CHAPTER II

REVIEW OF LITERATURE

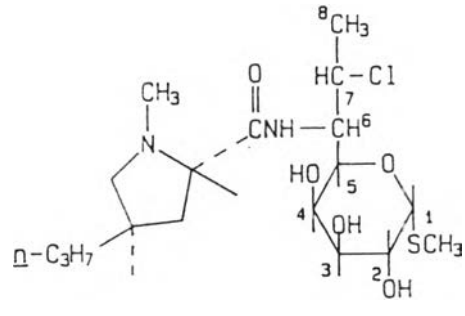
There are varieties of topical or systemic antibiotics that are capable of controlling the proliferation of *P. acnes*. The most frequently used agents include topical benzoyl peroxide, erythromycin or clindamycin, and systemic tetracycline or erythromycin (DiPiro, Talbert, Hayes, Yee, and Posey, 1989). However, long-term treatment of systemic antibiotics may alter normal flora in gastro-intestinal tract (Borglund,1984). Topical treatment is widely used because a drug reaches the affected area directly resulting in clinical efficacy without significant systemic side effect (Borglund, 1984; Siegle, et al., 1986).

Topical clindamycin is the most frequently employed in the therapy of mild acne with inflammatory papules and pustules, but not cysts. A group of patients treated with 1% clindamycin phosphate solution twice daily was compared with the other group treated with 250 - 500 mg twice daily. There were no significant different between the two treatment groups (Gratton, et al., 1982; Braathen, 1984; Katsambas, 1987). Sheehan-Dare, et al. (1990) compared the effect of topical clindamycin and oral minocycline in the treatment of acne vulgaris. The study had shown that the topical clindamycin twice daily was an effective alternative to 50 mg oral minocycline twice daily in the treatment of moderate to severe facial acne vulgaris. Leyden, et al.(1987) studied 2% erythromycin gel in comparison with 1% clindamycin phosphate solution. They found that no significant differences in lesion count reductions were detected between the two treatment groups after 8 and 12 weeks of treatment. The same results were also obtained by other researchers (Shahlita, Smith and Bauer, 1984; Thomas, Raimer and Smith, 1982). Resh and Stoughton (1976) had shown a 1% solution of clindamycin phosphate to decrease *Corynebacterium acnes* in open comedones and improved the acne caused by acne vulgaris clinically. Parker (1987) found that there was no significant difference between the two groups treated by 1% clindamycin

solution and 1% clindamycin gel.

Topical clindamycin caused no changes in the colon flora, whereas oral tetracycline suppressed the numbers of colon bacteria significantly (Borglund, 1984). No evidence of systemic clindamycin absorption was found (Algra, Rosen and Waismasn, 1977).

The synthesis and biological properties of clindamycin were first reported by Magerlein. Clindamycin is a highly effective antibiotic against Gram positive aerobes and both Gram negative and Gram positive anaerobic pathogens (Bevan and Thompson, 1983). It is synthesized from lincomycin, an antibiotic produced by microbial (*Streptomyces lincolnensis*) fermentation, by substituting chloride for the hydroxyl group which results in an increase in biological activity (Kucers, 1972; Graysin, 1982).



clindamycin hydrochloride, USP XX

Empirical Formula	$C_{18}H_{33}ClN_2O_5S.HCl$
Molecular Weight	461.44
Chemical Name	methyl 7(s)-chloro-6,7,8-trideoxy-6-trans-(l-methyl-4-propyl-L-2-pyrrolidinecarboxamidol)-l-thio-L-threo-D-galacto-octopyranoside monohydrochloride
Description	A white or practically white, crystalline powder, odorless or faint mercaptan like odor, bitter taste
Solubility	soluble in water and alcohol
Melting Range	141-143°C
pKa	7.72

Pharmacology

Clindamycin possesses a semibroad antibacterial spectrum. It is effective against most pathogenic streptococci (no *Strep. faecalis*), Staphylococci, Pneumococci, Corynebacteria, some strains of *Neisseria gonorrhoeae*, *H. influenzae* and norcardia, and most, if not all anaerobes (Bevan and Thompson, 1983). Clindamycin inhibits protein synthesis by binding exclusively to the 50S subunit of bacterial ribosomes, inhibiting peptidyl transferase activity and blocking protein synthesis.

