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Investigation Of Lanolin Alcohol As A Film-Forming Delivery System: In Vitro Kinetics Of Triamcinolone Acetonide Release And Skin Penetration.

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INVESTIGATION OF LANOLIN ALCOHOL AS A FILM-FORMING
DELIVERY SYSTEM: IN VITRO KINETICS OF TRIAMCINOLONE
ACETONIDE RELEASE AND SKIN PENETRATION

A Dissertation
Presented to
the Faculty of the Graduate School
University of the Pacific

In Partial Fulfillment
of the Requirements for the Degree
Doctor of Philosophy

by
Balasubramanian Venkataraman Iyer
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ABSTRACT

The nonpolymeric high molecular weight substances such as lanolin alcohol, stearyl alcohol, and cetyl alcohol have been investigated for their film-forming potential. Furthermore, the application of this potential in the design of topical drug delivery systems has also been explored. The utilization of such film-forming systems may offer certain unique advantages not associated with the polymeric systems.

Stearyl alcohol and cetyl alcohol do not form films of acceptable integrity. Lanolin alcohol forms thin films capable of being isolated. Inclusion of appropriate amounts of ethyl cellulose and propylene glycol or hexadecyl alcohol in lanolin alcohol was found to improve the integrity, hardness and modulus of elasticity of these films. These lanolin alcohol films have been shown to reduce the normal in vivo transepidermal moisture loss.

The in vitro release of ^3H -triamcinolone acetonide from selected films was investigated. The results suggest that the drug release follows a diffusion-controlled granular matrix model with the release profile proportional to the square root of time. The release rate constants were found to be proportional to the concentration of the

dispersed drug. Drug release was found to be maximal from a system containing the drug in a near-saturated solution.

In vitro penetration experiments using human cadaver skin were also conducted from the same matrix-forming compositions. The analysis of the penetration data revealed that the release from the matrix acted as the rate-limiting step for the penetration process. The maximum rate of penetration was observed from a system containing slightly excess solubilizer than required to completely solubilize the steroid. The importance of this finding has been explained.

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Chapter I

INTRODUCTION

The study of the passage of medicinal substances through the skin offers a great deal of challenge to research workers in the fields of pharmacy and dermatology. The choice of an optimum vehicle for a particular medication depends on the physical and chemical properties of the drug alone, its properties in the vehicle, as well as the nature of the skin condition being treated. In the human the skin's physiological function as a protective organ is served by its effective barrier properties against the penetration of a wide variety of substances. Compared to most other tissues of the body, the skin surface is only slightly permeable.

Barrier Properties of Skin

The skin's barrier properties reside essentially in the stratum corneum layer of epidermis. Studies have shown that there is no significant difference between rate-limiting properties in isolated stratum corneum and full thickness epidermis (1). The absence of metabolic processes in the dead keratinized layers precludes any role for active transport processes. Consequently, the stratum corneum

acts as a passive diffusion medium, but one which displays considerable diffusional resistance. The usual diffusion laws of physics (Ficks's law) apply to the phenomena of cutaneous penetration. The underlying epidermal cell layers, the dermis, and the capillary walls are relatively permeable. For a few substances like thorium chloride, the dermal-epidermal basement membrane may constitute a second barrier (2) but, in general, once the stratum corneum is traversed, penetration onto the dermis and systemic circulation is assured.

In humans, the skin surface is pierced by follicular orifices and sweat gland ducts. These provide alternate or additional pathways for absorption, although the significance of sweat ducts in this process is questionable. For substances penetrating the follicles, subsequent absorption may occur through the follicular wall, sebaceous gland duct, or sebaceous gland epithelium. The pilosebaceous apparatus is an important route of entry for some substances and, in part, is more permeable than the interfollicular stratum corneum. For most substances, both transepidermal and trans-appendageal routes play some role in absorption. Overall, however, the transepidermal route must be the principal portal of entry for most materials, since sweat gland ducts and hair follicle orifices account for only 0.1-0.2% of the total skin surface.

In the initial phase of percutaneous absorption after topical application, there may be transient but far greater

diffusion occurring through the transappendageal route than through the transepidermal pathway (3). After steady-state diffusion is attained, the dominant diffusion pathway is transepidermal rather than transappendageal. However, for certain slowly diffusing large molecules, transappendageal penetration may be the predominant route throughout.

In regard to transepidermal absorption, the sites of diffusional resistance in the stratum corneum layer reside both in its cell membrane and, predominantly, in the poorly hydrated intracellular filament-matrix contents. The membranes of cells of stratum corneum are structurally rigid and show remarkable chemical resistance. These membranes are about 150 Å thick, almost double the 80 Å thickness found in basal cell membranes. This change apparently results from surface deposition on, or inclusion of resistant material in or just beneath, the stratum corneum cell membranes (4). This thickness may contribute to the decreased cell permeability. Intracellularly the keratin matrix comprises about 65% of cell mass, and the overall water content is about 10%. For substances diffusing through these cells, keratin, lipids, and nonfibrous proteins constitute major "roadblocks" to passage. The keratin-matrix structure is a mosaic of polar (bound water) and nonpolar (interfilamentous lipid) regions which comprise parallel but distinct diffusional pathways for water- and lipid-soluble molecules (3).

Overall percutaneous absorption is a considerably more complicated process than the prevailing passive diffusion factors would suggest. The principal conditions affecting such an absorption can be discussed as follows.

Hydration

Hydration of the stratum corneum is possibly the most important factor in skin penetration, increasing the rate of passage of all substances which penetrate the skin. Hydration results from water diffusing from underlying epidermal layers or from the perspiration that accumulates after application of an occlusive vehicle or covering on the surface. Under occlusive conditions, the stratum corneum is changed from a tissue which normally contains very little water (5-15%) to one which may contain as much as 50% water (5).

The importance of hydration can be found in those investigations employing occlusive plastic film in steroid therapy. Here, the prevention of water loss from the stratum corneum and the subsequent increased water concentration in the skin layer enhances the penetration of the steroid (6-10). McKenzie and Stoughton (7) have shown that penetration of corticosteroids may be increased 100-fold by occluding the site of application and thus hydrating the stratum corneum. This enhanced activity of the steroid applied under a plastic barrier also has been attributed to increased contact between vehicle and skin, the accurate

localization of vehicle and increased skin temperature.

Wurster and Kramer (11) measured the rate of penetration of esters of salicylic acid across dry and hydrated stratum corneum. They found that when the tissue was hydrated the rate of penetration of the water-soluble esters increased more than that of the other esters studied. Working with aspirin in a temperature-humidity chamber, Fritsch and Stoughton (12) showed the importance of these factors on the penetration of excised skin. Full hydration of the keratin (accomplished by layering water over acetylsalicylic acid on the epidermal surface) dramatically increased the penetration when compared to conditions of lower humidity at the same temperature. Although under the conditions employed, aspirin may have hydrolyzed to salicylic acid, the conclusions drawn regarding the effect of humidity on penetration are still valid.

The mechanism of transport of a drug through hydrated stratum corneum may be quite different from that through normal stratum corneum. Any vehicle that alters the hydration state of the stratum corneum will affect its diffusional resistance. Increased hydration of the membrane appears to open up its dense, closely packed cells and increase its porosity. The magnitude of this effect depends on the degree of hydration and the specific penetrant. Schleuplein and Ross (13) indicated that hydration can increase the permeability of skin as much as eightfold even though some of Schleuplein's data (14) with water and polar alcohols suggested that hydration does not significantly alter

the barrier function of stratum corneum.

