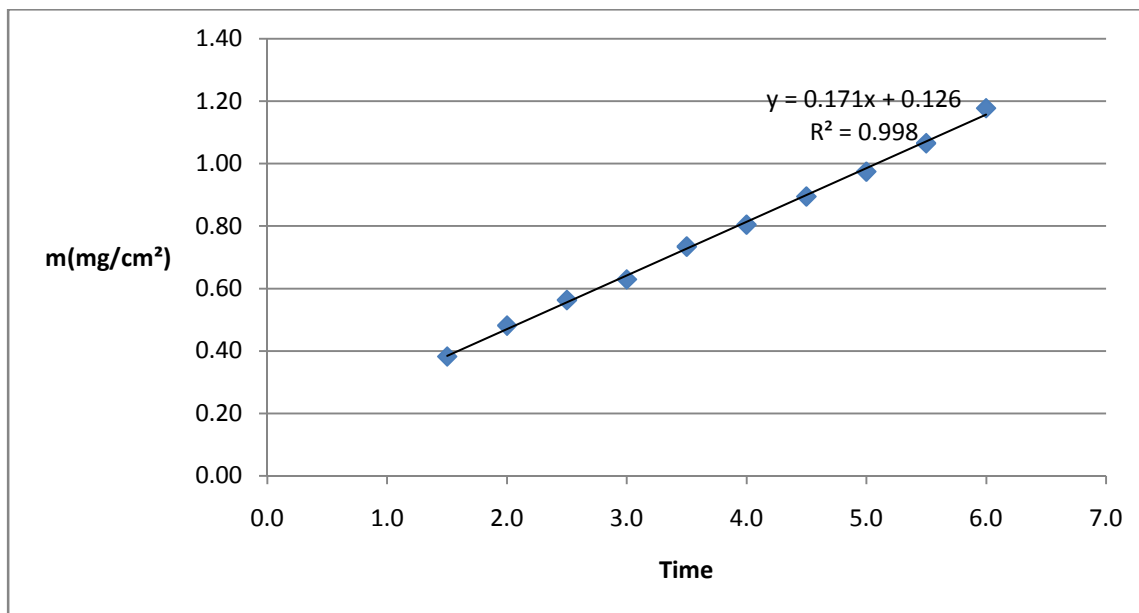


Figure 3.2: Linear In vitro flux diagram for the cumulative amount of O.C penetrated per unit area of synthetic membrane (mg/cm²) for solution no. 1 with no penetration enhancer.

Table 3.5: Diffusion parameters for solution no. 1, no penetration enhancer introduced.

Item	Slope	Intercept	T _L	D	P	K	ER
API	0.174	0.126	0.644h	0.0000534	0.0112	3.96	1.00

The permeability parameter of this solution is used as a reference for penetration enhancement comparison.

Orphenadrine citrate solution showed some penetration through the synthetic membrane due to its solubility in hydrophilic and hydrophobic parts of the synthetic membrane.

3.3.2. Solution 2 Orphenadrine citrate 1% and cremophore RH40 1% penetration enhancer in water

Cremophore RH40 is a Polyoxyethylene castor oil derivative of complex mixtures of various hydrophobic and hydrophilic components. Approximately 75% of the components of the mixture are hydrophobic. These comprise mainly fatty acid esters of glycerol, polyethylene glycol and fatty acid esters of polyethylene glycol. The hydrophilic portion consists of polyethylene glycols and glycerol ethoxylates.(Rowe, 2005). It is an Emulsifying agent, solubilizing agent and wetting agent. It can be used for oral and topical preparations. (Rowe, 2005).

A solution of orphenadrine citrate 1% was prepared by dissolving 1g of O.C in 80g of water, Cremophore RH40 1g is added, stirred and the volume is made up to 100ml. The permeability results of drug through the synthetic membrane is shown in table (3.6) and Fig. (3.3).

The cumulative amount of API permeated through unit area of membrane was then calculated as mentioned before, and the linear section, i.e. the steady state flux was plotted versus time Fig. (3.4).

Table 3.6: Data obtained by the diffusion of solution No. 2 through a synthetic membrane, using a Modified Franz diffusion cell, and cremophor RH40 as a penetration enhancer.

hrs	Area 1	Amount1 mg/ml	Area 2	Amount2 mg/ml	Area 3	Amoun3 mg/ml	Mean mg/ml	SD	RSD	cumulative mg/ml	mg/25.8ml	Q [mg]	m [mg/cm ²]
0.0	0	0	0		0	0	0	0	0	0	0	0	0
1.0	5489846	0.0934	1293549	0.0248	1730899	0.0332	0.0504	0.0374	74.1	0.0504	1.3015	1.3015	0.4145
1.5	7865607	0.1338	2303759	0.0442	2981187	0.0571	0.0784	0.0484	61.8	0.1288	2.0216	2.0720	0.6599
2.0	8884861	0.1511	3278716	0.0629	4221800	0.0809	0.0983	0.0466	47.4	0.2270	2.5359	2.6646	0.8486
2.5	10976152	0.1867	4799678	0.0920	5446122	0.1044	0.1277	0.0514	40.3	0.3547	3.2943	3.5213	1.1214
3.0	12855829	0.2186	6830268	0.1309	7392471	0.1417	0.1638	0.0478	29.2	0.5185	4.2248	4.5795	1.4585
3.5	15042933	0.2558	9400958	0.1802	11519899	0.2208	0.2189	0.0378	17.3	0.7374	5.6489	6.1674	1.9641
4.0	15886153	0.2701	12507328	0.2398	15195174	0.2913	0.2671	0.0259	9.7	1.0045	6.8902	7.6276	2.4292
4.5	17476605	0.2972	16311802	0.3127	22095085	0.4235	0.3445	0.0689	20.0	1.3490	8.8875	9.8920	3.1503
5.0	19116998	0.3251	20518380	0.3933	29188624	0.5595	0.4260	0.1206	28.3	1.7750	10.9903	12.3393	3.9297
5.5	20627842	0.3508	25187124	0.4828	36070242	0.6914	0.5083	0.1718	33.8	2.2833	13.1154	14.8903	4.7421
6.0	22464336	0.3820	30469215	0.5841	42565213	0.8159	0.5940	0.2171	36.6	2.8773	15.3255	17.6088	5.6079

In vitro permeation profile for the cumulative amount of O.C penetrated per unit area for solution no.2 is shown in figure (3.3); the Steady state flux diagram is shown in figure (3.4)

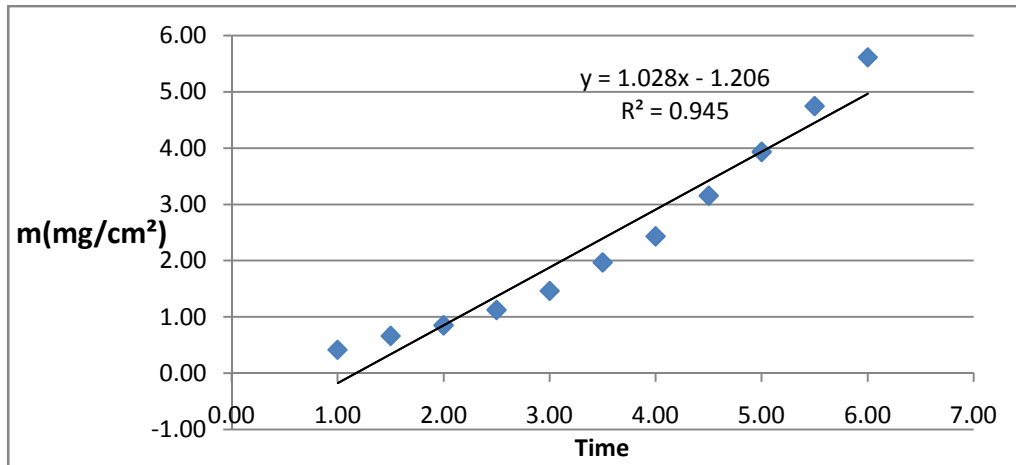


Figure 3.3: In vitro permeation profile for the cumulative amount of O.C penetrated per unit area of synthetic membrane (mg/cm²) for solution no. 2, Cremophor RH40 is used as a penetration enhancer.

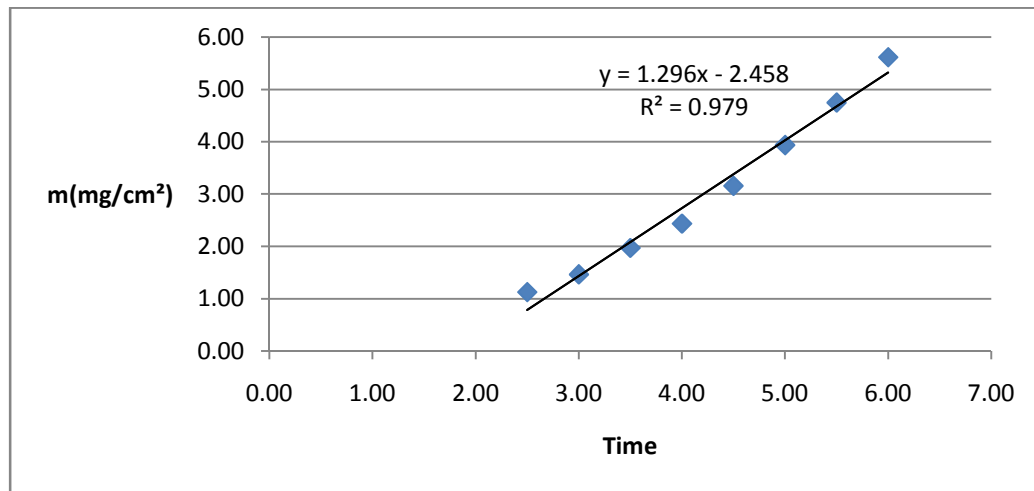


Figure 3.4: In vitro Steady steady permeation profile for the cumulative amount of O.C penetrated per unit area of synthetic membrane (mg/cm²) for solution no. 2, Cremophor RH40 is used as a penetration enhancer.

The diffusion parameters are calculated according to table (2.3) and the enhancement ratio is determined. (See table 3.6).

Table 3.7: Diffusion parameters for solution no.2. Cremophor RH40 is used as a penetration enhancer.

Item	Slope	Intercept	T_L	D	P	K	ER
API	1.296	2.458	1.89 h	0.0000318	0.1269	77.43	11.33

The rate of diffusion of API in the presence of Cremophor RH40 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 (11.33).

This great value of permeation coefficient of Cremophor RH40 is probably due to its ability to create miniscule holes in the intact membrane by removing some parts of oil through which the API can shuttle.

3.3.3. Solution No. 3 Cyclodextrin 1% and orphenadrine 1% in water

Cyclodextrins are crystalline, nonhygroscopic, cyclic oligosaccharides (7 glucose units) derived from starch. Cyclodextrins are ‘bucketlike’ or ‘conelike’ toroid molecules, with a rigid structure and a central cavity. The internal surface of the cavity is hydrophobic and the outside of the torus is hydrophilic; this is due to the arrangement of hydroxyl groups within the molecule. This arrangement permits the cyclodextrin to accommodate a guest molecule within the cavity, forming an inclusion complex. Cyclodextrins may be used to form inclusion complexes with a variety of drug molecules, resulting primarily in improvements to dissolution and bioavailability owing to enhanced solubility and improved chemical and physical stability (Rowe, 2005).