Adverse Effects Caused by Topical Preparation.

A case of pseudomembranous colitis after a topical administration of clindamycin was reported by Milstone et al (1981). Abdominal cramping and diarrhea developed in 24-year-old woman with facial acne vulgaris five days after treating with 1% clindamycin hydrochloride solution (Parry and Rha, 1986).

Topical Dosage Forms.

Solution: 1% clindamycin phosphate in mixed solvent (pH 5) of 50% isopropyl alcohol, propylene glycol and water (Dalacin T(R)).

Stability Studies of Clindamycin.

The kinetics and mechanisms of degradation of the antibiotic clindamycin were studied by Oesterling, 1970. Clindamycin undergoes several hydrolytic reactions. There are three reactions depending on the pH of the aqueous medium. Clindamycin has shown maximum stability at pH 3-5 (Figure 1). At lower pH levels the major degradation pathway is the hydrolysis of thioglycoside groups. Above pH 5, the predominant route is hydrolysis of the 7-chloro to yield lincomycin. Amide hydrolysis is also an important decomposition reaction. Reaction (1), (2) and (3) express major degradation pathways mentioned above.

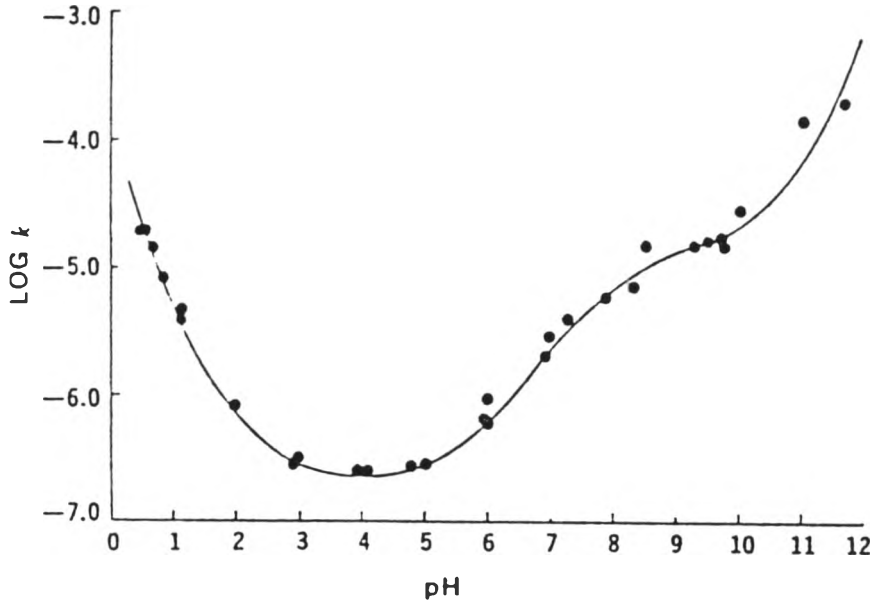
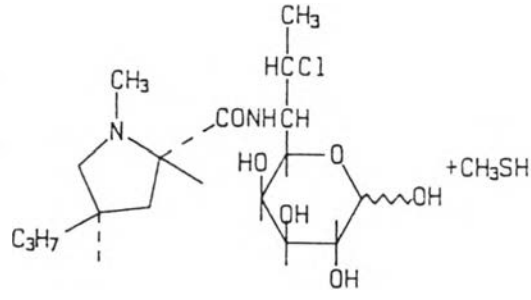
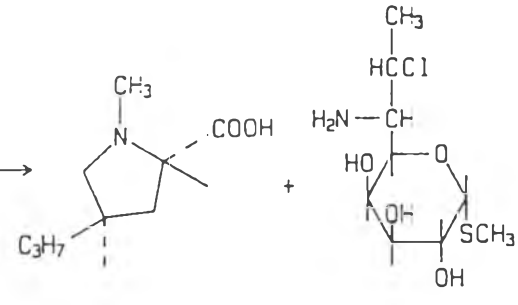