Vehicles

Vehicles do significantly affect the penetration of substances through stratum corneum (15). In general, the activity of the drug within the vehicle is more important than the properties of vehicle itself in terms of drug bioavailability.

The physical characteristics of the vehicle are a major consideration in vehicle selection. In case of solutions, substances will be more rapidly released from vehicles having a low affinity for the penetrant. Pertinent factors here are related to the solubility of the penetrant in the vehicle and the rate of release of the penetrant from its base. In general, it has been shown that the diffusion of the drug from the vehicle into the skin surface and subsequent penetration of the drug through stratum corneum are functions of the partition coefficient of the drug between stratum corneum and vehicle and of the relative solubility of the drug in the vehicle (16, 17). Particle size, drug concentration in the vehicle, and to some extent, viscosity of the vehicle are significant factors.

The thermodynamic activity of the incorporated material is an important factor. Thermodynamic activity is the product of the concentration and its activity coefficient in the vehicle. Rapid release of the drug is dependent on

its high thermodynamic activity in the vehicle, as the direction of flow is always from the higher to lower thermodynamic potential. For specific concentrations of certain substances, it has been shown that thermodynamic activities may vary as much as 1000-fold from one vehicle to another (18). Activity coefficients are reduced by such factors as pH changes that will shift the equilibria of ionizable compounds towards ionized state and complex formation with vehicular contents. Vehicles with relatively low solvent power for incorporated compounds will induce more rapid penetration (18). In general, a compound must be at least partially soluble in its vehicle so that it can be readily released into the receptor phase (skin barrier). High solubility may result in preferential retention of the drug in the vehicle.

The physical properties of vehicles are also important in the degree of occlusion they produce leading to water retention in the stratum corneum layer. The efficiency of various types of vehicles in aiding penetration can be reasonably explained on the basis of their effect on hydration of the stratum corneum or how the vehicle alters the activity of water in the stratum corneum and influences the stratum corneum/vehicle partition coefficient. Greases and oils are the most occlusive vehicles and induce the greatest hydration through sweat accumulation at the skin-vehicle interface (19). This can be accentuated

by covering with occlusive bandages or plastic. Emulsions of the water-in-oil type are less occlusive than greases. Substances in the vehicle, such as humectants, which have a high affinity for water, would act in proportion to the relative humidity of the environment. If the latter is low, the humectant would tend to dehydrate the stratum corneum and decrease penetration. Similarly, powders increase surface area, increase the rate of evaporation of water, and so decrease the extent of hydration (19).

Some vehicles have pronounced effects when applied to the skin surface. Certain nonaqueous bases promote penetration by producing structural change or chemical damage in the barrier layer (20). Dimethyl sulfoxide, dimethyl acetamide, and dimethyl formamide are examples of these.

Low molecular weight volatile solvents such as ether, methanol, ethanol and acetone may also damage skin's barrier layer. Substantial lipid extraction from stratum corneum cells, which leaves a more porous barrier, is most likely responsible for this effect.

Concentration of Drug in Donor (Vehicle) Phase

Tregear (21) has tabulated a number of experimental studies involving several compounds where Fick's law was found to hold. The penetration rate of butanol, for example, through isolated epidermis is directly proportional

to its concentration in saline solution at dilute concentrations (22). Scheuplein and Blank (3) have stated that unless the applied substance can damage the tissue, Fick's law seems to hold fairly well for all penetrating substances. When high concentrations of penetrant are present in the donor phase, positive or negative deviations from Fick's law ordinarily occur as a consequence of membrane changes induced by the high concentrations or because the partition coefficient between the donor phase and the skin barrier is not constant over the entire concentration range. An increase in penetration in proportion to the square root of concentration in the case of aliphatic alcohols has also been recently reported (23).

In addition, other factors such as fluctuations in skin temperature, peripheral circulation, species variation, age and drug binding and metabolism have been found to affect percutaneous absorption.

Polymers in Drug Delivery Systems

Protective films containing therapeutically active agents have been used for dermatological applications. Schertler has formulated a film-forming preparation using polyacrylate and hydroxypropyl cellulose, suitable for the topical application of corticosteroids (24). Conrady et al. have developed a long lasting insect repellent film intended for application on skin using unsaturated carbonyl

monomers (25). In recent years, great interest has developed regarding the use of medicated polymeric films in managing contaminated wounds (26). Spray-on bandages using polyvinyl alcohol in combination with polyvinyl acetate or acrylic resins and other water soluble resins have been investigated (26). In one study (27), optimal therapeutic responses were seen in the contaminated soft tissue wounds in rats when an antibiotic was administered to the affected area and covered with a spray dressing of isobutyl cyanoacrylate. Chloramphenicol and nitrofurazone have been incorporated into solutions of ethyl cellulose and sodium carboxymethyl cellulose (28). More recently, an inert polymeric matrix impregnated with the drug has been utilized to achieve prolonged and steady release of pilocarpine for administration to the eye (29) and in long-term buccal absorption of drugs (30). Shaw et al. (31) have recently described a transdermal system which delivers the drug scopolamine at a rate that prevents motion-induced nausea, but minimizes the other parasympatholytic effects of the drug.

In certain specific dermatological applications, the use of drug-containing polymeric films could offer certain unique advantages not associated with conventional dosage forms. These advantages include enhanced therapeutic effect combined with predictable control over the rate and extent of absorption of the drug. If the vehicle is a polymeric

film, it has been shown that it is possible to control the rate of release of a therapeutically active substance for subsequent penetration through the skin, depending upon the nature of the polymeric film and therapeutic agent (32). Coldman et al. (33) have studied the penetration of fluocinolone acetonide and its acetate ester through human skin with vehicle mixtures of isopropanol and isopropyl myristate or propylene glycol. The eight- to ten-fold increase in skin penetration is accounted for by the increase in the thermodynamic activity of the steroid brought about by the evaporation of isopropanol. A drug carried in a non-volatile polymeric solvent-vehicle containing a volatile solvent would also be expected to follow a similar pathway, depending on its thermodynamic activity in the film after evaporation of the solvent.

Relationships for Systems Where Diffusion from the Vehicle is Rate-Limiting

There is only one situation in which the properties of the skin can be ignored entirely as far as topical drug diffusion is concerned. This occurs when the release rate of the drug from the vehicle is exclusively rate-limiting for the absorption process. In this instance, the skin can be regarded as a perfect sink which plays no part in determining the rate at which the drug penetrates the skin. Generally it has been stated to be possible in cases involving absorption by injured skin or where highly insoluble,

suspension-type ointments are used where large concentration gradients may develop in the applied phase. Mathematical relationships useful in describing such cases have been given by T. Higuchi (18).

a. Penetrant Release from Solutions

If the penetrating substance initially is uniformly dissolved in a homogeneous base, the following equation derived from Fick's law of diffusion (34) applies:

$$Q = h C_o \left\{ 1 - \frac{8}{\pi^2} \sum_{m=0}^{\infty} \frac{1}{(2m+1)^2} \exp \left[\frac{-D(2m+1)^2 \pi^2 t}{4h^2} \right] \right\} \quad (\text{eq. 1})$$

where

- Q = quantity of drug released to the skin surface per unit area of application.
- C_o = initial concentration of drug in vehicle,
- h = thickness of vehicle layer,
- D = diffusion constant of the drug in the vehicle,
- t = time after application,
- m = integer with values from 0 to ∞.

This equation is a valid expression for release from one side of a layer of vehicle if the following restrictions are met (35):

- (i) A single drug is in true solution and (initially) uniformly distributed throughout the vehicle.
- (ii) The composition of the vehicle remains fixed during the diffusion process, i.e., components other than drug do not leave or enter the vehicle phase.
- (iii) The diffusion constant of the drug is independent of time and position in the vehicle.
- (iv) Drug reaching the receptor phase (skin) is absorbed instantaneously.

