CHAPTER II
LITERATURE REVIEW

1. Transdermal drug delivery system

A transdermal drug product is intended to deliver the drug systemically to treat or prevent disorder in location distant from the site of topical application. Drug released from the transdermal drug delivery system is absorbed through the various skin layer, the stratum corneum, epidermis and dermis into blood circulation and transported to target lissue to achieve therapeutic effect (Shah, 1994).

Now the transdermal drug product in the markets are in many dosage forms such as solution, gel, cream, ointment, emulsion, microemulsion, liposome and transdermal patch.

Advantages of transdermal delivery

Drug delivered via oral route can encounter the first-pass metabolism is minimized, which can often limit the tolerability and efficacy of many orally. Furthermore, some drugs degrade in the acidic environment of the stomach, and other drugs, such as NSIADS, can cause gastrointestinal bleeding through irritation. The mixing of drugs with food in the stomach, and the pulsed, often erratic delivery of drugs to the intestine leads to variability in the plasma concentration-time profiles achieved for many drugs. The transdermal route provides a more-controlled, non-invasive method of delivery, with the added advantage of being able to increase absorption. Furthermore, patient compliance may be improved because of the reduced frequency of administration for short half-life medications or avoidance of the trauma associated with parenteral therapy.

Disadvantages of transdermal delivery

Transport across the skin is also associated with several disadvantages, the main drawback being that not all compounds are suitable candidates. Variation in permeation rates can occur between individuals, different races and between the old and young. Furthermore, skin disease, can also affect permeation rates. The metabolic enzyme in the skin can also pose a problem and some drugs are almost completely metabolized before they reach the cutaneous vasculature. Another problem that can arise, which is sometimes overlooked, is that some drugs can be broken down before penetration through the stratum corneum by the bacteria that live on the skin surface (Roberts et al., 1994).

1.1 Skin structure

Skin is essentially composed of two major layers : an outer, unvascularized epithelial layer (the epidermis), and an inner layer (the dermis), which contains a rich supply of capillaries, nerve, sweat glands, sebaceous glands, and hair follicles that are supported by connective tissue (Suhonen, Bouwstra and Urtti, 1999).

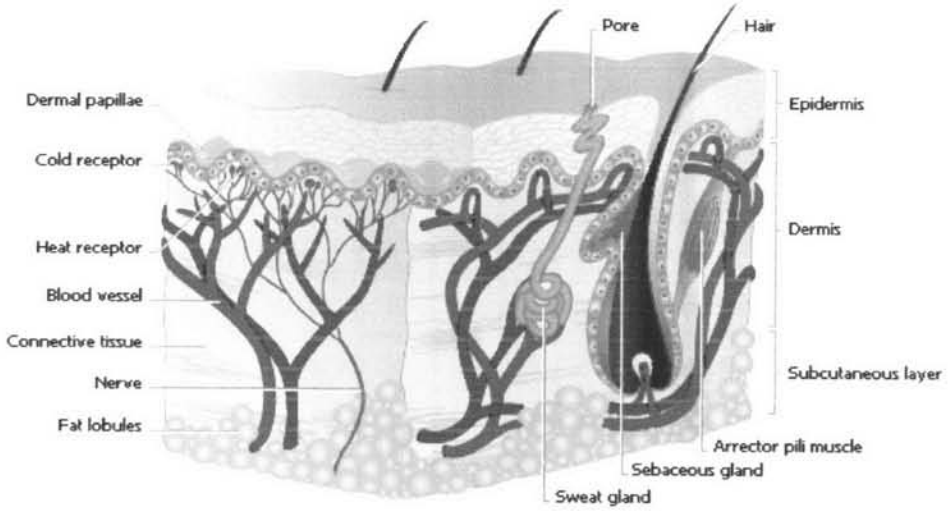


Figure 2-1 structure of the skin (Obtained from http://www.naturalrussia.com/nr/images/skinstructure.gif)

The epidermis

The epidermis performs a number of functions, one of the most important being the generation of the stratum corneum, which is the heterogenous outermost layer of the epidermis and is approximately 10-20 μm thick. It is nonviable epidermis and consists, in a given crosssection, of 15-25 flattened, stacked, hexagonal, and cornified cells embedded in a mortar of intercellular lipid. Each cells approximately 40 μm in diameter and 0.5 μm thick. The thickness varies, however, and may be a magnitude of order larger in areas such as the palms of the hand and soles of the feet, areas of the body associated with frequent direct and substantial physical interaction with the physical interaction with the physical environment. The stratum corneum barrier properties may be partly related to its very high density (1.4 g/cm^3 in the dry state), its low hydration of 15-20%, compared with the usual 70% for the body , and its low surface area for solute transport (it is now recognized that most solutes enter the body through the less than 0.1-μm-wide intercellular regions of the stratum corneum).

The dermis

The dermis, critical component of the body, not only provides the nutritive, immune, and other support systems for the epidermis, through a thin papillary later adjacent to the epidermis, but also plays a role in temperature, pressure, and pain regulation. The main structural component of the dermis is referred to as a coarse reticular layer. The dermis is about 0.1-0.5 cm thick and consists of collagenuos fiber (70%), providing a scaffold of support and crushining, and elastic connective tissue, elasticity, in a semigel matrix of mucopolysaccharides. In general, the dermis has a sparse cell population. The main cells present are the fibroblasts, which produce the connective tissue component of collagen, laminin, fibronectin, and vitronectin, mast cell, which are involved in the immune and inflammatory responses, and melanocytes involved in the production of the pigment melanin.

The subcutis

The deepest layer of the skin is the subcutaneous tissue or hypodermis. The hypodermis acts as a heat insulator, a shock absorber, and an energy storage region. This layer is a network of fat cells arranged in lobules and linked to the dermis by interconnecting collagen and elastic fibers. As well as fat cells (possibly 50% of the body's fat), the other main cells in the hypodermis are fibroblasts and adipocytes can be stimulated by the accumulation of interstitial and lymphatic fluid within the skin and subcutaneous tissue.

Skin appendages

There are four skin appendages: the hair follicles with their associated sebaceous glands, eccrine sweat glands, apocrine sweat glands, and the nails. Each appendage has a different function as outlined in Table 2-1.

Table 2-1 Appendages associated with the skin (Obtained from Walters and Robert, 2002)

	Appendage			
Parameter	**Hair follicle and sebaceous gland**	**Eccrine gland**	**Apocrine gland**	**Nails**
Function	Protection (hair) and lubrication (sebum)	Cooling	Vestigal secondary sex gland?	Protection
Distribution	Most of the body	Most of the body	Axillae, nipples, anogenital	Ends of fingers and toes
Average/cm^2	57-100	100-200	Variable	-
Fractional area	2.7×10^3	10^4	Variable	-
Secretions	Sebum	Sweat (dilute saline)	"Milk" protein, lipoproteins, lipid	Nil
Secretions stimulated by	Heat (minor)	Heat, cholinergic	Heat	-
Biochemical innervations of gland response	-	Cholinergic	Cholinergic (?)	-
Control	Hormonal	Sympathetic nerves	Sympathetic nerves	-

1.2 Transport pathway through the stratum corneum

The major limitation to transdermal drug delivery is the skin itself, and the major barrier to penetration of matter is provided by a superfacial layer of the skin, the stratum corneum and its compact structure (Suhonen, Bowstra and Urtti,1999).

