Applied and Industrial Technology

Department of Chemistry & Chemical Technology

Deanship of Graduate Studies

Preparation of topical Azithromycin gel for the treatment of Acne and investigation of the effect of different penetration enhancers on drug permeation rate

Waleed Yousef M. Kamil

M.Sc. Thesis

Jerusalem – Palestine

2006

Introduction

Drug delivery to or via skin has provided an effective route for local or systemic administration of therapeutically active agents. However, skin and in particular the stratum corneum provides an efficient protective barrier for drug absorption. Stratum corneum (horny layer) has a thickness of about 15-20 μm, and is a layer of compressed, overlapping keratinized cells that form a flexible, tough and coherent membrane. This layer contains dead cells with keratin filaments in a matrix of proteins with lipids and water-soluble substances. The thickness and penetration properties of the stratum corneum depend upon its hydration, which is normally around 20% water.

Gels for dermatological use have several favorable properties such as being thixotropic, greaseless, easily spreadable, easily removable, emollient, nonstaining, compatible with several excipients, and water-soluble or miscible.

Emulgels are emulsions, either of the oil-in-water or water-in-oil type, which are gelled by mixing with a gelling agent. They have a high patient acceptability since they possess the previously mentioned advantages of both emulsions and gels. Therefore, they have been recently used as vehicles to deliver various drugs to the skin (13).

Hence, one of the major challenges facing formulation scientists is to enhance and improve drug absorption through this rather elaborated barrier. A useful approach for increasing percutaneous absorption of drugs is to employ penetration or permeation enhancers.

The term penetration enhancer is applied to materials that have a direct effect on the permeability of the skin barrier. Some materials may act by a direct chemical action on the skin while others may not have a specific barrier effect. The latter may affect the solubility and/or dispersibility of the medicament and/or its delivery system (the vehicle) (51).

The active pharmaceutical ingredient this research deals with is Azithromycin, its trade name is Zithromax.

This antibiotic is classified as a semi-synthetic macrolide antibiotic chemically related to erythromycin and clarithromycin (39).

Azithromycin differs from other macrolides in its azalide structure., a methylated nitrogen atom in the number 9a position on the macrolide lactone ring (39).

Acne vulgaris is a common skin disease that affects 85-100% of people at some time during their lives. It is characterized by noninflammatory follicular papules or comedones and by inflammatory papules, pustules, and nodules in its more severe forms. Acne vulgaris affects the areas of skin with the densest population of sebaceous follicles; these areas include the face, the upper part of the chest, and the back (53).

The pathogenesis of acne vulgaris is multifactorial. Four key factors are responsible for the development of an acne lesion (53):

1-Follicular epidermal hyperproliferation and hyperkeratinization.

2-Excess sebum.

3-Propionibacterium acnes.

4- Inflammation.

Propionibacterium acnes is a microaerophilic organism present in many acne lesions. Although, it has not been shown to be present in the earliest lesions of acne, the microcomedo, its presence in later lesions is almost certain. P acnes stimulates inflammation by producing proinflammatory mediators that diffuse through the follicle wall. Recent studies have shown that P acnes binds to the toll-like receptor on monocytes and neutrophils (14).

Binding of the toll-like receptor then leads to the production of multiple proinflammatory cytokines, including interleukin 12 (IL-12), interleukin 8 (IL-8),

and tumor necrosis factor (TNF). Hypersensitivity to *P. acnes* may also explain why some individuals develop inflammatory acne vulgaris (53).

Topical antibiotics are mainly used for their role against *P. acnes.* They may also have anti-inflammatory properties. Topical antibiotics are not comedolytic, and bacterial resistance may develop to any of these agents.

Commonly prescribed topical antibiotics include erythromycin and clindamycin alone or in combination with benzoyl peroxide. Clindamycin and erythromycin are available in a variety of topical agents. They may be applied once or twice a day(53).

The more lipophilic antibiotics, such as minocycline, are generally more effective than tetracycline. Greater efficacy may also be due to less *P. acnes* resistance to minocycline. However, *P. acnes* resistance is becoming more common with all classes of antibiotics currently used to treat acne vulgaris. *P. acnes* resistance to erythromycin has greatly reduced its usefulness in the treatment of acne. Other antibiotics such as azithromycin, is reportedly helpful.

Achieving a desirable percutaneous absorption of drug molecule is a major concern in formulating dermatological products. The use of penetration enhancers could provide a successful mean for this purpose.

Different formulations of topical gels shall be prepared, where gels with different penetration enhancers shall be investigated to study the effect of these penetration enhancers on the permeability rate of the drug through a synthetic membrane in a Franz-type diffusion cells.

1.1- Human skin

The term skin (figure 1) is commonly used to describe the body covering of any animal but technically refers only to the body covering of vertebrates (animals that have a backbone). The skin has the same basic structure in all vertebrates, including fish, reptiles, birds, and humans and other mammals(52).

The skin is essential to a person's survival (27);

1- It forms a barrier that helps prevent harmful microorganisms and chemicals from entering the body.

2- It also prevents the loss of life-sustaining body fluids.

3- It protects the vital structures inside the body from injury and from the potentially damaging ultraviolet rays of the sun.

4- The skin also helps regulate body temperature, excretes some waste products.

5- It is an important sensory organ, it contains various types of specialized nerve cells responsible for the sense of touch.

The skin is made up of two layers, the epidermis and the dermis. The epidermis, the upper or outer layer of the skin, is a tough, waterproof, protective layer, about 90 percent of the cells in the epidermis are keratinocytes, named because they produce a tough, fibrous protein called keratin. This protein is the main structural protein of the epidermis, and it provides many of the skin's protective properties. Keratinocytes in the epidermis are arranged in layers, with the youngest cells in the lower layers and the oldest cells in the upper layers (52).

The old keratinocytes at the surface of the skin constantly slough off. Meanwhile, cells in the lower layers of the epidermis divide continually, producing new keratinocytes to replace those that have sloughed off.

As keratinocytes push up through the layers of the epidermis, they age and, in the process, produce keratin. By the time the cells reach the uppermost layer of the epidermis, they are dead and completely filled with the tough protein. Healthy epidermis replaces itself in a neatly orchestrated way every month (1).

Scattered among the keratinocytes in the epidermis are melanocytes, cells that produce a dark pigment called melanin. This pigment gives color to the skin and protects it from the sun’s ultraviolet rays.

The dermis, or inner layer, is thicker than the epidermis and gives the skin its strength and elasticity. The dermis or lower layer of the skin is richly supplied with blood vessels and sensory nerve endings.

The dermis is a complex structure. It consists mainly of a dense network of structural protein fibers, collagen, reticulum, and elastin, embedded in a semigel matrix of mucopoly-saccharidic "ground substance" .

It ranges from 0.1 to 0.5 cm in thickness, it also exhibits distinct layers, the finely structured, thin papillary layer adjacent to the epidermis and the coarse reticular layer, which is the main structural body of the skin of considerable importance, the microcirculation that sub serves the entire skin is located in the dermis.

The dermis is also penetrated by a network of sensory nerves (pressure, temperature, and pain) and lymphatics (43,44,52).

The healthy dermis is sparsely populated with cells. Fibroblasts, which synthesize the fibrous structural materials, are found here and there.

One also finds mast cells, whose functions presumably include conversion of the carbohydrate secretions of the fibroblasts into the ground substance.

These cells and Langerhans cells have been linked to the allergic responses of the skin.

Melanocytes lie within and beneath the epidermal-dermal interface, these are irregularly distributed.

They produce a pigment, melanin, which is deposited into neighboring keratinocytes through their dendritic projections, which are pinched off after puncturing a keratinocyte's cell wall. Pigment is continuously formed and deposited in brown-black skins in response to the presence of melanocyte stimulating hormone (MSH).

The two layers of the skin are anchored to one another by a thin but complex layer of tissue, known as the basement membrane. This tissue is composed of a series of elaborately interconnecting molecules that act as ropes and grappling hooks to hold the skin together. Below the dermis is the subcutaneous layer, a layer of tissue composed of protein fibers and adipose tissue (fat). Although not part of the skin itself, the subcutaneous layer contains glands and other skin structures, as well as sensory receptors involved in the sense of touch, see figure 1.

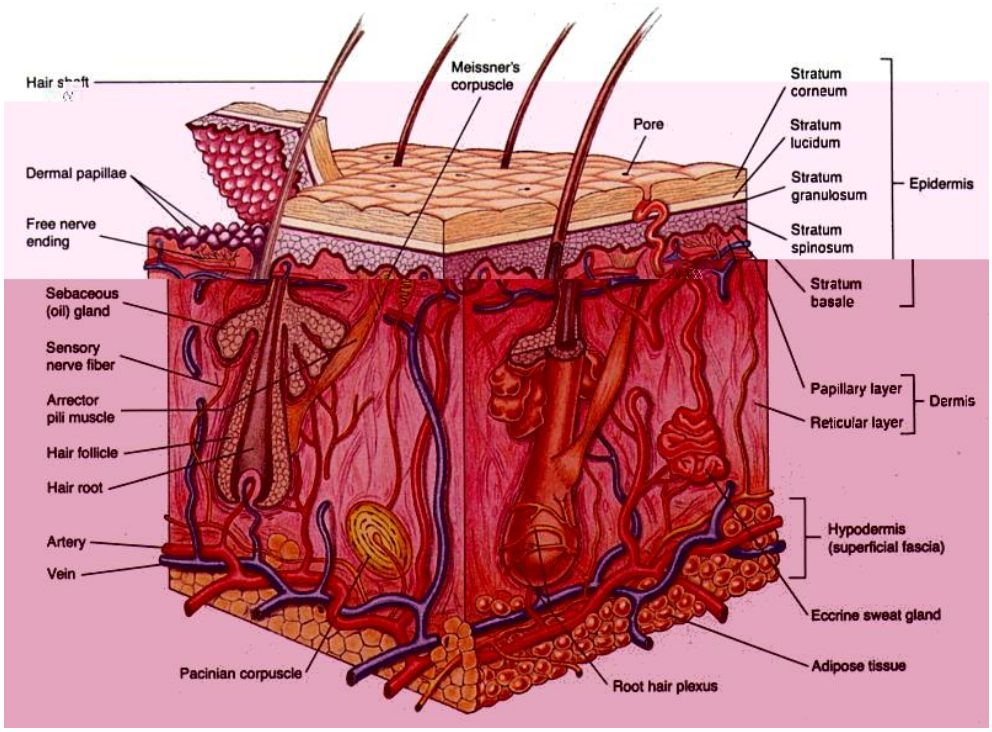

Figure 1: Model of the skin layers and composition (52).

1.2 - Stratum corneum

The stratum corneum ("the horny layer") is the outermost layer of the epidermis, and comprises the surface of the skin. It is composed mainly of dead cells that lack nuclei. As these dead cells slough off, they are continuously replaced by new cells from the stratum germinativum (52).

The stratum corneum is under continuous formation, cells replace from beneath those that are worn off the surface.

A turnover of cells of the stratum corneum occurs about every 2 weeks in normal adults (6).

The cell units that are eventually form the stratum corneum layer is therefore referred to as the germinal (proliferative) layer because, at least in humans, cell division occurs here exclusively.

Mitosis begins an extraordinary process in which daughter cells are pushed up into the next-higher layer, the spinous layer, and from there they continue to migrate to the body's surface. During their transit the cells flatten acutely and inwardly synthesize the protein and lipid which determine the nature of the fully differentiated horny layer. Protegenesis begins in the basal layer, where protein strands are formed. As the cells progress through the epidermis to take positions in the stratum corneum, these strands become more numerous. Massed proteins of different kinds can be distinguished by the time the cells reach the granular layer. A darkly staining material, which gives the granular layer its characteristic appearance, is a basic protein, filagrin. It is believed to induce filament aggregation when released in one of the culminating events of formation of the cornified structure. Under the influence of filagrin, the individual helical strands are formed into multistranded fibers which also have helical geometries. These, in turn, are organized into complex bundles (a-keratin) (52).

The finished keratinocyte's intracellular space is packed mainly with this semicrystalline a-keratin.

Keratin in the cells of the stratum corneum helps keep the skin hydrated by preventing water evaporation. In addition, these cells can also absorb water, further aiding in hydration and explaining why humans and other animals experience finger and toe wrinkling (colloquially called "pruning") when immersed in water for prolonged period (52).

The thickness of the stratum corneum varies according to the amount of protection and/or grip required by a region of the body. For example, the hands are typically used to grasp objects, requiring the palms to be covered with a thick stratum corneum. Similarly, the sole of the foot is prone to injury, and so it is protected with a thick stratum corneum layer (figure 2).

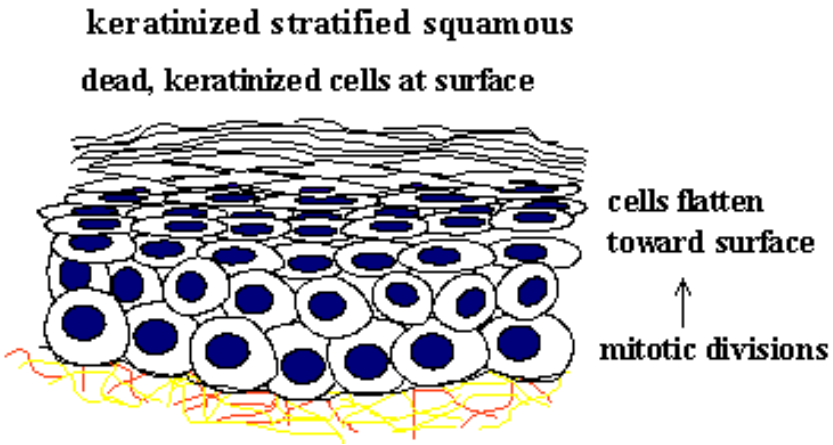

Figure 2: Stratum corneum structure (6).

1.3- Percutaneous absorption

Different factors can affect percutaneous absorption, some are mentioned bellow:

1.3.1- Gender and Race is associated with obvious appearance differences human skin.

There is little evidence that the skins of male and female differ in permeability in any consequential way, however, there are differences in permeability of skin across human races.

While the horny layers of Caucasian and black skin are of equal thickness, the latter contains more cell layers and is more dense. As a consequence, black skin has proven to be several fold less permeable than white skin (27).

1.3.2- Environmental factors may have different effects on healthy skin which affect permeability. Moisture has been recognized as a plasticizer of stratum corneum.

This means that it reduces crystallinity in the internal regimes of the horny matrix. It has long been known that hydrated stratum corneum is more permeable than dry stratum corneum (27).

1.3.3- Skin temperature also influences permeability. There are both kinetic and physiological influences associated with temperature.

Additionally, the perfusion of blood through the skin both in terms of amount and closeness of approach to the skin surface is determined by external temperature and an individual's immediate requirement to retain or release body heat to maintain the body's 37°C isothermal state. Since clearance into the systemic circulation is sensitive to blood flow patterns, the normal fluctuations in surface blood flow should influence the uptake of transdermally delivered chemicals (27).

1.3.4- Health state of the skin determines its physical and physiological condition and thus its permeability, broken skin, where the skin has a physically disrupted stratum corneum represents a high permeability state (27).

1.3.5- Polar solutes may have their absorption increased by several log orders in broken skin while hydrophobic solutes pass through the intact stratum corneum with greater ease due to favorable partitioning (27).

When a drug system is applied topically, the drug diffuses passively out of its carrier or vehicle and into the stratum corneum and the sebum-filled pilosebaceous gland ducts. Inward diffusive movement continues through the full thickness of the stratum corneum and ducts and into the viable epidermal and dermal strata. A concentration gradient is thus established across the skin up to the outer reaches of the skin's microcirculation in the dermal layer. The systemic circulation acts as a reservoir or "sinks" for the drug, and a near-zero concentration of the drug is maintained at the plane formed at the edge of the capillaries where the drug is passed into the general system and diluted (8).

A flow diagram of the steps or events requisite to percutaneous absorption following application of a drug in a thin vehicle film is shown in Figure 3. The important kinetic processes of dissolution and diffusion within the vehicle layer have been added to processes already described to complete the drug delivery picture. Each step in the diagram is potentially rate limiting, depending on the drug and how it interacts within its vehicle and with the skin.