When the percentage of drug released from vehicles is less than 30-50% and the vehicle layer is sufficiently thick, then the following simplified equation proposed by T.

Higuchi (18) can be used:

$$Q = 2 C_0 \left[\frac{D t}{\pi} \right]^{\frac{1}{2}} \quad (\text{eq. 2})$$

b. Penetrant Release from Solid Matrix Containing Dispersed Drug

1. Release From a Planar System Having a Homogeneous Matrix:

The extraction of the medicament by a simple diffusional process through and from an enveloping homogeneous matrix follows this case. The drug is presumed to go successively from the crystal surfaces into the uniform matrix and out into the bathing solvent which in turn acts as a perfect sink. The amount of total drug released from such a system

into a bathing medium acting essentially as a perfect sink would be determined by the relationship (36):

$$Q = \sqrt{D t (2A - C_s) C_s} \quad (\text{eq. 3})$$

where

Q = quantity of drug released after time t per unit exposed area,

D = the diffusivity of the drug in the homogeneous matrix media,

A = the total amount of drug present in the matrix per unit volume,

C_s = the solubility of the drug in the matrix substance.

2. Release From a Planar System Having a Granular Matrix:

Here the medicament is leached by the bathing fluid which is able to enter the drug-matrix phase through pores, cracks, and intergranular spaces. The drug is presumed to dissolve slowly into the permeating fluid phase and to diffuse from the system along the cracks and capillary channels filled with the extracting solvent. Intragranular diffusion is assumed, in this instance, to be insignificant. Here the equation 3 is modified for the effective volume where diffusion can occur and the effective diffusional path. For this system:

$$Q = \sqrt{\frac{D \epsilon}{t} (2A - \epsilon C_s) C_s t} \quad (\text{eq. 4})$$

where

D = diffusivity of the drug in the permeating fluid,

τ = the tortuosity factor of the capillary system $\cong 3$,

ϵ = the porosity of the matrix,

C_s = the solubility of the drug in the permeating fluid.

For both equations 3 and 4 the derivation is based on the existence of a pseudo-steady state condition during the release process and on the assumption that the drug particles are quite small relative to the average distance of diffusion and are uniformly distributed in the matrix. These equations would be essentially valid for systems in which A is greater than C_s or ϵC_s by a factor of three or four.

The Case Where Diffusion Across Skin Barrier is Rate-Limiting

The majority of topical preparations on application to an intact skin fall in this category. This is due to the fact that the skin is a highly resistant barrier and it is penetrated only with difficulty by most noncaustic substances.

Fick's law specifies that the flux (moles $\text{cm}^{-2} \text{sec}^{-1}$) of a substance through a plane perpendicular to the direction of diffusion is directly proportional to the concentration gradient,

$$J = - D \frac{dc}{dx} \quad (\text{eq. 5})$$

where J is the flux and $\frac{dc}{dx}$ is the concentration gradient.

A modified form of equation 5, applicable to biological membranes, has been developed by T. Higuchi (13):

$$\frac{dQ}{dt} = \frac{P C_v D_s A}{h} \quad (\text{eq. 6})$$

where

$\frac{dQ}{dt}$ = steady-state rate of penetration,

P = effective partition coefficient between skin barrier and vehicle,

C_v = concentration of drug dissolved in the vehicle,

D_s = average diffusion constant of the drug in skin barrier,

A = area of skin to which drug is applied,

h = effective thickness of skin barrier.

A number of limitations must be borne in mind when applying equation 6. In general, it applies only for relatively low concentrations of penetrant in the vehicle, and deviations from Fick's law are usual at high concentrations. Good compliance with this equation could be observed when following constraints were imposed upon experimental conditions (15).

- (i) a constant concentration of solute (penetrant) was maintained in the donor (vehicle) phase,
- (ii) sink conditions were maintained on the receptor side of the membrane,
- (iii) the composition of the donor phase was maintained

constant and loss by evaporation or diffusion does not occur, and when

- (iv) both donor and receptor phases were stirred.

Scope of the Present Study

Medicated occlusive dressings capable of forming a film at the site of application could be considered superior to conventional dressings which necessitate the use of "Saran Wrap" to achieve occlusivity. These medicated occlusive dressings:

- (i) could be designed to provide an optimum level of occlusivity which does not hamper the normal physiological function of the skin,
- (ii) could be expected to have greater acceptance due to ease of application and the invisible nature of the film, and
- (iii) as an auxilliary role, could be expected to prevent the mechanical removal of topically-applied drug.

Although several polymeric substances have been studied for their film characteristics and potential application in topical delivery systems, the nonpolymeric high molecular weight substances such as lanolin alcohol, stearyl alcohol and cetyl alcohol do not appear to have been investigated for their film-forming potential. Furthermore, the application of this concept in the design of topical drug delivery

systems has also not been fully explored. The utilization of such systems could be expected to minimize the hazard associated with the monomeric impurities present in the high molecular weight polymers. Moreover, these high molecular weight alcohols can be obtained in a state of more definable composition than polymers. The films formed by these substances can be easily washed off from the skin by soap and water.

In the present study, a preliminary screening of the film-forming potential of lanolin alcohol, cetyl alcohol and stearyl alcohol has been made. Propylene glycol or hexadecyl alcohol was used as the plasticizer for the film. The film-forming potential of lanolin alcohol, cetyl alcohol, and stearyl alcohol was also tested in combination with a known film former, ethyl cellulose (37), in presence of the plasticizers mentioned above. The films formed by lanolin alcohol either alone or in combination with ethyl cellulose in combination with the plasticizers mentioned above were then characterized. The evaluation of the films was carried out by measuring the hardness and modulus of elasticity at a given film thickness. Selected films of lanolin alcohol were also tested for their in vivo water vapor transmission characteristics.

This work describes the kinetics of drug release and in vitro skin penetration from selected compositions of films of lanolin alcohol. For the purpose of these studies,

triamcinolone acetonide was chosen as the model drug. Triamcinolone acetonide belongs to the family of corticosteroids with potent topical antiinflammatory activity. Furthermore, this drug was readily available in radiolabeled form which provided a highly sensitive technique to study the kinetics of release and skin penetration.

Chapter II

SCREENING AND EVALUATION OF THE PHYSICAL CHARACTERISTICS OF THE FILMS

This chapter describes results of initial screening of potential film formers, evaluating screened films for integrity, and occlusive characteristics. For the initial screening of the film forming capability, the following nonpolymeric high molecular weight alcohols were selected:

- (i) lanolin alcohol B.P. (mp 61°-64°),¹
- (ii) cetyl alcohol NF,²
- (iii) stearyl alcohol USP.³

Ethyl cellulose⁴ with an ethoxyl content of 48.5 was selected as polymeric film former. The selection of these compounds was based upon earlier observations of potential film-forming capability and upon their pharmaceutical acceptability. Propylene glycol USP⁵ and hexadecyl alcohol (cosmetic grade, d 0.84)⁶ were used as plasticizers to

¹Super Hartolan, Croda, Inc., New York, N.Y.

²Ruger Chemical Company, Inc., Irvington, N.J.

³Ruger Chemical Company, Inc., Irvington, N.J.

⁴Ethyl Cellulose N-50, Hercules Inc., Wilmington, Delaware.

⁵Ruger Chemical Company, Inc., Irvington, N.J.