Transport across the stratum corneum barrier might occur by any combination of the three pathways as shown in Figure 2-2 (1) a transcellular pathway (2) a paracellular pathway or (3) an appendageal pathway (via the sweat gland, hair follicle or possibly the sebaceous glands).

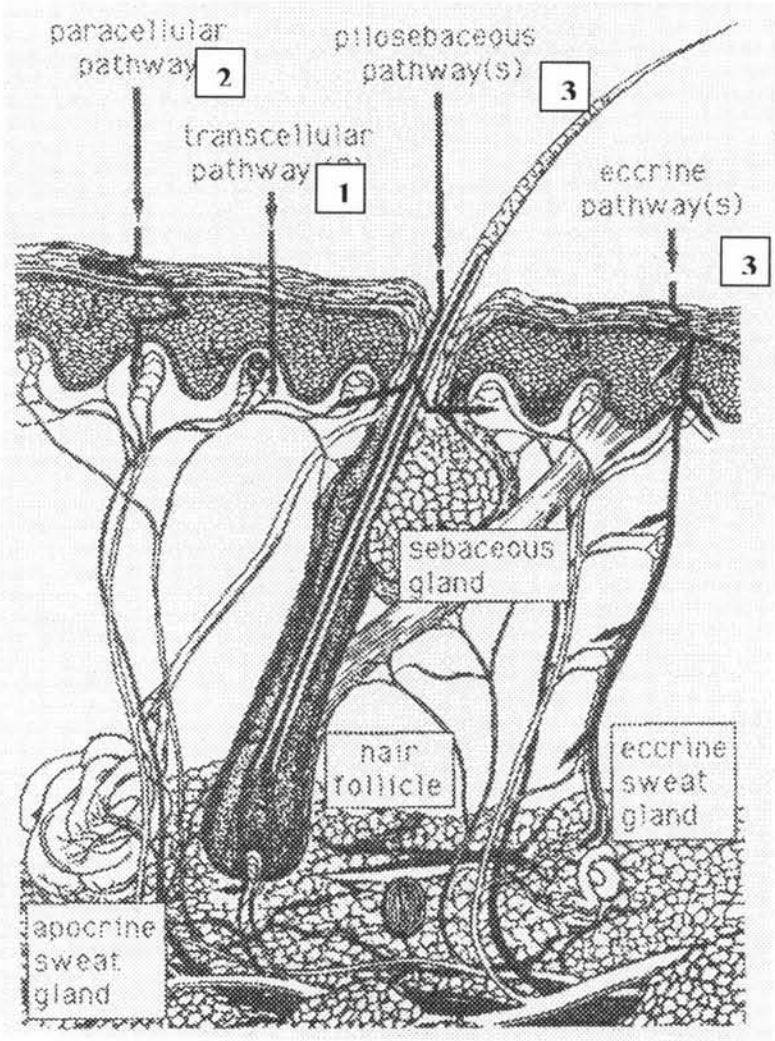


Figure 2-2 Potential pathways of permeation through human skin (Obtained from Cullander, 1999)

Transcellular pathway

This pathway include passive transport of small molecules, active transport of inonic and polar compounds, and endocytosis and transytosis of macromolecules. Passive transport is the movement of a solute along its concentration gradient. Active transport differs from passive transport in that the transport process is mediated by membrane transport against a concentration gradient. The transport of macromolecules is different from the transport of small molecules across epithelial cell. Most of the transport of macromolecules is progressively enclosed by the cell membranes. They then migrate within membrane-bound vehicles coalesce with lysosomes in which intracellular digestion occurs. In transcytosis, macromolecule bind to receptors on the surface of epithelial cells, then the receptor-macromolecule complex is incorporated into vehicles and carried into the cell. The complex remains intact in endosomes and is retrieved in transport vehicles that fuse with the membranes of the opposite side of the cell monolayer. Therefore, intact macromolecules can be transported across the epithelial barrier.

Paracellular pathway

This pathway is the transport of molecules around or between the cells. At the tight junctions, the cell membranes are brought into extremely close opposition, but are not fused, so as to acclude the extracellular space. Consequently, ion or molecules may not be able to pass through the membranes. Because of the perception that proteolytic activity is deficient in the paracellular space, it was once commonly is deficient in the paracellular space, it was once commonly assumed that peptides and proteins could not be absorbed via the paracellular pathway (Hsieh, 1994)

Appendageal pathway

This pathway is the transport of molecules through pores in the stratum corneum, it is a dominate role for hydrophilic solutes. In addition to pores, transport of hydrophilic solutes may also occur through hair follicles and sweat ducts. These

pathways, collectively referred to as shunt pathways, allow diffusion of solutes not only across the stratum corneum (Mitragotri, 2003).

2. Permeation enhancement

The pathway for drug penetration through the skin can also be divided by the type of drug molecules into three pathways: polar, nonpolar, and polar/nonporlar pathway. The enhancers act by altering one of these pathways. The key to altering the polar pathway is to cause protein conformational change or solvent swelling. The key to altering the nonpolar pathway is to alter the rigidity of the lipid structure and fluidize the crystalline pathway (this substantially increases diffusion). The fatty acid enhancers increase the fluidity of the lipid portion of the stratum corneum. Some enhancers (binary vehicles) act on both polar and nonpolar pathways by altering the multilaminate pathway for penetrants. Enhancers can increase the drug diffusivity in the stratum corneum by dissolving the skin lipids or by denaturing skin proteins. The type of enhancer employed has a significant impact on the design and development of the product.

The success of dermatological drug products that are intended for systemic drug delivery, such as the transdermals, depends on the ability of the drug to penetrate through skin in sufficient quantities to achieve its desired therapeutic effect. The methods employed for modifying the barrier properties of the SC to enhance drug penetration (and absorption) through the skin can be divided as physical enhancement and chemical enhancement.

2.1 Permeation enhancement by physical techniques

Physical enhancement of absorption of drug molecules through skin includes Iontophoresis, Electroporation, Sonoporation, Thermal poration, and Microneedles (chan, 2005).

The iontophoresis and ultrasound (also known as phonophoresis or sonophoresis) techniques are examples of physical means of enhancement that have been used for enhancing percutaneous penetration (and absorption) of various

therapeutic agents. One of the major concerns in the usage of iontophoresis is that the device may cause painful destruction of the skin with high current settings. It is essential to use high quality electrodes with adequate skin adhesion, uniform current distribution, and well-controlled ionic properties. The mechanism of transdermal penetration by this technology is still not clear (Shah, 1994).

Microneedle arrays, one of the latest physical method, hold the promise of delivering through the stratum corneum drug with properties incompatible with delivery via chemical enhancers. This technology has limitations, the most pronounced being limited throughput, but with knowledge gained from chemical enhanced, a future combination product may well be useful (Chan, 2005).

2.2 Permeation enhancement by dermatological vehicles

Permeation enhancement by dermatological vehicles includes such as group of chemical enhancer and dermatological drug delivery systems.