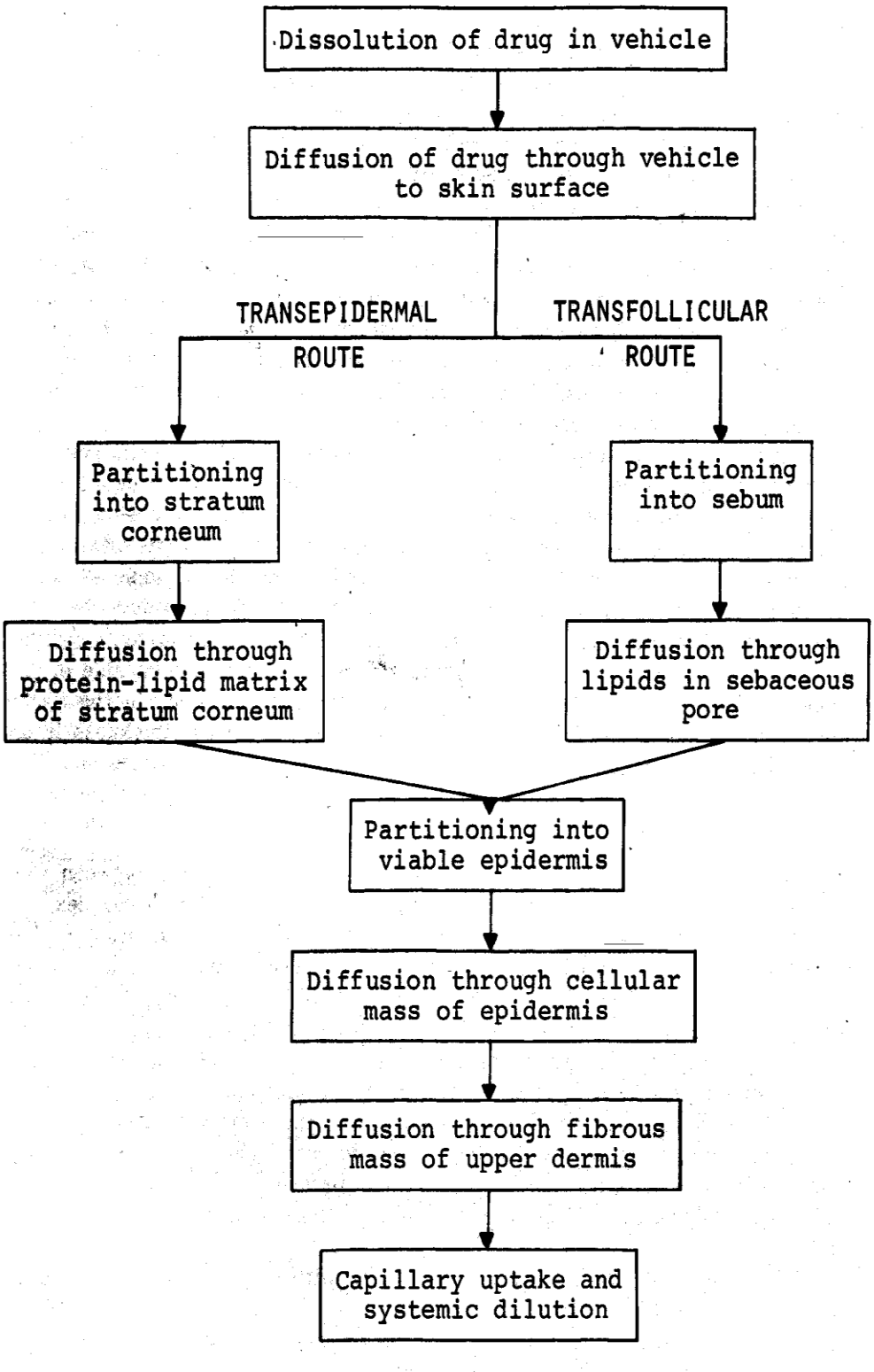

Figure 3: A schematic diagram showing the events for percutaneous absorption (27).

The process consists consecutively of distributing or partitioning events at strata interfaces and diffusive events through strata. Whether the transepidermal route or transfollicular route is followed depends on the relative affinities of the respective tissues for the drug, the fractional areas of the routes, and the ease of diffusion through the respective phases.

Regardless of which pathway is followed, the drug must partition into and diffuse through underlying viable tissues to be effective. Note that the first kinetic event, dissolution, is eliminated as a rate-determining factor if the applied vehicle film contains the drug as a solution.

Two principal absorption routes are identified (27):

1- The trans-epidermal route, correspondent to diffusion directly across the stratum corneum.

2- The trans-follicular route, corresponding to diffusion through the follicular pore.

It is true that the stratum corneum is a source of high diffusional resistance for most compounds, and when it is broken, many drugs penetrate the residual tissue with ease, and thus in a sense, the stratum corneum is the barrier layer. Exceptions exist, however, and extremely hydrophobic chemicals have as much or more trouble passing across the viable tissues (5,7,8).

We have come to know, for instance, that ointments can be used over open wounds safely, for their hydrocarbon constituents are not transported significantly across aqueous phases. Similarly, the full-thickness skin is considerably more impermeable to Octanol in diffucion experiments in vitro than is the stratum corneum alone. On the other hand, physical analysis indicates that corticosteroids breach the stratum corneum so slowly that clinical responses, which are seen promptly, may be due to follicular diffusion.

Because of the dense nature of the stratum corneum, values of diffusion coefficients in this tissue are 1000 times smaller than anywhere else in the skin.

The partition coefficient also varies as the composition of the stratum corneum varies, up to a point, percutaneous absorption can be improved by chemically modifying and increasing hydrophobicity of drugs.

1.4- Penetration enhancers

Different approaches are known to reduce the skin barrier properties like

1- Physical approach which acts on the stratum corneum by stripping and hydration.

2- Chemical approach by synthesis of lipophilic analogs, delipidization of the stratum corneum and coadministration of skin penetration enhancer.

3- Biochemical approach by the synthesis of bioconvertible prodrugs and coadministration of skin metabolism inhibitors (8).

Penetration enhancers are substances which temporarily diminish the impermeability of the skin, such material can be used clinically to enhance the penetration rate of drugs.

Attributes for ideal penetration enhancer (3):

1- The material shall be pharmacologically inert.

2- The material shall not affect the skin normal barrier properties upon removal.

3- The material shall not cause losses of body fluids or any endogenous material.

4- The material shall not be toxic, allergic or irritating.

5- It shall be compatible with other ingredients in the formula.

6- The action of this material shall be predictable.

Classification of penetration enhancers (7,8):

1- Ionic surfactants such as sodium lauryl sulphate, sodium laurate, sodiun dodecyl sulphate (SDS), and dioctyl sodium sulfosuccinate.
2- Non ionic surfactants such as polyoxyethylene sorbate (Tween 80) and polyoxyethylene 9 lauryl ether (PLE).
3- Bile salts and derivatives such as sodium glycocholate, sodium deoxycholate and sodium glycodihydrofusidate.
4- Fatty acids and derivatives such as oleic acid, caprylic acid and luric acid.
5- Sulfoxides such as DMSO.
6- Polyols such as propylene glycol, polyethylene glycol and glycerol.
7- Chelating agents such as EDTA.
8- Monohydric alcohols such as ethanol and isopropyl alcohol.
9- Others like urea , azone (1-dodecylazacycloheptane-2-one), cyclodextrin, terpenes and liposomes.

1.5- Modes of action of skin penetration enhancers (8,12,27) :

The action of penetration enhancers can be simplified as follows:

1- Disruption of the stratum corneum lipids, enhancers may disrupt the polar head groups of the lipid bilayer, the aqueous region between the polar head groups and the lipophilic bilayer site in the non polar tail groups.

For example, oleic acid can disrupt the structure of intercellular lipids because of its structure , also oleic acid can penetrate the skin but does not homogenously mix with the stratum corneum lipids, it shall exist as a pool of fluid within the stratum corneum leading to defects in the barrier function by providing an alternative penetration route.

Rigid packed lipid structure of long saturated hydrocarbon chains are also disrupted by the incorporation of shorter saturated hydrocarbon chain or those with branched side chains, this will explain the optimum penetration enhancement for the C10-C12 unsaturated fatty acid and alcohols and justify why isostearic is better penetration enhancer than stearic acid., see figure 4 & 5 for explanation.

2- Interaction with intercellular protein in the proteinaceous domain which result in the swelling of the protein matrix and change in the keratin helices.

3- Increased partitioning of the drug into the stratum corneum

The partitioning coefficient of any drug determine its affinity to pass from the vehicle through the stratum corneum.

In a case where the API have a very high affinity to the solvent and the solvent have a high penetratability 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.

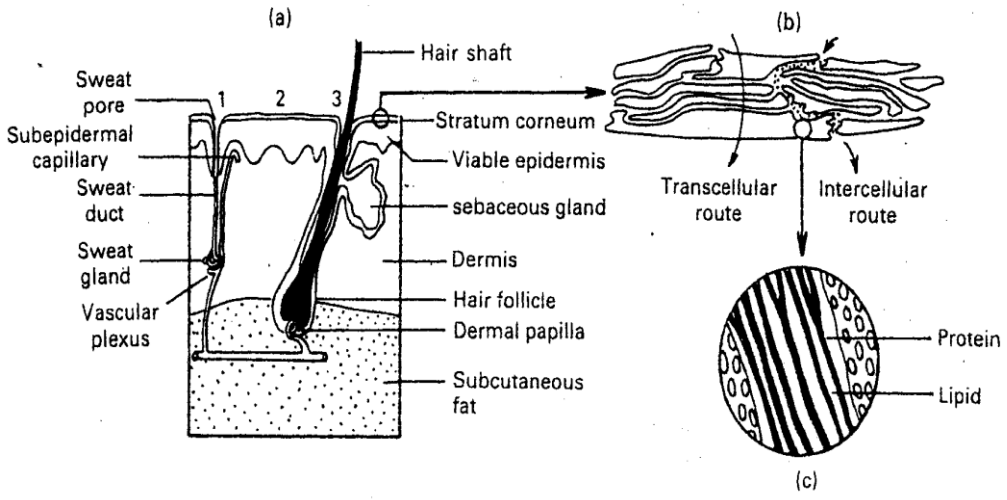

Figure 4: simplified diagram of skin structure and routes of drug penetration.

(a): Route (1) via the sweat ducts; route (2) across the continuous stratum corneum; or route (3) through the hair follicles with their associated sebaceous glands. (b) Representation of the stratum corneum membrane, illustrating two possible subroutes for diffusion.(c) Idealized representation of protein filaments in lipid matrix within stratum corneum cell (8).

(b) and (c) routes can be clarified by Figure 5.

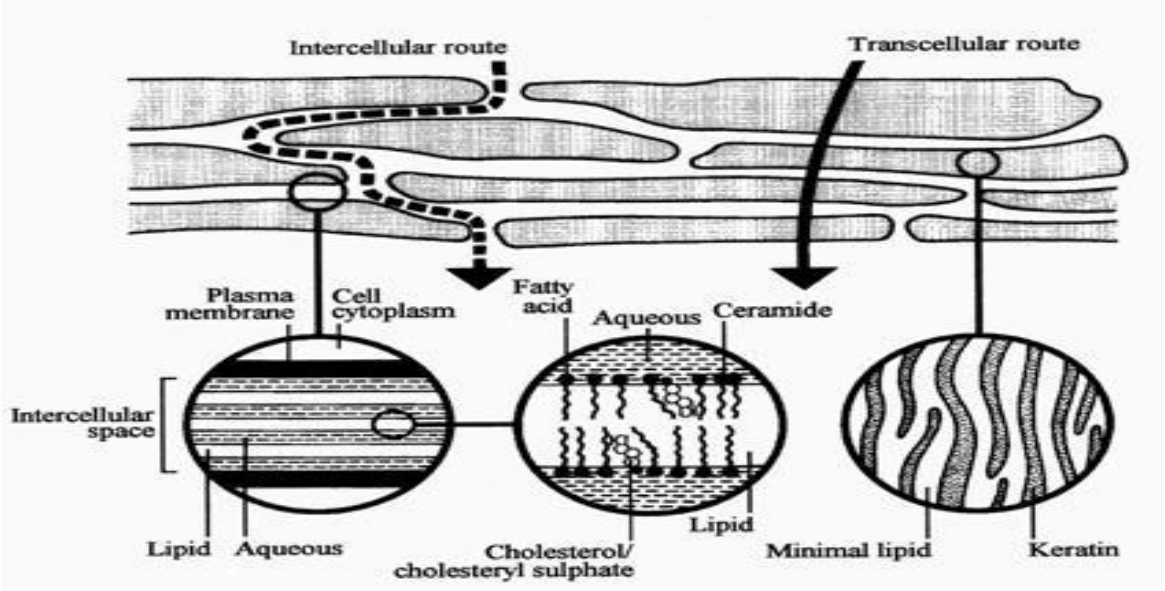

Figure 5: Schematic representation of stratum corneum and its intercellular and transcellular pathways of drug permeation (27).

1.6- Topical Gel preparations

Gels are semisolid systems in which a liquid phase is constrained within an interlocked, three-dimensional polymeric matrix of a natural or synthetic gum.

The gelling polymer is normally present in low percentage concentration, certainly less than 10%, and usually on the order of 0.5 to 2.0%. Some of these systems are clear as water in appearance and visually aesthetically pleasing.

Others are turbid, as the polymer is either not fully molecularly dissolved or forms aggregates, which disperse light.

Gels are watery when warm but solidify near room temperature to form gels that are semisolid to rubbery, depending on the source and concentration of the gelling agent.

The gelling is not simply due to increased chain-chain entanglements; otherwise, the system would merely become viscid. Rather, the system sets up as the result of the entwining of the polymer ends into double helices, a crystallization phenomenon that fixes the polymeric strands in place, yielding a stable, yet deformable structure (11,16,17).

The structure should persist to temperatures exceeding body temperature for such systems to be most topically useful. The polymers used to prepare pharmaceutical gels include natural gums such as tragacanth, pectin, carrageen, agar, and alginic acid, and synthetic and semisynthetic materials such as methylcellulose, hydroethylcellulose, carboxymethylcellulose, Polyacrylamide Iso-

paraffin Laureth-7, and the Carbopols.

Gels are used pharmaceutically as lubricants and as carriers for active pharmaceutical agents (26,27).

The preparation of gels may involve a fusion process or may require a

special procedure, depending on the gelling agent involved.

Tragacanth systems must be prepared at low temperature due to the extreme heat liability of this natural gum. On the other hand, it is easier to disperse methyl cellulose in hot than in cold water. The Carbopols are gelled by a unique procedure, the polymer is dispersed in an acidic medium, when the dispersion is uniform, gelling is induced by neutralizing the system with an inorganic base (aqueous systems) or with an amine such as triethanolamine. This ionizes the acidic functional groups on the polymer, drawing the polymer into colloidal solution, in which state it forms the requisite structural matrix.

Several prototype gel formulations are available where the design of specific systems tailored to meet predetermined, demanding performance criteria, particularly with respect to bioavailability.

1.7- Acne

Is an inflammatory disease of the skin, caused by changes in the pilosebaceous units (skin structures consisting of a hair follicle and its associated sebaceous gland). The most common form of acne is known as "acne vulgaris", which means common acne. Excessive secretion of oils from the glands combine with naturally occurring dead skin cells to block the hair follicles. Oil secretions build up beneath the blocked pore, providing a perfect environment for the skin bacteria Propionibacterium acnes to multiply uncontrolled. In response, the skin inflames, producing the visible lesion. The face, chest, back, shoulders and upper arms are especially affected (52,53).

The typical acne lesions are: comedones, papules, pustules, nodules and inflammatory cysts. These are the more inflamed form of pus-filled or reddish bumps, even boil-like tender swellings. Non-inflamed 'sebaceous cysts', more properly called epidermoid cysts, occur either in asssociation with acne or alone but are not a constant feature. After resolution of acne lesions, prominent unsightly scars may remain.

The condition is common in puberty as a result of an abnormal response to normal levels of the male hormone testosterone. The response for most people diminishes over time and acne thus tends to disappear, or at least decrease, after one reaches about the age of thirty. There is, however, no way to predict how long it will take for it to disappear entirely, and some individuals will continue to suffer from acne decades later, into their thirties and forties and even beyond.

1- Acne vulgaris is more common in males than in females during adolescence.

2- It is more common in women than in men during adulthood.

3- Three categories of legions formed during acne are non inflammatory legions, inflammatory lesions, and scars.

4- Three non inflammatory legions include microcomedos Fig 6, open comedos Fig.7, which have a central impaction of keratin and lipid and might or might not be slightly raised, and closed comedos Fig 8, which are slightly elevated papules that could possibly form into a larger inflammatory lesion (30).

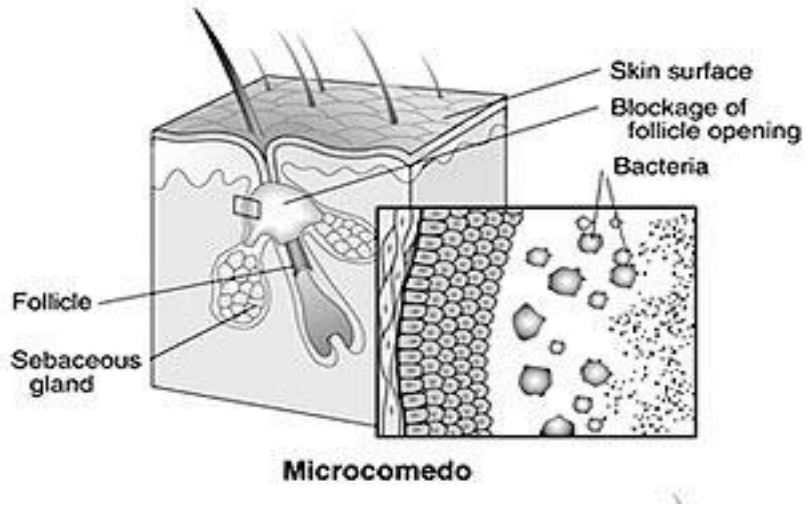

Figure 6: Microcomed structure (30).

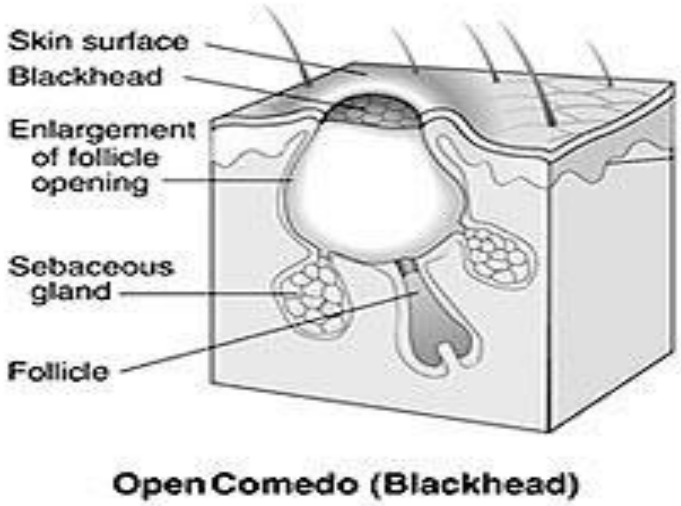

Figure 7: Open comedo structure (30).