⁶M. Michel and Company, Inc., New York, N.Y.

enhance the film-forming characteristics of these materials. Isopropyl alcohol NF⁷ was found to be a good solvent for selected alcohols and ethyl cellulose and was selected for preparing the compositions studied in view of its wide acceptability in topical applications.

Test for Film-Forming Capability

For the initial screening, all the model films were cast from 10% w/v solution of film former(s) and plasticizer in isopropyl alcohol. The films were cast by the mercury-substrate technique. Five milliliters of the solution was poured within a stainless steel ring (9.2 cm internal diameter) which had been placed on a mercury surface contained in a 140 x 10 mm glass Petri dish. The Petri dish was then partially covered with its lid and the solvent was allowed to evaporate overnight. The whole operation was carried out in a humidity controlled room at 30° and 40% relative humidity. Partially covering the Petri dish was helpful in controlling the rate of evaporation of the solvent and reduced the blistering of the surface of the deposited film. Any film formation was easily noted by observing the mercury surface after the complete evaporation of the solvent, and by slowly lifting the ring from the mercury support.

⁷Mallinckrodt Chemical Works, St. Louis, MO.

For the initial screening, the concentration of the plasticizer (propylene glycol or hexadecyl alcohol) was kept at 5% w/w of the film composition, after the evaporation of the solvent. The concentration of ethyl cellulose was increased from 0 to 30% w/w with a corresponding decrease in lanolin alcohol, cetyl alcohol or stearyl alcohol concentration. The increment in ethyl cellulose concentration was done at 5% w/w intervals.

In the second stage, the concentration of ethyl cellulose was kept as a constant at 15% w/w. The concentration of propylene glycol (plasticizer) was varied from 0 to 30% w/w in 5% increments, with a corresponding decrease in high molecular weight alcohol concentration.

Results and Discussion

Based on this initial screening, it was observed that the compositions containing cetyl or stearyl alcohol were not able to form films of acceptable integrity. These compositions were, therefore, not subjected to any further evaluation.

Solutions of lanolin alcohol in isopropyl alcohol were found to form a translucent thin film on the mercury. A film thickness of 65 μm or more ensured proper isolation. The inclusion of solvent-plasticizer propylene glycol or hexadecyl alcohol reduced the tackiness of the film. The integrity of the lanolin alcohol film was further enhanced

by inclusion of ethyl cellulose with a corresponding decrease in lanolin alcohol concentration. The films formed by lanolin alcohol at 5% w/w of propylene glycol or hexadecyl alcohol and at 15 to 20% w/w/ of ethyl cellulose were very smooth and flexible. The films with higher than 20% w/w concentration of ethyl cellulose showed surface roughness. Increase in concentration of propylene glycol beyond 25% w/w with a corresponding decrease in lanolin alcohol concentration, resulted in tacky films at the 15% w/w ethyl cellulose concentration.

Determination of Film Hardness and Modulus of Elasticity

Selected films of lanolin alcohol with or without ethyl cellulose were characterized by measurement of hardness and modulus of elasticity. It was thought necessary to evaluate these physical properties since these film-forming compositions were intended for potential application to the skin which has its own mechanical properties. The film hardness was determined on films cast on polished aluminum plates. For each composition tested, 10% w/v solution of film former(s) and plasticizer was prepared in isopropyl alcohol and was cast on a polished aluminum plate (20 cm x 20 cm) using a Multiple-Clearance Applicator⁸ with a wet

⁸Gardner Laboratory, Inc., Bethesda, Maryland.

film thickness of about 1 mm. The Multiple-Clearance Applicator was chosen over other types of film-forming devices, since it offered the advantages of simplicity in changing the thickness and in operation in casting the films.

The Multiple-Clearance Applicator (square shaped) has a dimension of 10.2 x 10.2 x 1 cm and permits formation of films of up to 8 different thicknesses by the openings on each of its eight edges. In the applicator used, the thickness can be varied from 5 to 50 mils, a mil thickness being equivalent to 25 μ m. The plates were then dried in a humidity-controlled room at 30° and 40% relative humidity. The exact thickness of the dried films was determined after complete evaporation of the solvent, using a Minitector (Model-N) thickness measuring gauge.⁹

The Minitector (Model-N) is an instrument for measuring non-conductive coatings on a non-ferrous metallic base, utilizing eddy current principles. A small AC voltage is fed to a coil within the measuring probe to create an alternating field. This field causes minute currents to flow within the conductive base in concentric paths giving rise to eddy currents. These currents, in turn create their own field which is in opposition to the original field and has the effect of reducing the voltage across the coil. This change in voltage is dependent upon the

⁹Gardner Laboratory, Inc., Bethesda, Maryland.

distance of the probe tip from the base material (i.e. the coating thickness) and can, after suitable amplification, be displayed on the large scale meter on the instrument which is calibrated directly in units of μm thickness in the range of 0-2500 μm .

Before each measurement, it is necessary to zero the instrument on the same aluminum plate in which film casting was done. The instrument was regularly calibrated using the standard foils provided with the instrument.

Film hardness was then determined using an I.C.I. Automatic Sward Hardness Rocker.¹⁰ The Sward Hardness Rocker has been used for many years to measure the Sward Hardness of paint films (38) and of some polymeric films used in pharmaceutical applications (37). The rocker consists of two 4-inch metal rings spaced an inch apart, a gravity bob for regulating the oscillation frequency or period of the moving system at 1.2 seconds, and a rider on the horizontal nameplate for adjusting the zero point of the balanced rocker.

All automatic rockers are fitted with a light-chopping shutter on the left to interrupt a focused beam of light during automatic counting. Two tilted glass bubble tubes are located below the horizontal nameplate for visually

¹⁰Gardner Laboratory, Inc., Bethesda, Maryland.

indicating the start and finish of the test. Thus the rocker can be used, without change, for either automatic or visual counting.

To make a hardness test, the rocker with clean edges was placed on the coated plate kept in a leveled holder and set in motion. The total number of oscillations made by the rocker is given by the automatic counter which is then multiplied by 2 to give the number of rocks. The number of rocks was measured as the average of three determinations, rounded off to the nearest whole number. Before each measurement, the rocker was calibrated to show 100 rocks with the standard polished glass plate provided with the instrument. The measurements were made at the ambient conditions of the room.

From the Sward Hardness R (number of rocks of the rocker on the test film) the modulus of elasticity E was calculated by the following equation:

$$E = \frac{K R^3}{T^3} \quad (\text{eq. 7})$$

The values of constant K, for different thicknesses T, were obtained by plotting the different T values against standard K values on a semi-logarithmic paper. The standard values of K for different thicknesses are as shown below (38):

Thickness	K
0.0012"	1.73×10^{-9}

0.0024"	2.10×10^{-8}
0.0040"	1.30×10^{-7}
0.1250"	2.50×10^{-2}

The values of Sward Hardness were shown to be affected by factors such as high variation in temperature and surface roughness of the films (38).

Results and Discussion

The results of modulus of elasticity determinations confirmed observations made earlier that the integrity of the lanolin alcohol film was enhanced by inclusion of ethyl cellulose. When the concentration of the solvent-plasticizer was kept constant at 5% w/w, the modulus of elasticity increased with increasing proportions of ethyl cellulose and decreasing proportions of lanolin alcohol. The modulus of elasticity reached a maximum value at an ethyl cellulose concentration of 15% w/w (Tables I and II). The increase in ethyl cellulose concentration to 30% w/w with propylene glycol as plasticizer reduced the modulus of elasticity to 2.95 kg/cm^2 (Table I). With hexadecyl alcohol as plasticizer, the modulus of elasticity started to decline, at 20% w/w concentration of ethyl cellulose (Table II).