2.2.1 Chemical enhancer

Chemical enhancement of absorption of drug molecules through skin includes group of organic solvents, fatty acids and alcohols, detergents and surfactants and proprietary chemical enhancers.

Table 2-2 A partial list of some chemical enhancers (Obtained from Chan, 2005)

• Ethanol • Glyceryl monoethyl ether • Monoglycerides • Isopropylmyristate • Lauryl alcohol (also, lauric acid, lauryl lactate) • DMSO (dimethyl sulfoxide)	• 1-Dodecylaza cycloheptan-2-one (Azone®) • Polysorbates • Fatty acids (e.g. oleic) • Bile salts • N-methylpyrrolidone • Polyglycosylated glycerides

Chemicals enhancers that promote the penetration of topically applied drugs are commonly referred to as accelerants, absorption promoters, or penetration enhancers. A prime research objective is to identify chemicals that significantly enhance drug penetration through the epidermis but do not severely irritate or damage the skin. These chemicals ideally should be safe and nontoxic, pharmacologically inert, nonirritating, and nonallergic. Inaddition, the skin tissue should revert to its normal integrity and barrier properties upon removal of the chemical.

The mechanism of the chemical enhancers is to increase drug permeability through the skin by causing reversible damage to the stratum corneum are ; increase (and optimize) thermodynamic activity of the drug when functioning as cosolvent, increase the partition coefficient of the drug to promote its release from the vehicle into the skin, operate by conditioning the stratum corneum to promote drug diffusion, and promote penetration and establish drug reservoir in the stratum corneum.

Many of the vehicles, in spite of being great enhancers, are limited in their functions as vehicles because of their deleterious effect on the skin; an example is dimerhyl sulfoxide (DMSO) is a powerful solvent, and it increases drug penetration, but at the same time, it alters the biochemical and structural integrity of the skin and operates by direct insult to the stratum corneum (Shah, 1997). And one of the chemical enhancer, that attractive and often used as an enhancer/cosolvent, to increase drug penetration is ethanol.

Ethanol

Ethanol is an inactive or active ingredient in numerous prescription and nonprescription drug products, particularly those for topical application. Its role has been primarily to solubilize drugs. Some topical contain ethanol at concentrations of up to 90%.

Ethanol is used to enhance the permeation of drugs from products such as creame, gels, and ointments, as well as controlled-release transdermal products.

Ethanol has a relatively low incidence of topical reactions such as contact dermatitis; even under occlusion, levels of skin irritation (mainly erythema) on human

subjects are acceptable when the duration of applications is short and the ethanol concentration in the formulation is low.

The mechanism of action of ethanol on the skin permeation is to change in the structures of the stratum corneum, which is occurred in other permeation enhancer. Concentrated aqueous ethanol can alter structure of the stratum corneum in ways that appreciably increase solute diffusivity. The mechanisms by which ethanol induces damage and thereby elicits microstructural changes and increase diffusivity are not fully understood. Lipid extraction and osmotic expansion may be the most plausible explanations.

With dilute ethanolic solutions, ethanol appears primarily to increase drug solubility in the epidermis; its effect on solute diffusivity seems to be relatively small. This drug-partitioning effect may operate equally well for neutral and ionizable molecules. The octanol/water partition coefficient of solutes can be used as a measure by which ethanol's effectiveness may be estimated (Yum et al., 1994).

From the *In vitro* permeation study about the mechanism of ethanol on permeation of hydrocortisone, the model of hydrophobic drug, through male hairless mice shown the mechanism of ethanol to enhance the permeation of drug by reduction of lipid polar head interaction, an interfacial transport pathway between limited population of interdigitated and nonnterdigitated gel phase within the membrane or further disordering of possible liquid-crystalline phase within the membrane (Knutson, Krill and Zhang, 1990). For hydrophilic drug, the *in vitro* permeation study the mechanism of ethanol on permeation through human skin found that ethanol can increase the volume of the polar pathway, creating new pore or expanding the existing one which result in better permeability of stratum corneum to ions. And in the same study shown the concentration of ethanol up to 70% can change the nature of the porous pathway slowly, while the effect of 95% ethanol is rapid and maximum (Sznitowska, 1996).

Now ethanol has been reported to increase the flux of many drugs through skin such as ibuprofen, flurbiprofen, indomethacin, isosorbide dinitrate, cyclobarbital, zalcitabine, didanosine, zidovudine and fluoxetine (Parikh and Ghosh, 2005).

Surfactants

In addition to the skin permeation enhancers as describe aboved, the chemical enhancer that important and widely used is the surfactants. The surfactants can be classified into several categories based on their physicochemical properties: (1) ionic surfactants, (2) nonionic surfactants include ; cationic surfactant, anionic surfactant and zwittering ionic surfactant.

The mechanism of action of these compounds as permeation enhancers must be considered in conjunction with an appropriate model of the stratum corneum, which is the rate-limiting barrier in mass transport processes across the skin. The stratum corneum is the external layer of the epidermis, a heterogeneous structure composed of approximately 40% dead cells (keratinocytes), which are relatively polar; about 40% water; and about 20% lipids (triglycerides, free fatty acids, free sterols, and ceramides), which are nonpolar. Protein is present in both the inter-and intracellular phases, whereas lipid is concentrated largely in the intercellular phase, particularly in the continuous membranes surrounding the keratinocytes.

"Brick-and-mortar" model for the normal stratum corneum in which the intercellular lipids are the mortar and the keratinized cells are the bricks. In this model, the fatty acid and other lipids surrounding the keratinocytes are the major barrier to permeation of solutes through the stratum corneum. Keratinocytes represent a minor barrier to most compounds.

Molecules penetrate the stratum corneum via two distinct paths, both governed by Fickian solution-diffusion processes. One route of penetration is through the two phases in series-that is, transcellular penetration. The other pathway consists of transport solely through the lipid phase. Permeation enhancers could affect either of both of these routes; in other words, both intracellular protein and intercellular lipids may be physically or chemically altered by permeation enhancers.

It is generally recognized that protein denaturation or conformational changes in the keratin may contribute to enhanced permeability of the stratum corneum. Epidermal protein can be denatured by ionic surfactants; however the denature does not quantitatively correlate with changes in epidermis permeability, the surfactant can denature keratin, the keratinocytes, may impair the barrier properties of

stratum corneum by "opening up" the proteinaceous polar pathway. These structural changes in the stratum corneum induced by surfactants may be similar to those effected by some of the hydrogen-bonding solvents.

In the other hand, the expansion of stratum corneum caused by surfactants, the greatest swelling of tissue with anionic surfactants and very little with cationic surfactant, increased with time, was concentration-dependent, and was partially reversible, can increased the permeability of the skin (Yum et al., 1994).

2.2.2 Transdermal drug delivery systems

Microemulsions

Microemulsions are colloidal dispersions composed of an oil phase aqueous phase, surfactant and cosurfactant at appropriate ratio (Peltola et al., 2002). Microemulsion are thermodynamically, stabilized by an interfacial film of surfactant molecules. The surfactant may be pure, a mixture, or combined with other additives. The role of surfactant is stabilization of the microemulsion for instance, by decrease the interfacial tension (D' cruz and Uckun, 2001), which makes the microemulsions transparent liquids because the droplet size in the dispersed phase is very small, usually below 140 nm in diameter (Peltola et al., 2002).