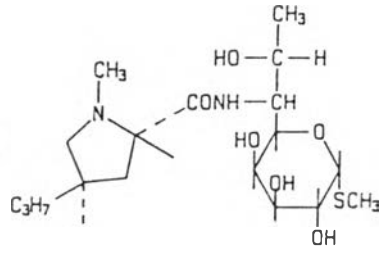
Figure 1. pH-Rate Profile of Clindamycin Degradation at 70°C.

clindamycin ⟶  +CH_3SH .....(reaction 1)

Reaction (1) shows hydrolysis of the thioglycoside to give 1-dethiomethyl-1-hydroxyclindamycin and methyl mercaptan. This is the predominant degradation pathway at pH below 4.

clindamycin ⟶  .....(reaction 2)

Reaction (2) shows amide hydrolysis occurring through the entire pH range, especially, in a solution of very high or very low pH.

clindamycin ⟶  + HCl .....(reaction 3)

Reaction (3) is the most important reaction in the pH 5 to 9 region. The drug is converted into another active antibiotic, lincomycin.

The decomposition reaction of clindamycin in solution is a first order process. If C represents clindamycin base and CH^+ is the protonated form of clindamycin, the rate equation for the overall degradation can be written as equation (1).

$$\textbf{Rate} = k_H[CH^+][H^+] + k'[CH^+] + k''[C] + k_{OH}[C][OH^-] \text{ .....(eq 1)}$$

This equation does not partition the rate into contribution from the several routes shown in reaction (1) through (3). For example, k_H term in equation (1) includes both the thioglycoside hydrolysis and the amide hydrolysis. The effect of pH on the rate of clindamycin degradation in the pH range 0.40-12 at 70°C is shown in Figure 1.

The smooth curve in Figure 1 was calculated with the appropriate form of equation (1) and the values $k_H = 5.28 \times 10^5\ M^{-1}S^{-1}$, $k' = 2.4 \times 10^{-7}\ S^{-1}$, $k'' = 8.0 \times 10^{-6}\ S^{-1}$ and $k_{OH} = 1.00 \times 10^{-1}\ M^{-1}S^{-1}$. The activation energy for clindamycin degradation in 0.1 M HCl, where thioglycoside hydrolysis is predominant, is 38.0 $\pm$ 1.2 kcal/mol, and the activation energy in 0.2 M citrate buffer adjusted to pH 5, where conversion to lincomycin is predominant, is 29.1 $\pm$ 0.6 kcal/mol.

Dermatological Dosage Forms

The purpose of dermatological dosage forms is to conveniently deliver drug across a localized area of the skin. To develop an ideal dosage form, one must consider amount of drug across skin, retention of the dosage form on the skin surface, the reservoir capacity of the dosage form and the patients' acceptability of the formulation. Solutions, which is a very simple dosage form, let the drug contact the skin surface easily leading to a successful treatment. However, this preparation presents a short duration of action owing to a low reservoir capacity and easily clearing out of the skin surface (Osborne, 1990). Semi-solid dosage forms are widely used in both pharmaceutical and cosmetic fields, especially gel preparation because of its elegant appearance. The gel dosage form gives a sustained action of drug, a high reservoir capacity and an

easy application and has a high ability to release medicaments.

Gel Dosage Form

Gels are transparent to opaque semi-solid containing a high ratio of solvent. When a gelling agent is dispersed in an appropiate solvent, it merges or entangles to form a three-dimensional colloidal network structure. This network limits fluid flow by entrapment and immobilization of the solvent molecules (Yalkawsky, 1979). For optimum appearance, the gel should have good optical clarity and sparkle.

Main Compositions of Gel Formula (Collett, 1990)

Gelling agent: Gelling agents are either organic hydrocolloids or hydrophilic inorganic substances. The viscosity of the gel produced may be varied to suit the requirement of the final product. Some of gelling agents frequently used are carbomer and cellulose derivatives such as methylcellulose, carboxymethyl cellulose sodium, hydroxyethyl cellulose, hydroxypropyl methylcellulose, etc. Polyoxyethylene-polyoxypropylene block copolymers series, poloxamer groups, are also widely used to form gels because it gives a clear gel and an air bubble-free gel can be prepared easily.