The composition with 15% w/w ethyl cellulose and 7.5 to 10% w/w propylene glycol gave films with high modulus of elasticity (Table III). Further increases in proportion of propylene glycol up to 25% w/w, with a proportionate

Table I

Effect of Change in Lanolin Alcohol-Ethyl Cellulose Ratio on Sward Hardness and Modulus of Elasticity of Films Containing Propylene Glycol at Room Temperature ($22^{\circ} + 0.5^{\circ}$)

Lanolin Alcohol: Ethyl Cellulose: Propylene Glycol Ratio ^a	Mean Dry Film Thickness ($\times 10^3$) ^b , inches	Constant for a Given Thickness $K_t \times 10^{10}$	Sward Hardness (R) ^c , rocks	Modulus of Elasticity (E), psi (kg/cm ²)
9.50 : 0.00 : 0.50	0.189	0.030	2	3.6 (0.2)
9.00 : 0.50 : 0.50	0.252	0.068	3	11.5 (0.8)
8.50 : 1.00 : 0.50	0.402	0.450	4	44.2 (3.1)
8.00 : 1.50 : 0.50	0.724	3.200	4	53.9 (3.8)
7.50 : 2.00 : 0.50	1.047	9.000	4	50.2 (3.5)
7.00 : 2.50 : 0.50	1.063	9.200	4	51.3 (3.6)
6.50 : 3.00 : 0.50	1.425	45.000	3	42.0 (3.0)

^aAll films were cast from 10% w/v solution of film formers in isopropyl alcohol.

^bThicknesses are expressed as mean values of five measurements.

^cSward Hardnesses are expressed as the mean value of three measurements rounded to the nearest whole number.

Table II

Effect of Change in Lanolin Alcohol-Ethyl Cellulose Ratio on Sward Hardness and Modulus of Elasticity of Films Containing Hexadecyl Alcohol at Room Temperature ($22^{\circ} \pm 0.5^{\circ}$)

Lanolin Alcohol: Ethyl Cellulose: Hexadecyl Alcohol Ratio ^a	Mean Dry Film Thickness ($\times 10^3$) ^b , inches	Constant for a Given Thickness $K_t \times 10^{10}$	Sward Hardness (R) ^c , rocks	Modulus of Elasticity (E), psi (kg/cm ²)
9.50 : 0.00 : 0.50	0.189	0.030	2	3.6 (0.2)
9.00 : 0.50 : 0.50	0.193	0.031	3	11.7 (0.9)
8.50 : 1.00 : 0.50	0.504	0.850	4	42.5 (3.0)
8.00 : 1.50 : 0.50	0.650	2.200	4	51.4 (3.6)
7.50 : 2.00 : 0.50	0.480	0.700	4	40.4 (2.8)
7.00 : 2.50 : 0.50	0.441	0.600	4	44.8 (3.2)
7.50 : 3.00 : 0.50	1.284	30.000	3	38.31 (2.7)

^aAll films were cast from 10% w/v solution of film formers in isopropyl alcohol.

^bThicknesses are expressed as mean values of five measurements.

^cSward Hardnesses are expressed as the mean value of three measurements rounded to the nearest whole number.

Table III

Effect of Change in Lanolin Alcohol-Propylene Glycol Ratio on Sward Hardness
and Modulus of Elasticity of Films at Room Temperature ($22^{\circ} \pm 0.5^{\circ}$)

Lanolin Alcohol: Ethyl Cellulose: Propylene Glycol Ratio ^a	Mean Dry Film Thickness ($\times 10^3$) ^b ,	Constant for a Given Thickness $K_t \times 10^{10}$	Sward Hardness (R) ^c rocks	Modulus of Elasticity (E), psi (kg/cm ²)
8.50 : 1.50 : 0.00	0.669	2.80	3	25.2 (1.8)
8.25 : 1.50 : 0.25	0.606	1.12	4	31.6 (2.2)
8.00 : 1.50 : 0.50	0.724	3.20	4	53.9 (3.8)
7.75 : 1.50 : 0.75	0.755	4.54	4	66.7 (4.7)
7.50 : 1.50 : 1.00	0.809	5.52	4	66.6 (4.7)
7.25 : 1.50 : 1.25	0.693	3.13	4	59.6 (4.2)
7.00 : 1.50 : 1.50	0.874	6.54	4	62.3 (4.4)
6.50 : 1.50 : 2.00	0.740	4.05	4	63.1 (4.4)
6.00 : 1.50 : 2.50	0.724	3.91	4	65.7 (4.6)
5.50 : 1.50 : 3.00	0.622	1.52	4	39.9 (2.8)
5.00 : 1.50 : 3.50	0.622	1.52	4	39.9 (2.8)

^aAll films were cast from 10% w/v solution of film formers in isorpopyl alcohol.

^bThicknesses are expressed as mean values of five measurements.

^cSward Hardnesses are expressed as the mean value of three measurements rounded to the nearest whole number.

decreases in lanolin alcohol concentration did not substantially alter the film characteristics. However, an increase in concentration of propylene glycol beyond 25% w/w resulted in films which were visibly tacky and with a low modulus of elasticity.

Test for Occlusive Property of the Films

The importance of occlusion on steroidal penetration has been discussed earlier. The occlusive nature of a film-forming material can be measured by considering the water vapor transmission characteristics of these materials. Since the lanolin alcohol films under investigation were to be tested as vehicles for topical application of the steroid, triamcinolone acetonide, it was considered more appropriate to measure the effect of these films on the normal in vivo transepidermal moisture loss (TEML) rather than studying the occlusive effects in vitro by one or more techniques reported (37, 39).

The normal stratum corneum is a very efficient but not an absolutely perfect barrier to water loss. Under normal conditions, water passes from the underlying tissues to the stratum corneum, and diffuses through it to the exterior surface where it evaporates. This process is known as transepidermal moisture loss (TEML) or insensible perspiration, and is governed by laws of diffusion as discussed in detail by Scheuplein (40). Hydration by occlusion is

achieved by placing onto the surface of the skin a topical substance which has high diffusional resistance to water, thereby reducing the net water loss from the stratum corneum. The reduction of TEML after the use of a topical agent has been used as a criteria to evaluate moisturizing capability (41).

A variety of methods have been developed to measure the moisture loss in vivo. Noninstrumental methods such as desiccator method of Powers and Fox (42) and cold trapping of vapor from a stream of gas passing over a surface of the skin have been used with only a limited degree of success. Thiele and Shutter (43) have developed an instrumental approach utilizing a salt crystal method. Later, these same authors presented a critical review concerning an improved salt crystal method and electrolytic methods (44). Electrolytic methods were also used by Spruit and Malten (45, 46). Spruit (47) also applied a thermal conductivity cell to in vivo measurements. Baker and Kligman (48) used electrohygrometry to make moisture loss measurements.

Measurement of In Vivo Moisture Loss

Lanolin alcohol films used in this study were prepared by using 10% w/v solutions and the mercury support technique discussed earlier. The dry film thicknesses were maintained in the range of 30 to 40 μm in order to avoid the effect of variation in thickness of TEML (41). This

was achieved by changing the amount of isopropanolic solution poured onto the mercury in the range of 4 to 7 ml. Films of uniform thicknesses were chosen and kept in a desiccator containing anhydrous calcium chloride. The film was placed on a polished aluminum plate and the exact thickness was measured at five different places using the Minitector described earlier before TEML measurements.