Depending on properties of the involved components microemulsions can potentially appear over a wide range of oil-water-surfactant compositions. However, with given oil-water-surfactant components, microemulsions are usually only formed in narrow specific concentration ranges. The region of existence is typically presented in pseudoternaty phase diagrams, as ratios between oil, water and a fixed mixture of surfactant-co-surfactant.

The primary determinant for the range of microemulsion formation is the physicochemical properties of the aqueous phase, oil phase and surfactants. The physicchemical interaction between the components is too complex to provide a functional general mathematical guideline for prediction of microemulsion formation as a function of component properties. A few essential conditions are the production of a very low interfacial tension at the water-oil interface, the formation of a highly

fluid interfacial surfactant film and the penetration and association of the molecules of the oil phase with the interfacial surfactant film.

Lowering of the interfacial tension and fluidization of the interfacial surfactant film, is usually done by introducing a short chain co-surfactant to the surfactant film. The introduction of a co-surfactant may also expand the field of existence for system already capable of forming microemulsions without a co-surfactant, due to the more flexible interfacial film. If a single surfactant system is desired, the lipophilic chains of the surfactant should be sufficiently short, or contain fluidizing groups (e.g., unsaturated bonds). To enable integration of the oil with the interfacial film, the size of the oil molecules should not be too large.

In contrast to regular emulsions, microemulsion vehicles are formed spontaneously when admixing the appropriate quantities of the components, without requiring additional mechanical energy, and they are 'infinitely' physically stable due to their thermodynamic nature. Furthermore, they are transparent and have low viscosity (can principally be thickened by appropriate choice of noninteracting polymer, or gel forming surfactant, if desired), which facilitates filtration and visual inspection for particles. The characteristics of microemulsions make them very straight forward to prepare for pharmaceutical formulations, and the wide range of oil-water-surfactant compositions, which principally can form microemulsions, enable solubilisation of a wide range of both lipophilic and hydrophilic drugs-potentially even in the same vehicle (Malmsten, 2002). There are many studies about microemulsion, shown the various composition which can produced microemulsion such as the system of cinnamic alcohol/Aerosol OT/water in ratio of oil/surfactants/ water are 75-80/5-13/6-15%, cinnamic alcohol/Tween 20/water in ratio of oil/ surfactants/water are 50-89/4-33/7-17% (Mukhopadhyay et al., 1996), captex 300/cremophore EL/phospholipon/water/the other component in ratio of oil/surfactants/water/the other component are 10.8/7.6/5.1/66.5/ 10% (D'Cruz and Uckun, 2001), oleic acid/Tween 80/ethanol/PBS 7.4 in ratio of oil/surfactants/water are 22.2/18.5/27.8/31.5%, isopropyl myristate/soy bean phospatidylcholine/isopropanol/PBS 7.4 in ratio of oil/surfactants/ water are 25/25/25/25% (Peltola et al., 2003), isopropyl myristate/span 20/Tween 40/isobutanol/water in ratio of oil/surfactants/water are 36.3/4.5/10.2/30.7/18.3%

(Nandi, Bari and Joshi., 2003) Labrafil M 1944/cremophore ELP/ethanol/PBS 7.4 in ratio of oil/surfactants/water are 6-30/14-70/0-80% (Lee et al., 2005) and lauroglycol/ labrasol/transcutol/water/ the other component in ratio of oil/surfactants/water/ the other component are 7.5/32.5/58.26/22% (Parikh and Ghosh, 2005).

Microemulsions can accommodate a number of different microstructure, depending on the nature of the surfactant, the system composition, temperature, and presence of the cosurfactant and cosolute (Malmsten, 2002). Microemulsion microstructure are

1. Micellar ('o/w') droplet structure : water diffusion will be rapid, in the same order of magnitude as that of neat water. Oil and surfactant diffusion will be slow and within the same order of magnitude.
2. Inverted micellar ('w/o') droplet structure : oil deffusion will be rapid, in the same order of magnitude as that of neat oil. Water and surfactant diffusion will be slow and within the same order of magnitude.
3. Bicontinuous structures : both water and oil diffusion will be rapid, only slightly slower than those of the neat liquids. Surfactant diffusion will be low due to the constitution of the interfacial film, but slightly higher than cases 1 and 2 as the interface is fluctuating and less restricted than in the micellar shapes (Kreilgaard, 2002).