Humectant: Loss of water can lead to the surface dryness of gels. Humectant such as glycerin, propylene glycol or sorbitol solution was added to retain water. It also increases stratum corneum hydration which may result in an increase in the penetration rate of ingredients across the skin. However, excess ten percent of glycerin in a formula may cause irritation or sensitization.

Preservative: All gels have high water content therefore, they can support microbial growth unless a suitable preservative is added. The chosen preservative must be compatible with the gelling agent.

Stability Testing of Gel Dosage Form

Instability in any pharmaceutical product can be attributed to one of three

mechanisms of degradation: chemical, physical and microbiological. (Grimm, 1987)

Physical changes within the system will have three major consequences : They may affect

1. the appearances of the product.
2. dose uniformity.
3. the bioavailability of the product.

These changes may lead to poor patient compliance or reduce efficacy in the treatment. In the case of gel preparation, changes can occur in the structure of gels leading to an alteration in rheological properties. This may in turn affect the stability of a suspended medicament.

Some of the physical accelerated testing that have been proposed include elevated temperature, temperature cycling (Freeze-Thaw cycle, 4°C - 40°C), centrifugation and shaking tests. These high stress conditions may be useful in raking formulation on an comparative basis but they are not usually predictive of performance under condition of normal storage. The accelerated test for physical stability may introduce conditions that do not operate under normal storage. As a general rule, it can be stated that systems that withstand stress condition should be stable under normal storage conditions. The important characteristics that should be examined during physical test of semi-solid dosage forms are appearances (clarity, color, homogeneity), odor, pH, consistency and viscosity (Connors, 1990).

Chemical stability is limited of not more than a 10% change in the active ingredients. In a typical long-term study a batch would be tested at time of manufacture and the test should be continued at least once per year until the expiration date (Norwood, 1986). In these studies, the product is stored at the conditions specified on the product labeling (ambient temperature).

Accelerated test is performed to estimate the quality of a drug in a certain condition and peroid by a short-term test. This test shall be performed for three months or more, in principle, under 37-40°C, 75-100% RH (The Joel-Davis Test) (Carstensen, 1990). The results of this test is often how FDA justifies a two-year expiration peroid.

Experimental Designs of *In Vitro* Release Studies

Drug delivery from a topical formulation for local or systemic effects involves passive diffusion of drug. The main process is that the drug molecules release from a vehicle into the skin then diffuse across the skin. The physicochemical characteristics of drug molecule, the vehicle and the skin are the important factors influencing the drug efficacy.

For topical antiseptic and antibiotic preparations, the actual targets are surface bacteria and other microorganism. It is desirable to have penetration of the chemical agents into microcracks and fisures in the skin surface where these organism reside, as well as into the microorganism themselves. The drug efficacy depends upon the uptake of the antibacterials into the membranes and cytoplasms of the surface microbes. This is accomplished by diffusion from the applied film into the target organisms. A poor formulation can lead to a non release or retainment of the active chemical in the base and this will result in therapeutic inactivity.

The assessment of a vehicle potential for delivering a drug to the skin is determined by *in vitro* release study. There are several methods to perform the release of drug from its vehicle. Flow of molecules through a barrier such as a polymeric membrane is a particularly convenient way to study this process.

The first experiments performed on the release of drugs from ointments were those developed sixty years ago using agar diffusion method. (Flynn, 1972). The other method that has been used most frequently involves the release of the drug from the vehicle directly into a stirred solvent, which is presented in a volume that allows sink condition throughout the course of an experiment and in direct contact with the vehicle by using nonaqueous solution, then the samples of solvent are withdrawn to assay. This method is known as membraneless model. Many investigators have utilized this system. Chen-Chow and Frank (1981) comparatively evaluated of the in vitro efficacy of lidocaine release from Pluronic F-127 and other gel bases. Su Wu and Miller (1990) studied the effect of poloxamer lipophilicity, concentration and temperature on the *in vitro* release rate of nicotinic acid alkyl esters from its formulations. This experimental model was also used by Chi and Jun (1991).