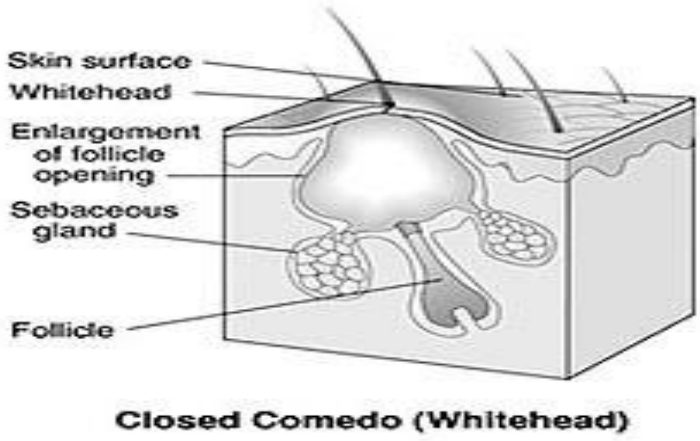

Figure 8: Closed comedo structure (30).

5- Moderate inflammatory acne has Inflammatory legions include more aggravated legions such as pustules and large, fluctuant nodules.

6- Nodulocystic acne is characterized by comedones, inflammatory lesions, and large nodules greater than 5 mm in diameter. Scarring is often evident.

Acne can be treated at different levels (53):

1- Topical and oral antibiotics treatment with the tetracyclines , clindamycin, erythromycin and Benzoyl peroxide topically.

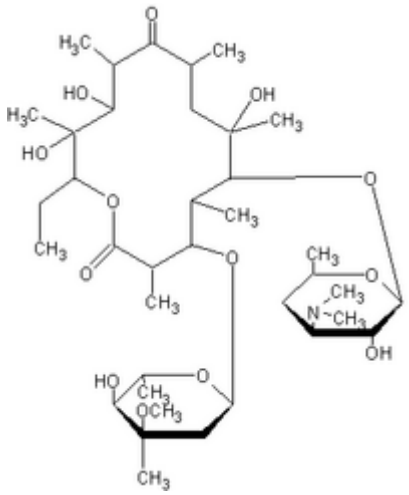

Figure 9: Erythromycin A structure (52).

Erythromycin prevents bacteria from growing, by interfering with their protein synthesis. Erythromycin binds to the subunit 50S of the bacterial ribosome, and thus inhibits the translation of peptides.

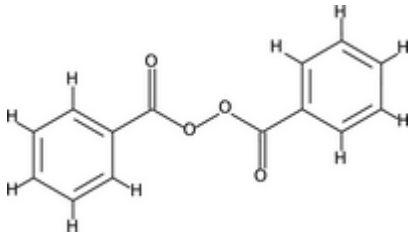

Figure 10: Benzoyl peroxide structure (52).

While it is not fully known how benzoyl peroxide works in fighting acne, it is presumed that benzoyl peroxide is easily absorbed into pores where it works by interfering with acne bacterial metabolism through oxidation.

2- Systemic treatment with Vitamin E derivatives like Isotretinoin to reduce the secretion of oils from the glands

3- Normalizing the follicle cell lifecycle by a group of medications like topical retinoids such as tretinoin (brand name Retin-A), adapalene (brand name Differin) and tazarotene (brand name Tazorac).

4- Surgical treatment by exfoliating the skin. This can be done either mechanically, using an abrasive cloth or a liquid scrub, or chemically. Common chemical exfoliating agents include salicylic acid and glycolic acid, which encourage the peeling of the top layer of skin to prevent a build-up of dead skin cells which combine with skin oil to block pores.

5- Phototherapy using red light or blue light and photodynamic therapy are being assessed as potential treatments for acne.

6- The usefulness of some laser treatments in the management of acne is also being evaluated.

7- Hormonal treatments for females, acne can be improved with a combined oestrogen/progestogen contraceptive pill. Cyproterone brande name (Diane 35) is particularly effective at reducing androgenic hormone levels and until recently was the best oral contaceptive treatment.

1.8- Propionibacterium acne (14)

Table 1: Propionibacterium acne classifications

Kingdom	Bacteria
Phylum	Actinobacteria
Order	Actinomycetales
Family	Propionibacteriaceae
Genus	Propionibacterium
Species	P. acnes

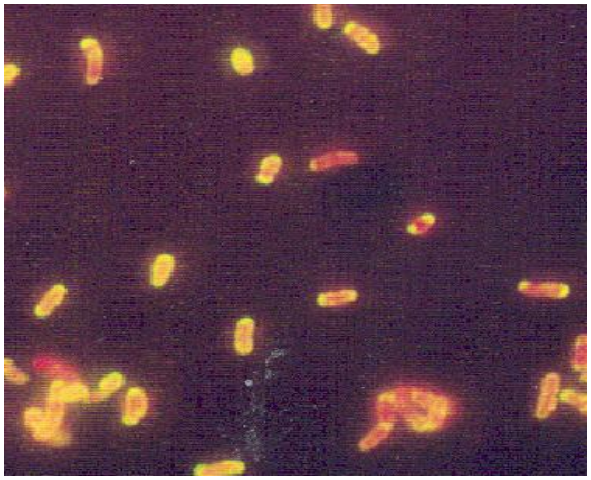

Figure 11: Propionibacterium acnes. (by courtsy of inflammation research center) (14).

Description and Significance:

Species of *Propionibacteria* can be found all over the body. *Propionibacteria* are generally nonpathogenic; however, when certain species of *Propionibacteria* contaminate blood and other body fluid, they can cause a number of infections

including the common skin disease acne vulgaris (caused by *P. acnes*). Some species of *Propionibacteria* are found in food like cheeses and other dairy products. *P. freudenreichii* is used in Swiss cheese manufacturing to produce its flavor and characteristic holes (14).

Genome Structure:

Currently there is one genome project that is complete on Propionibacterium acnes KPA1202 and there is one in progress on Nocardioides sp. JS324. The entire genome sequence of P. acnes encodes 2333 putative genes and revealed numerous gene products involved in degrading host molecules, including sialidases, neuraminidases, endoglycoceramidases, lipases, and pore-forming factors (14).

Cell Structure and Metabolism:

Propionibacteria are slow-growing, nonsporeforming, Gram-positive, anaerobic bacteria. They can be rod-shaped or branched and can occur singularly, in pairs, or in groups. They generally produce lactic acid, propionic acid, and acetic acid from glucose.

1.9- Azithromycin

Description:

Azithromycin is an azalide, a subclass of macrolide antibiotics, for oral administration.

Azithromycin has the chemical name (2R,3S,4R,5R,8R,10R,11R,12S,13S,14R)-13-[(2,6-dideoxy-3-*C*-methyl-3-*O*-methyl-□-*L*-*ribo*-hexopyranosyl)oxy]-2-ethyl-3,4,10-trihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3,4,6-trideoxy-3-(dimethylamino)-□-*D*-*xylo*-hexopyranosyl]oxy]-1-oxa-6-azacyclopentadecan-15-one (39).

Azithromycin is derived from erythromycin; however, it differs chemically from erythromycin in that a methyl-substituted nitrogen atom is incorporated into the lactone ring. Its molecular formula is C38H72N2O12, and its molecular weight is 749.00.

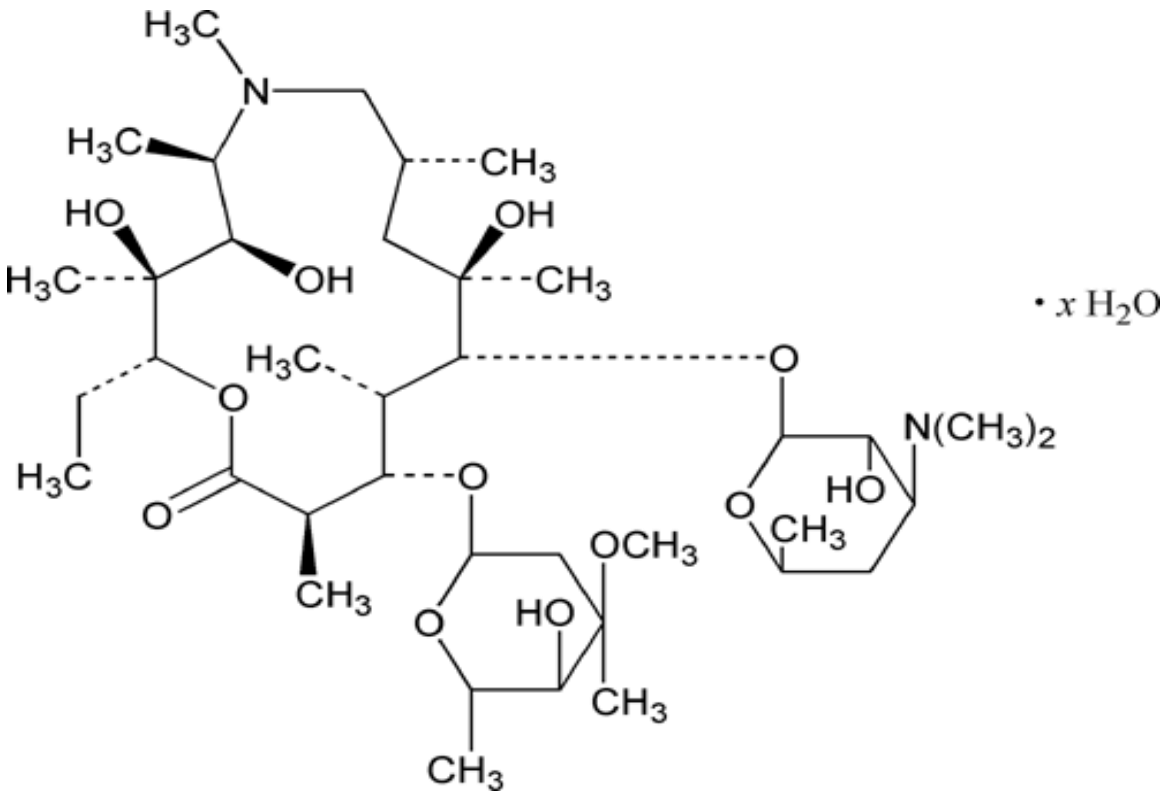

Figure 12: Azithromycin structure (32).

Azithromycin, as the dihydrate, is a white crystalline powder with a molecular formula of C38H72N2O12□ 2H2O and a molecular weight of 785.0.

Pharmacokinetics:

Following oral administration of a single 500 mg dose (two 250 mg tablets) to 36 fasted healthy male volunteers, the mean (SD) pharmacokinetic parameters were AUC from 0-72 = 4.3 (1.2) mcg ·h / ml; C max = 0.5 (0.2) mcg/ml; T max = 2.2 (0.9) hours (39).

Absorption:

The absolute bioavailability of azithromycin 250 mg capsules is 38%.

Distribution:

The serum protein binding of azithromycin is variable in the concentration range approximating human exposure, decreasing from 51% at 0.02 mcg/ml to 7% at 2 mcg/ml.

Following oral administration, azithromycin is widely distributed throughout the body with an apparent steady-state volume of distribution of 31.1 L/kg. Greater azithromycin concentrations in tissues than in plasma or serum were observed. High tissue concentrations should not be interpreted to be quantitatively related to clinical efficacy. The antimicrobial activity of azithromycin is pH related and appears to be reduced with decreasing pH. However, the extensive distribution of drug to tissues may be relevant to clinical activity (39).

Metabolism:

In vitro and in vivo studies to assess the metabolism of azithromycin have not been performed.

Elimination:

Plasma concentrations of azithromycin following single 500 mg oral and i.v. doses declined in a polyphasic pattern with a mean apparent plasma clearance of 630 mL/min and terminal elimination half-life of 68 hours. The prolonged terminal half-life is thought to be due to extensive uptake and subsequent release of drug from tissues.

Mechanism of action:

Azithromycin acts by binding to the 50S ribosomal subunit of susceptible

Microorganisms and, thus, interfering with microbial protein synthesis, nucleic acid synthesis is not affected.

Biological activity:

Azithromycin has been shown to be active against most isolates of the following microorganisms (39):

1-Aerobic and facultative gram-positive microorganisms

Staphylococcus aureus

Streptococcus agalactiae

Streptococcus pneumoniae

Streptococcus pyogenes

2-Aerobic and facultative gram-negative microorganisms

Haemophilus ducreyi

Haemophilus influenzae

Moraxella catarrhalis

Neisseria gonorrhoeae

3-Other microorganisms

Chlamydia pneumoniae

Chlamydia trachomatis

Mycoplasma pneumoniae

Beta-lactamase production should have no effect on azithromycin activity.

Indication and usage:

Acute bacterial exacerbations of chronic obstructive pulmonary disease due to Haemophilus influenzae, Moraxella catarrhalis or Streptococcus pneumoniae.

Acute bacterial sinusitis due to Haemophilus influenzae, Moraxella catarrhalis or Streptococcus pneumoniae.

Community-acquired pneumonia due to: Chlamydia pneumoniae, Haemophilus influenzae, Mycoplasma pneumoniae or Streptococcus pneumoniae in patients appropriate for oral therapy (14).

Uncomplicated skin and skin structure infections due to Staphylococcus aureus,

Streptococcus pyogenes, or Streptococcus agalactiae. Abscesses usually require surgical drainage.

Urethritis and cervicitis due to Chlamydia trachomatis or Neisseria gonorrhoeae.

Genital ulcer disease in men due to Haemophilus ducreyi, (chancroid).

1.10- Modified Franz diffusion cell (MFDC)

Modified Franz diffusion cell as shown in Figure 12 is used for permeation studies as described bellow.

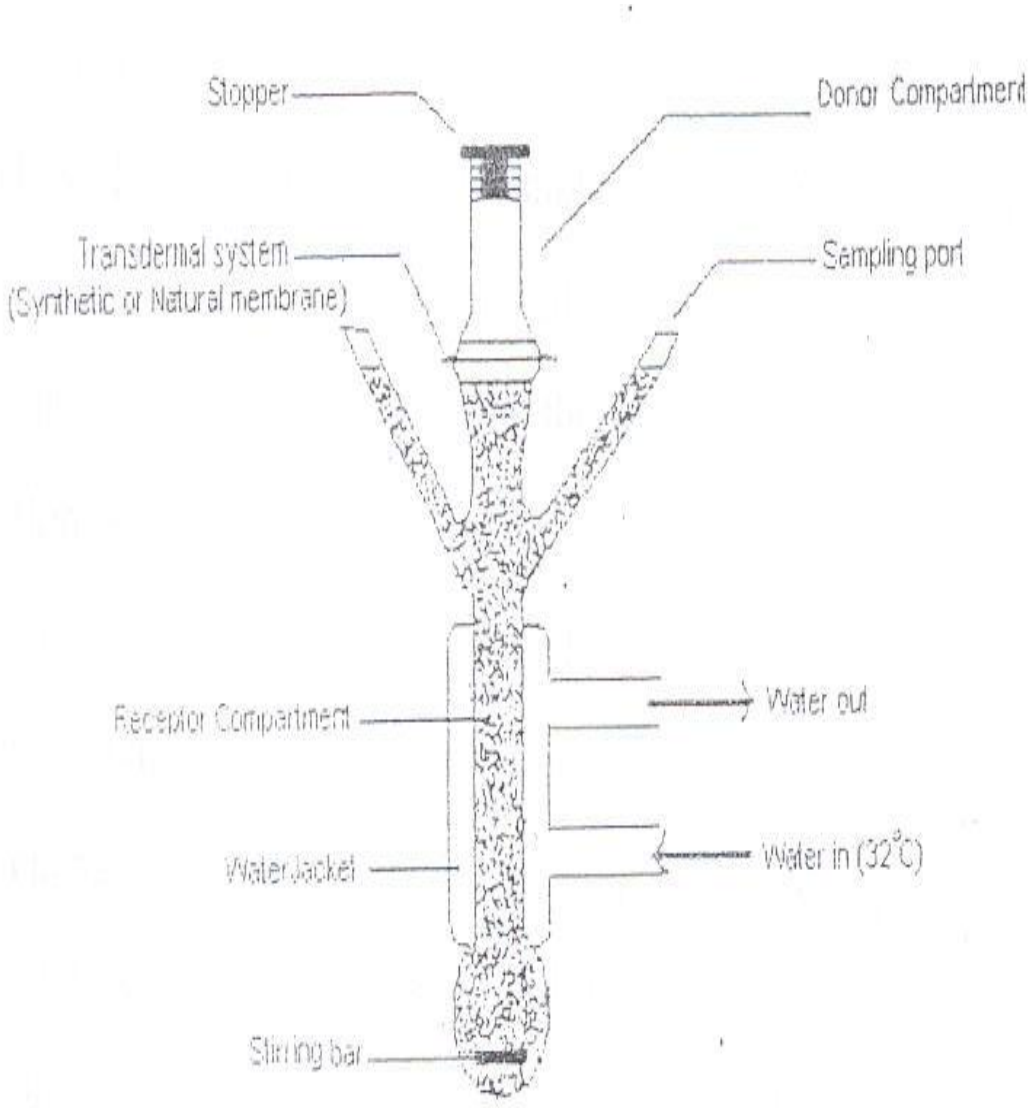

Figure 13: Modified Franz Diffusion Cell (MFDC) (5).

The cell is composed of 3 main parts (38):

1- The donor compartment

The donor compartment filled with the gel dosage form which contains the Azithromycin and a particular penetration enhancer (PE).

2- The receiver compartment

The receiver compartment was filled with degassed 30% Hydro alcoholic solution.

3- The synthetic membrane

The synthetic membrane was mounted between the donor and the receiver compartments of the diffusion cell with effective surface area of 3 cm2.

At the junction of the donor and receiver compartments, the diffusion cell wrapped with a waterproof parafilm to prevent solution leakage during the diffusion experiment and connected tightly together using rubber ring and a metal clamp.

In order to avoid the evaporation of the solvent from the donor compartment during the diffusion experiment, it is closed by waterproof parafilm.

The diffusion experiment study lasted for 6 hours.

The diffusion system especially the receiver compartment was kept at a temperature of 32 (temperature of the human skin) by using a water jacket.

The receiver fluid was mixed continuously during diffusion experiment using a magnetic stirring at 500 rpm in order to keep sink condition in the receiver media as is the case of blood circulation in the human body (27).