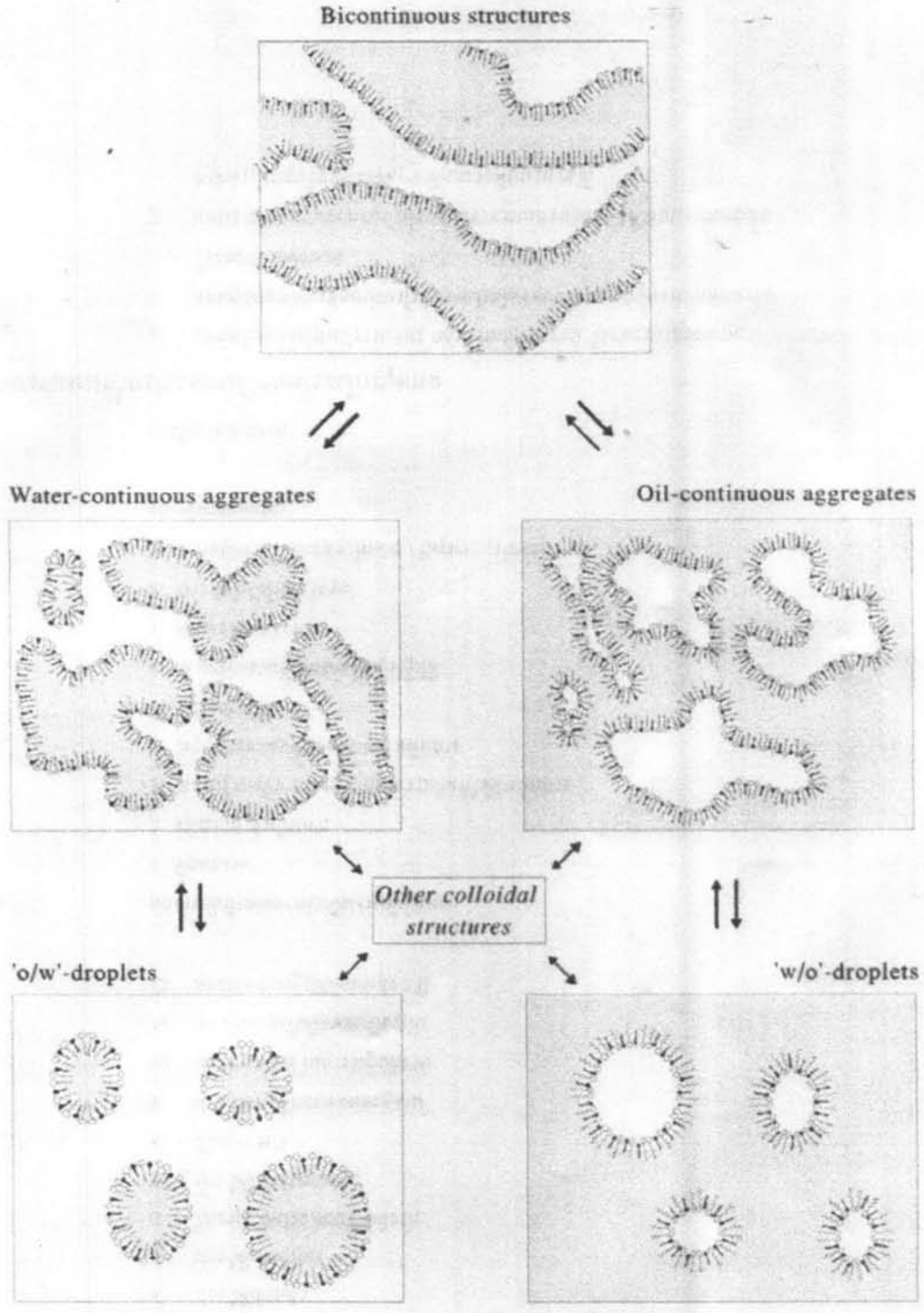


Figure 2-3 Basic dynamic microemulsion formed by oil phase (grey), aqueous phase (white) and surfactant/co-surfactant interfacial film, and plausible transition between the structure (indicated by arrows) by increase oil fraction (clockwise from left to right) and water fraction(anti-clockwise from right to left), respectively (Obtain from Kreilgaard, 2002)

The microemulsion structure is important for the rate of drug release. The wide range of possible structures means that microemulsions can release the solubilised drug at different rates. In an o/w microemulsion, hydrophobic drugs, solubilised mainly in the oil droplets, experience hindered diffusion and are therefore released rather slowly (depending on the oil/water partitioning). The diffusion of water soluble drugs, on the other hand, is less restrained and they are release rapidly. The reverse behaviour is expected in w/o type. For balanced microemulsion due to the bicontinous structure, relatively fast diffusion and release occur for both water-soluble and oil-soluble drugs (Podlogar et al., 2004).There have many studies shown the microemulsion system can enhance drug permeability such as ; the *in vitro* permeation across rat skin of aceclofenac, microemulsion system was 5-fold higher than that of the ethanol vehicle (Lee et al., 2005), the *in vitro* permeation across human skin of amethocaine, microemulsion system was higher permeation than that commercial amethocaine gel product (Escribano et al., 2005), the *in vitro* permeation across rat skin of celecoxib, microemulsion system increase the permeation rate of celecoxib up to 5 and 11 times compared with those of microemulsion gel and cream (Subramanian, Ghosal and Moulik, 2005), the *in vitro* permeation across male Swiss albino mice skin of 5-fluorouracil, the skin flux of the drug in microemulsion system could be enhanced up to 6-fold compared to aqueous solution and the skin flux of drug is dependent on the concentration of water and surfactant in microemulsion (Gupta et al., 2005), the *in vitro* permeation across rat skin of vinpocetine, which drug solubility in microemulsion system was 3160-fold higher than solution vehicle then the permeation in microemulsion system was higher (Hua et al., 2004), and the same result was found in the *in vitro* permeation across human skin of estradiol ,which drug solubility in microemulsion system was 1500-fold higher than control solution vehicle then the estradiol flux in microemulsion system was increased 200-700-fold over the control (Peltola et al., 2002).

Microemulsions have been considered as drugs delivery system in many route such as oral route, ophthalmic route, parenteral route, rectal route (Djordjevic, Michniak and Uhrich, 2003), vaginal route and transdermal route (Nandi, Bari and Joshi, 2003). For transdermal route, it had been, shown that microemulsion can interact with the stratum corneum changing structural rearrangement of its lipid layers

and consequently increasing transdermal drug permeation and so act as penetration enhancer (Zabka and Skoviera, 2003). Another that mobility of drugs in microemulsions is more facile (Peltola et al., 2003). And when compare microemulsion with the common method to improve drug permeation through to skin such as use penetration enhancers i.e. organic solvents and fatty acid. Iontophoresis and ultrasound shown that microemulsion drugs delivery system have many more benefit, namely higher storage stability, lower preparation cost, good production feasibility, absence of organic solvents, and no necessity of intensive sonication (Paolino et al., 2002).

3. Method for studying percutaneous absorption

There is an increasing demand for data describing the rate, degree and of penetration of compounds across human skin. Although drug delivery and toxicological considerations are perhaps the most important factors. The need for relevant data, produced under reproducible and reliable conditions, has led to an increase in both the development and the standardization of *in vitro* and *in vivo* test produces.

In vitro techniques to assess skin penetration and permeation are used extensively in industry and academia. Although at present, *in vitro* permeation data are not a requirement of regulatory bodies, there is an increasing trend for such data to be submitted, either alone or together with *in vivo* data. In some ways, *in vitro* techniques have advantages over *in vivo* testing. For the example, permeation through the below the skin surface. This contrasts with most *in vivo* methods, which rely on the measurement of systemic (or at least nonlocal) levels of permeant. Some form of *in vitro* diffusion cell experiment is often the most appropriate method for assessment of percutaneous penetration in a developmental drug-delivery (transdermal or topical) program or in a dermal toxicology screen.

3.1 *In vitro* skin diffusion cells

Most common methods for evaluation of *in vitro* skin penetration is a use of diffusion cells. The major advantage of *in vitro* investigations is that the experimental condition can be controlled precisely, such that the only variables are the skin and the test material. Although a potential disadvantage is that little information on the metabolism, distribution and effects of blood flow on permeation can be obtained.

Diffusion cell design

In vitro systems range in complexity from a simple two-compartment 'static' diffusion cell to multijacketed "flow-though" cells. Construction materials must be inert, and glass is most common, although Teflon and stainless steel are also used. Excised skin is always mounted as a barrier between a donor chamber and a receptor chamber, and the amount of compound permeating from the donor to the receptor sides is determined as a function of time. Efficient mixing of the receptor phase (and sometimes the donor phase) is essential, and sample removal should be simple. Neither of these processes should interfere with diffusion of the permeant. Comprehensive reviews on diffusion cell design are available. Continuous agitation of the receptor medium, sampling from the bulk liquid rather than the side arm, and accurate replenishment after sampling, are important practical considerations. It is essential that air bubbles are not introduced below the membrane during sampling.

Static diffusion cells are usually of upright ("Franz") or side-by-side type, with receptor chamber volumes of about 2-10 mL and surface areas of exposed membranes of near 0.2-2 cm^2. Cell dimensions should be accurately measured, and precise values should be used in subsequent calculations, with due attention to analyte dilution resulting from sampling and replenishment. The main difference in the application of these two static cell types is that side-by-side cells can be used for the measurement of permeation from one stirred solution, through a membrane, and into another stirred solution. This is of particular advantage when examining flux from saturated solutions in the presence of excess solid if accumulation of solid on the membrane surface must be prevented. This type of cell can also be modified to allow

the absorption of permeants in the donor chamber so that the membrane is exposed to only the permeant in the gaseous state. Upright cells are particularly useful for studying absorption from semisolid formulations spread on the membrane. Surface and membrane are optimal for simulating *in vivo* performance. The donor compartments can be capped to provide occlusive conditions, or left open, according to the objectives of the particular study.