Using β -cyclodextrin 1% as potential penetration enhancer and following the same general procedure, the data measured are shown in table (3.8).

Table 3.8: Data obtained by the diffusion of solution No. 3, Using Cyclodextrin as penetration enhancer.

hrs	Area 1	Amount1 mg/ml	Area 2	Amount2 mg/ml	Area 3	Amoun3 mg/ml	Mean mg/ml	SD	RSD	cumulative mg/ml	mg/25.8ml	Q [mg]	m [mg/cm ²]
0.0	0	0	0	0	0	0	0	0	0	0	0	0	0
1.0	3194707	0.0561	1663511	0.0319	492408	0.0094	0.0325	0.0233	71.9	0.0325	0.8380	0.8380	0.2669
1.5	4319249	0.0759	2205082	0.0423	810020	0.0155	0.0446	0.0302	67.9	0.0771	1.1495	1.1820	0.3764
2.0	5433989	0.0954	2809820	0.0539	1151436	0.0221	0.0571	0.0368	64.4	0.1342	1.4739	1.5509	0.4939
2.5	6434722	0.1130	4540559	0.0870	1684304	0.0323	0.0775	0.0412	53.2	0.2116	1.9982	2.1324	0.6791
3.0	7522734	0.1321	6055446	0.1161	2212421	0.0424	0.0969	0.0478	49.4	0.3085	2.4994	2.7110	0.8634
3.5	8348101	0.1466	7864420	0.1508	2766066	0.0530	0.1168	0.0553	47.3	0.4253	3.0135	3.3221	1.0580
4.0	9599551	0.1686	9829646	0.1884	3317956	0.0636	0.1402	0.0671	47.8	0.5655	3.6176	4.0429	1.2875
4.5	10106399	0.1775	11735205	0.2250	4218305	0.0809	0.1611	0.0734	45.6	0.7266	4.1567	4.7222	1.5039
5.0	11338049	0.1992	12601477	0.2416	4956821	0.0950	0.1786	0.0754	42.2	0.9052	4.6073	5.3339	1.6987
5.5	12451307	0.2187	14097264	0.2702	5727370	0.1098	0.1996	0.0819	41.0	1.1048	5.1491	6.0543	1.9281
6.0	12851639	0.2257	16200048	0.3105	6773976	0.1299	0.2220	0.0904	40.7	1.3268	5.7288	6.8335	2.1763

In vitro permeation profile for the cumulative amount of O.C penetrated per unit area for solution no3 is shown in figure (3.5); the Steady state diagram is shown in figure (3.6)

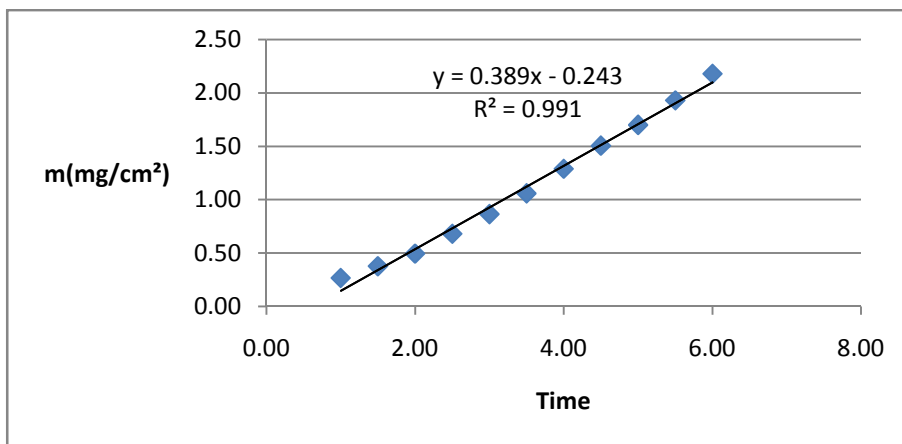


Figure 3.5 : In vitro permeation profile for the cumulative amount of O.C penetrated per unit area of synthetic membrane (mg/cm²) for solution no.3, using Cyclodextrin as penetration enhancer

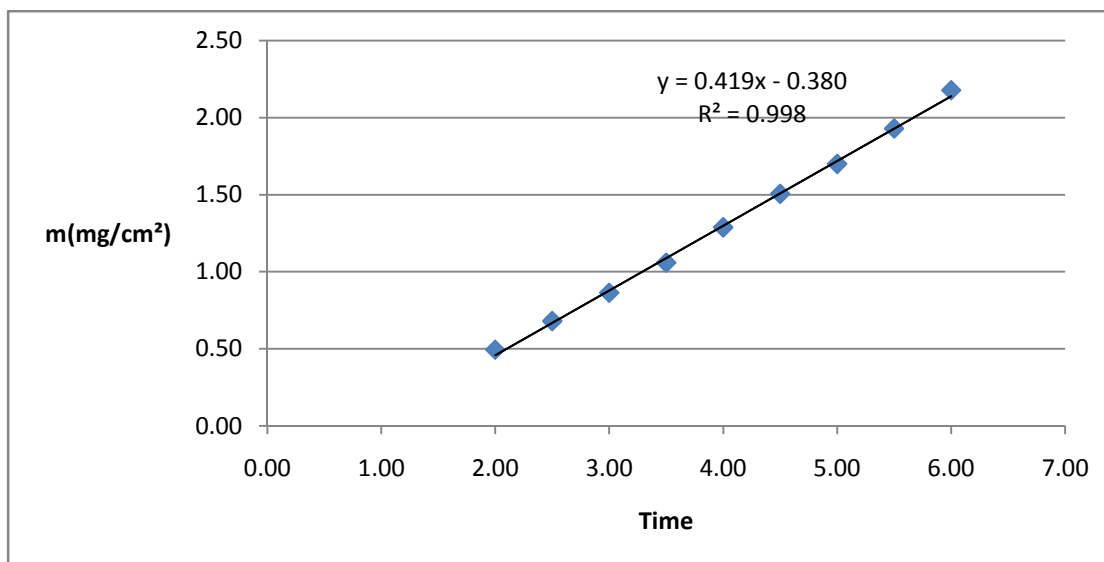


Figure 3.6: In Steady state vitro permeation profile for the cumulative amount of O.C penetrated per unit area for solution no3, using Cyclodextrin as penetration enhancer.

Table 3.9: Diffusion parameters for solution no.3, using Cyclodextrin is used as a penetration enhancer.

Item	Slope	Intercept	T _L	D	P	K	ER
API	1.296	0.380	0.9 hh	0.0000669	0.0419	11.9	3.74

The calculated enhancement ratio for β -cyclodextrin was more than triple, but not to the extent of Cremophor RH40,.

3.3.4. Solution no. 4 Urea 1% and orphendrine citrate 1% in water

Urea promotes transdermal permeation by facilitating hydration of the stratum corneum and by the formation of hydrophilic diffusion channels within the barrier.

The data of HPLC analysis of solution no. 4 is shown in table (3.10) The cumulative amount of O.C penetrated per unit area is shown in figure (3.7); the Steady state flux diagram is shown in figure (3.8)

The enhancement ratio of Urea was found to lie between Cremophor RH 40 and β -cyclodextrin. The calculated diffusion parameters are presented in table (3.11).

Table 3.10: Data obtained by the diffusion of solution No. 4 through a synthetic membrane using a Modified Franz diffusion cell. Urea is used as penetration enhancer.

hrs	Area 1	Amount1 mg/ml	Area 2	Amount2 mg/ml	Area 3	Amoun3 mg/ml	Mean mg/ml	SD	RSD	cumulative mg/ml	mg/25.8ml	Q [mg]	m [mg/cm ²]
0.0	0	0	0	0	0	0	0	0	0	0	0	0	0
1.0	1635010	0.0278	3109159	0.0554	2848654	0.0507	0.0446	0.0148	33.1	0.0446	1.1514	1.1514	0.3667
1.5	2530591	0.0430	4891549	0.0938	4520314	0.0805	0.0724	0.0263	36.3	0.1170	1.8687	1.9133	0.6093
2.0	3480743	0.0592	6714606	0.1287	6181406	0.1101	0.0993	0.0360	36.2	0.2164	2.5625	2.6795	0.8534
2.5	4246353	0.0722	8338956	0.1599	8092108	0.1441	0.1254	0.0467	37.3	0.3417	3.2348	3.4512	1.0991
3.0	4953174	0.0842	10030286	0.1923	9230824	0.1644	0.1470	0.0561	38.2	0.4887	3.7914	4.1331	1.3163
3.5	5495417	0.0935	11644495	0.2232	10573876	0.1883	0.1683	0.0671	39.9	0.6570	4.3425	4.8311	1.5386
4.0	6187368	0.1052	12935269	0.2480	11681390	0.2080	0.1871	0.0736	39.4	0.8441	4.8260	5.4830	1.7462
4.5	6681278	0.1136	14314367	0.2744	12973600	0.2310	0.2063	0.0832	40.3	1.0504	5.3235	6.1675	1.9642
5.0	7289575	0.1240	15492687	0.2970	14129198	0.2516	0.2242	0.0897	40.0	1.2746	5.7836	6.8340	2.1764
5.5	8037361	0.1367	16799356	0.3220	15372173	0.2737	0.2441	0.0961	39.4	1.5187	6.2987	7.5733	2.4119
6.0	8977908	0.1527	17963476	0.3443	16443073	0.2928	0.2633	0.0992	37.7	1.7820	6.7922	8.3109	2.6468

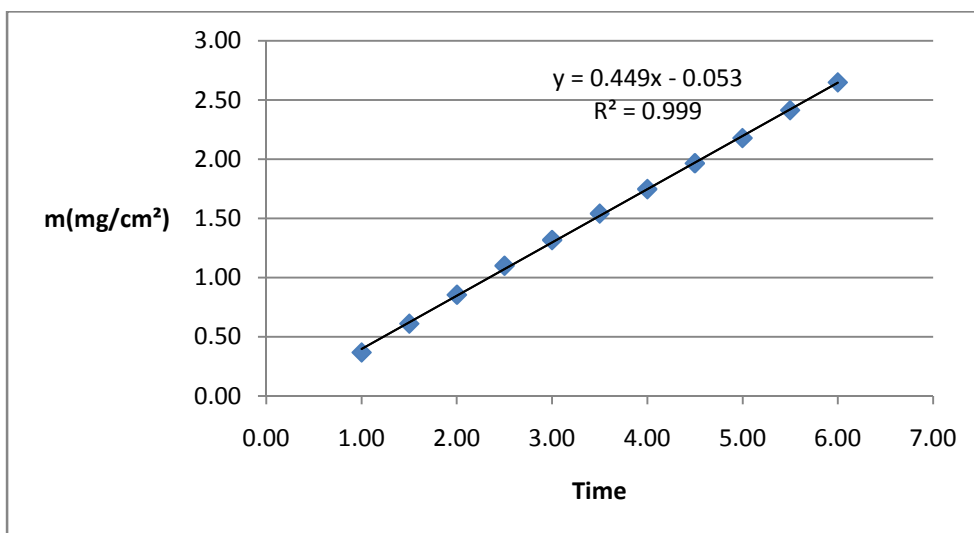


Figure 3.7: In vitro permeation profile for the cumulative amount of O.C penetrated per unit area of synthetic membrane (mg/cm²) for solution no. 4, using urea as penetration enhancer.