Serial sampling from the receptor phase was performed for 6 hrs every O.5hr intervals and the amount of the absorbed drug was analyzed using high performance liquid chromatography (HPLC), or by a biological assay.

The cumulative amount of the permeated drug was plotted against time and the slope of the linear part of the graph (permeation flux) was measured, from which the permeability coefficient was calculated using first Fick's Law.

1.11- Diffusion theory :

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

$J = d M / S . d t$ …….. 1

The flux in turn is proportional to the concentration gradient, d C / d x

$J = -D (d C / d x)$ ……...2

In which D is the diffusion coefficient of a penetrant or diffusant in cm2 / sec, C is concentration in g / cm3 , and x is the distance in cm of movement perpendicular to the surface of the barrier.

In equation (1), the mass, M, is usually given in grams, the barrier surface, S, in cm2, and the time, t, in seconds.

The negative sign of equation (2) signifies that diffusion occurs in a direction (the positive x direction) opposite to that of increasing concentration.

That is to say, diffusion occurs in the direction of decreasing concentration of diffusant; thus, the flux is always a positive quantity.

The diffusion constant, D, or diffusivity as it is often called, does not ordinarily remain constant, for it may change in value at higher concentrations.

D is also affected by temperature, pressure, solvent properties, and the chemical nature of the diffusant.

Therefore, D is referred to more correctly as a diffusion coefficient rather than as a constant.

Fick's second law represents the equation for mass transport that emphasizes the change in concentration with time at a definite location.

This diffusion equation is derived as follows:

The concentration C, in a particular volume element, (see Figures13), changes only as a result of net flow of diffusing molecules into or out of the region.

A difference in concentration results from a difference in input and output. The concentration of diffusant in the volume element changes with time, i.e., d C / d t, as the flux or amount diffusing changes with distance, d J / d x, in the x direction, or

$$d c / d J = - d J / d x \qquad \text{…….....3}$$

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

$$d J / d x = D (d2C / d x2) \qquad \text{……..4}$$

Substituting d C/ d t from equation (3) into equation (4) results in Fick's second law, namely

$$d C / d t = D (d2C/ dx2) \qquad \text{……5}$$

Equation (5) represents diffusion only in the x direction. If one wishes to express concentration changes of diffusant in three dimensions,

Fick's second law is written in the general form:

$$d C/ dt = D (d2C/dx2 + d2C/ dy2 + d2C/ dz2) \qquad \text{……..6}$$

This expression is not ordinarily needed in pharmaceutical problems of diffusion, however, since movement in one direction is sufficient to describe most cases.

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

Fick's first law, equation (2), gives the flux (or rate of diffusion through unit area) in the steady state of flow. The second law refers in general to a change in concentration of diffusant with time, at any distance, x, i.e., a non steady state of flow.

Steady state may be described, however, in terms of the second law, equation (5).

Consider the diffusant originally dissolved in a solvent in the left-hand compartment of the cell shown in Figure 14. Solvent alone is placed on the right-hand side of the barrier, and the solute or penetrant diffuses through the central barrier from solution to solvent side (donor to receptor compartment). In diffusion experiments, the solution in the receptor compartment is constantly removed and replaced with fresh solvent so as to keep the concentration at a low level.

This is referred to as "sink conditions," the left compartment being the source and the right compartment the sink.

Originally, the diffusant concentration will fall in the left compartment and rise in the right compartment until the system comes to an equilibrium, based on the rate of removal of the diffusant from the sink and the nature of the barrier.

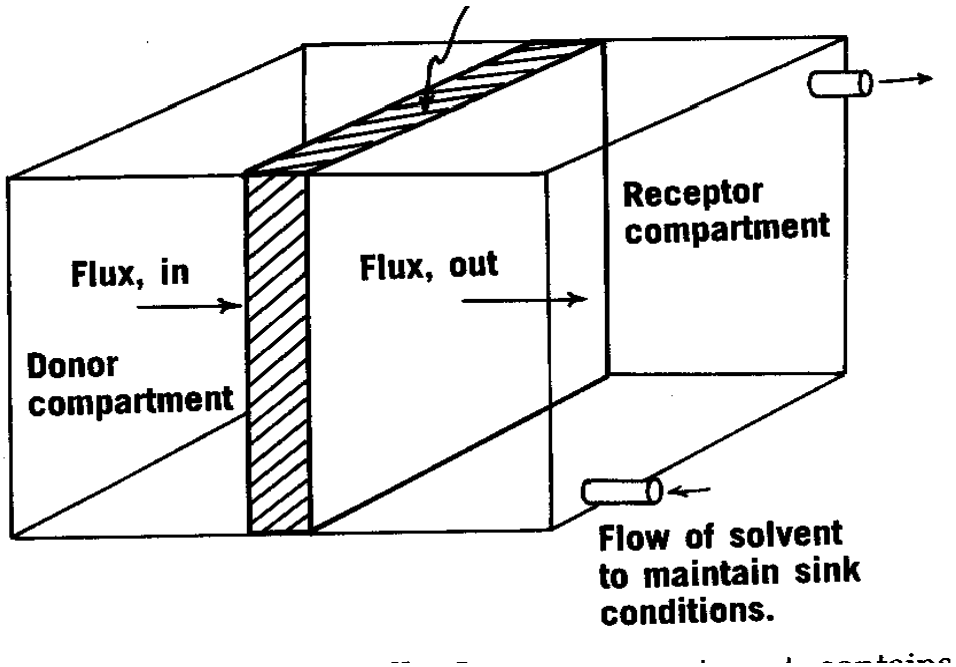

Figure 14: Diffusion cell, donor and receiver compartments

When the system has been in existence for a sufficient period of time, the concentration of the diffusant in the solutions at the left and the right of the barrier become constant with respect to time, but obviously not the same in the two compartments.

Then within each diffusional slice perpendicular to the direction of flow, the rate of change of concentration dC / dt, will be zero , and by the second law, C is the concentration of the permeant in the barrier expressed in mass/cm3.

D C / d t = D (d2C/ dx2) = 0 …….7

Equation (7) demonstrates that since D is not equal to zero,

d2C/d x2 = O.

In other words, the concentration gradient across the membrane,

dC/dx is constant, signifying a linear relationship between concentration, C, and distance x.

This is shown in Figure 15 (in which the distance x is equal to h) for drug diffusing from left to right in the cell of figure 13.

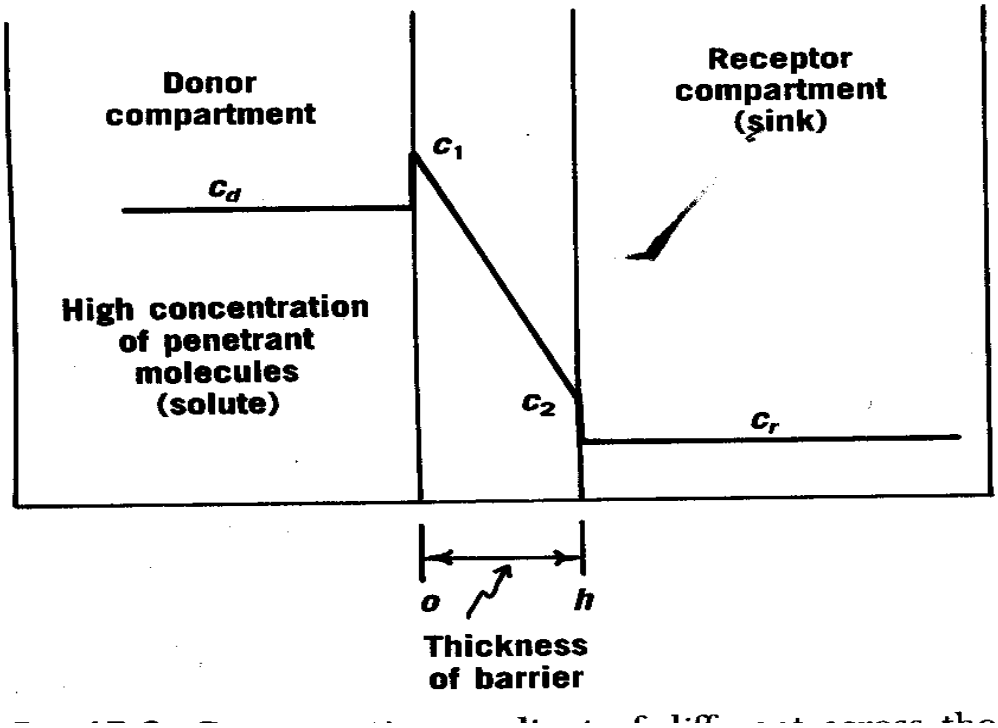

Figure 15: Concentration gradient of diffusant across the diaphragm of a diffusion cell.

The concentration will not be rigidly constant but rather is likely to vary slightly with time, and then dC / dt is not exactly zero.

Fick adapted the two diffusion equations, (2) and (5), to the transport of matter from the laws of heat conduction.

If a diaphragm separates the two compartments of a diffusion cell of cross-sectional area S, and thickness h, and if the concentrations in the membrane on the left (donor) and on the right (receptor) sides are C 1 and C 2, respectively, (Figure14), the first law of Fick may be written:

$$J = d M/ S dt = D ((C1-C2) / h) \quad \text{……..8}$$

in which (C1 -C2)/h approximates dC/dx.

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 Cd on the donor side or Cr in the receiver side as follows. The distribution or partition coefficient, K, is given by

$$K = C1/Cd = C2/Cr \quad \text{…… 9}$$

Hence,

$$d M/ dt = DSK (Cd-Cr) / h \quad \text{……10}$$

and, if sink conditions hold in the receptor compartment, Cr nearly zero, then

$$dM /dt = DSKCd / h = PSCd \quad \text{……11}$$

in which

$$P = D k / h \quad (cm / second) \quad \text{…….12}$$

It is noteworthy that the permeability coefficient, P, has units of linear velocity.

In some cases, it is not possible to determine D, K, or h independently and thereby to calculate P, it is a relatively simple matter, however, to measure the rate of barrier permeation and to obtain the surface area S and concentration Cd in the donor phase and the amount of permeant M in the receiving sink.

One can then obtain P from the slope of a linear plot of M/cm2 versus t:

$$M = PSCdt \quad \text{……13}$$

A constant-activity dosage form may not exhibit a steady-state process from the initial time of release. Figure15 is a plot of the amount of butylparaben penetrating through guinea pig skin from a dilute aqueous solution of the penetrant. It is observed that the curve of Figure 16 is convex to the time axis in the early stage and then becomes linear.

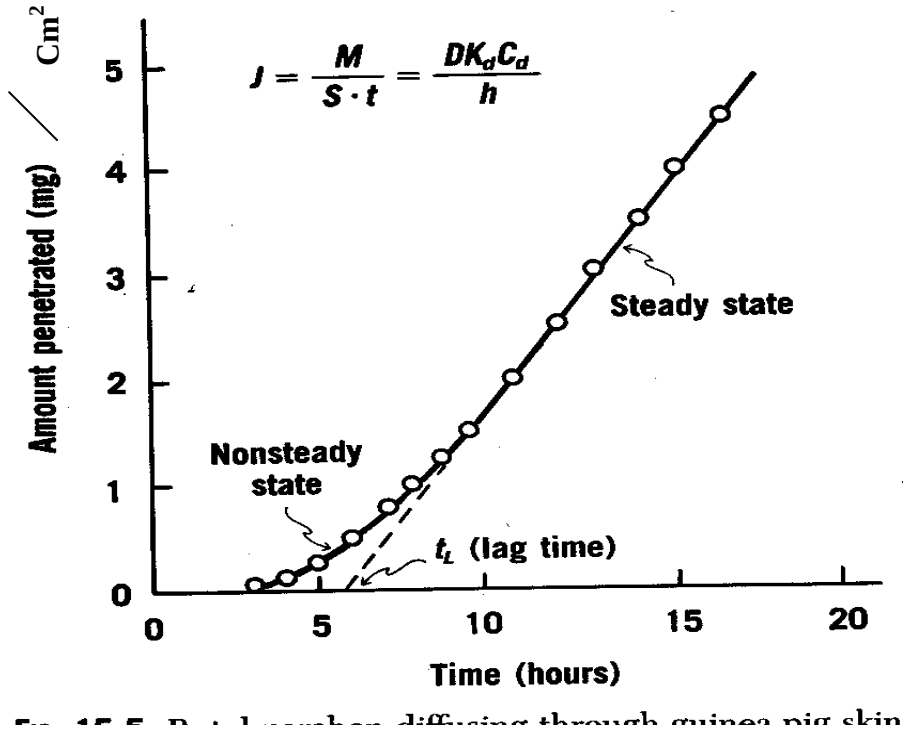

Figure 16: Butylparaben penetrating through guinea pig skin from a dilute aqueous solution of the penetrant.

The early stage is the non steady-state condition. At later times, the rate of diffusion is constant, the curve is essentially linear, and the system is at steady state.

When the steady-state portion of the line is extrapolated to the time axis, as shown in Figure 16, the point of intersection is known as the lag time, T_L.

This is the time required for a penetrant to establish a uniform concentration gradient within the membrane separating the donor from the receptor compartments.

In the case of a time lag, the straight line of Figure 15 may be represented by a modification of equation (10):

$M = (SDKCd / h)(t – t_L)$ ……..14

The lag time, t L, is given by

$T_L = h2 / 6D$ ……15

and its measurement provides a means of calculating the diffusivity D, presuming a knowledge of the membrane thickness h.

Also, knowing P, the thickness h can be calculated from

$T_L = h K / 6P$ …… 16

Diffusivity depends on the resistance to passage of a diffusing molecule" (38).

Important equations :

T_L = intercept / slop, from M / S v s Time curve

$T_L = h K / 6P$

$D = h2 / 6 T_L$

P = slop / concentration, from M / S v s Time curve

$P = D K / h$

$K = P .h / D$

Chapter Two

Experimental part

Experimental part

The main objective of this study is:

1- Dissolving Azithromycin in a suitable solution that conserve stability over a certain period of time.

2-Preparation of a gel structure, containing a constant concentration (2% w/w) of the active pharmaceutical ingredient (API) and known concentration of each penetration enhancer.

3- Study the permeability behavior of Azithromycin in the presence of different penetration enhancers using modified Franz diffusion cell through a synthetic membrane.

3-Depending on the diffusion study, the most effective penetration enhancers will be selected to determine the final formula of the drug.

2.1- Materials

Table 2: List of material used in the study

No,	**Item**	**Source**
1	Azithromycin	Pfizer company
2	Bacteria	*Micrococus luteus* ATCC #9341 organism
3	Carbomer 940	Noveon / 1504G187A
4	Cremophore Rh40	BASF / 16428368E0
5	Ethyl alcohol	Pharmco / 6365
6	IPM	Industrial Quimisalase / 309022
7	Methyl paraben	Clariant / GBGA029313
8	Octanol	Merck
9	Oleic acid	Merck
10	Propylene Glycol	Dow / SL271920D4
11	Propyl paraben	Clariant / GEGA023832
12	Sepigel (Polyacrylamide, Iso-paraffin, Laureth-7).	Seppic / T51215
13	Tween 80	Croda 2473
14	Triethanolamine	Merck 22006665
15	Urea	Riedel / 32270

2.2- Equipment

Table 3: List of equipment used in the study

No.	Equipment	Source / Model
1	Computer	Acer
2	Incubator	Tuttnawer
3	Modified Franz diffusion cell	Jordan University of science and technology work shop
4	Microscope	Leica
5	Polyamide membrane	Sartolon polyamide -Germany, Pore size = 0.2mc.m.
6	pH meter	Inolab
7	Refrigerator	Leonard
8	Stirrer-Heater	Fried
9	Synthetic Membranes.	dialysis membrane (32/32A, 0.2 mc.m pores).
10	UV/ HPLC.	Unicam / Merck-Hitachi
11	Viscometer	Brook-Field
12	Water bath and pump.	Jolabo / Millipore

2.3- Methodology

2.3.1-Solubilization of Azithromycin in a suitable solvent to obtain chemically and physically stable solution:

Different solvents, co-solvents and combinations of them shall be tried such as purified water, ethyl alcohol, tween80 and propylene glycol taking into consideration that the solvent is acceptable for the formulation of topical dosage forms and attain stability of the API during the period of usage.

2.3.2- Preparation of the gel vehicle with the following characteristics.

1- Acceptable as a topical dosage form.
2- Non irritating to the skin.
3- Compatible with all ingredients forming the product.
4- Having a physically stable structure during the shelf life period.
5- Attain the API in soluble form during the period of treatment.
6- Keep the API stable during the period of treatment

Different formulations were prepared as indicated below:

Table 4: Different trials of gel formulations

Ingredient	Formula A	Formula B	Formula C
Azithromycin (API)	*	*	*
Purified water (Solvent)	*	*	*
Ethyl alcohol (Solvent)		*	
Tween 80(Polyoxy sorbate 80) (Solubilizer)	*		*
Cremophore RH40 (Solubilizer)		*	*
Sepigel (Gelling agent)		*	*
Carbomer (gelling agent)	*		
Triethanolamine (alkalinizing agent)	*		
Methyl paraben 0.1% (preservative)	*	*	*
Propyl paraben 0.01% (Preservative)	*	*	*

2.3.3- Formulations of Azithromycin gel containing different penetration enhancers. The final gel structure that achieves the requirements are prepared with different penetration enhancers to run the permeability study and an ongoing shelf life stability study.

The following formulations were prepared for further study:

Table 5: Different trials of Azithromycin formulations with different penetration enhancers

Ingredient	F1 (Balnk)	F2	F3	F4	F5
Gel vehicle	98%	88%	88%	88%	96%
Azithromycin (API)	2%	2%	2%	2%	2%
Urea		10%			
Propylen Glycol			10%		
IPM				10%	
Oleic acid					2%

2.3.4 – Manufacturing procedure:

1- Add 30 g ethyl alcohol to a mixing vessel.

2- Add 33.89 g purified water to the mixing vessel, and mix well.