Flow-through cells can be useful when the permeant has a very low solubility in the receptor medium, and designs are continuously improving. Sink conditions are maximized as the fluid is continually replaced using a suitable pump (at a rate about 1.5 ml/hr). However, the dilution produced by the continuous flow can raise problems with analytical sensitivity, particularly if the permeation is low. Flow-through systems static system have produced equivalent result. Automated flow-through system can allow unattended sampling and commercial systems are available.

To summarize, a well-designed skin diffusion cell should ; be inert, robust and easy to handle, allow the use of membranes of different thicknesses, provide thorough mixing of the receptor chamber contents, ensure intimate contact between membrane and receptor phase, maintainable at constant temperature, have precisely calibrated volumes and diffusional areas, maintain membrane integrity, provide easy sampling and replenishment of receptor phase and available at reasonable cost.

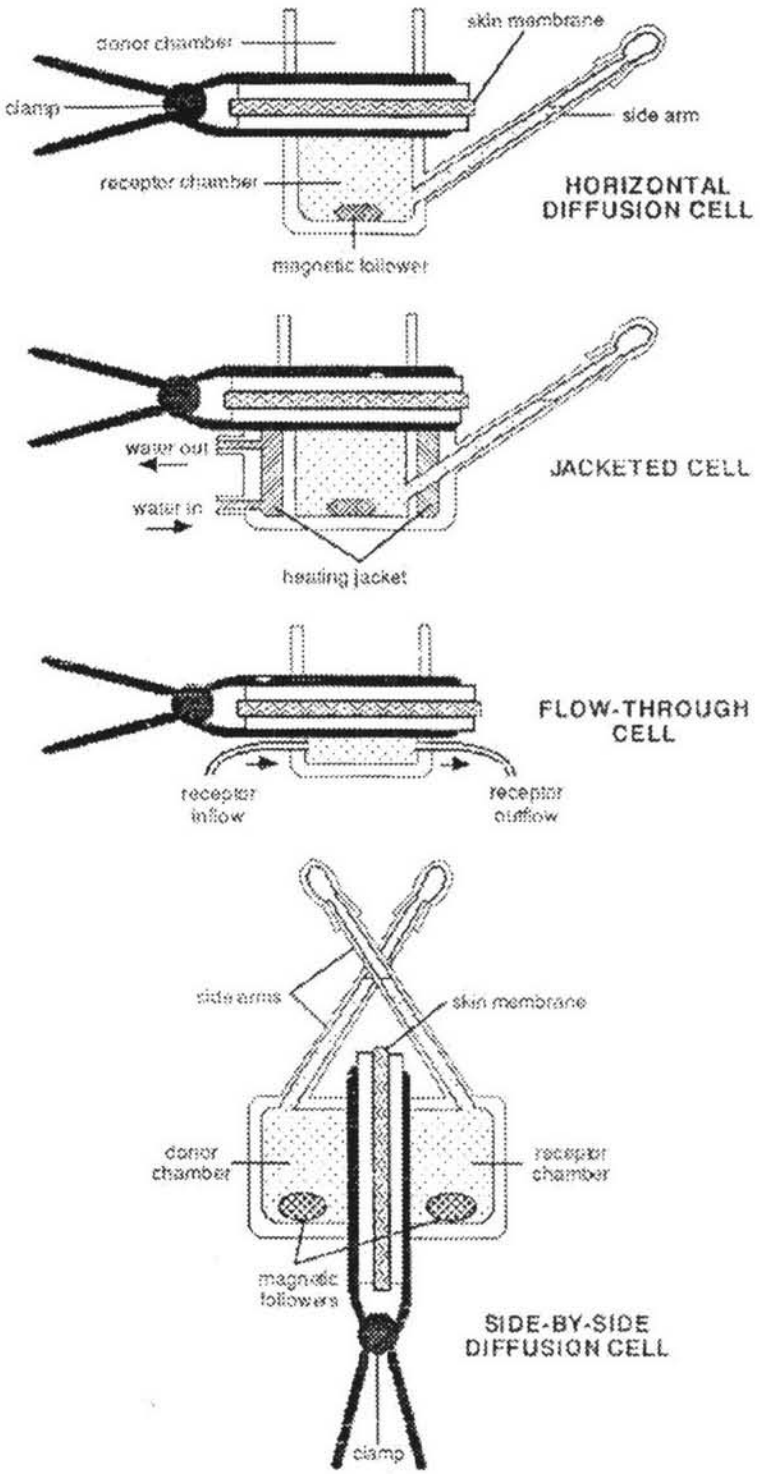


Figure 2-4 Basic diffusion cell designs: static horizontal cells may be jacketed (as in the Franz – type) or unjacketed (and temperature – controlled using a water bath or Heating block). Flow – though cells usually have a small receptor chamber to maximize mixing. Side-by-side cells are used mainly for solution vehicles (Obtained from Brain, Walters and Watkins, 2002).

Receptor Chamber and Medium

Receptor chamber dimensions are constrained by the conflicting requirements of guaranteeing that the receptor phase can act as a sink, while ensuring that sample dilution does not preclude analysis. A large receptor volume may ensure sink conditions, but will reduce analytical sensitivity unless large samples can be taken and subsequently concentrated. Concentration of permeant in an aqueous receptor phase may be possible by lyophilization, or by techniques such as solid-phase extraction.

The ideal receptor phase provides an accurate simulation of the conditions pertaining to *in vivo* permeation of the test compound. As a general rule the concentration of the permeant in the receptor fluid should not be allowed to exceed approximately 10% of saturation solubility. Excessive receptor-phase concentration can lead to a decrease in the rate of absorption, which may result in an underestimate of bioavailability. The most commonly used receptor fluid is pH 7.4 phosphate-buffered saline (PBS), although this is not always the most appropriate material. It has been postulated that if a compound has a water solubility of less than about 10 μg/mL, then a wholly aqueous receptor phase is unsuitable, and the addition of solubilizers becomes necessary (Brain, Walters and Watkinson, 2002).

Animal models for human skin for *in vitro* percutaneous absorption studies.

A major potential variant in the design of *in vitro* skin diffusion is the nature for the skin membrane. The skin membranes used in *in vitro* permeation study includes ; (1) natural skin membrane such as surgically-obtained human skin, cadaver skin, animal skin model (2) biological material membrane such as egg shell membrane (3) synthetic or artificial membrane such as silastic membrane (Panomsuk, Ngawhirunpat and Opanasopit, 2002).

From the skin membrane described above, the animal skin membrane are widely used as substitutes for human skin.