The TEML was measured on a single volunteer using the method described by Spruit and Malten (46, 47). Before starting the measurements, the volunteer was asked to wash his left hand with soap and water and the hand was allowed to dry completely with the help of a soft cloth towel. The volunteer was a 29-year-old healthy male of Asian origin. The volunteer was then comfortably seated in the temperature- and humidity-controlled room for a period of 1 hour before starting the measurement. In this method, a stream of dry nitrogen was passed over the skin through a specially designed cup on the volar aspect of the forearm. The cup was kept in a specially designed plastic holder, which was then held in position on the skin by means of self-adhesive tapes attached to it as shown in Figure 1. The cup had two openings--nitrogen entered the cup through one and left the cup through the other and passed to an electrolytic water analyzer.¹¹ Thus the water present on the skin area

¹¹Electrolytic Moisture Analyzer (Model W), Mfrs. Eng. and Equip. Corp., Warrington, Pa.

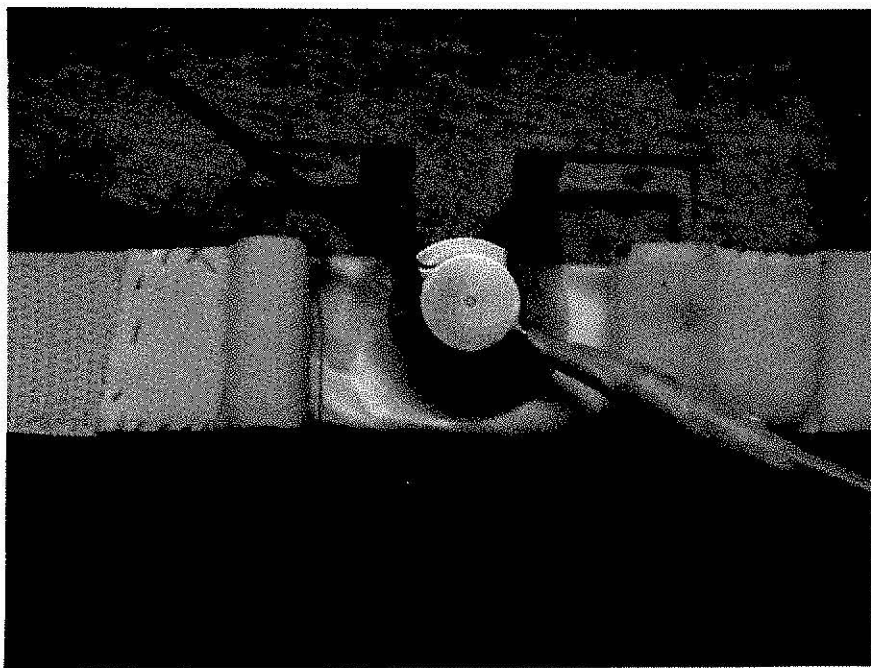


Figure 1. Skin Cup (0.62 cm^2) with Plastic Holder.

covered by the cup (area: 0.62 cm^2) entered the nitrogen flow and was conducted through the teflon tubing into the electrolytic water analyzer. The amount of water in the nitrogen stream could be recorded by a recorder attached to the water analyzer. The measurements were carried out at 22°C and 40% relative humidity, in a temperature- and humidity-controlled room. The gas stream was allowed to flow through the cell at a controlled rate of 100 standard cubic centimeters per minute and the metering circuitry was chosen so that the meter read in parts per million by volume of moisture in the gas.

In the electrolytic water analyzer, the moisture was absorbed from the gas stream flowing through it. By virtue of the cell design with its associated circuitry, a current was established whose magnitude depended on the rate of absorption of moisture.

The exact amount of moisture in the nitrogen gas from the cylinder was determined by passing nitrogen through the analyzer before the experiment. A square mark was made in the volar aspect of the forearm of the volunteer and the skin cup was secured in position tightly in the marked area. Then the hand was allowed to rest in position, until a steady value was noted in the recorder which usually took 15 to 25 minutes. Attainment of steady-state was assumed when a constant value was noted for a period of 15 minutes. After deducting

the amount of initial moisture in the gas from this value, the normal transepidermal moisture loss for the volunteer was calculated in $\text{mg}/\text{cm}^2/\text{hr}$ from the ppm/volume values as follows:

Temperature of measurement = $22^\circ\text{C} = 295^\circ\text{K}$

Observed atmospheric pressure = 768 mm of mercury

ppm (by volume) value observed = 48.1 (sample value)

Gas flow rate = 100 ml/min

Therefore, transepidermal moisture loss = 48.1×10^{-4}
ml/min

For ideal behavior:

$$\frac{P_1 V_1}{T_1} = \frac{P_2 V_2}{T_2}$$

$$\frac{760 \times 22.4}{273} = \frac{768 \times V_2}{295}$$

Therefore, $V_2 = 23.95$ liters

1 mole of water vapor = 23.95 liters

i.e. 18 g of water vapor = 23.98 liters

Therefore, rate of transepidermal moisture loss

$$\begin{aligned} &= \frac{18 \times 48.1 \times 10^{-7}}{23.95 \times 0.62} \\ &= 3494.04 \times 10^{-7} \text{ g}/\text{cm}^2/\text{hr} \\ &= 0.349 \text{ mg}/\text{cm}^2/\text{hr} \end{aligned}$$

The normal TEML was measured on each day of measurement.

The test film was then placed on the marked site of the skin after removing the cup, and the cup was then held

in position over the film as shown in Figure 2. The ppm value was noted once the steady-state was reached. The measurement was repeated thrice for each test film at three different locations within same marked area. At the end of three measurements, the film was removed from the skin site and a 20-minute rest period was given before starting with the next film.

Results and Discussion

Various films of lanolin alcohol were tested for their effect on transepidermal moisture loss as shown in Tables IV, V and VI. As can be seen from Table IV, the occlusive capability decreased with an increase in propylene glycol concentration in the matrix, when the concentration was varied from 0 to 25% w/w with a corresponding decrease in lanolin alcohol concentration.

The increase in ethyl cellulose concentration, with a corresponding decrease in lanolin alcohol concentration of the films at 5% w/w propylene glycol concentration, decreased the normal water vapor loss in the compositions tested as shown in Table V. Similarly, increasing the ethyl cellulose concentration resulted in the decrease in normal water vapor loss at 5% w/w concentration of hexadecyl alcohol as plasticizer, as shown in Table VI.

These studies have demonstrated that at the thicknesses studied, the films are relatively occlusive. It

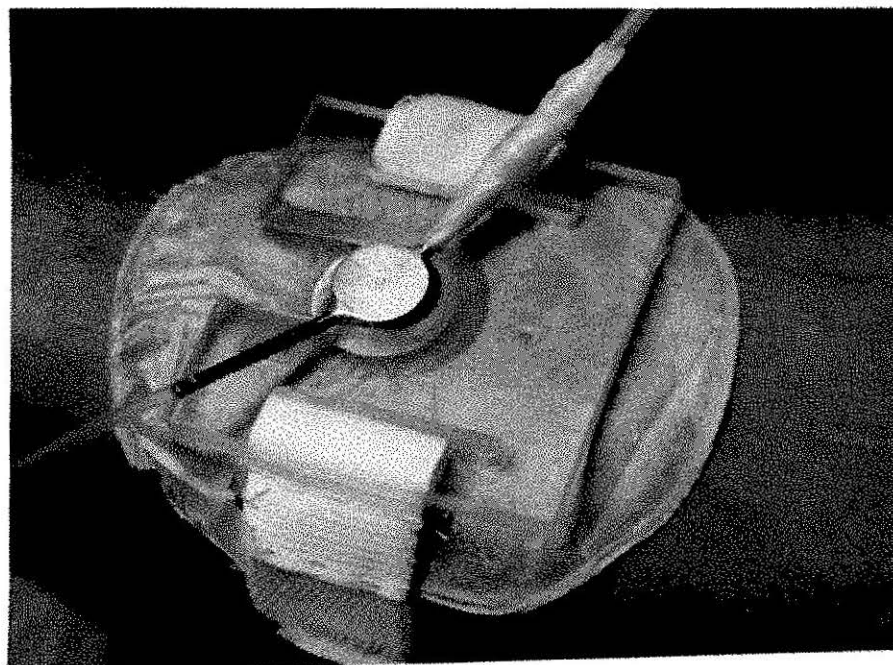


Figure 2. Skin cup (0.62 cm^2) placed on the film position on the volar aspect of the forearm of the volunteer.