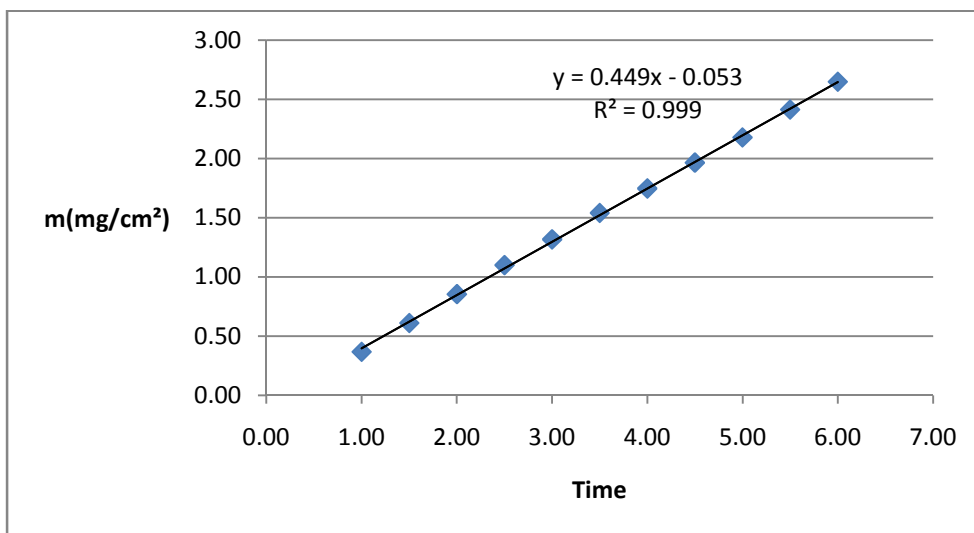


Figure 3.8: In vitro Steady state permeation profile for the cumulative amount of O.C penetrated per unit area of synthetic membrane (mg/cm²) for solution no. 4, using urea as penetration enhancer.

Table 3.11: Diffusion parameters for solution number 4, using Urea as a penetration enhancer.

Item	Slope	Intercept	T_L	D	P	K	ER
API	0.449	0.053	0.118	0.000510	0.0449	1.67	4.01

3.3.5. Solution no. 5 Tween 80 1% and orphenadrine citrate 1% in water

The effect of nonionic surfactant Tween 80 in amount of 1% on the permeation of API is presented in table (3.12). The cumulative amount of O.C penetrated per unit area and Steady state flux diagram are figures (3.9) and (3.10), respectively.

The diffusion parameters are presented in table (3.13)

Table 3.12: Data obtained by the diffusion of solution No. 5 through a synthetic membrane using a Modified Franz diffusion cell. Tween 80 is used as a penetration enhancer.

hrs	Area 1	Amount1 mg/ml	Area 2	Amount2 mg/ml	Area 3	Amoun3 mg/ml	Mean mg/ml	SD	RSD	cumulative mg/ml	mg/25.8ml	Q [mg]	m [mg/cm ²]
0.0	0	0	0		0	0	0	0	0	0	0	0	0
1.0	3563634	0.0626	1300536	0.0232	1300536	0.0232	0.0363	0.0228	62.7	0.0363	0.9366	0.9366	0.2983
1.5	4602857	0.0808	2061750	0.0367	2061750	0.0367	0.0514	0.0255	49.6	0.0877	1.3267	1.3630	0.4341
2.0	5774812	0.1014	2810767	0.0500	2810767	0.0500	0.0672	0.0297	44.2	0.1549	1.7331	1.8208	0.5799
2.5	7368450	0.1294	3564058	0.0635	3564058	0.0635	0.0854	0.0381	44.6	0.2403	2.2045	2.3594	0.7514
3.0	8608224	0.1512	4329833	0.0771	4329833	0.0771	0.1018	0.0428	42.0	0.3421	2.6263	2.8667	0.9130
3.5	9810147	0.1723	5178468	0.0922	5178468	0.0922	0.1189	0.0463	38.9	0.4610	3.0678	3.4099	1.0860
4.0	11321277	0.1989	5900220	0.1051	5900220	0.1051	0.1363	0.0542	39.7	0.5974	3.5171	3.9781	1.2669
4.5	12528679	0.2201	6688859	0.1191	6688859	0.1191	0.1528	0.0583	38.2	0.7501	3.9410	4.5384	1.4453
5.0	13651980	0.2398	7746557	0.1379	7746557	0.1379	0.1719	0.0588	34.2	0.9220	4.4346	5.1847	1.6512
5.5	14690766	0.2580	8239991	0.1467	8239991	0.1467	0.1838	0.0643	35.0	1.1058	4.7426	5.6646	1.8040
6.0	15796869	0.2775	9317057	0.1659	9317057	0.1659	0.2031	0.0644	31.7	1.3089	5.2395	6.3454	2.0208

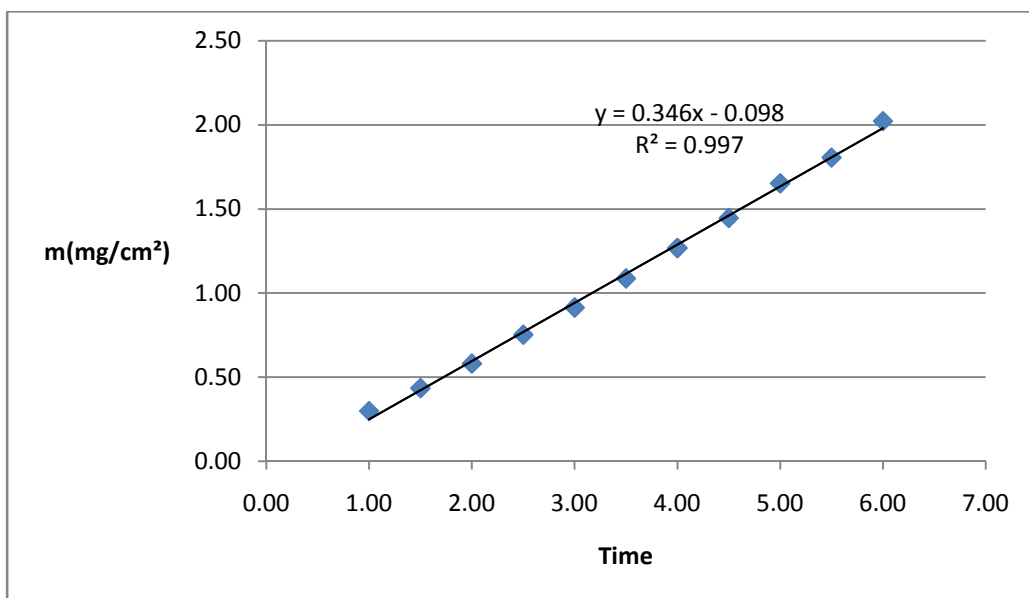


Figure 3.9: In vitro permeation profile for the cumulative amount of O.C penetrated per unit area of synthetic membrane (mg/cm²) for solution no.5, using Tween 80 as a penetration enhancer.

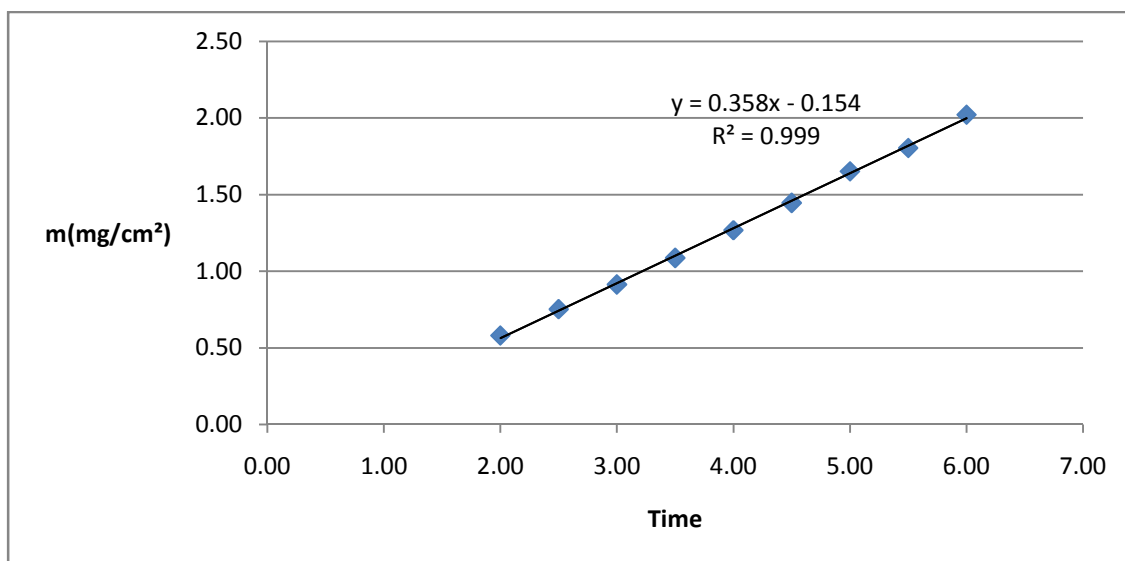


Figure 3.10: In vitro steady state flux diagram of orphenadrine citrate (mg/cm²) through a synthetic membrane for solution no. 5., using Tween 80 used as a penetration enhancer.

Table: 3.13: Diffusion parameters for solution no. 5, using Tween80 as a penetration enhancer.

Item	Slope	Intercept	T _L	D	P	K	ER
API	0.358	0.154	0.43 h	0.000140	0.0358	4.86	3.20

3.3.6. Solution no. 6 Propylene glycol 1% and orphenadrine citrate 1% in water

In general propylene glycol increases the permeant partitioning and solubility within the stratum corneum.

The data obtained from the diffusion study of solution No.6 is presented in table (3.14), the cumulative amount of O.C penetrated per unit area is calculated and presented in figure (3.11); the Steady state flux diagram is shown in figure (3.12). The diffusion parameters are tabulated in table (3.15). It is obvious that propylene glycol has the lowest effect on penetration enhancement, increasing the percentage of Propylene glycol to 10% caused a total blockage of penetration.