3- Dissolve Methyl paraben, Propyl paraben in the alcoholic solution and mix well for 2 minutes.

4- Add cremophore RH40 to the mixing vessel and mix for 3 minutes.

5- Add Azithromycin to the above solution and mix for 5 minutes. make sure of complete dissolution of Azithromycin powder.

6- Add the PE and mix well for 3 minutes.

7- Add sepigel slowly with continuous mixing till a gel structure is formed.

2.4- Permeability test methodology:

1- Preparation of the synthetic membrane:

The synthetic membrane is composed of three layers; polyamide filter membrane inserted between two layers of dialysis membrane. The two layers of dialysis membrane were soaked for half an hour in distilled water for hydration before use. The polyamide filter membrane was soaked in octanol for 24 hours to simulate the lipophilic barrier in the skin and captured between the dialysis membrane to prevent octanol from floating or leaving the filter membrane during the diffusion experiments (13).

This design of the synthetic membrane was proved to be useful in a previous study performed by Mr. Al-Hindi in the University of Jordan in his thesis about Diclofenac complexes with some metals as candidate system for drug delivery in the year 2002, the correlation between diffusion through the SC and the synthetic membrane was detected (34).

2- Receptor and donor phase:

A 30% hydro alcoholic medium is used as the receptor phase in our skin permeation studies based on the fact that Azithromycin (API) is freely soluble in this medium.

The receptor solution was thoroughly degassed to prevent the formation of bubbles beneath the synthetic membrane, and samples were withdrawn at specified time intervals.

Thus plots could be generated showing the amount of drug released versus time. Most often the receptor solvent is present in a volume that allows sink conditions to be approximated throughout the course of the experiment.

In all instances, the thermodynamic activity of the drug in the receptor fluid should

not exceed 10 %of its thermodynamic activity in the donor medium to maintain a favorable driving force for permeation and to assure reasonable and efficient collection of diffusant.

Diffusion test parameters:

Sample weight :	1.8 gm 2% Azithromycin gel.
Sample content of Azithromycin:	36 mg.
Total diffusion area available:	3 cm^2
Temperature controlled at:	32-34C
Total diffusion time:	6 hours.
Total volume of MFD cell:	20.6 ml.
Total membrane height:	0.016 cm.
Sample size:	1.0 ml

2.5- Calculation methods:

1- From the biological assay procedure, we can measure the concentration of Azithromycin in the 1 ml sample taken during the diffusion test by measuring the diameter of the inhibition zones for the different concentrations prepared and by using certain pharmacopeial equations to convert the diameter into concentration, the equations available in part 2.7.

2- According to the table no. 12

Sampling time	Sample conc.mg/ml	Qc mg/20.6 ml	Qa (mg)	M (mg/cm^2)	J (mg/cm2/hour)	% diffused

-Qc is the current quantity of the drug diffused at any time which equals to the sample concentration of the drug at that time multiplied by the volume of the receiver solution (20.6ml).

-Qa is the accumulative quantity diffused equals to the total quantity diffused at certain time plus the quantity removed during all last sampling.

Example:

The accumulative quantity after 4 hours of diffusion equals to the concentration of drug (mg/ml)at time 4 hours *20.6 ml+mg/1 ml sample at 3 hours+ mg/1 ml sample at 2 hours+ mg/1 ml sample at 1 hours+ mg/1 ml sample at 0.5 hours.

- M equals to the accumulative amount diffused divided by area of the synthetic membrane ($3cm^2$).
- J represent the accumulative amount diffused divided by area of the synthetic membrane ($3cm^2$) divided by time.

- % diffused equals to the amount of drug diffused divided by the total available amount in the donor compartment (36 mg).

3- According to the figure 16, we can plot time versus M, and then we determine the steady state area and plot again the steady state area versus time.

- By chart options on the excel software, one can choose equation and R^2 to determine the intercept and the slope of the steady state area.

4- According to the table no.13

Formula	Slope	Intercept	T_L =intercept /slope	$D=h^2/6\ T_L$	P = slope / concentration	K=P.h/D

- Lag time is calculated by dividing the intercept / the slope.(hr)
- Diffusion coefficient (D) = $h^2/6\ T_L$ (cm^2 / hr)
- The permeability (p) = slope / concentration in the donor compartment. P = (cm / hr)
- The partition coefficient (K) = Ph/D
- The Enhancement ratio (ER) = P after the addition of PE / P before the addition of PE.

2.6- Chemical assay of Azithromycin as the USP28

"**Note :** Use water that has a resistivity of not less than 18 Mohm/cm.

Mobile phase:

Dissolve 5.8 g of monobasic potassium phosphate in 130 mL of water, add 870 mL of acetonitrile, and mix. Adjust with about 6 mL of 10 N potassium hydroxide to a pH of 11.0 ± 0.1, and pass through a filter having a 0.5-µm or finer porosity, and degas, make adjustments if necessary.

Standard stock preparation:

Transfer about 16.5 mg of USP Azithromycin RS, accurately weighed, to a 100-mL volumetric flask, add 10 mL of acetonitrile, and dissolve by swirling and with the aid of brief sonication. Dilute with acetonitrile to volume, and mix.

Standard preparation:

Transfer 2.0 mL of the Standard stock preparation to a 100-mL volumetric flask, dilute with Mobile phase to volume, and mix to obtain a Standard preparation having a known concentration of about 0.0033 mg of USP Azithromycin RS per mL.

Assay preparation:

Transfer about 16.5 mg of Azithromycin, accurately weighed, to a 100-mL volumetric flask, add 10 mL of acetonitrile, and dissolve by swirling and with the aid of brief sonication. Dilute with acetonitrile to volume, and mix. Transfer 2.0 mL of the solution so obtained to a 100-mL volumetric flask, dilute with Mobile phase to volume, and mix.

Chromatographic system:

The liquid chromatograph is equipped with an amperometric electrochemical detector with dual glassy carbon electrodes operated in the oxidative screen mode with electrode 1 set at +0.70 ± 0.05 V and electrode 2 set at +0.82 ± 0.05 V, and the background current optimized to 85 ± 15 nanoamperes, a 4.6-mm × 5-cm guard

column that contains 5-µm packing L29 and a 4.6-mm × 15-cm analytical column that contains 5-µm packing L29 or 3-µm packing L49 without the guard column. The flow rate is about 1.5 mL per minute. Chromatograph the Resolution solution, and record the responses as directed for Procedure: the relative retention times are about 0.7 for azaerythromycin A and 1.0 for Azithromycin with the L29 column and about 0.8 for azaerythromycin A and 1.0 for Azithromycin with the L49 column; and the resolution, R, between azaerythromycin A and Azithromycin is not less than 2.5. Chromatograph the Standard preparation, and record the responses as directed for procedure.

The tailing factor for the Azithromycin peak is not less than 0.9 and not more than 1.5; the column efficiency is not less than 1000 theoretical plates; and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure:

Separately inject equal volumes (about 50 µL) of the Standard preparation and the Assay preparation into the chromatograph, record the chromatograms, and measure the areas for the major peaks. Calculate the quantity, in µg, of Azithromycin ($C_{38}H_{72}N_2O_{12}$) in each mg of Azithromycin taken by the formula:

$$(WP/w)(r_U / r_S),$$

in which W is the quantity, in mg, of USP Azithromycin RS taken to prepare the Standard preparation; P is the potency, in µg of Azithromycin per mg, of USP Azithromycin RS; w is the quantity, in mg, of Azithromycin taken to prepare the Assay preparation; and r_U and r_S are the Azithromycin peak responses obtained from the Assay preparation and the Standard preparation, respectively." (32)

2.7- Biological assay of Azithromycin

Procedure:

1. Preparation of Standard Stock Solution

1.1 Dissolve 20-100 mg quantity of USP Reference Standard (working Standard of the tested antibiotic (accurately weighed) to prepare a stock solution in a certain concentration (Table 1 – USP28).

1.2. Make a proper dilutions in a solvent specified, to obtain the target concentrations Dilute to the required concentration.

1.3. Store in a refrigerator (where applicable) and use within the period indicated according to the validation method recommendations for each antibiotic

2. on the day of assay: using the stock solution,

2.1 Prepare from the standard stock solution, three standard target concentrations

Standard low = lowest concentration

Standard Median = median concentration

Standard High = highest concentration

Note: Dose ratio between the two adjacent doses is 1.25

3. Preparation of Sample

3.1. Assign to the unknown sample an assumed potency per unit weight or volume, depending on the declared potency by the manufacturer.

3.2. On this assumption, prepare on the day of assay: A stock solution and a test solutions as specified for each antibiotic using the same final diluent as used for standard.

3.3. From a sample stock solution, prepare three test sample target concentrations as well as standard concentrations:

Test low = lowest concentration

Test median = median concentration

Test high = highest concentration

Note: Dose ratio between the two adjacent doses is 1.25

4. Microorganisms and inoculum:

4.1 Test Organism:

The test organism is Micrococus Luteus ATCC # 9341

4.2. Preparation of the Inoculum's:

4.2.1. Take 1 loopful of frozen culture (at – 80 °C) and transfer to 2 ml SCDM or BHIM. , mix gently on vortex

4.2.2. Pipette 1 ml from the SCDM or BHIM and inoculate on SCDA or BHIA plate or one loopful from slant culture and streak on SCDA or BHIA plate.

4.2.3. Remove the growth from a recently grown slant or culture of the organism with 3 mL of sterile saline TS and sterile glass beads.

4.2.4. Inoculate by streaking on a surface of BHIA or SCDA agar medium specified for this microorganism.

4.2.5. Incubate at 35 C$\pm$2 for the time indicated (18-24 hour).

4.2.6. At the end of incubation, prepare the stock suspension by collecting the surface growth in 50 ml of sterile saline TS.

4.2.7. Measure the Transmittance % (T) of the bacterial suspension using visible

Spectrophotometer at wavelength = 140 nm. The transmittance of the suspension shall be around 23%-27%

4.2.8. Add a portion of bacterial stock suspension to a sufficient amount of agar medium melted and cooled to 45^{o} - 50^{o} C and swirl to homogenize.

5. Calculation of potency estimate by three dose level

To calculate the potency of antibiotic from the data obtained by the cylinder plate method, using the following equations provided by USP

5.1. E = The difference in response between adjacent dose levels

E = ¼ (S3+T3) – (S1+T1)

5.2. F= The difference in response between the two preparations (Standard and sample)

F= ⅓ (T3 + T2 + T1) – (S3 + S2 + S1)

5.3. I= Log ratio of the doses (log ratio between two adjacent doses).

I= Log dilution factor.

5.4. b= the slope of response

b= E/I

5.5. M = the log of the potency ratio

M=F/b

5. 6. Potency ratio = Anti log10 M X 100 %.

* Potency ratio = antilog10 MX100%

S1 = Standard low concentration T1 = Sample low concentration

S2 = Standard medium concentration T2 = Sample medium concentration

S3 = Standard high concentration T3 = Sample high concentration

Chapter Three

Results and discussion

Results and discussion

3.1- Assay method:

This product can be analyzed chemically or biologically, but we managed to determine the potency of the product biologically where potency can be determined by measuring the diameter of inhibition on the culture plate for organism *Micrococcus luteus* ATCC #9341.

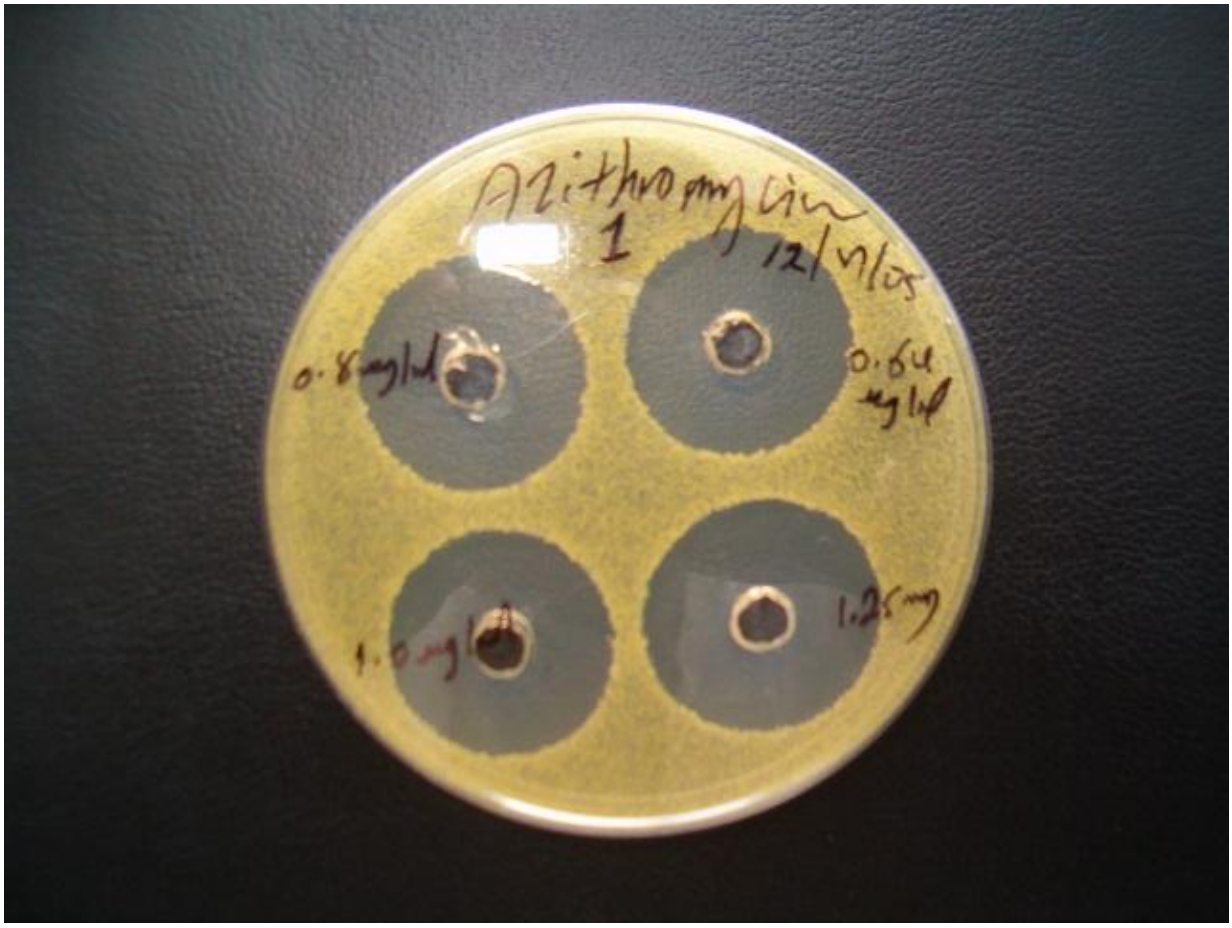

Figure 17: sample of the biological assay plates where the zone of inhibition is calculated and compared to standard plate using *Micrococcus luteus* ATCC #9341 organism as our reference.

3.2- In the first object of this study, it was found that Azithromycin is soluble in 30% ethanol/water, a 2% azithromycin in 30% ethanol/water solution maintained stable at room temperature for up to 6 months but at 37°C there was some decay (16%) at 26 weeks. The stability was greatest at pH 6.8 and was unaffected by ambient light exposure, this result was assured by a previous study done by RC McHugh [A1], A Rice [A1], ND Sangha [A1], MA McCarty [A1], R Utterback [A1], JM Rohrback [A1], BE Osborne [A1], AB Fleischer Jr [A1], SR Feldman in the Department of Dermatology Wake Forest School of Medicine Winston-Salem North Carolina USA.(?)

3.3- In the second part of this study, a stable gel structure was prepared according to formula B which consist of :

Table 6: Gel formula B

Material name	Function	Concentration
Azithromycin	API	2%
Purified water	Solvent	33.89%
Ethyl alcohol	Solvent	30%
Cremophore RH40	Solubilizer	1%
Sepigel (Polyacrylamide and C13-14 Isoparaffin and Laureth-7)	Gelling agent	3%
Methyl paraben	preservative	0.1%
Propyl paraben	Preservative	0.01%

The 2% Azithromycin gel structure obtained by gel base Formula B without adding any penetration enhancer achieves all the predetermined specifications which are:

Table 7 : Specification data for 2% Azithromycin in Formula B.

Specification	Limit	Result	Verification method
Color	Transparent	Transparent	visual test
pH	9.0 – 10.5	9.9	pH meter
Viscosity at 23C	3000-3500 cps	3400 cps	Brookfield viscometer
Crystals	None	None	Microscopically exam.
Irritation	None	None	Human skin application
Compatibility	Compatible	Compatible	Chemical assay

The in-use stability of the 2% Azithromycin gel was detected for 2 months where the active ingredient was mixed directly with the gel media before use where the Azithromycin dose shall be provided unmixed in a hard gelatin capsule and the normal period of treatment is mainly 7 days.

3.4-The third part of this study includes the introduction of different penetration enhancers into the 2% Azithromycin gel structure to prepare different formulations where 2 studies have been run on these formulations:

3.4.1- A 2 months shelf life stability study, to investigate most of the chemical and physical characteristics of the preparations as presented in the tables below:

Storage conditions: at room temperature.

Packaging material: polyethylene well closed gar.

1-Formula 2: Penetration enhancer 10% Urea

Table 8: Specification data for Formula 2 with 10% urea.

		0 Month	Month 1	Month 2
Specification	Limit	Result	Result	Result
Color	Transparent	Transparent	Transparent	Transparent
pH	9.0 – 10.5	9.7	9.5	9.3
Viscosity at 23C	3000-3500 cps	3250 cps	3000 cps	3000 cps
Crystals	None	None	None	None
Irritation	None	None	None	None
Compatibility	Compatible	Comply	Comply	Comply
Assay of Azithromycin	95-105%	98%	97%	97%

2- Formula 3: Penetration enhancer 10% Propylene glycol

Table 9 : Specification data for Formula 3with 10% propylene glycol.