Table IV

The Decrease in Transepidermal Moisture Loss of Human Forearm Skin After Application of Various Films Containing Differing Concentrations of Lanolin Alcohol and Propylene Glycol at 22° and 40% Relative Humidity

Lanolin Alcohol:		Water Vapor Loss ^c	
Ethyl Cellulose:	Film Thickness,	mg cm ⁻² hr ⁻¹	Occlusive
Propylene Glycol	μm ^b	x 10 ²	Capability,
Ratio ^a			Percent ^d
8.5 : 1.5 : 0.0	32 ± 4	0.4 ± 0.1	98.9
8.0 : 1.5 : 0.5	36 ± 3	1.3 ± 0.1	96.3
7.5 : 1.5 : 1.0	33 ± 4	2.5 ± 0.1	92.9
6.0 : 1.5 : 2.5	36 ± 4	4.6 ± 0.2	87.8

^a All films were prepared from 10% w/v solution of film formers in isopropyl alcohol.

^b Thicknesses are expressed as the mean ± standard deviation of five measurements.

^c Water vapor loss is expressed as the mean ± standard deviation of three measurements.

^d Occlusive capability was computed from the normal water vapor loss of 0.351 mg cm⁻² hr⁻¹ from the forearm skin.

Table V

The Decrease in Transepidermal Moisture Loss of Human Forearm Skin After Application of Various Films Containing Differing Concentrations of Lanolin Alcohol and Ethyl Cellulose at 22° and 40% Relative Humidity

Lanolin Alcohol:		Water Vapor Loss ^c	
Ethyl Cellulose:	Film Thickness,	mg cm ⁻² hr ⁻¹	Occlusive
Propylene Glycol	μm ^b	x 10 ²	Capability
Ratio ^a			Percent ^d
9.5 : 0.0 : 0.5	34 ± 1	3.6 ± 0.2	89.7
8.5 : 1.0 : 0.5	36 ± 3	2.5 ± 0.2	92.9
7.5 : 2.0 : 0.5	38 ± 1	0.8 ± 0.1	97.7
6.5 : 3.0 : 0.5	39 ± 2	0.5 ± 0.1	98.6

^a All films were prepared from 10% w/v solution of film formers in isopropyl alcohol.

^b Thicknesses are expressed as the mean ± standard deviation of five measurements.

^c Water vapor loss is expressed as the mean ± standard deviation of three measurements.

^d Occlusive capability was computed from the normal water vapor loss of 0.351 mg cm⁻² hr⁻¹ from the forearm skin.

Table VI

The Decrease in Transepidermal Moisture Loss of Human Forearm Skin After Application of Various Films Containing Differing Concentrations of Lanolin Alcohol and Ethyl Cellulose at 22° and 40% Relative Humidity

Lanolin Alcohol:		Water Vapor Loss ^c	
Ethyl Cellulose:	Film Thickness	mg cm ⁻² hr ⁻¹	Occlusive
Hexadecyl Alcohol	μm^b	$\times 10^2$	Capability
Ratio ^a			Percent ^d
9.0 : 0.5 : 0.5	35 \pm 4	2.4 \pm 0.2	93.2
8.5 : 1.0 : 0.5	33 \pm 4	2.0 \pm 0.2	94.3
8.0 : 1.5 : 0.5	38 \pm 3	0.9 \pm 0.2	97.4
7.5 : 2.0 : 0.5	37 \pm 1	0.6 \pm 0.1	98.3
6.5 : 3.0 : 0.5	39 \pm 1	0.3 \pm 0.1	99.1

^aAll films were prepared from 10% w/v solutions of film formers in isopropyl alcohol.

^bThicknesses are expressed as the mean \pm standard deviation of five measurements.

^cWater vapor loss is expressed as the mean \pm standard deviation of three measurements.

^dOcclusive capability was computed from the normal water vapor loss of 0.351 mg cm⁻² hr⁻¹ from the forearm skin.

would be of interest to determine their relative occlusive capability at lesser thicknesses (10 μm or less).

Chapter III

SOLUBILITY AND IN VITRO RELEASE STUDIES

After evaluating the physical characteristics of the films formed by lanolin alcohol with or without ethyl cellulose, it was decided to establish the release kinetics of the steroid triamcinolone acetonide¹² from the films of selected compositions, showing the effect of change in proportion of film formers and the solvent-plasticizer, and the nature of the solvent-plasticizer. As in the case of previous studies, propylene glycol or hexadecyl alcohol was used as the solvent-plasticizer.

The solubility profile of the steroid in the components of the matrix system was defined before establishing the release characteristics of the drug from selected films. For this purpose, the solubility of the steroid in propylene glycol, hexadecyl alcohol, and lanolin alcohol was individually determined. The solubility of the steroid in ethyl cellulose was assumed to be negligible.

Solubility Studies

The solubilities of triamcinolone acetonide in propylene glycol and in hexadecyl alcohol were determined

¹²Johnson and Johnson, Dermatological Div., New Brunswick, N.J.

at room temperature ($22^{\circ} \pm 0.5^{\circ}$) and at $37^{\circ} \pm 0.5^{\circ}$.

Solubility was determined by adding excess steroid to 25 ml of propylene glycol or hexadecyl alcohol in 50-ml screw-capped glass bottles. A teflon-coated magnetic bar was placed in each of the bottles prior to capping them tightly. The bottles were then kept stirred in water baths on magnetic stirrers for a period of six days. All studies were conducted in triplicate in a constant temperature room.

Prior to sampling, the stirring was stopped and the excess steroid was allowed to settle. An aliquot was then filtered using a filter¹³ with 0.22 μ filter paper. The filtration and sampling apparatus were allowed to equilibrate to room condition for a period of 48 hours prior to use. The first 5 ml of filtrate was rejected in each case to avoid any reduction in solubility values due to the adsorption of the steroid to the filter paper (49).

The concentration of the steroid in the solvent was determined using a scanning spectrophotometer,¹⁴ after appropriate dilutions of the solvent in spectral grade anhydrous methanol¹⁵ and by using a Beer's law plot prepared

¹³Swinnex-25, Millipore Filter Corp., Bradford, Mass.

¹⁴Perkin-Elmer (Model-202), Coleman Instruments Div., Oak Brook, Ill.

¹⁵Mallinckrodt Chemical Works, St. Louis, Mo.

in that concentration range using known concentrations of the steroid. The steroid solubility in propylene glycol was determined at 239 nm and in hexadecyl alcohol at 265 nm.

Apparent Solubility in Lanolin Alcohol

The apparent solubility was estimated by determining the effective partition coefficient of the steroid in lanolin alcohol-water system at $22^{\circ} \pm 0.5^{\circ}$ and $37^{\circ} \pm 0.5^{\circ}$.