Table 3.14 : Data obtained by the diffusion of solution No. 6 through a synthetic membrane using a Modified Franz diffusion cell.
Using propylene glycol.

hrs	Area 1	Area 2	Area 3	Mean	SD	RSD	mg/ml	cumulative mg/ml	mg/25.8ml	Q [mg]	m [mg/cm ²]
0.0	0	0	0	0	0	0	0	0	0	0	0
1.0	937953	607549	277145	607549	330404	54.4	0.0116	0.0116	0.3005	0.3005	0.0957
1.5	1077802	796946	516089	796946	280857	35.2	0.0135	0.0251	0.3481	0.3597	0.1146
2.0	1302541	1048965	795388	1048965	253577	24.2	0.0178	0.0429	0.4582	0.4833	0.1539
2.5	1852615	1480579	1108543	1480579	372036	25.1	0.0251	0.0680	0.6467	0.6896	0.2196
3.0	2585932	1987897	1389861	1987897	598036	30.1	0.0337	0.1016	0.8683	0.9363	0.2982
3.5	3462287	2620584	1778880	2620584	841704	32.1	0.0444	0.1460	1.1447	1.2463	0.3969
4.0	4291869	3227101	2162333	3227101	1064768	33.0	0.0546	0.2006	1.4096	1.5556	0.4954
4.5	5309112	3893464	2477815	3893464	1415649	36.4	0.0659	0.2665	1.7006	1.9013	0.6055
5.0	6490985	4717606	2944226	4717606	1773380	37.6	0.0799	0.3464	2.0606	2.3272	0.7411
5.5	7854147	5750400	3646652	5750400	2103748	36.6	0.0974	0.4438	2.5117	2.8581	0.9102
6.0	8210189	6077263	3944336	6077263	2132927	35.1	0.1029	0.5466	2.6545	3.0983	0.9867

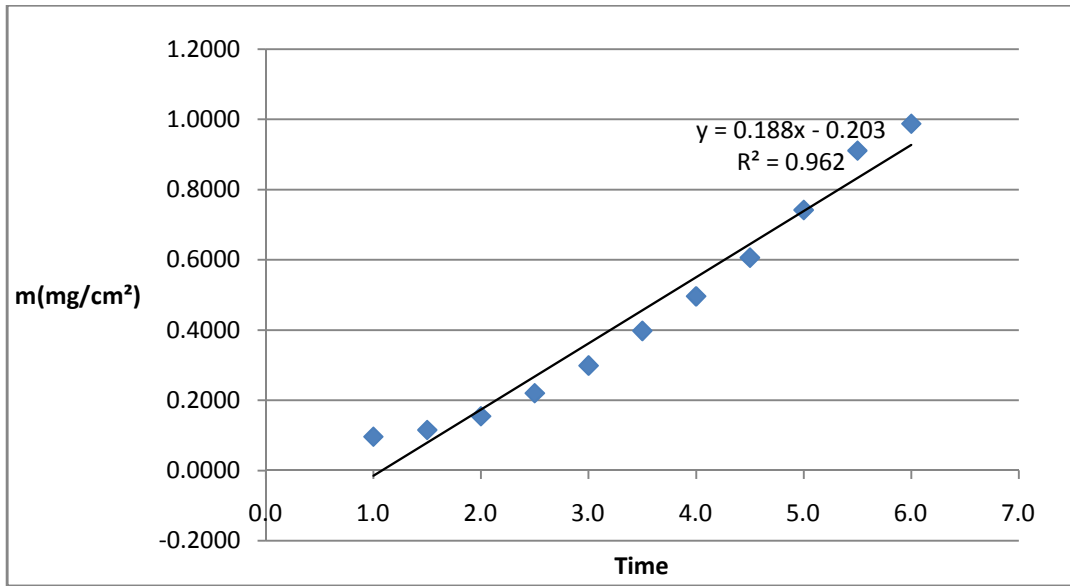


Figure 3.11: In vitro permeation profile for the cumulative amount of O.C penetrated per unit area of synthetic membrane (mg/cm²) for solution no. 6, using propylene glycol as penetration enhancer.

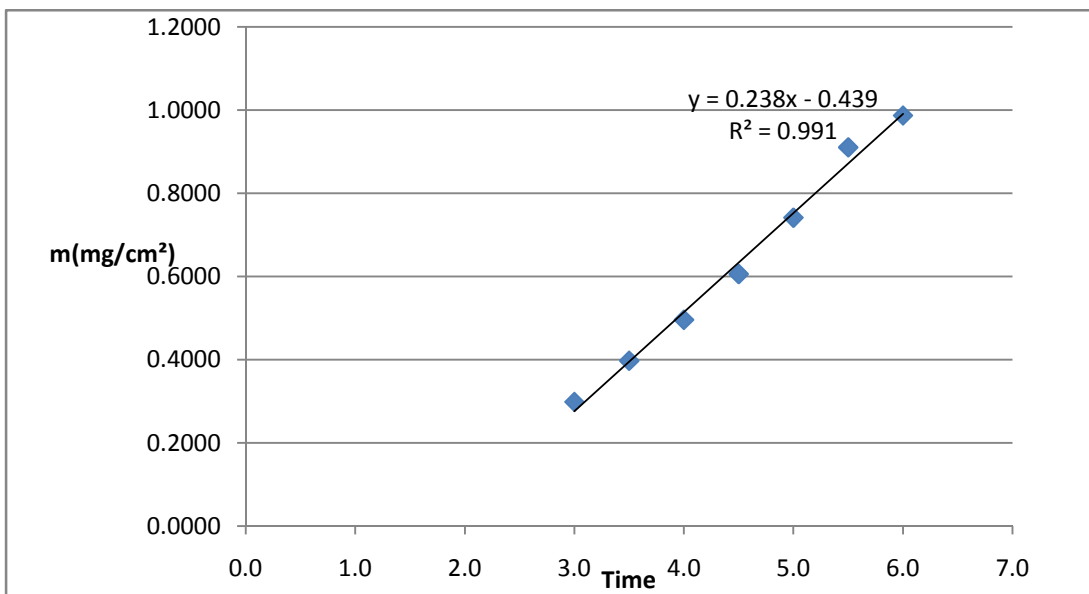


Figure 3.12: In vitro steady state flux diagram of orphenadrine citrate (mg/cm²) through a synthetic membrane for solution no. 6, using propylene glycol as penetration enhancer.

Table: 3.15: Diffusion parameters for solution no. 6, using Propylene glycol as a penetration enhancer.

Item	Slope	Intercept	T _L	D	P	K	ER
API	0.238	0.154	1.84 h	0.0000327	0.0238	13.8	2.13

3.4. Gel Permeation study using a MFDC and the best penetration enhancer “Cremophor RH40” and a synthetic membrane.

The recovered amounts of API in the receiver compartment from Gel no.1 by time are illustrated in table (3.16).

The cumulative amount and flux diagram are shown in figure (3.13), (3.14) respectively.

This gel formula (Permeability coefficient) was considered a reference for other CMC Na. gel formulations to compare with. That is the enhancement ratio of this gel is 1.

3.4.1 Gel Formulas using CMC Na as gelling agent.

3.4.1.1 Gel no. 1: Orphenarine citrate 1%, Cremophore RH40 0.2%, CMC Na 2%, glycerin 5%, methyl paraben 0.1%

The recovered amounts of API in the receiver compartment from Gel no.1 by time are illustrated in table (3.16).

The cumulative amount and flux diagram are shown in figure (3.13), (3.14) respectively.

This gel formula (Permeability coefficient) was considered a reference for other CMC Na. gel formulations to compare with. That is the enhancement ratio of this gel is 1.

Table 3.16: Data obtained by the diffusion of gel No. 1 through a synthetic membrane using a Modified Franz diffusion cell. Cremophore RH40 0.2% is used as a penetration enhancer , CMC Na as gelling agent.

hrs	Area	mg/ml	cumulative mg/ml	mg/25.8ml	Q [mg]	m [mg/cm ²]
0.0	0.000	0	0	0	0	0
1.0	2.004	0.0058	0.0058	0.1493	0.1493	0.0476
1.5	2.892	0.0084	0.0141	0.2155	0.2213	0.0705
2.0	3.821	0.0110	0.0252	0.2847	0.2989	0.0952
2.5	4.717	0.0136	0.0388	0.3515	0.3767	0.1200
3.0	5.652	0.0163	0.0551	0.4212	0.4600	0.1465
3.5	6.342	0.0183	0.0734	0.4726	0.5277	0.1681
4.0	7.405	0.0214	0.0948	0.5518	0.6252	0.1991
4.5	8.104	0.0234	0.1182	0.6039	0.6987	0.2225
5.0	8.939	0.0258	0.1441	0.6661	0.7843	0.2498
5.5	9.795	0.0283	0.1723	0.7299	0.8739	0.2783
6.0	10.578	0.0306	0.2029	0.7882	0.9606	0.3059

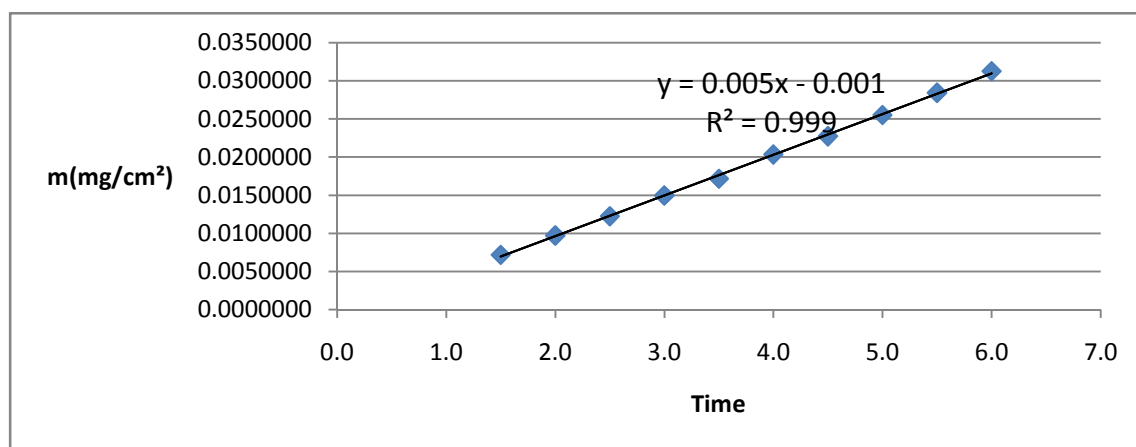


Figure 3.13: In vitro permeation profile for the cumulative amount of O.C penetrated per unit area of synthetic membrane (mg/cm²) for gel no. 1. Cremophor RH 40 0.2% was used as a penetration enhancer, and CMC Na 2% as gelling agent.

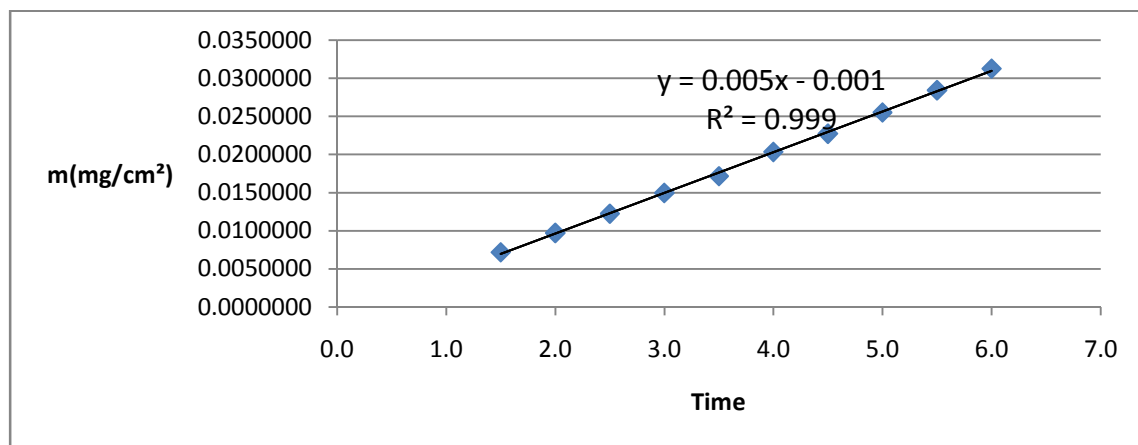


Figure 3.14: In vitro steady state flux diagram of orphenadrine citrate (mg/cm²) through a synthetic membrane for gel no. 1. Cremophor RH 40 0.2% was used as a penetration enhancer and CMC Na 2% as gelling agent

Table 3.17: Diffusion parameters for gel no. 1. Cremophor RH 40 0.2% was used as a penetration enhancer and CMC Na 2% as gelling agent

Gelling Agent	PE	Slope	T_L	D	P	K	ER
CMC Sodium	0.2% Cremophor RH40	0.005	0.2	0.000301	0.0005	0.0316	1.00

The concentration of the penetration enhancer cremophore RH40 was kept as low as possible to give an acceptable gel formulation.