The studies involves drug release from a topical formulation into a solvent receptor phase, it is preferable to reduce the probability that cross-contamination of the two phase will occur. On occasion, a synthetic membrane has been used to seperate the donor from the receiver phase. Wood et al. (1962) introduced a diffusion cell for the study of the release of a drug within a vehicle. The cell consisted of donor and receptor compartments separated by a cellophane membrane. The donor phase contained drug incorporated into the vehicle, whereas the receptor phase contained only the vehicle. Both phases were stirred, and the cell was kept at a constant temperature. In a modification of the previous method, Howze and Billups (1966) also used a cellophane membrane to separate the donor and receiver phase. However, in this method, the donor phase contained drug in the vehicle and the receiver compartment was filled with aqueous solution which was stirred thoughout the experiment. The sample were withdrawn to assay at the specified time intervals (Davis, 1973; Parikh, Babar and Plakogiannis, 1986). Babar et al (1990) used both cellulose membrane and hairless mouse skin. This study supports the evidence that the *in vitro* diffusion method served as a useful model for screening formulation with optimum drug release for use in *in vivo* evaluations. With the use of similar experimental designs, this group of researchers have investigated the release of other topical drugs from various vehicles (Babar,1991). Macedo et al. (1993) studied the effect on telmotin release from gel formulations prepared with different types of carbomers using Franz diffusion cells with cellulose acetate membrane. The experiment indicated that no significant differences in drug release characteristics were observed between all types of carbomer gels. This procedure has been used frequently by others as a means of assessing the relative ability of formulation bases to release drug (Miyazaki., 1984; 1986).

Although membranes composed of cellulose ester have generally been chosen for release studies, some investigators have used other membranes. Bottari et al. (1974; 1977) used silicone rubber membranes in release studies involving drug suspended gels and homogeneous ointments. Similar studies have been described by Colo (1990). Bottari, et al. (1975) studied permeation rate of drug-macromolecule interactions using nonporous nylon membranes. The permeability of nylon to drug is not similar to a porous membrane. Nylon is relatively impermeable to small molecules and ions, but many less polar, higher

molecular weight, and unionized species, such as ethylpyridinium bromides and sodium naphthalene sulfonate diffuse readily through nylon films (Kostenbander, Boxenbaum and Deluca, 1969).

The passage of matter through a barrier may occur by simple molecular permeation or by movement through pores. Molecular diffusion or permeation through nonporous media depends on dissolution of the permeating molecules in the bulk membrane (Figure 2a), whereas the substance passes through solvent filled pores of a pore membrane type. (Figure 2b) and is influenced by the relative size of the penetrating molecules and the diameter of the pores. The transport of a drug through a polymeric membrane involves dissolution of the drug in the matrix of the membrane (Figure 2c). Depending on the size and shape of the diffusing molecules, they may pass through the tortuous pores formed by the overlapping strands of polymer. If too large for such channel transport, the diffusant may dissolve in the polymer matrix and pass through the film by simple diffusion. (Martin, 1983)

The type of membrane utilized for the *in vitro* evaluation of drug release is of primary significant since the permeability depends on the nature of membrane materials (Walkow, 1987). Synthetic membranes are commonly employed for *in vitro* release studies due to their accessibility and reproducibility. It was demonstrated previously by other investigators that the membrane type and receptor phase composition can alter the diffusion profile (Walkow and McGinity, 1987). This experiment will study the differences observed in clindamycin hydrochloride gel diffusion using three types of synthetic membranes and two different receiving media.