Table 2-3 Skin membrane widely used in permeation study from the most permeability to the less (Obtained from Panomsuk, Ngawhirunpat and Opanasopit, 2002)

Tregear	Marzulli et al.	Mcgreesh
Rabbit	Mouse	Rabbit
Rat	Guinea ping	Rat
Guinea pig	Goat	Guinea pig
Man	Rabbit	Cat
	Horse	Goat
	Cat	Monkey
	Dog	Dog
	Monkey	Pig
	Weanling pig	
	Man	
	Chimpanzee	

Table 2-4 Human and animal skin thickness measurement[a] (Obtained from Bronaugh et al., 1981)

Type of skin[b]	Stratum corneum (μm)	Epidermis (μm)	Whole skin (mm)
Human (16)	16.8 ± 0.7	46.9 ± 2.3	2.97 ± 0.28
Pig (35)	26.4 ± 0.4	65.8 ± 1.8	3.43 ± 0.05
Rat (9)	18.4 ± 0.5	32.1 ± 1.3	2.09 ± 0.07
Hairless mouse (12)	8.9 ± 0.4	28.6 ± 0.9	0.70 ± 0.02
Mouse (9)	5.8 ± 0.3	12.6 ± 0.8	0.84 ± 0.02

[a] values are $\bar{x}$ ± SE of the thickness of the number of section in parentheses. Three to six sections were taken from each skin sample.

[b] Skin was obtained from humans and pigs of either sex and from female rats, hairless mice, and mice.

Table 2-5 Density and size of hair follicles[a] (Bronaugh et al., 1981)

Species	Area of skin	Number of follicles/cm^2	Diameter of follicles (μm)
Human	Abdomen	11 ± 1	97 ± 3
Pig	Back	11 ± 1	177 ± 4
Rat	Back	289 ± 21	25 ± 1
Mouse	Back	658 ± 38	26 ± 1
Hairless mouse	Back	75 ± 6	46 ± 1

[a] values are $\bar{x}$ ± SE readings taken three to six sections.

In preview studies of permeability of animal skin, the pig or miniature pig has often proved to be a good animal model (Bronaugh et al., 1987). Pig and men are both backbone animals in the subphylum vertebrata of the phylum chordata. Within the vertebrates, pig and men belong to the same class, the mamalia since they have a high level of metabolic actively (warm-bloodedness) to control body temperature and they nurse their young with milk secreted by mammary glands (Suwanpidokkul, 2002).

Pig ear skin was selected to be used in this study because the use of it in human dermatological research has been discussed extensively in regard to the testing of transdermal system, or skin permeability. There had many study about *in vitro* permeation of drug in microemulsion dosage form using pig skin membrane such as 8-metrosalen, metrotrexate and sodium salicylate (Kreilgaard, 2002). Recent study of the outer ear skin of female pigs by light microscopy, transmission electron microscopy and cryo scanning electron microscopy methods exhibited that the stratum superfacial dermis of the pig ear skin had a very homogeneous and compact construction, also it could generally be applied to the human integument, provided that the ear material had been handle correctly before use (Meyer et al., 2007).

The permeation process

Considering that the skin is such a heterogeneous membrane, it is surprising that simple diffusion laws can be used to describe the percutaneous absorption process. Since transdermal delivery involves the application of a device over a long period of time, it is generally assumed that steady-state conditions have been reached and that the most relevant low of diffusion is therefore Fick's first law. The second law describes non-steady state diffusion and can be used to analyze the rates of release from matrix type transdermal patches, to evaluate the lag phase prior to the establishment of steady-state conditions, and to describe concentration profiles across the skin as they evolve towards linearity.

The most quoted form of Fick's first law of diffusion describes steady-state diffusion through a membrane :

$$J = \frac{KD}{h} \times (c_o - c_i) \qquad (1)$$

Where J is the flux per unit area, K is the stratum corneum-formulation partition coefficient of the drug, and D is its diffusion coefficient in the stratum corneum of path length h; c_o is the concentration of drug applied to the skin surface, and c_i is the concentration inside the skin. In most practical situations, $c_o >> c_i$, and Eq. (1) simplifies to

$$J = k_p c_i \qquad (2)$$

Where k_p (= KD/h) is the permeability coefficient, which has units of velocity (often quoted as cm h^{-1}), i.e., it is a heterogeneous rate constant and encodes both partition and diffusional characteristics. The input rate of the drug into the systemic circulation, from a patch of area A, is therefore given by the product

$$\text{Input rate} = A \times k_p \times c_o \qquad (3)$$

The output or elimination rate from the systemic circulation equals the clarance (Cl) multiplied by the plasma concentration at steady state ($c_{p,ss}$)

$$\text{Output rate} = Cl \times c_{p,ss} \quad (4)$$

Hence Eqs. (3) and (4) may be combined to predict the drug's plasma concentration following transdermal delivery:

$$c_{p,ss} = \frac{Ak_p c_o}{Cl} \quad (5)$$

The plasma concentration achieved therefore depends directly on the area of the device, the skin permeability, and the applied concentration and is inversely related to the drug's clearance (4).

For a given, the clearance and the target plasma level are likely to be known, so to examine the feasibility of delivery, one needs the drug's skin permeability and its solubility, as this will give an indication of the maximum concentration that can be applied. These parameters can be estimated from basic physicochemical properties, which are typically measured during preformulation (Hadgraft and Guy, 2003).

4. Model drug and components

4.1 Model drug : Glucosamine

Physiochemical properties of Glucosamine hydrochloride (USP XXVII)

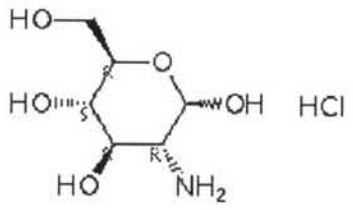


Figure 2-5 Chemical structure of glucosamine hydrochloride

Chemical name	:	2-Amino-2-deoxy-D-glucopyranose hydrochloride
Generic name	:	Glucosamine hydrochloride
Molecular formula	:	$C_6H_{13}NO_5 \cdot HCl$
Molecular weight	:	215.63 g/mol
Appearance	:	A white powder, odorless
Therapeutic category	:	Antiarthrosic-arthrotrophic in osteoarthritis
pK_a	:	7.75

Pharmacotherapeutic properties of Glucosamine

Machanism of action (Laurie and Nixon, 2006)

Glucosamine is an amino monosaccharide derived from chitin in crustacean shells. It appears to result in an elevation of intracellular UDP-N-acetylglucosamine, which is critical to the formation of glycosaminoglycans found in cartilage such as hyaluronic acid, heparin sulfate and keratin sulfate.

Glucosamine's mechanism of action has yet to be elucidate ; however, evidence of its multifaceted benefits is mounting. It's anti-inflammatory effects include: protease inhibitor, nitric oxide and MMP inhibition and, modification of PLA_2 and collagenase activity; and inhibition of IL 1ß induce NF-kB and matrix metalloproteinases. The matrix enhancing effects reportedly are elevated protein synthesis and increased mRNA production of aggregate core protein, which augments cartilage's ability to repair. Glucosamine is available in various form, including glucosamine hydrochloride, glucosamine sulfate, N-acetyl-D-glucosamine. Of the three products available glucosamine hydrochloride and glucosamine sulfate were found to inhibit cartilage degradation in similar manner, whereas N-acetyl-D-glucosamine had no beneficial effect.