3.4.1.2. Gel no. 2: Orphenarine citrate 1%, Cremophore RH40 1%, CMC Na 2%, glycerin 5%, methyl paraben 0.1%

Table 3.18: Data obtained by the diffusion of gel No. 2 through a synthetic membrane using a Modified Franz diffusion cell. Cremophore RH40 1% is used as a penetration enhancer and CMC Na 2% as a gelling agent.

Area	mg/ml	cumulative mg/ml	mg/25.8ml	Q [mg]	m [mg/cm ²]
0.000	0	0	0	0	0
4.977	0.0015	0.0015	0.0389	0.0389	0.0124
8.662	0.0026	0.0041	0.0677	0.0692	0.0220
10.660	0.0032	0.0074	0.0833	0.0874	0.0278
10.926	0.0033	0.0107	0.0854	0.0928	0.0295
12.007	0.0036	0.0143	0.0938	0.1045	0.0333
13.276	0.0040	0.0183	0.1038	0.1181	0.0376
14.545	0.0044	0.0227	0.1137	0.1320	0.0420
16.161	0.0049	0.0276	0.1263	0.1490	0.0475
18.676	0.0057	0.0333	0.1460	0.1736	0.0553
20.544	0.0062	0.0395	0.1606	0.1939	0.0617
22.920	0.0069	0.0465	0.1791	0.2186	0.0696

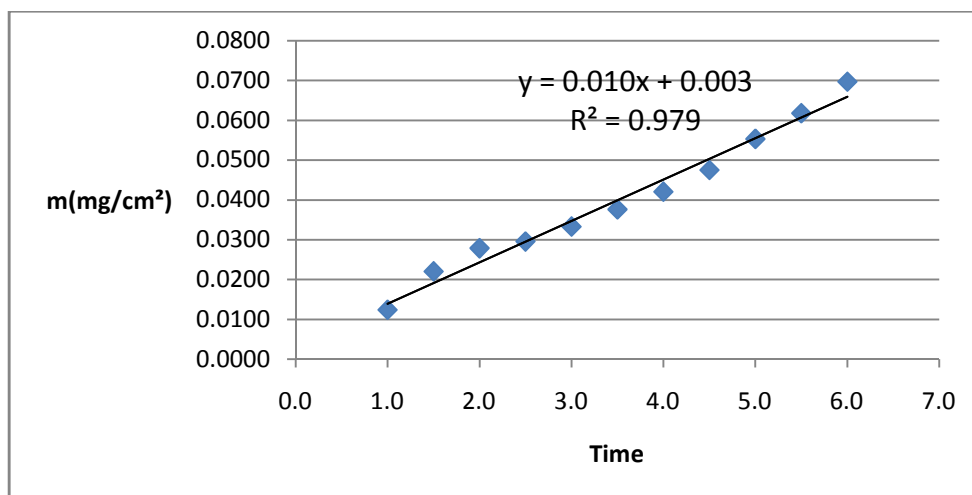


Figure 3.15: In vitro permeation profile for the cumulative amount of O.C penetrated per unit area of synthetic membrane (mg/cm²) for gel no. 2. Cremophore RH40 1% is used as a penetration enhancer and CMC Na 2% as a gelling agent.

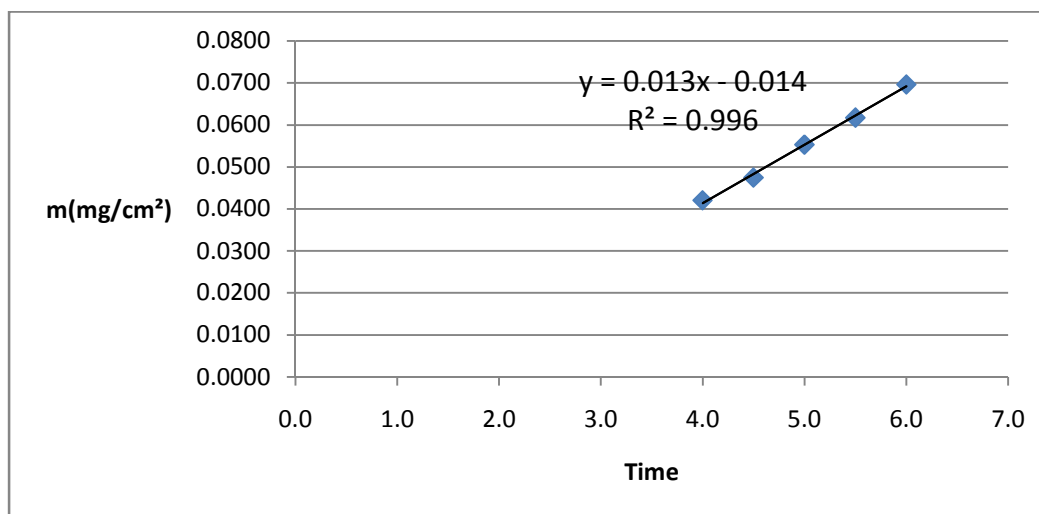


Figure 3.16: In vitro steady state flux diagram of orphenadrine citrate (mg/cm²) through a synthetic membrane for gel no.2. Cremophore RH40 1% is used as a penetration enhancer and CMC Na 2% as a gelling agent.

Table: 3.19: Diffusion parameters for gel no. 2. Cremophore RH40 1% is used as a penetration enhancer and CMC Na 2% as a gelling agent.

Gelling Agent	PE	Slope	T_L	D	P	K	ER
CMC Sodium	1 % Cremophor RH40	0.0113	1.1 h	0.0000547	0.00113	0.0316	2.26

Increasing the concentration of penetration enhancer from 0.2% to 1% increased the permeability of the gel

3.4.2. Gels using HPMC as gelling agent

3.4.2.1. Gel no. 3: HPMC 3%+ cremophore 0.2%+orphendrine citrate 1%+ glycerine 5%+Methyl paraben 0.1%

This gel formula is considered a reference for other gel formulas that use the same gelling agent (HPMC), that is the enhancement ratio is one.

In this formula the concentration of Cremophor was kept as low as possible to have an acceptable gel formula.

The data collected is presented in table (3.20). The steady state flux diagram is shown in figure (3.17) and the diffusion parameters are calculated in table (3.21).

Table 3.20: Data obtained by the diffusion of gel No. 3 through a synthetic membrane using a Modified Franz diffusion cell. Cremophore RH40 0.2% is used as a penetration enhancer and HPMC 3% as gelling agent.

hrs	Area	mg/ml	cumulative mg/ml	mg/25.8ml	Q [mg]	m [mg/cm ²]
0.0	0.000	0	0	0	0	0
1.0	3.438	0.0010	0.0010	0.0262	0.0262	0.0083
1.5	5.316	0.0016	0.0026	0.0405	0.0415	0.0132
2.0	7.436	0.0022	0.0048	0.0566	0.0592	0.0189
2.5	10.055	0.0030	0.0077	0.0765	0.0813	0.0259
3.0	11.886	0.0035	0.0113	0.0905	0.0982	0.0313
3.5	14.000	0.0041	0.0154	0.1066	0.1178	0.0375
4.0	15.927	0.0047	0.0201	0.1213	0.1366	0.0435
4.5	18.008	0.0053	0.0254	0.1371	0.1572	0.0501
5.0	19.779	0.0058	0.0312	0.1506	0.1760	0.0560
5.5	21.890	0.0065	0.0377	0.1667	0.1979	0.0630
6.0	24.246	0.0072	0.0448	0.1846	0.2223	0.0708

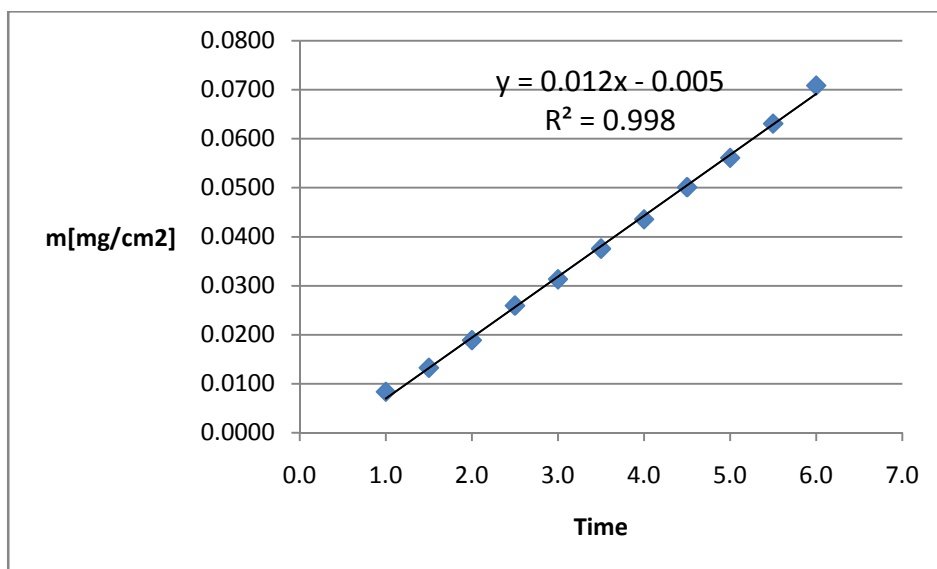


Figure 3.17: In vitro steady state flux for the cumulative amount of O.C penetrated per unit area of synthetic membrane (mg/cm²) for gel no.3. Cremophore RH40 0.2% is used as a penetration enhancer and HPMC 3% as gelling agent.

Table 3.21: Diffusion parameters for gel no.3. Cremophore RH40 0.2% is used as a penetration enhancer and HPMC 3% as gelling agent.

Gelling Agent	PE	Slope	T _L	D	P	K	ER
HPMC 3%	Cremophor RH40 0.2%	0.012	0.416	0.000145	0.0012	0.066	1

3.4.2.2: Gel no. 4: HPMC 3%+ cremophore 1%+orphendrine citrate 1%+ glycerine5% + Methyl paraben 0.1%

This study was repeated two times using a MFDC. The data are shown in table (3.22).