		0 Month	Month 1	Month 2
Specification	Limit	Result	Result	Result
Color	Transparent	Transparent	Transparent	Transparent
pH	9.0 – 10.5	10.3	9.8	9.8
Viscosity at 23C	3000-3500 cps	3500 cps	3200 cps	3200 cps
Crystals	None	None	None	None
Irritation	None	None	None	None
Compatibility	Compatible	comply	comply	comply
Assay of Azithromycin	95-105%	101%	97%	97%

3 – Formula 4: Penetration enhancer 10% IPM

Table 10 : Specification data for Formula 4 with 10% IPM.

		0 Month	Month 1	Month 2
Specification	Limit	Result	Result	Result
Color	White	White	White	White
pH	9.0 – 10.5	10.1	9.7	9.7
Viscosity at 23C	3000-3500 cps	3270 cps	3000 cps	3000 cps
Crystals	None	None	None	None
Irritation	None	None	None	None
Compatibility	Compatible	comply	comply	comply
Assay of Azithromycin	95-105%	100.5%	97%	97%

4-Formula 5: Penetration enhancer 2% Oleic acid

Table 11 : Specification data for Formula 5 with 2% oleic acid.

		0 Month	Month 1	Month 2
Specification	Limit	Result	Result	Result
Color	White	White	White	White
pH	9.0- 10.5	8.7	8.19	8.19
Viscosity at 23C	3000-3500 cps	2700 cps	2500 cps	2500 cps
Crystals	None	None	None	None
Irritation	None	None	None	None
Compatibility	Compatible	comply	comply	comply
Assay of Azithromycin	95-105%	97%	97%	96%

* pH and viscosity fail to achieve specification.

3.4.2 - A diffusion study has been done to the different preparations using MFDC for each penetration enhancer according to the predetermined conditions in point no.2.4 and the results were found as follows:

1- Formula No. 1: 2% Azithromycin gel without PE

Table 12: Data obtained by the diffusion of 2% Azithromycin gel formula 1 through a synthetic membrane in a Modified Franz diffusion cell in the absence of penetration enhancer.

Sampling time	Diameter mm	Sample conc. mg/ml	Qc mg/20.6 ml	Qa (mg)	M (mg/cm2)	J (mg/cm2/hour)	% diffused
0	0.00	0.0000	0.000	0.000	0.000	0.000	0
0.5	15.66	0.0480	0.989	0.989	0.330	0.627	3%
1	14.47	0.0740	1.524	1.132	0.524	0.524	4%
2	14.39	0.1280	2.637	2.727	0.920	0.430	8%
3	15.23	0.2220	4.133	4.823	1.308	0.536	13%
4	16.33	0.2750	5.665	6.137	2.046	0.511	17%
5	17.10	0.3050	6.283	7.030	2.343	0.469	20%
6	17.38	0.3100	6.386	7.438	2.479	0.413	21%

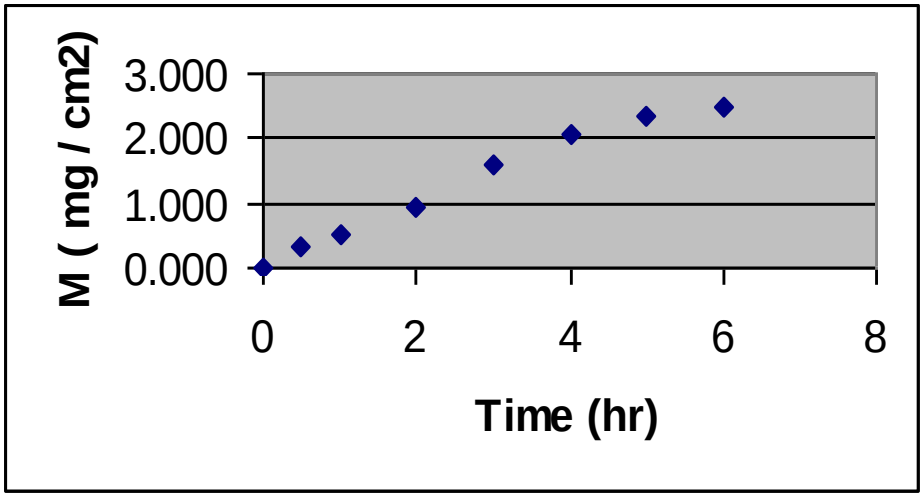

Figure 18: In vitro permeation profile for the cumulative amount of Azithromycin penetrated per unit area of synthetic membrane (mg/cm2) in formula no. 1 as a function of time (hr) in the absence of Penetration enhancer.

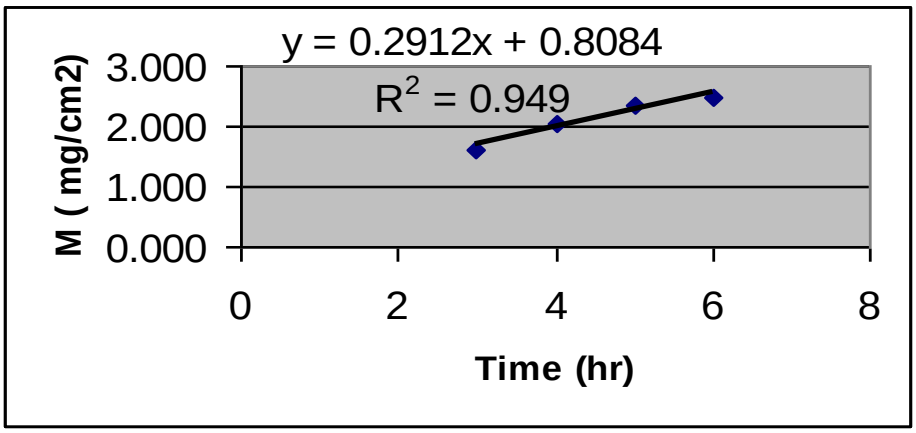

Figure 19: In vitro Steady state permeation profile for the cumulative amount of Azithromycin penetrated per unit area of synthetic membrane (mg/cm2) in formula no.1 as a function of time (hr) in the absence of Penetration enhancer.

Diffusion results:

Table 13: Diffusion results for formula 1 obtained by MFDC study.

Formula	Slope	Intercept	T_L=intercept /slope	D=h2/6T_L	P = slope / concentration	K=P.h/D
1	0.2912	0.8084	2.77	1.45E-05	**0.0146**	15.16

2- With 10% Urea

Formula No. 2: 2% Azithromycin gel with 10% Urea

Table 14 : Data obtained by the diffusion of 2% Azithromycin gel formula no 2 through a synthetic membrane in a Modified Franz diffusion cell in the presence of 10% Urea as penetration enhancer.

Sample time	Diameter mm	Sample conc. mg/ml	Qc mg/20.6 ml	Qa (mg)	M (mg/cm2)	J (mg/cm2/hour)	% diffused
0	0	0.0000	0.000	0.000	0.000	0.000	0%
0.5	15.63	0.1800	3.708	3.708	1.236	2.472	10%
1	18.70	0.6320	13.019	13.199	4.400	4.400	37%
2	18.96	0.7000	14.420	15.232	5.077	2.539	42%
3	19.03	0.8000	16.480	17.992	5.997	1.999	50%
4	19.00	0.8000	16.480	18.792	6.264	1.386	52%
5	20.33	1.0000	20.300	23.712	7.904	1.141	66%
6	20.47	1.0500	21.630	25.742	8.141	1.430	72%

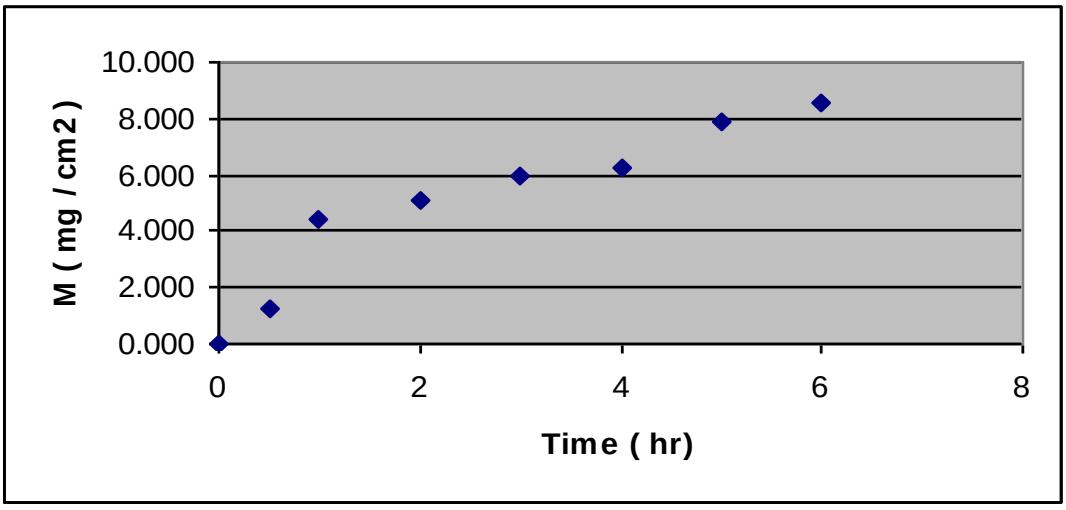

Figure 20: In vitro permeation profile for the cumulative amount of Azithromycin penetrated per unit area of synthetic membrane (mg/cm2) in formula no.2 as a function of time (hr) in the presence of 10% Urea as Penetration enhancer.

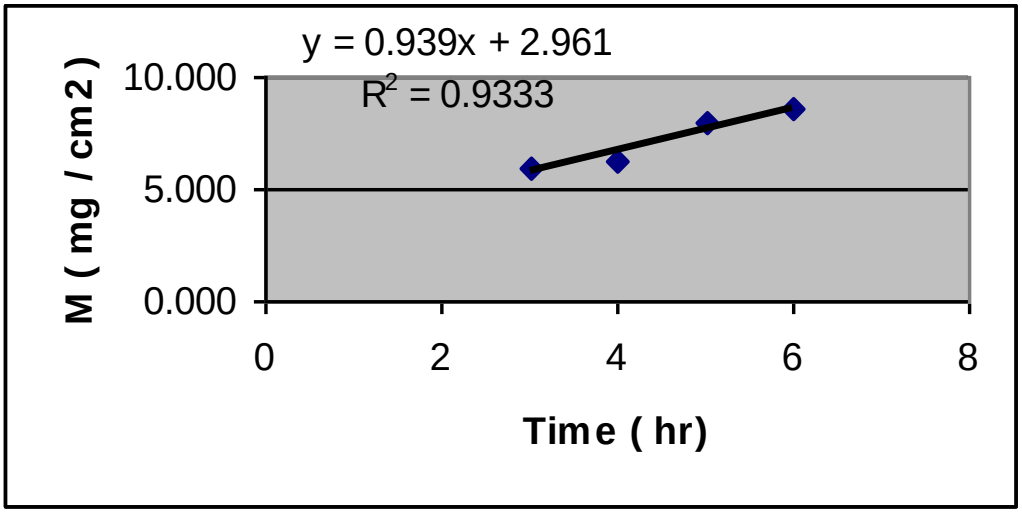

Figure 21: In vitro Steady state permeation profile for the cumulative amount of Azithromycin penetrated per unit area of synthetic membrane (mg/cm2) in formula no.2 as a function of time (hr) in the presence of 10% Urea as penetration enhancer.

Diffusion parameters:

Table15: Diffusion parameters for formula 2 obtained by MFDC study.

Formula	Slope	Intercept	T_L =intercept /slope	D=h2/6 T_L	P = slope / concentration	K=P.h/D
2	0.939	2.932	3.15	1.35E-05	**0.047**	39.52

Enhancement ratio for 10% Urea =0.047 / 0.0146 = 3.22

Urea is among the amide compounds and is widely used in amounts of around 5-10% in moisturizing creams and topical steroids (??).

Urea is a hygroscopic compound, which hydrates the stratum corneum and hence improves permeation of drug molecules. By increasing the amount of water present within stratum corneum, this layer swells and even though the passageway of drug molecules gets longer but nevertheless because of the decompression of cells and an increase in the rate of passage of drug molecules, the extent of drug permeation increases

3- with 10% propylene glycol

Formula No. 3: 2% Azithromycin gel with 10% PG

Table 16 : Data obtained by the diffusion of 2% Azithromycin gel formula no 3 through a synthetic membrane in a Modified Franz diffusion cell in the presence of 10% propylene glycol as penetration enhancer.

Sample time	Diameter mm	Sample conc. mg/ml	Qc mg/20.6 ml	Qa (mg)	M (mg/cm2)	J (mg/cm2/hour)	% diffused
0	0	0.0000	0.000	0.000	0.000	0.000	0
0.5	17.65	0.3140	6.468	6.468	2.138	4.312	18%
1	17.67	0.3130	6.510	6.824	2.275	2.275	19%
2	19.33	0.3000	12.330	12.990	4.330	2.165	36%
3	19.27	0.6720	13.843	15.073	5.024	1.675	42%
4	19.39	0.7430	15.368	17.270	5.713	1.439	48%
5	19.14	0.7270	15.635	18.283	6.094	1.219	51%
6	19.32	0.7630	15.718	19.125	6.375	1.062	53%

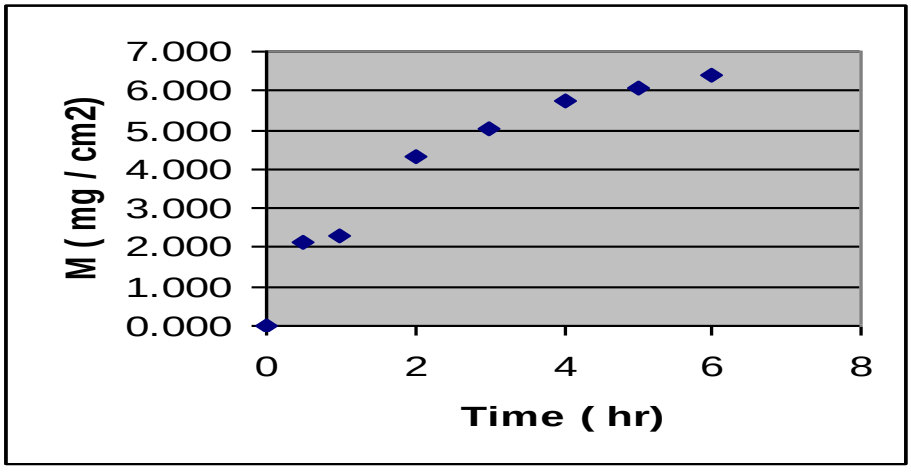

Figure 22: In vitro permeation profile for the cumulative amount of Azithromycin penetrated per unit area of synthetic membrane (mg/cm2) in formula no. 3 as a function of time (hr) in the presence of 10% Propylene glycol as a penetration enhancer.

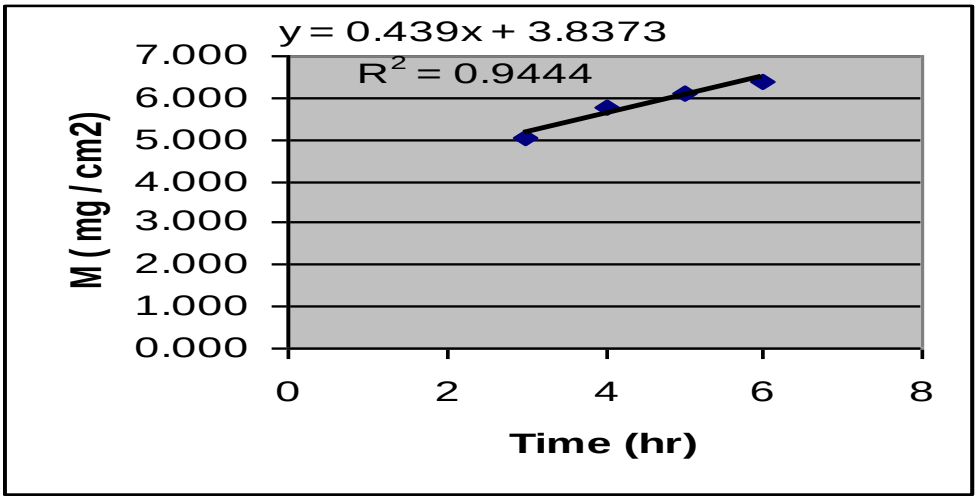

Figure 23: In vitro Steady state permeation profile for the cumulative amount of Azithromycin penetrated per unit area of synthetic membrane (mg/cm2) in formula no. 3 as a function of time (hr) in the presence of 10% Propylene glycol as a Penetration enhancer.

Diffusion parameters:

Table 17 : Diffusion parameters for formula 3 obtained by MFDC study.

Formula	Slope	Intercept	T_L =intercept /slope	D=h2/6 T_L	P = slope / concentration	K=P.h/D
3	0.439	3.8373	8.74	4.88E-06	**0.022**	71.95

Enhancement ratio for 10% Propylene glycol =0.022 / 0.0146 = 1.51

4-With 10% IPM

Formula No. 4: 2% Azithromycin gel with 10% IPM

Table 18 : Data obtained by the diffusion of 2% Azithromycin gel formula no 4 through a synthetic membrane in a Modified Franz diffusion cell in the presence of 10% IPM as penetration enhancer.