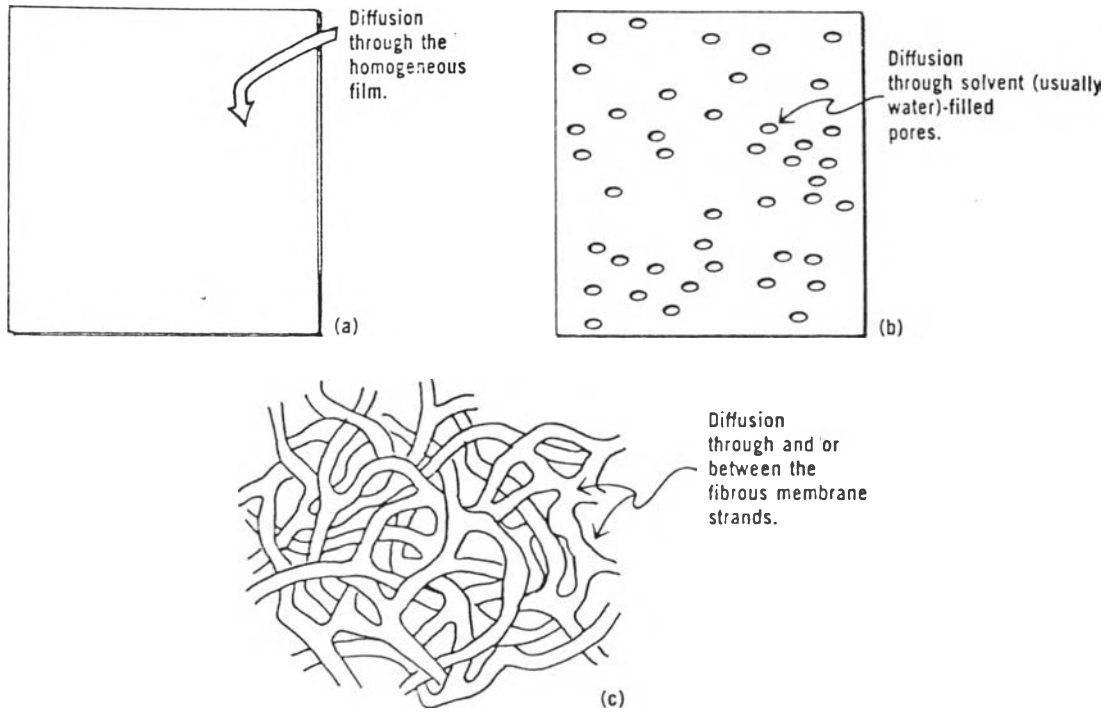


Figure 2. Schematic Diagram of Membrane Model.
(a) homogeneous membrane without pores.
(b) membrane of dense material with straight-through pores.
(c) cellulose membrane showed interwining nature of fibers and tortuous channels.

Theory of Drug Release

Many researchers have attempted to seek methods to determine a drug release rate from its vehicle. It is generally assumed that the results obtained in such experiment at least qualitatively relate to the release of the drug to the skin in a clinical situation (Osborne,1990). A theoretical basis for the study of the release kinetics of drugs from suspension and homogeneous ointments for the case in which release from the vehicle matrix is rate-limiting was established by Higuchi (1961). Higuchi first depicted the situation that the excess solute uniformly suspended as small particles in the ointment vehicle. The exact assumptions for this situation are as follows :

1. The total concentration (mass/volume) of dissolved and undissolved drug (Q) is much greater than the solubility (mass/volume) of the drug in the vehicle (C_S).
2. A sink condition prevails at the formulation receiver phase interface.
3. Release occurs through a planar surface.
4. There is no significant boundary layer adjacent to the formulation.
5. Quasi-steady-state diffusion exists between the dissolution interface at the edge of the particle field and the interface with the sink.
6. The model is semi-infinite, as in the original derivation no limit was placed on how far the boundary could recede.
7. The drugs are in a fine particle state so that dissolution of the particle is not rate-limiting.

The equation describing the release of solute was derived :

$$M = \sqrt{2DC_SA\left(Q - \frac{C_S t}{2}\right)} \qquad \text{.....(eq.2)}$$

where M is the amount of drug release (mg), t is the time after application (sec), A is a surface area of diffusion cell (cm^2) and D is the diffusion coefficient (cm^2/sec) of the drug in the ointment. After differentiation with respect to time, a rate of release is obtained :

$$\frac{dM}{dt} = \frac{1}{2}\sqrt{\frac{DA(2Q - C_S)C_S}{t}} \qquad \text{.....(eq.3)}$$

When $Q >> C_S$, the amount of drug released into a sink bears the following relationship to time :

$$M = \sqrt{2QDAC_S t} \qquad \text{..... (eq.4)}$$

and the rate becomes :

$$\frac{dM}{dt} = \sqrt{\frac{QDAC_S}{2t}} \qquad \text{..... (eq.5)}$$

Equations (4) and (5) predicts that a plot of the amount of drug release versus the square root of time should be linear, whereas the rate of drug release is proportional to the reciprocal of the square root of time.