Table 3.22: Data obtained by the diffusion of gel No. 4 through a synthetic membrane using a Modified Franz diffusion cell. Cremophor RH40 1% is used as a penetration enhancer and HPMC 3% as gelling agent

hrs	Amount1 mg/ml	Amount2 mg/ml	Mean mg/ml	cumulative mg/ml	mg/25.8ml	Q [mg]	m [mg/cm ²]
0.0	0	0		0	0	0	0
1.0	0.0014	0.0017	0.0015	0.0015	0.0398	0.0398	0.0127
1.5	0.0022	0.0029	0.0026	0.0041	0.0664	0.0679	0.0216
2.0	0.0032	0.0050	0.0041	0.0082	0.1060	0.1102	0.0351
2.5	0.0042	0.0092	0.0067	0.0149	0.1730	0.1812	0.0577
3.0	0.0050	0.0127	0.0088	0.0238	0.2279	0.2428	0.0773
3.5	0.0059	0.0152	0.0106	0.0343	0.2727	0.2964	0.0944
4.0	0.0068	0.0191	0.0129	0.0473	0.3341	0.3684	0.1173
4.5	0.0077	0.0233	0.0155	0.0627	0.3990	0.4463	0.1421
5.0	0.0085	0.0274	0.0179	0.0807	0.4628	0.5255	0.1674
5.5	0.0092	0.0339	0.0215	0.1022	0.5548	0.6355	0.2024
6.0	0.0101	0.0373	0.0237	0.1259	0.6109	0.7130	0.2271

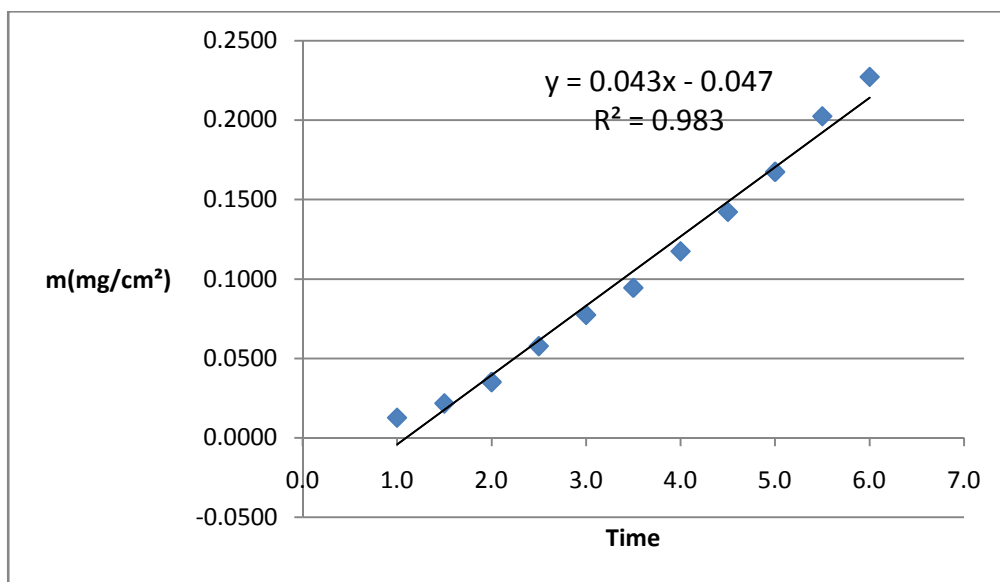


Figure 3.18: In vitro permeation profile for the cumulative amount of O.C penetrated per unit area of synthetic membrane (mg/cm²) for gel no. 4. Cremophor RH40 1% is used as a penetration enhancer and HPMC 3% as gelling agent

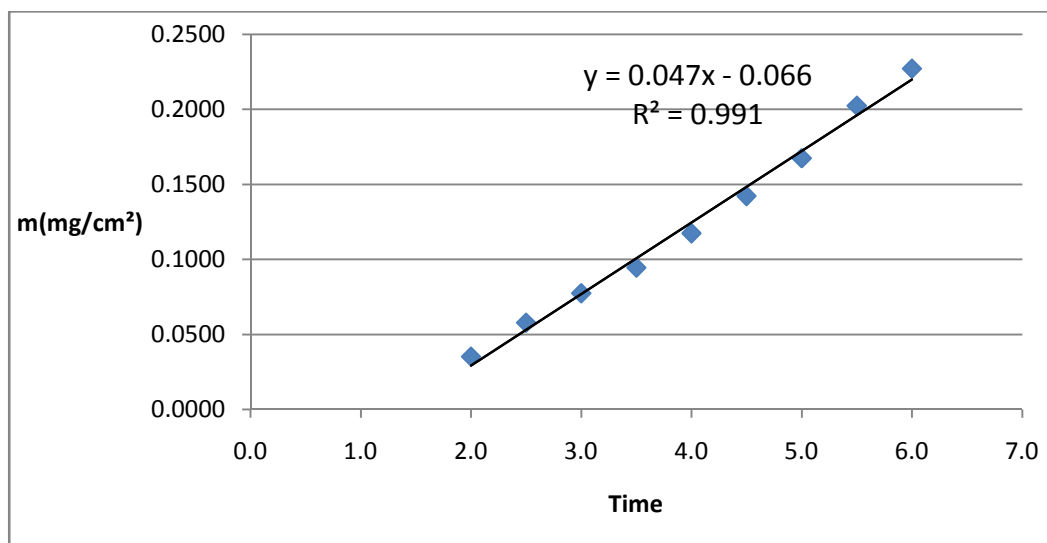


Figure 3.19: In vitro steady state flux diagram of orphenadrine citrate (mg/cm²) through a synthetic membrane for gel no. 4. Cremophor RH40 1% is used as a penetration enhancer and HPMC 3% as gelling agent

Table 3.23: Diffusion parameters of gel no.4. Cremophor RH40 1% is used as a penetration enhancer and HPMC 3% as gelling agent

Gelling Agent	PE	Slope	T_L	D	P	K	ER
HPMC 3%	Cremophor RH40 1%	0.047	1.4 h	0.0000429	0.0047	2.1	3.9

Increasing the concentration of Cremophor RH40 to 1% resulted in about 4-fold penetration enhancement of the API.

3.5. The effect of gelling agent and the concentration of penetration enhancer on API permeability

The diffusion parameters and the permeability coefficients of gels numbers 1, 2, 3, and 4 are shown in table (3.24). Considering gel no. 1 as a reference with enhancement ratio equals one. The enhancement ratio is calculated by dividing the permeability of the gel over the permeability of gel no. 1.

Table 3.24: Summary of the Diffusion results for gels numbers 1, 2, 3, and 4. Cremophor RH40 was used as penetration enhancer, HPMC and CMC Na as gelling agents.

Gel no.	Gelling Agent	PE Conc.	T_L	D	P	K	ER
1	CMC Na. 2%	0.2%	0.2	0.0003010	0.00050	0.0316	1.0
2	CMC Na 2%	1%	1.1 h	0.0000547	0.00113	0.0316	2.3
3	HPMC 3%	0.2%	0.4	0.0001450	0.00120	0.0660	2.4
4	HPMC 3%	1%	1.4 h	0.0000429	0.00470	2.1000	9.4

When concentration of penetration enhancer is equal and the only difference is the type of gelling agent, HPMC showed a better permeation results than CMC Na.

The HPMC gel formula no. 4 is selected for further diffusion study on human skin.

3.6. The effect of viscosity on the permeation of API through a synthetic membrane using a MFDC.

To study the effect viscosity on API diffusion through a synthetic membrane, a new formula is prepared with less viscosity than gel no. 4 and the permeability coefficient is determined.

The permeability coefficient of gel no.4 is used as a reference, that is the permeability coefficient is one.

Gel formula no. 5: HPMC 2%, Cermophor 1%, orphenadrine citrate 1%, glycerin 5%, methyl paraben 0.1%: Viscosity 7×10^3 cps

The gel formula no. 5 is studied for diffusion through a synthetic membrane using a MFDC. The data are shown in table (3.25) .

Table 3.25: Data obtained by the diffusion of gel No. 5 through a synthetic membrane cell, using HPMC 2% as gelling agent and Cremophor RH40 1% as a penetration enhancer

hrs	Area	mg/ml	cumulative mg/ml	mg/25.8ml	Q [mg]	m [mg/cm ²]
0.0	0	0	0	0	0	0
1.0	235029	0.0031	0.0031	0.0812	0.0812	0.0259
1.5	526718	0.0071	0.0102	0.1819	0.1851	0.0589
2.0	772767	0.0103	0.0205	0.2669	0.2771	0.0883
2.5	1183818	0.0158	0.0364	0.4089	0.4294	0.1368
3.0	1579673	0.0211	0.0575	0.5456	0.5820	0.1854
3.5	2014659	0.0270	0.0845	0.6958	0.7534	0.2399
4.0	2333564	0.0312	0.1158	0.8060	0.8905	0.2836
4.5	2787282	0.0373	0.1531	0.9627	1.0785	0.3435
5.0	3187721	0.0427	0.1957	1.1010	1.2541	0.3994
5.5	3540175	0.0474	0.2431	1.2228	1.4185	0.4517
6.0	3907396	0.0523	0.2954	1.3496	1.5927	0.5072

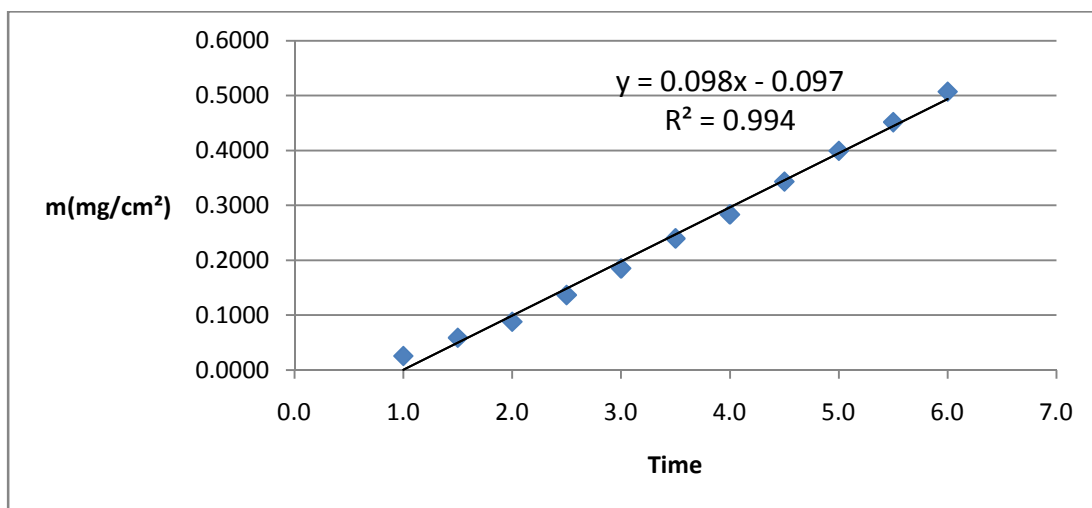


Figure 3.20: In vitro permeation profile for the cumulative amount of O.C penetrated per unit area of synthetic membrane (mg/cm²) for gel no. 5 through a synthetic membrane using HPMC 2% as gelling agent and Cremophor RH40 1% as a penetration enhancer