Sample time	Diameter mm	Sample conc. mg/ml	Qc mg/20.6 ml	Qa (mg)	M (mg/cm2)	J (mg/cm2/hour)	% diffused
0	0.0	0.0000	0.000	0.000	0.000	0.000	0%
0.5	0.0	0.0000	0.000	0.000	0.000	0.000	0%
1	0.0	0.0000	0.000	0.000	0.000	0.000	0%
2	0.5	0.0012	0.025	0.025	0.008	0.004	0%
3	0.5	0.0012	0.025	0.026	0.009	0.003	0%
4	0.5	0.0012	0.025	0.027	0.009	0.002	0%
5	0.5	0.0012	0.025	0.028	0.009	0.002	0%
6	0.5	0.0012	0.025	0.030	0.010	0.002	0%

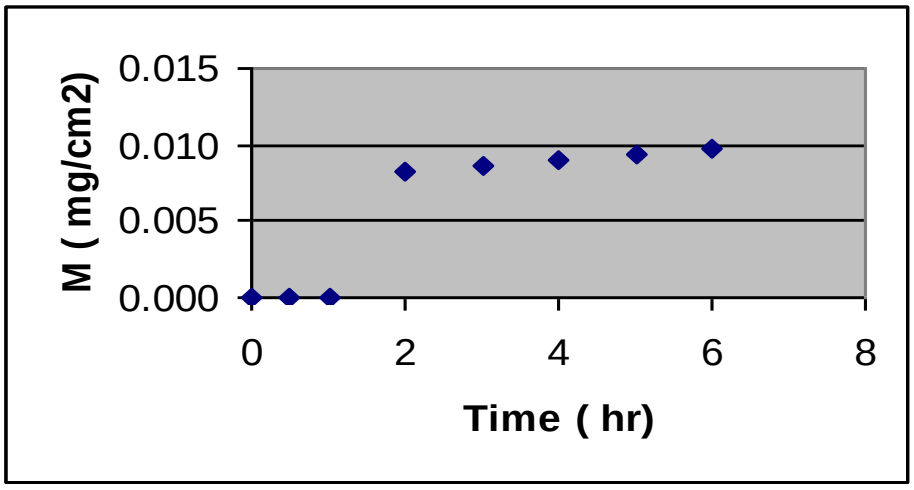

Figure 24: In vitro permeation profile for the cumulative amount of Azithromycin penetrated per unit area of synthetic membrane (mg/cm2) in formula no. 4 as a function of time (hr) in the presence of 10% IPM as a Penetration enhancer.

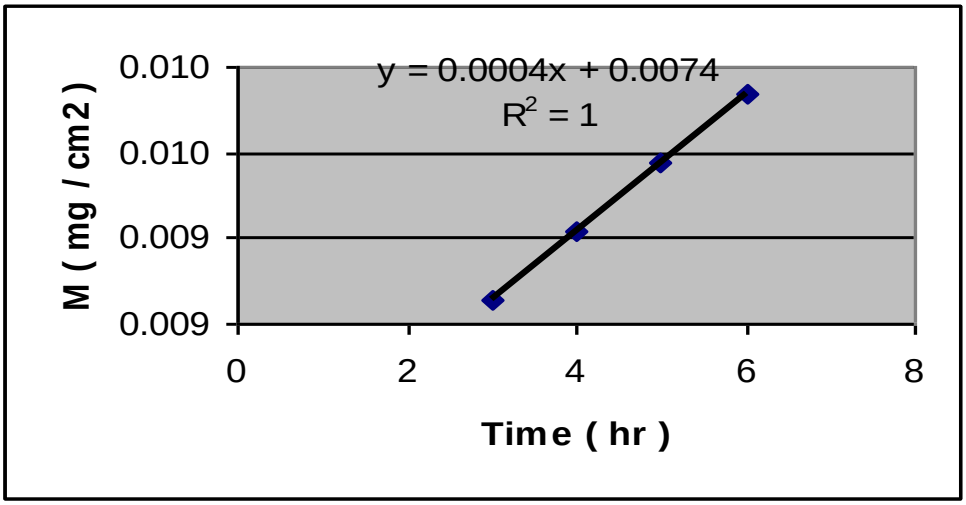

Figure 25: In vitro Steady state permeation profile for the cumulative amount of Azithromycin penetrated per unit area of synthetic membrane (mg/cm2) in formula no. 4 as a function of time (hr) in the presence of 10% IPM as a penetration enhancer.

Diffusion parameters:

Table 19: Diffusion parameters for formula 4 obtained by MFDC study

Formula	Slope	Intercept	T_L =intercept /slope	D=h2/6 T_L	P = slope / concentration	K=P.h/D
4	0.0004	0.007	17.5	2.44E-06	**0.00002**	0.14

Enhancement ratio for 10% IPM =0.00002 / 0.01438 = 0.001

IPM is an aliphatic ester, it penetrates between the lipid bilayers of stratum corneum and due to its' chain structure, disrupts the order and arrangement of lipid bilayers of stratum corneum and hence improves drug permeation into this layer.

5-With 2 % Oleic acid

Formula No. 5: 2%Azithromycin gel with 2% Oleic acid

Table 20 : Data obtained by the diffusion of 2% Azithromycin gel formula no 5, through a synthetic membrane in a Modified Franz diffusion cell, in the presence of 2% Oleic acid as penetration enhancer.

Sample time	Diameter mm	Sample conc. mg/ml	Qc mg/20.6 ml	Qa (mg)	M (mg/cm2)	J (mg/cm2/hour)	% diffused
0	0.0	0.0000	0.000	0.000	0.000	0.000	0%
0.5	0.0	0.0000	0.000	0.000	0.000	0.000	0%
1	0.0	0.0000	0.000	0.000	0.000	0.000	0%
2	0.0	0.0000	0.000	0.000	0.000	0.000	0%
3	0.0	0.0000	0.000	0.000	0.000	0.000	0%
4	0.0	0.0000	0.000	0.000	0.000	0.000	0%
5	0.0	0.0000	0.000	0.000	0.000	0.000	0%
6	0.0	0.0000	0.000	0.000	0.000	0.000	0%

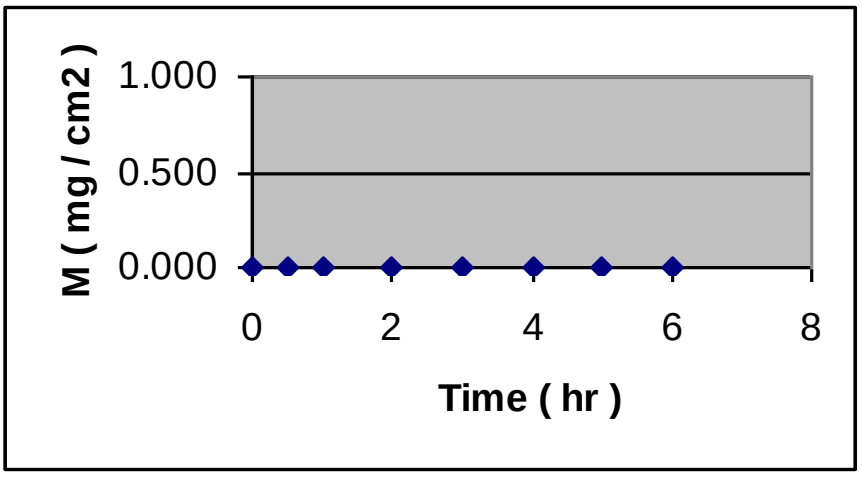

Figure 26: In vitro permeation profile for the cumulative amount of Azithromycin penetrated per unit area of synthetic membrane (mg/cm2) in formula no.5 as a function of time (hr) in the presence of 2 % Oleic acid as a Penetration enhancer.

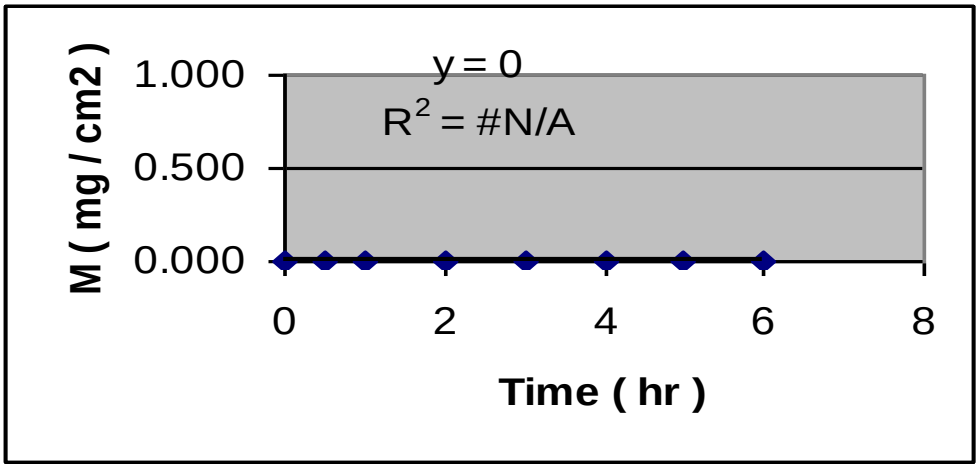

Figure 27: In vitro Steady state permeation profile for the cumulative amount of Azithromycin penetrated per unit area of synthetic membrane (mg/cm2) in formula no.5 as a function of time (hr) in the presence of 2% Oleic acid as a Penetration enhancer.

Diffusion parameters:

Table 21: Diffusion parameters for formula 5 obtained by MFDC study.

Formula	Slope	Intercept	T_L =intercept /slope	D=h2/6 T_L	P = slope / concentration	K=P.h/D
5	0	0	0	0	**0**	0

Enhancement ratio for 2 % Oleic acid =0.0000 / 0.01438 = 0

The effect of oleic acid in amount of 2% on the permeability of Azithromycin is shown in table 22 and the calculated permeability equal to zero.

Even oleic acid is a popular penetration enhancer and penetrates into the stratum corneum and decompresses this layer and hence reduces its' resistance to drug penetration, it can also accumulate within the lipid bilayers of stratum corneum cells and hence increase their flowability and penetration ability, but in high concentrations like used in this study (2%), oleic acid inhibit permeability to zero.

The reason for this inhibition is may be due to the formation of oleic acid layer on the membrane because of its low solubility in the aqueous domain and consequently increases the diffusion coefficient.

3.5- Selection of the best penetration enhancer

By determining all diffusion parameters for the different penetration enhancers from the above experiments as the table below, we can make certain comparison such as table 22 and 23:

Table 22: Diffusion parameters for the different penetration enhancers

Penetration enhancer	Slope	Intercept	T_L (hr)	D (cm^2/hr)	**P (cm /hr)**	K	Enhancement ratio (ER)
Without penetration enhancer	0.2912	0.8084	2.77	1.45E-05	**0.0146**	15.16	1
Urea	0.939	2.932	3.15	1.35E-05	**0.047**	39.52	3.22
Propylene glycol	0.439	3.8373	8.74	4.88E-06	**0.022**	71.95	1.51
IPM	0.0004	0.007	17.5	2.44E-06	**0.00002**	0.14	0.001
Oleic acid	0	0	0	0	**0**	0	0

By plotting Time versus M for each penetration enhancer

Table 23: Time v s M for all penetration enhancers

Time (hr)	Without	Urea	Propylene glycol	IPM	Oleic acid
0	0	0	0	0	0
0.5	0.33	1.236	2.138	0	0
1	0.524	4.4	2.275	0	0
2	0.92	5.077	4.33	0.008	0
3	1.308	5.997	5.024	0.009	0
4	2.046	6.264	5.713	0.009	0
5	2.343	7.904	6.094	0.009	0
6	2.479	8.141	6.375	0.01	0

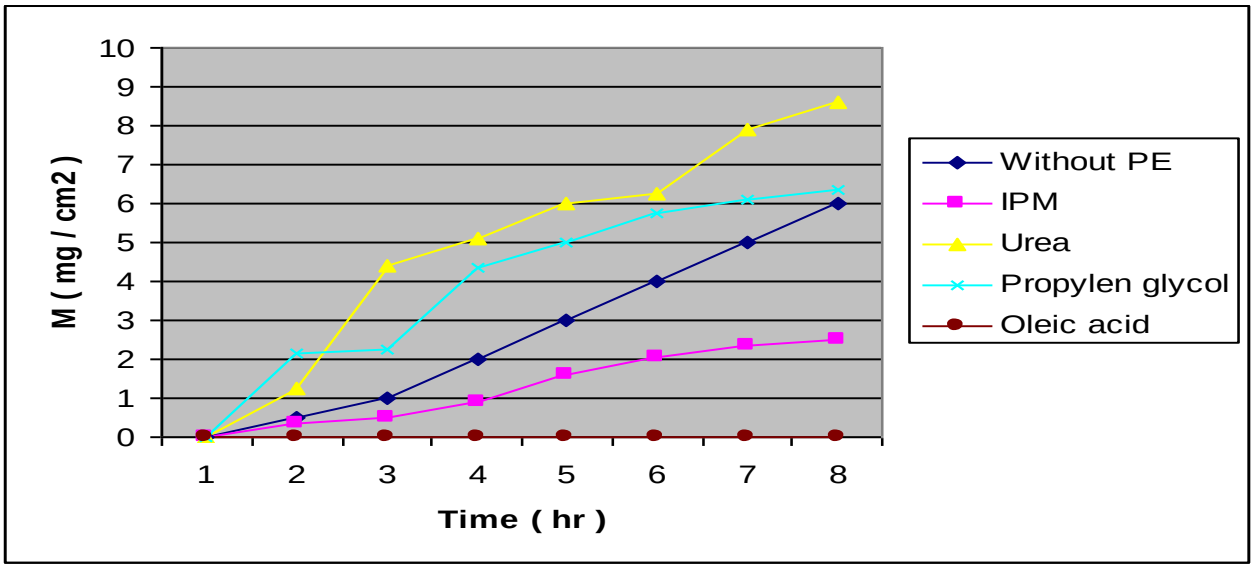

Figure 28: Plot of Time versus M for all penetration enhancers

Enhancement ratio for each penetration enhancer can be plotted as figure 29 for comparison such as:

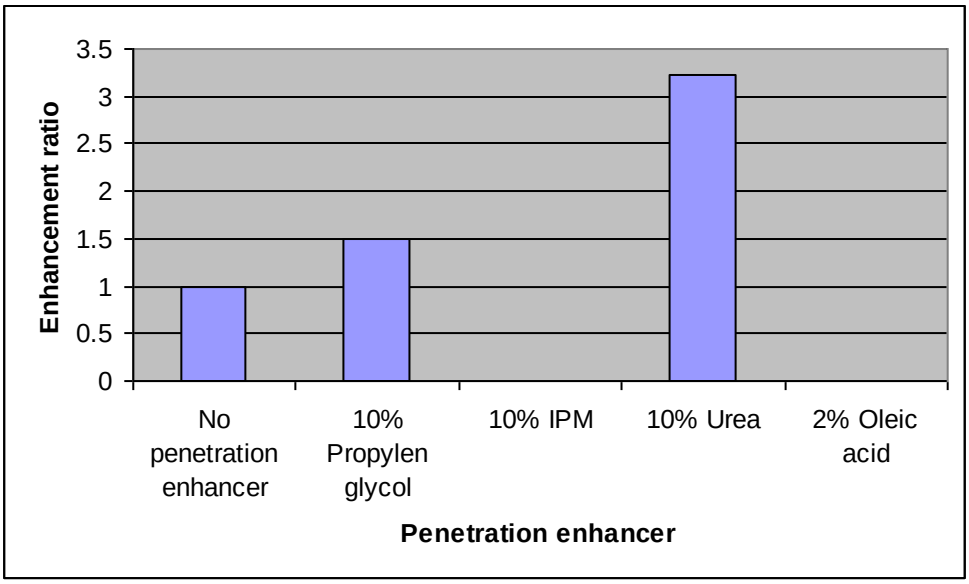

Figure 29: Enhancement ratio for each penetration enhancer

Lag time (T_L) for each penetration enhancer can be plotted as shown in figure 30 for comparison such as:

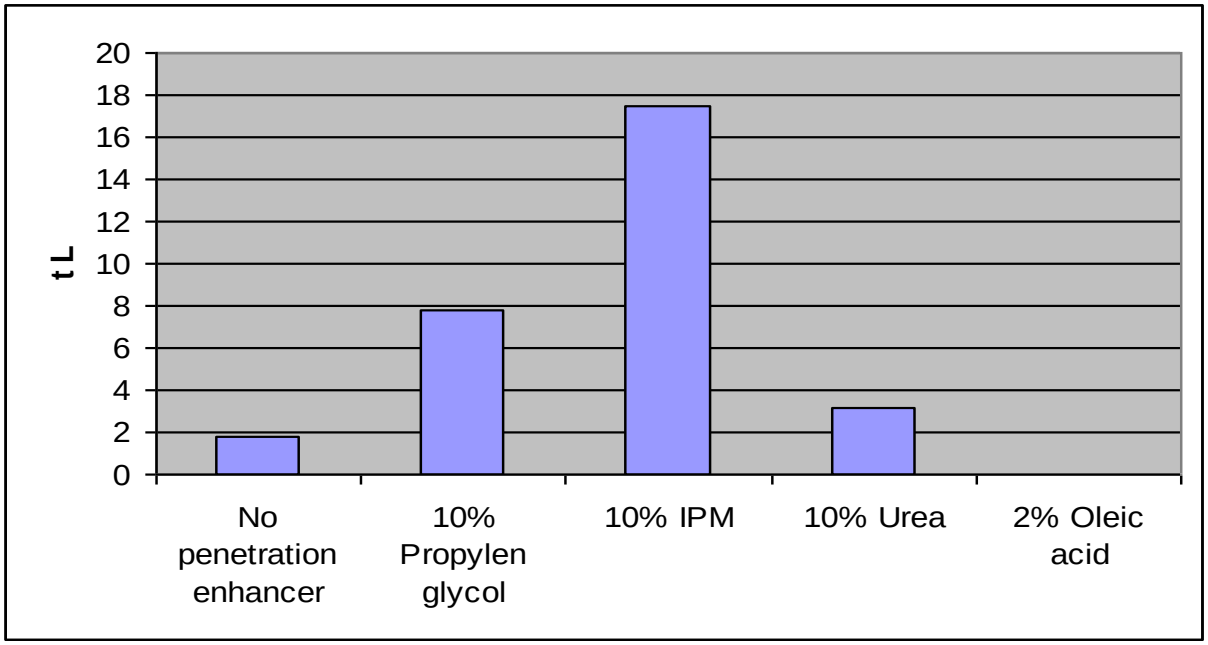

Figure 30: A plot of T_L of different penetration enhancers

Diffusion coefficient (D) for each penetration enhancer can be plotted in Figure 31for comparison such.