Pharmacokinetic and side effect

The pharmacokinetic parameters of glucosamine are summarized in Table 2-6. Although orally administered glucosamine is absorbed from intestinal mucosa over

90% but absolute bioavailabity is only 20% because it loses considerable potency during its first pass effect (kulnawan, 2004).

The reported adverse effects predominantly affected the gastrointestinal tract causing epigastric pain, heartburn and diarrhea (Henrolin, sanchez and Baligand, 2004).

Because most glucosamine is derived from shellfish, it is still possible that people who are allergic to shellfish can have an allergic reaction (The Arthritis & Glucosamine Information center, 2005).

Table 2-6 Pharmacokinetic parameter of Glucosamine

Parameter	**Glucosamine**
Oral bioavailability	20%
Plasma $t_{½elim}$	58 h.
Plasma protein binding (max)	8-10 h.
Excretion	In the urine 10% In the feces 11.3%

Therapeutic uses

Several human studies have demonstrated that orally administered glucosamine 1,500 mg/day are effective in alleviating the symptoms of osteoarthritis and improving joint function (Henrotin et al., 2004). Long term study (1-3 years) has shown that the patients with the less severe radiographic knee osteoarthritis, will experiences, the most dramatic reduction in disease progression, in term of joint space narrowing. Such patients may be particularly responsive to structure-modifying drug (Bruyere et al, 2003). Another benefit of glucosamine for treatment of osteoarthritis is ; glucosamine can be expected to enhance hyaluronic acid production in the wound, promoting swifter healing and possibly diminishing complications related to scarring (McCarty, 1996).

Administration

The recommended oral starting dose is two 250 mg capsules taken three times a day, or the content of one 1884 mg sachet dissolved in water, taken once daily for at least six weeks. The recommended duration of the therapy is three months and the treatment course may be repeated at intervals of two months. Capsule or the content of the sachets should be taken 15 minutes before each meal. The oral administration can be usefully combined with the intramuscular administration of parenteral solution for injections to accelerate and improve the therapeutic effects. These can only be seen after approximately one week after the treatment has been started.

Figure 2-6 Commercial products oral dosage form of glucosamine (Obtained from http://www.glucosamine-arthritis.org/glucosamine/glucosamine-productguide.html)

Figure 2-7 Commercial products transdermal patch dosage form of glucosamine (Obtained from http://www.glucosamine-arthritis.org/glucosamine/glucosamine-product-guide.html)

Figure 2-8 Commercial products topical dosage form of glucosamine (Obtained from http://www.glucosaminearthritis.org/glucosamine/glucosamine-product-guide.html)

4.2 The components in formulation

4.2.1 Surfactant

Cetyltrimethylammonium bromide (CTAB)

Figure 2-9 Chemical structure of cetyltrimethylammonium bromide

Chemical name	:	hexadecyl-trimethylazanium
Generic name	:	cetrimonium bromide, CTAB cetyltrimethyammonium bromide
Molecular formula	:	$C_{19}H_{42}N^+$
Molecular weight	:	284.54 g/mol
Appearance	:	a white powder
Functional category	:	anti-infective agent, local detergent, cationic surfactant (Obtained from http://www.pubchem.mcbi.nlm.nih.gov/summary/summary)

Phosphatidylcholine (PC, lecithin)

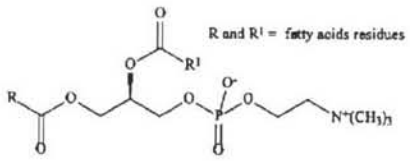


Figure 2-10 Chemical structure of phosphatidylcholine

Chemical name	:	lecithin, phosphatidylcholine
Generic name	:	egg lecithin, soybean lecithin, vegetable Lecithin, soybean phospholipid
Appearance	:	viscous semiliquid to powder, may also vary in color from brown to light yellow, no odor , those derived from vegetable source have bland or nut-like taste, similar to soy bean oil.
Functional category	:	emollient, emulsifting agent, solubilizing Agent (pharmaceutical excipient, 1994)

Polyoxyethelene 20 sorbitan monooleate (Tween 80)

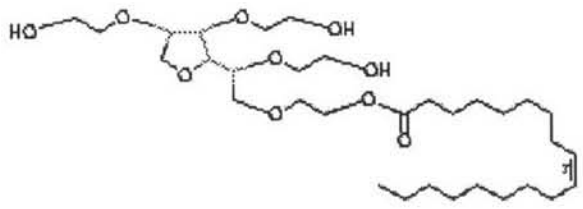


Figure 2-11 Chemical structure of polyoxyethelene 20 sorbitan monooleate

Chemical name	:	polyoxyethelene 20 sorbitan monooleate
Generic name	:	Tween 80
Molecular formula	:	$C_{64}H_{124}O_{26}$
Molecular weight	:	1310 g/mol
Appearance	:	odor and a warm, somewhere bitter taste yellow oily liquid
Functional category	:	emulsifying agent, non-ionic surfactant, solubilising agent, wetting agent (pharmaceutical excipient, 1994)

Soduim bis (2-ethylhexyl) sulfosuccinate (AOT)

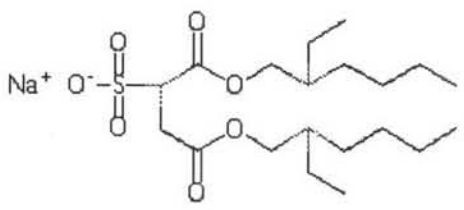
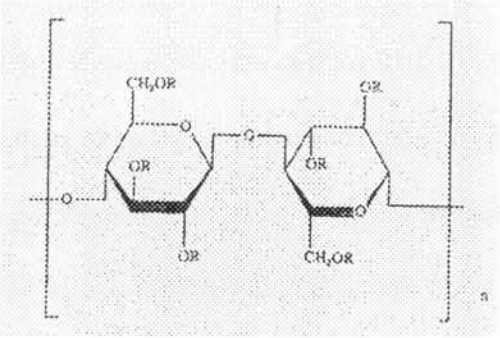


Figure 2-12 Chemical structure of Soduim bis (2-ethylhexyl) sulfosuccinate

Chemical name	:	Soduim bis (2-ethylhexyl) sulfosuccinate
Generic name	:	docusate sodium salt
Molecular formula	:	$C_{20}H_{37}Na0_7S$
Molecular weight	:	444.56 g/mol
Appearance	:	colorless wax-like mass
Functional category	:	anionic surfactant (Obtained from http://www.sigmaaldrich.com/catalog/search/CerOfAnalysisPage)

4.2.2 Gelling agent

Hydroxypropyl methylcellulose (HPMC)



Where R is H, CH_3 or [CH_3CH (OH)CH_2]

Figure 2-13 Chemical structure of hydroxypropyl methylcellulose

Chemical name	:	cellulose, 2-hydroxypropyl methyl ether
Generic name	:	hydroxypropyl methylcellulose
Molecular weight	:	10,000-1,500,000 g/mol
Appearance	:	an adorless and tasteless white or creamy-white colored fibrous or granular powder
Functional category	:	coating agent, film-former, stabilizing agent, tablet binder, viscosity-iccresing agent (pharmaceutical excipient, 1994)