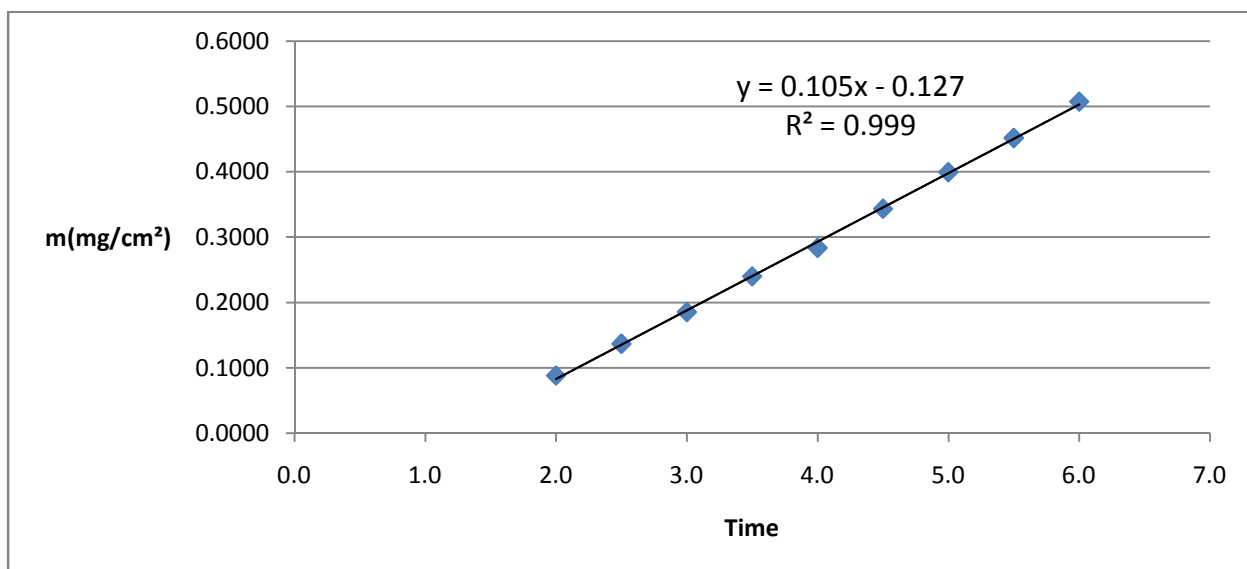


Figure 3.21: In vitro steady state flux diagram of orphenadrine citrate (mg/cm²) through a synthetic membrane for gel no. 5 through a synthetic membrane using HPMC 2% as gelling agent and Cremophor RH40 1% as a penetration enhancer

Table 3.26: Diffusion parameters for Gel no. 5 through a synthetic membrane using HPMC 2% as gelling agent and Cremophor RH40 1% as a penetration enhancer

Gelling Agent	PE	Slope	T_L	D	P	K
HPMC 2%	Cremophor 1%	0.105	1.2 h	0.0000501	0.0105	3.98

The permeability coefficient of gel no. 5 is divided over the permeability coefficient of gel no. 4 to determine the enhancement ratio. (See table 3.27)

Table 3.27: The effect of viscosity on drug permeation using cremophore RH40 as penetration enhancer and HPMC as gelling agent

Gel No.	Gelling Agent	PE	Viscosity ×10³ cps	Slope	T_L	D	P	K	ER
4	HPMC 3%	Cremophor 1%	30	0.047	1.4 h	0.0000429	0.0047	2.1	1
5	HPMC 2%	Cremophor 1%	7	0.105	1.2 h	0.0000501	0.0105	3.98	2.23

As the viscosity decreased the permeation of the API increased.

3.7. Human Skin Permeation results

The best penetration enhancer (Cremophor RH40) was used with orphenadrine citrate in the gel formula and it was tested for permeation through the stratum corneum of human skin using a MFDC. The gel formula used was gel formula no. 4:

Gel formula no. 4: Orphenadrine citrate 1%, Cremophore RH40 1%, HPMC 3%, Glycerin 5%, Methylparaben 0.1%

The gel specifications are shown in table (3.28), and the data obtained are shown in table (3.29)

Table (3.28): Specifications of final gel formula (no.4) that was tested for permeation through human skin.

Specification	Description
Color	Colorless
pH	3.61
Viscosity	30×10^3 cps
Spreadability	Easily spreadable
Crystals	Clear

Table 3.29: The Data obtained from the diffusion study through human skin using HPMC 3% as gelling agent and Cremophore 1% as penetration enhancer

hrs	Area 1	Amount1 mg/ml	Area 2	Amount2 mg/ml	Area 3	Amount3 mg/ml	Mean mg/ml	SD	RSD	cumulative mg/ml	mg/25.8ml	Q [mg]	m [mg/cm ²]
0	0	0	0	0	0	0	0	0	0	0	0	0	0
1.0	0.278	0.0011	0.276	0.0011	0.288	0.0012	0.0011	0.000026	2.3	0.0011	0.0290	0.0290	0.0092
1.5	0.315	0.0013	0.317	0.0013	0.315	0.0013	0.0013	0.000005	0.4	0.0024	0.0326	0.0337	0.0107
2.0	0.479	0.0019	0.479	0.0019	0.483	0.0019	0.0019	0.000009	0.5	0.0043	0.0496	0.0519	0.0165
2.5	0.590	0.0024	0.598	0.0024	0.594	0.0024	0.0024	0.000016	0.7	0.0067	0.0613	0.0656	0.0209
3.0	0.693	0.0028	0.700	0.0028	0.694	0.0028	0.0028	0.000015	0.5	0.0094	0.0718	0.0785	0.0250
3.5	0.828	0.0033	0.823	0.0033	0.826	0.0033	0.0033	0.000010	0.3	0.0127	0.0852	0.0947	0.0301
4.0	0.895	0.0036	0.908	0.0036	0.899	0.0036	0.0036	0.000027	0.7	0.0163	0.0929	0.1057	0.0337
4.5	1.030	0.0041	1.027	0.0041	1.029	0.0041	0.0041	0.000006	0.1	0.0205	0.1062	0.1225	0.0390
5.0	1.081	0.0043	1.088	0.0044	1.084	0.0043	0.0043	0.000014	0.3	0.0248	0.1119	0.1324	0.0422
5.5	1.215	0.0049	1.220	0.0049	1.214	0.0049	0.0049	0.000013	0.3	0.0297	0.1255	0.1503	0.0479
6.0	1.301	0.0052	1.299	0.0052	1.302	0.0052	0.0052	0.000006	0.1	0.0349	0.1342	0.1639	0.0522

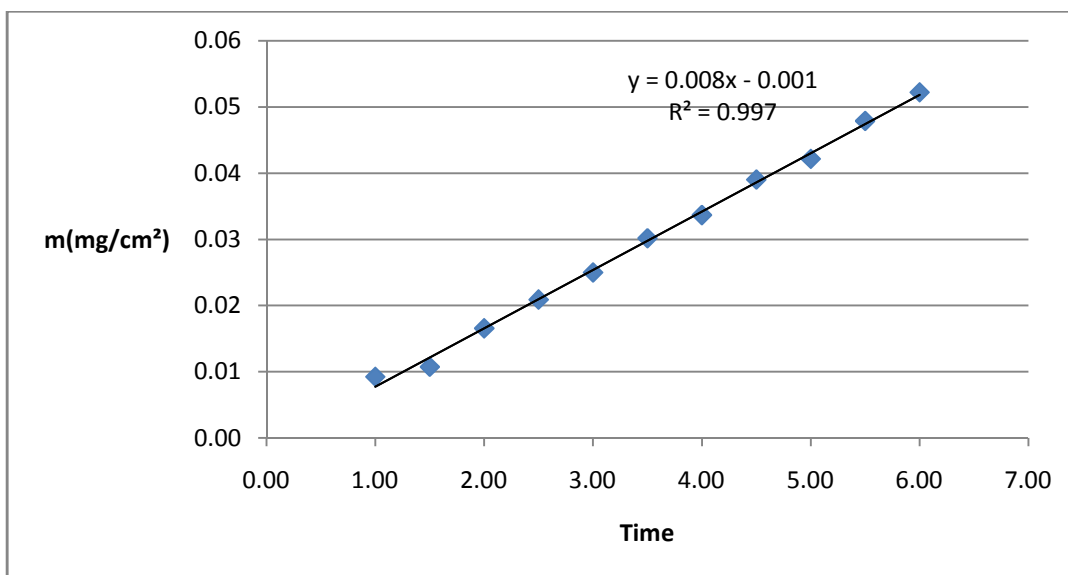


Figure 3.22: In vitro permeation profile for the cumulative amount of O.C penetrated per unit area of Human skin (mg/cm²) for gel no. 4 using HPMC 3% as gelling agent and CremophoreRH40 1% as penetration enhancer

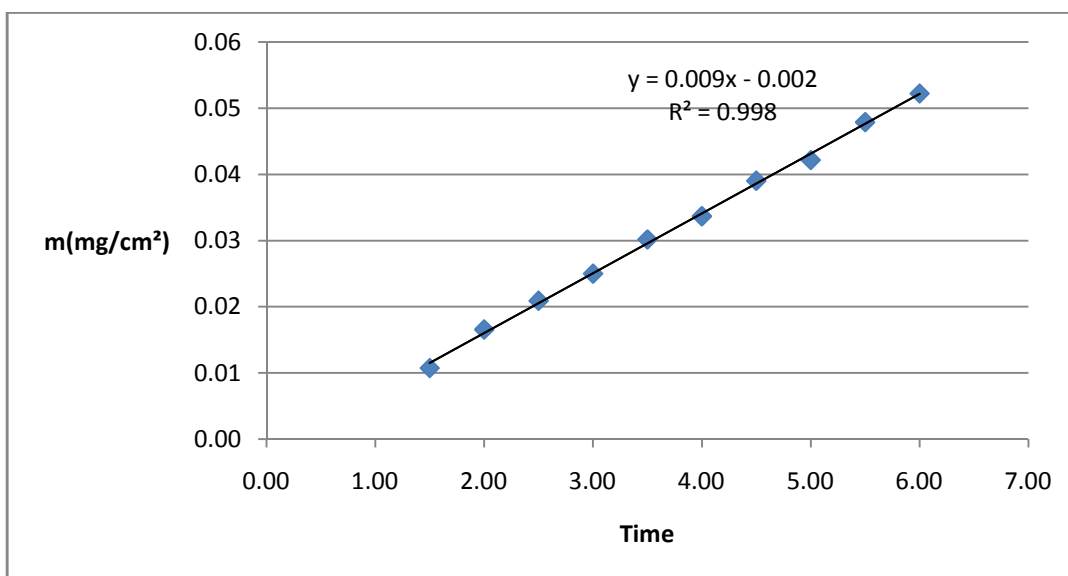


Figure 3.23: In vitro steady state flux diagram of orphenadrine citrate through a human skin for gel no. 4, using HPMC 3% as gelling agent and Cremophore RH40 1% as penetration enhancer

Table 3.30: Diffusion parameters for gel no. 4 through human skin using HPMC 3% as gelling agent and Cremophore RH40 1% as penetration enhancer

Gel No.	Gelling Agent	PE	Slope	T _L	D	P	K
4	HPMC3%	Cremophore1%	0.009	0.22	0.00000170	0.0009	0.794

The effect of Cremophor RH 40 1% on the permeation of orphenadrine citrate through synthetic membrane and human skin is shown in table (3.31)

Table 3.31 Summary of the effect of Cremophor RH40 on the permeation of Orphenadrince citrate using MFDC, through synthetic and Human skin for gel no. 4

Gel no.	Gelling Agent	PE 1%	Membrane Type	Slope	T _L	D	P	ER
4	HPMC3%	Cremophor	Human Skin	0.009	0.22 h	0.00000170	0.0009	1
4	HPMC 3%	Cremophor	Synthetic	0.047	1.40 h	0.0000429	0.0047	5.2

The API showed less penetration through human skin than through the synthetic membrane because the skin has a more complex structure. In order for the API to penetrate through the skin it must penetrate the intercellular lipid matrix which is composed of bi-layer lipid matrix with hydrophilic and hydrophobic channels. The lipid bi-layer contains ceramides, fatty acids, cholesterol and cholesteryl esters. The synthetic membrane on the other hand is composed of octanol soaked filter membrane (hydrophobic layer) sandwiched between two dialysis membranes (hydrophilic layer).