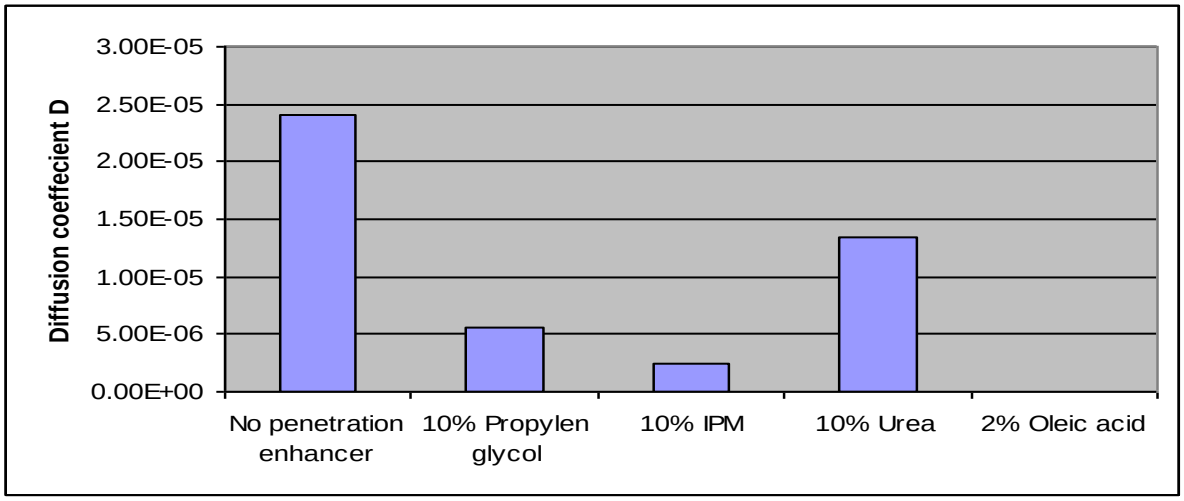

Figure 31: A plot of diffusion coefficient of different penetration enhancer

Chapter Four

Conclusion

Conclusion

Results show that among the penetration enhancers used, the use of urea at a concentration of 10% w/w had the greatest effect on the permeability rate of Azithromycin, and produce the highest enhancement ratio among all the penetration enhancers examined, followed by propylene glycol 10%.

The other penetration enhancers used like oleic acid 2% and IPM 10% were found to have an inhibitory effect on the permeability rate of Azithromycin through the synthetic membrane.

The results of this study on Azithromycin shows that penetration enhancers like IPM and Oleic acid whom work to increase the fluidity of the lipid domain always decrease or inhibit the permeability of the drug, while other penetration enhancers like Urea and Propylene glycol whom work to increase the fluidity of the aqueous domain always increase the permeability of the drug.

UR is a hygroscopic compound, which hydrates the stratum corneum and hence improves permeation of drug molecules. By increasing the amount of water present within stratum corneum, this layer swells and even though the passageway of drug molecules gets longer but nevertheless because of the decompression of cells and an increase in the rate of passage of drug molecules, the extent of drug permeation increases

The rate of permeability enhancement was found to be as follow

Urea > Propylene glycol > without penetration enhancer > IPM > Oleic acid

The lag time T_L was found on the opposite order

Oleic acid > IPM > Propylene glycol > Urea

This finding suggests that the type and concentration of a penetration enhancer for incorporation into a specific formulation containing a particular drug should be

selected carefully and following extensive initial studies, in order to achieve a formulation with desirable drug permeability rate and efficacy.

Further work shall be done on this product in future if a topical form of Azithromycin to investigate the effect of different concentrations of different penetration enhancers.

Chapter Five

References

References

1- Rona, M.M., Clinical Dermatology, 3rd ed., Oxford university press, USA, (1991), 2:22.

2- Yie W. Chien, Transdermal Controlled Sysrtemic Medications, Marcel Dekker, Inc., New York and Basel, 1987.

3- Barry, B. W., Dermatological Formulation, Percutaneous Absorption, Marcel Dekker, New York, 1983.

4- Leon Lachman, Herbert A. Liberman, Joseph L. Kanig, The Theory and Practice of Industrial Pharmacy, 3rd Ed., USA, 1986.

5- Chien, Yie W., Novel Drug Delivery System, 2nd Ed., Marcel Dekker Inc., USA, (1992), pg.301 -302.

6- Banker, G. S. and Rhodes, Ch.T., Modern Pharmaceuticals, 3rd Ed., USA, (1996), pg. 239-240.

7- Alfonson R. Gennaro. Remington -The Science and Practice of Pharmacy, 20th Edition, USA, 2000.

8- Aulton, M.E., Pharmaceutics, The Science Of Dosage From Design, 5th ed, Churchill Living Tone, New York, (1995), pg.384.

9- Martin Malmsten, Surfactants and Polymers in Drug Delivery, USA, Vol. 122,2002.

10- Y. W. Chien and C. S. Lee; in: Controlled Release Technology: Pharmaceutical Application (P.l:Lee and W. R. Good, Eds.) American Chemical Society Washington, D. C, 1987, ch. 21.

11-Champion, R.H.; Burton, J.L. and Ebling F.J.G., Textbook of Dermatology, Vol. 1, 5th ed, Blackwell press, (1992),3:27 -30.

12-Barry, B. W., 1988. Mode of action of penetration enhancers in human skin. J. Contr. ReI., 6, 85 -97.

13- Habes, Mohamad, Investigation the effect of some penetration enhancers on permeability of Chlorpheneramine Maleate (M.Sc. thesis), Al- Quds university, (2005).

14- Propionibacterium Acne ……..

15 -Francis, N.M.; Howard, I.M., Dermato-toxicology, 3rd ed., Hemispere publishing corporation, London, (1988), 1 : 1-3.

16-Barry, B. W., 1988 chap. 22, Topical preparation in: Aulton, M. E., (editor). The Science of Dosage form Design. Churchill- Living stone. New York.

17- Carr, M. G., Corish, I. m, Corrigan, O. I., Drug Delivery From a Liquid Crystalline Base Across Visking and Human Stratum Corneum. International Journal of Pharmaceutics, : 1997, 113: 35 -42.

18- Goa, S., Singh, J. Mechanism of transdermal transport of 5-fluorouracil by terpenesi Carvon, 1, 8-cineole and thymol. International Journal of Pharmaceutic, 1997, 145: 67 -.77.

19-Loebl, S. and Spratto, G. R., The Nurse's Drug Handbook, 4th edition, John Wiley & Sons Inc., (1986), pg. 30.

20- Schipper NGM, Verhoef JC, Merkus FWHM. The nasal mucocilliary clearance: relevance to nasal drug delivery. Pharm Res. 1991; 8:807-814.

21- Van de Donk HJM, Merkus FWHM. Decreases in ciliary beat frequency due to instranasal administration ofpropanolol. J. Pharm Sci. 1982; 71 :275-276.

22- Griffith N, Howell S, Mason DG. Intranasal midazolam for premedication of children undergoing day-case anaesthesia: comparison of two delivery systems with assessment of intra-observer variability. Br J Anaesth. 1998; 81 :865-869.

23-Meltzer EO, Orgel HA, Bush RK, Haltom JR, Metzger J, Moss BA, Mitchell DQ, Ballas ZK, Seltzer JM, Shapiro GG, Van Bavel JH. Evaluation of symptom relief, nasal airflow, nasal cytology, and acceptability of two formulations of flunisolide nasal spray in patients with perennial allergic rhinitis. Ann Allergy. 1990; 64:536-450.

24-Katayoun Welin-Berger, 2001, Formulation, Release and skin penetration of Topical Anesthetics, PH.D. Thesis, 2001, p. 28.

25- Engstrom. S., and Engstrom, L. Phase behavior of the lidocaine-monoolein-water system. Int. J. Pharm. 1992 -79, 113-122.

26- Engstrom. S., Drug delivery from cubic and other lipid-water phases. Lipid Technol., 1990,2(2), 42-45.

27- Gilbert S. Banker, Christopher T. Rhodes, Modern Pharmaceutics , 2d edition, Marcel Dekker, New York, 1990.

28- Welin-Berger K., Formulation, release and skin penetration of topical anesthetics, ph. D. thesis, Uppsala University Sweden, Uppsala 2001. P. 36

29- Forslind, B. Engstrom, S., Engblom, J., Norlen, L., 1997. a novel approach to the understanding of human skin barrier function. J. Dermatol. Sci. 14, 115 -125.

30- Acne ……

31-Cornwell, P. A., Barry, B. W., 1993. The routes of penetration of ions and 5-fluorouracil across human skin and the mechanisms of action of terpene skin penetration enhancers, Int. J. Pharm., 94,189 -194.

32- United State Pharmacopoeia , USPC Inc. official , 2005.

33- Osborne D. W., Amann A. H., Topical Drug Delivery Formulations, Volume 42, Marcel Dekker, New York and Basel, 1990.

34- AI-Hindi Fawzi, Investigation of Diclofenac Complexes with some Metals as Candidate System for Drug Delivery (M. SC. Thesis), University of Jordan, 2002.

35-Hadgraft, J. 2001. Skin, the final frontier. International Journal of Pharmaceutics, 224: 1 - 18.

36- British pharmacopoeia Commission Office, British pharmacopoeia, the stationary office limited, London, 2001.

37- Seyed A. M. et. al., Iranian Journal of Pharmaceutical Research (2003), [135 -140].

38- Alfred Martin, James Swarbrick, Arthur Cammarata, Physical pharmacy 3d edition, LEA&FEBIGER, Philadelphia 1983.

39- ZITHROMAX®, Pfizer Labs, Division of Pfizer Inc, NY, NY 10017 Revised January 2004.

40-Balsam, M.S. and others, Cosmetics Science And Technology, Vol. 2, 2nd ed., Krieger publishing company, Florida, (1992), 36 :136 -165.

41- Thibodeau, G. and Pattou, K., Anatomy And Physiology, 4th ed., Mosby Inc. (1999), 2:164-165.

42- Kayali, Ibrahim, Stratum Corneum Lipids (Ph. D. thesis), Clarkson University, (1989).

43- Ansel, H. C., Poporich, N. C., and Allen, L. Pharmaceutical Dosage Forms and Drug Delivery System, 6th edition, Williams, and Wilkins, USA, 1995.

44- Sherwood, L., Human Physiology From Cells To Systems, 3rd Edition, Wadsworth Published Company, UK, (1997).

45- Bryan Mann, mann@ tiger.fhsu.edu.

46- Keeton, William, Biological Science, 2nd ed, Norton and Company Inc., New York, (1972),pg.92.

47-Guyton and Hall, Textbook of Medical Physiology, 9th edition, W.B. Saunders Company, USA, (1996).

48- Winfield, A. J. and Richards, R.M.E., Pharmaceutical Practice, 2nd Edition, Churchill Living Stone, China, (1999).

49- Thomas C.K. chan,PhD, Percutaneous Penetration Enhancer, 9th Biennial international conference of perspectives in percutaneous penetration , France, April 13,2004, published January 2005.

50- CRAIG E. PONCELET, LYNN K. PERSHING, LYSSA D. LAMBERT, AND JUDY CORLETT Department of Medicine (Dermatology),University of Utah, Vol.2,No.1 pp.11-16 .

51- Saunders James CJ1, Davis Henry J, Coetzee Linda, Botha Susan, Kruger Ansie E, Meyer Zall Laboratories, Drie Valleyen, Sedgefield, South Africa, Grobler Anne, Medical

Research Council, Cape Town, South Africa , J Pharm Pharmaceut Sci (www.ualberta.ca/~csps) 2(3):99-107, 1999.

52- Wikipedia, the free encyclopedia, "http://en.wikipedia.org/wiki/Epidermis_(skin)".

53- Julie C Harper, MD, Assistant Program Director, Assistant Professor, Department of Dermatology, University of Alabama at Birmingham, Acne Vulgaris, e- Medicine.com,Inc. July 29, 2004.

الملخص

إن تحقيق الامتصاص الكامل للأدوية التي تقدم على شكل مستحضرات للمسح الخارجي على الجلد أصبح من أهم الأمور التي يتم دراستها في مجال المستحضرات الجلدية.

إن هدف هذه الدراسة هو تحضير مستحضر للاستعمال الموضعي على الجلد لمادة المضاد الحيوي AZITHROMYCIN وللاستعمال بشكل مباشر في علاج المرض الجلدي المسمى حب الشباب ACNE VULGARISوالمتسبب من بكتيريا مسماة PROPIONIBACTERIUM ACNE وذلك للتغلب على مشكلة المناعة أو الحصانة التي يكونها الميكروب ضد الأدوية التقليدية المستعملة لعلاج هذا المرض.

إن تطوير ألصيغة المناسبة التي تحقق جميع المتطلبات الموضوعة لجعل العلاج فعالاً ومقبولاً هي احد أهم أهداف الدراسة.

تجارب مختلفة تم عملها لكي نقيم تأثير إدخال بعض المواد المحسنة للامتصاص أو النفاذية في تركيبة AZITHROMYCIN%2 على شكل جل للاستعمال الموضعي الجلدي وذلك بقياس نفاذية الدواء من خلال غشاء نفاذ صناعي يشابه في تركيبته الجلد الآدمي.

في هذه الدراسة، تم استعمال أنواع مختلفة من محسنات الامتصاص أو النفاذية مثل اليوريا، البر وبلين جلايكول، ايزوبروبل ميرستات وحامض الأوليك.

تم استعمال خلية محسنة من خلايا FRANZ CELL لفحص النفاذية حيث تم استعمال 30% محلول ايثيل الكحول والماء في الوسط المستقبل في الخلية على درجة حرارة 32 مئوية تقريباً.

تم سحب العينات من الوسط المستقبل على فترات مختلفة خلال الدراسة وقد تم حساب كمية المادة AZITHRO0MYCIN المتواجدة في العينة والتي نفذت من خلال الغشاء النفاذ حيث تم اعتماد طريقة معينة لدراسة التركيز باستخدام الفحص البيولوجي.

لقد أظهرت النتائج بان من بين المواد المحسنة للامتصاص فقد وجد أن مادة اليوريا بتركيز 10% لها اكبر تأثير على عملية نفاذية الدواء عبر الغشاء النفاذ وتحقق أعلى نسبة تخسين للنفاذية ثم تلاها في الترتيب حسب التأثير الأكبر مادة البر وبلين جلايكول بتركيز 10%.

- إن المواد الأخرى المحسنة للنفاذية التي استعملت في الدراسة مثل حامض الأوليك أو ايزوبروبل ميرستات فقد وجدت ذات تأثير سلبي على النفاذية لدواء AZITHROMYCIN حيث قامت بإحباط النفاذية إلى حدود تصل إلى الصفر.

في محاولة لتفسير النتائج التي حصلنا عليها في الدراسة وبمعرفتنا المسبقة بهذه المواد وبكيفية عملها ممكن أن نصل إلى الاستنتاج التالي.

إن المواد التي تحسن الامتصاص عن طريق عملها على زيادة السيولة والاندفاع في الوسط المائي داخل خلايا الجلد تحسن من نفاذية مادة AZITHROMYCIN بينما المواد التي يكون عملها على الوسط الزيتي فأنها تحبط نفاذية مادة AZITHROMYCINمن المستحضرات الحاوية لهذه المواد.

إقرار

اقر أنا مقدم الرسالة أنها قدمت لجامعة القدس لنيل درجة الماجستير و أنها نتيجة أبحاثي الخاصة باستثناء ما تم الإشارة له حيثما ورد، و أن هذه الرسالة أو أي جزء منها لم يقدم لنيل أية درجة عليا لأي جامعة أو معهد.

وليد يوسف كامل

التوقيع: ..... التاريخ: .....

برنامج الدراسات العليا في التكنولوجيا التطبيقية و الصناعية

عمادة الدراسات العليا

تحضير المضاد الحيوي أزثرومايسين على شكل جل موضعي لاستخدامه في علاج حب الشباب و فحص تأثير بعض المواد المحسنة للنفاذية على درجة نفاذية المضاد الحيوي

اسم الطالب: وليد يوسف كامل

الرقم الجامعي: 9910437

المشرف : الدكتور إبراهيم ألكيالي

نوقشت هذه الرسالة بتاريخ: 2006/1/23

لجنة المناقشة المدرجة أسمائهم و توقيعهم

1- الدكتور إبراهيم ألكيالي / رئيس اللجنة / مشرف رئيس..... توقيع .....

2 - الدكتور وديع سلطان / ممتحن داخلي / ..... توقيع .....

3- الدكتور نعمان مالكية / ممتحن خارجي/ ..... توقيع .....

جامعة القدس

العام الدراسي 2006

تحضير المضاد الحيوي أزثرومايسين على شكل جل موضعي لاستخدامه في علاج حب الشباب و فحص تأثير بعض المواد المحسنة للنفاذية على درجة نفاذية المضاد الحيوي

مقدمة من الطالب وليد يوسف كامل

بكالوريوس صيدلة / جامعة العلوم و التكنولوجيا الأردنية

قدمت هذه الرسالة استكمالا لمتطلبات درجة الماجستير
في
التكنولوجبا التطبيقية و الصناعية

برنامج الدراسات العليا في التكنولوجيا التطبيقية و الصناعية
كلية العلوم و التكنولوجيا

جامعة القدس

كانون الثاني 2006

عمادة الدراسات العليا
جامعة القدس
القدس – فلسطين

تحضير المضاد الحيوي أزثرومايسين على شكل جل موضعي لاستخدامه في علاج حب الشباب و فحص تأثير بعض المواد المحسنة للنفاذية على درجة نفاذية المضاد الحيوي

إعداد : وليد يوسف كامل

رسالة ماجستير

المشرف: الدكتور إبراهيم ألكيالي

2006