Higuchi also described a relationship characterizing the release of drug from an ointment with its drug totally in solution. This equation is a solution to Fick's second law. As in the previous assumption, uptake into a sink is assumed, with diffusion to the releasing interface being the rate-limiting step in the overall process. The equation of this system was presented :

$$M = hAC_0 \sqrt{1 - \frac{8}{\pi^2} \sum_{m=0}^{\infty} \frac{[\exp[-D(2m+1)^2 \pi^2 t]]}{\frac{4h^2}{(2m+1)^2}}} \qquad \text{..... (eq.6)}$$

In this expression, h is the thickness of the ointment phase and C_0 is the initial drug concentration in the ointment (mg/ml). The following simplified equation closely describes the release of the medicament from the base for the first 30 % :

$$M = 2AC_0 \sqrt{\frac{Dt}{\pi}} \qquad \text{..... (eq.7)}$$

Equation (7) is based on the following assumption :

1. only a single drug species is important in the ointment.
2. only the drug diffuses out of the vehicle.
3. the diffusion coefficient is in varient with respect to time or position within the ointment.
4. the drug reaching the receptor site is removed rapidly.

The diffusion coefficient (D) can then obtain from the slope of a linear plot of M versus square root of time, providing that C_o remains relatively constant throughout the time of study.

If the release of drug was controlled by membrane, the Fick's law is used. The membrane is a film seperating the phases, and material passes by passive, active, or facilitated transport across this film. The term barrier applies in a more general sense to the region or regions and offers resistance to passage of a diffusing material, the total barrier being the sum of individual substances of membranes interposed between a donor and receptor chamber. An equation for an amount (M) of material flowing through a unit cross-section (A) of a barrier in unit time (t) is known as the flux (J) . Therefore, the Fick's first law is derived as follow:

$$J = \frac{dM}{A.dt} \qquad \text{..... (eq.8)}$$

If a diaphragm seperates the two compartments of a diffusion cell of cross-sectional area (A) and thickness (h), and if the concentrations in the membranes on the donor and the receptor chamber are C_1 and C_2, respectively, the equation 8 may be written :

$$J = \frac{dM}{A\,dt} = \frac{D\,(C_1 - C_2)}{h} \qquad \text{..... (eq.9)}$$

which $(C_1-C_2)/h$ approximates dC/dx. The gradient $(C_1-C_2)/h$ within the diaphragm must be assumed to be constant for a quasi-stationary state to exist. Equation 9 presumes that the aqueous boundary layers on both sides of the membrane do not significantly affect the total transport process.

The concentrations C_1 and C_2 within the membrane ordinarily are not known but can be replaced by the partition coefficient multiplied by the concentration (C_d) in the donor side or C_r on the receiver side. The partition coefficient (K) is given by :

$$K = \frac{C_1}{C_d} = \frac{C_2}{C_r} \qquad \text{..... (eq.10)}$$

therefore, the equation 9 could be written as :

$$\frac{dM}{dt} = \frac{DAK\,(C_d - C_r)}{h} \qquad \text{.....(eq.11)}$$

and, if sink conditions hold in the receptor compartment $C_r = 0$,

$$\frac{dM}{dt} = \frac{DAKC_d}{h} = PAC_d \qquad \text{..... (eq.12)}$$

in which

$$P = \frac{DK}{h} \qquad \text{.....(eq.13)}$$

where P is a permeability coefficient (cm/sec). Eventually, the amount of drug permeating into a sink bears the following relationship to time :

$$M = PAC_dt \qquad \text{..... (eq.14)}$$

The permeability coefficient (P) can then obtain from the slope of a linear plot of M versus t, providing that C_d remains relatively constant throughout the time of study.