Part Four
Conclusion

Conclusion

In the present work, the solubility of orphenadrine citrate was determined in different solutions and was found to increase as the pH increases

Compatibility study of orphenadrine citrate with different excipients was carried out; almost all excipients were found to be compatible with orphenadrine citrate except carbopol, Urea and sodium lauryl sulfate

The influence of selected penetration enhancers included in a 1 % w/w orphenadrine citrate aqueous and gel preparation was investigated. The enhancement ratio was calculated for each penetration enhancer and found to be in the following order:

Cremophor RH40 > Urea > β -Cyclodextrin > Tween 80 > Propylene glycol

Topical muscle relaxant, orphenadrine citrate gel was formulated with the selected penetration enhancer cremophor RH40. The best gelling agent was found to be HPMC.

As the viscosity of the O.C gel decreased the drug permeability through the synthetic membrane increased and when the concentration of Cremophor RH40 is increased to 1% in the gel formula the permeability of O.C increased.

The synthetic membrane showed better permeation results than the skin, suggesting that the human skin is more complex than the synthetic bio-membrane

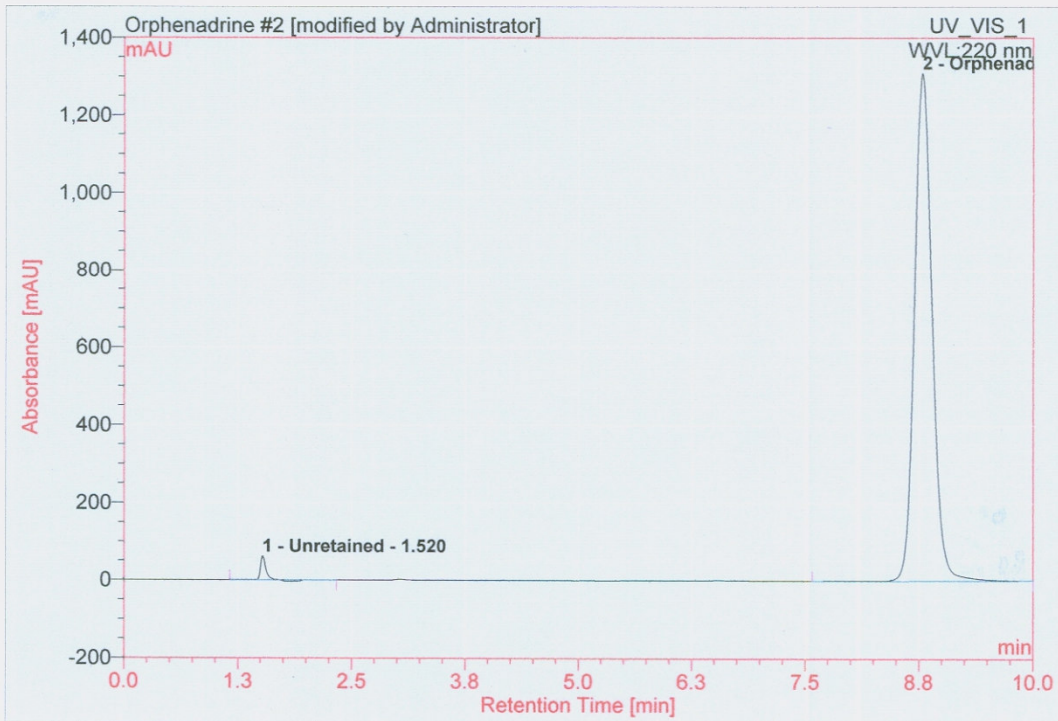
Some penetration enhancers increased the partition coefficient and others increased the diffusion coefficient, combinations of these penetration enhancers are recommended for further enhancement of O.C permeability.

Part Five
Appendix

2 Orphenadrine Citrate St

Orphenadrine Citrate Penetration Enhancing

Sample Name:	Orphenadrine Citrate St	Injection Volume:	20.0
Vial Number:	RA2	Channel:	UV_VIS_1
Sample Type:	standard	Wavelength:	220
Control Program:	Orphenadrine	Bandwidth:	n.a.
Quantif. Method:	Orphenadrine	Dilution Factor:	1.0000
Recording Time:	20-8-08 14:23	Sample Weight:	1.0000
Run Time (min):	10.00	Sample Amount:	1.0000



No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Amount	Type
1	1.52	Unretained	61.506	3.301	1.11		
2	8.79	Orphenadrine Citrate	1308.219	293.782	98.89		
Total:			1369.725	297.083	100.00		

Figure 5.1: A chromatograph of O.C reference standard using Dionex HPLC.

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متوافقة على درجة حرارة 25 درجة مئوية فقط. و كذلك فإن سيطرات الأورفينادرين أبدى ثباتية على نطاق واسع من درجات الحموضة.

محسّنات النفاذية تحت البحث هي: cremophor, SLS, Urea, PG, Cyclodextrin, oleic acid, Tween 80

لقد تم إضافتها بتركيز 1% إلى 1% سيطرات الأورفينادرين في الغرفة المانحة من خلية فرانز وتم تحديد قيم النفاذية و هي: الميل و التقاطع السيني للكمية التراكمية التي نفذت في وحدة الزمن ثم تم حساب ، الفترة الزمنية التي استغرقتها المادة الفعالة قبل النفاذ، معامل الانتشار، معامل النفاذية، معامل التجزئة و قورنت النفاذية من خلال حساب معدل تحسن النفاذية لمختلف العينات.

لقد كان معدل تحسن النفاذية لمختلف محسّنات النفاذية في المحلول المائي كالاتي:

PG < Tween 80 < Cyclodextrin < Urea < Cremophore RH 40.

لقد كانت هناك عدة محاولات لتركيبة جل موضعي باستخدام HPMC, CMC Na يحتوي على 1% سيطرات الأورفينادرين مع تراكيز تتراوح بين 0.2% - 1% من محسّنات النفاذية المختارة و قد تمت هذه الدراسة باستخدام نفس خلية فرانز و بنفس طريقة التحليل.

إن الجل المكون من HPMC و 1% من المادة الفعالة و 1% من Cremophor RH40 أبدى تحسن كبير في النفاذية مقارنة بالجل المكون من CMC Na وكان لزيادة تركيز محسن النفاذية في النوعين إلى زيادة في معدل النفاذية.

التركيبة الأخيرة للجل مع أفضل تركيز من محسن النفاذية تم دراسة نفاذيتها من خلال جلد الإنسان ، و قد أبدت المادة الفعالة نفاذية جيدة من خلال الجلد و كان معامل نفاذيتها يساوي 0.0009 سم/ساعة

في المرحلة الأولى من هذه التجربة تم تحديد قيم النفاذية لمحلول مائي مكون من 1% سبترات الأورفينادرين واستخدمت هذه القيم كمرجع للتقييم تأثير محسنات النفاذية على نفاذ سبترات الأورفينادرين. في المرحلة الثانية تم استخدام جل موضعي يحتوي على 1% سبترات الأورفينادرين كمرجع لقياس تأثير أفضل محسن نفاذية على نفاذيته و كذلك تم بحث تأثير درجة اللزوجة و نوع المادة المكونة للجل على سرعة نفاذية الدواء. في المرحلة الثالثة من البحث تم استخدام عينة سليمة من جلد الإنسان لفحص نفاذية أفضل نظام منتقى من الجل و محسنات النفاذية.

لقد تم استخدام خلية فرانز محسنة لقياس الانتشار في هذا البحث، ففي المرحلة الأولى و الثانية تم استخدام غشاء منقوع في الأوكتانول مثبت بين غشائي ديلزة كبديل عن جلد الإنسان و تم ملء الغرفة المستقبلية في خلية فرانز بمحلول فوسفات محافظ على درجة الحموضة على 7.4 و الغرفة المانحة احتوت على 5مل في حالة المحلول المائي و 5 جرام في حالة الجل .

في المرحلة الثالثة تم استبدال الغشاء الصناعي بجلد إنسان تم الحصول عليه من امرأة في الأربعينات من العمر وتم تثبيته ليفصل بين الغرفة المانحة و الغرفة المتلقية في خلية فرانز. تم أخذ عينات من 1مل من الغرفة المتلقية أول ساعة ثم عينة كل نصف ساعة لمدة ست ساعات لكل عينة بحثية ثم تم تحديد الكمية النافذة من سبترات الأورفينادرين في كل عينة وذلك من خلال تحليلها باستخدام جهاز HPLC على $\lambda = 220$ نانوميتر.

لقد تم تحديد ذائبية سبترات الأورفينادرين في الماء و في محلول فوسفاتي على عدة درجات من الحموضة و كانت أكثر ذائبية (2.43 جرام لكل 100مل) في درجة الحموضة 7.4.

لقد تم إجراء فحص للتوافق لسبترات الأورفينادرين مع مختلف المواد المضافة و هي:

(HPMC, CMC Na, PG, MP, Tween 80, Cyclodextrin, Cremophore, SLS, Urea, carbapol, Oleic acid, glycerin)

وذلك لمدة ثلاثة أشهر على درجة حرارة 25 و 40 درجة مئوية و تم التوصل على أن سبترات الأورفينادرين متوافقة مع غالبية المواد المضافة ما عدا ال Carbapol و ال SLS في حين كانت ال Urea

تحضير الأورفينادرين سترات على شكل جل موضعي و التحقق من تأثير محسنت النفاذية على درجة نفاذيته

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ملخص

الأورفينادرين سترات هو دواء يستخدم كمرخي للعضلات و يوجد على شكل حبوب و إبر، إن المستحضرات الموضعية تبدا و اعدة وذلك لسهولة الإستخدام و القبول لدى المرضى و لكن هذه المستحضرات ليست فعالة لعدم قدرتها على إختراق الجلد بفعالية. إن هدف هذه الرسالة هو بحث تأثير بعض محسنت النفاذية على نفاذ جل طور حديثا في هذا البحث كنموذج للمستحضرات الموضعية شبه الصلبة ذات التأثير المرخي للعضلات