About 100 to 200 mg of the melted lanolin alcohol was coated as a thin film in the bottom of a preweighed 50-ml flat-bottom polypropylene beaker and allowed to cool to room temperature. The beaker was weighed again to determine the exact weight of the film. Ten microliters of ^3H -triamcinolone acetonide¹⁶ in ethanol with a radioactivity concentration of 10 μCi was taken in a crystallization vial and the solvent was allowed to evaporate in a steady stream of dry nitrogen. The radioactive steroid was then dissolved in one ml of distilled water. The aqueous solution was then transferred to a 100-ml volumetric flask. Cold triamcinolone acetonide necessary to make a 0.002% w/v solution was weighed and added to the contents of the volumetric flask. The steroid was dissolved by addition of sufficient distilled water and warming the flask. This solution was then allowed to cool to room temperature

¹⁶Amersham/Searle Corp., Arlington Heights, Ill.

before making up the volume. Twenty milliliters of this solution was then added to the beaker in the lanolin alcohol film after verifying the activity of this solution. The beaker was covered with parafilm and was kept in a temperature controlled water bath at appropriate temperature until equilibrium was reached. At each time interval, the solution was briefly stirred and one milliliter sample was withdrawn from the aqueous phase into a scintillation vial and 10 ml of dioxane containing scintillation fluid¹⁷ was added to each vial. The vials were then counted in a scintillation counter.¹⁸ Quench corrections were done using the external standard ratio method and by using the standard quench curves prepared with standard tritiated water.¹⁹

The apparent partition coefficient was then determined using the equation proposed by Chowhan (50).

$$K_e = \frac{A_1/W_1}{A_a/W_a} = \frac{(A'_a - A_a) W_a}{A_a W_1} \quad (\text{eq. 8})$$

where K_e is the effective partition coefficient, A_1 and A_a are the amounts of drug in lanolin alcohol and in aqueous phase respectively, A'_a is the initial amount of drug in

¹⁷Hydroscint, ICN Chemical and Radioisotope Div., Irvine, CA.

¹⁸Bekman CPM-100 Liquid Scintillation Counter, Fullerton, CA.

¹⁹Amersham/Searle Corp., Arlington Heights, Ill.

water, and W_1 and W_a are the weights of lanolin alcohol and water respectively.

The apparent solubility was calculated from the relationship:

$$K_e = \frac{C_s^I}{C_s^W} \quad (\text{eq. 9})$$

where C_s^I is the apparent solubility in lanolin alcohol and C_s^W is the solubility in water.

Density Determination

Accurate determination of true powder densities of lanolin alcohol and ethyl cellulose was accomplished by using a simple attachment in the Quantasorb Surface Area and Pore Volume Instrument.²⁰

The attachment was a volume-calibrated glass cell with attached limbs. The glass cell was first cleaned, dried and weighed. It was then filled with lanolin alcohol or ethyl cellulose as full as possible while still allowing adequate passageway for the unimpeded flow of gas above the sample surface. The filling was accomplished through an opening at the bottom of the cell and the opening was closed tightly with a lightly greased ground glass stopper. The cell was weighed again and the difference in weight was noted. The limbs of the cell were then

²⁰Quantachrome Corp., Greenwale, N.Y.

inserted into a cell holder in the instrument and the cell was filled with nitrogen flowing through the Quantasorb circuit. When the cell was completely filled with nitrogen as indicated by the constant signal in the instrument, the cell was isolated from its limbs by using a 4-way stopcock placed at the junction of the cell and its limbs. At this stage the entire Quantasorb system and the limbs were purged by helium. When a constant signal was obtained, the digital integrator was zeroed. Now the 4-way stopcock was turned to its original position, thus allowing helium to purge the isolated sample cell. The signal due to this purged nitrogen was noted and it was calibrated by injecting definite quantities of nitrogen in the instrument using the gas sampling syringes supplied with the instrument. The calibration signal should match the sample signal as closely as possible.

The volume of nitrogen swept out of sample cell was calculated from the following equation:

$$V_{N_2} = V_{\text{calibration}} \times \frac{\text{Area sample cell}}{\text{Area calibration}} \quad (\text{eq. 10})$$

where,

$V_{\text{calibration}}$ = volume of nitrogen injected using the sampling syringe,

Area sample cell = the integrator reading when nitrogen was purged from the powder-filled cell with helium,

Area calibration = the integrator reading when the calibration volume was injected.

The true powder volume in the sample cell can be calculated from the following relationship:

$$V_{\text{powder}} = V_{\text{cell}} - V_{N_2} \quad (\text{eq. 11})$$

where V_{cell} is the empty cell volume and V_{N_2} can be calculated from equation 10.

From the volume and weight of the powder, the density was calculated since density = weight/volume.

Determination of Release Rate

Lanolin alcohol, ethyl cellulose, and propylene glycol or hexadecyl alcohol were weighed in required quantities to make 10 ml of 10% w/v solution in a 25-ml beaker. Five ml of isopropyl alcohol was added to this and the contents allowed to go into solution by gentle heating. The solution was cooled to room temperature and was transferred to a 10-ml volumetric flask. The required concentration of unlabeled triamcinolone acetonide was added to this and dissolved. The quantity of steroid used was in addition to the 10% w/v concentration of film formers and plasticizer. One half mCi of radiolabeled steroid in 0.5 ml of ethanol (specific activity 66 mCi/mg) was taken in a crystallization vial and the solvent was allowed to evaporate in a steady stream of dry nitrogen. The radioactive

steroid was then dissolved in 0.5 ml of isopropanol. Ten microliters of this radiolabeled steroid solution with a radioactive concentration of approximately 10 μ Ci was added to the solution in the volumetric flask using a precision pipette.²¹ The exact concentration of radioactivity in the solution was determined by adding 13 ml of an organic scintillant²² to 0.2 ml aliquot of this solution in a scintillation vial and counting the activity of the sample in a liquid scintillation counter. External standard ratio method was employed for calculating counting efficiency using a standard quench curve. After the addition of 10 μ l of radioactive steroid solution, the volume was made up to 10 ml. Two milliliters of this solution were pipetted into a preweighed aluminum Petri dish (diameter: 7.5 cm; area: 44.2 cm²), using the precision pipette and the solution was allowed to spread evenly thus completely covering the bottom surface.

The Petri dish was then kept on a flat surface for 24 hours and the solvent was allowed to evaporate to form a uniform film. The complete evaporation of the solvent was confirmed by weighing the Petri dish to a constant weight. The film coated Petri dish was stored in a

²¹ Finn timer, Markson Science Inc., Del Mar, CA.

²² OCSTM, Amersham Corp., Arlington Heights, Ill.

desiccator containing anhydrous calcium chloride at least 24 hours prior to release studies. The good adherence of the film formed to the Petri dish insured that only the exposed surface area was available for release. Each film was inspected at the end of release experiment. No visible pores and "peeling" of the film from the Petri dish were observed.

The release studies were conducted in a dissolution assembly²³ with following modifications. The dissolution flasks were replaced by 1000 ml flat-bottomed polypropylene beakers and the dissolution basket assemblies were replaced by stainless steel stirrers with propeller diameter of 4.5 cm. The film-coated Petri dishes were placed in the bottom of the beakers. The beakers were then held in position by means of plexiglass discs with central circular port for the stirrers and a small sampling port. The stirring assembly was then set in position so that stirring blade was approximately 2 cm above the film surface. Three hundred milliliters of preheated (37°) distilled water was carefully added to each, and the stirring was maintained at 40 rpm. The water bath in the assembly was maintained at 37°. The release apparatus described above is shown in Figures 3a and 3b.

During the release study, one milliliter samples were drawn at frequent time intervals over a period of 24 hours

²³Hanson Research Corp., Northridge, CA.

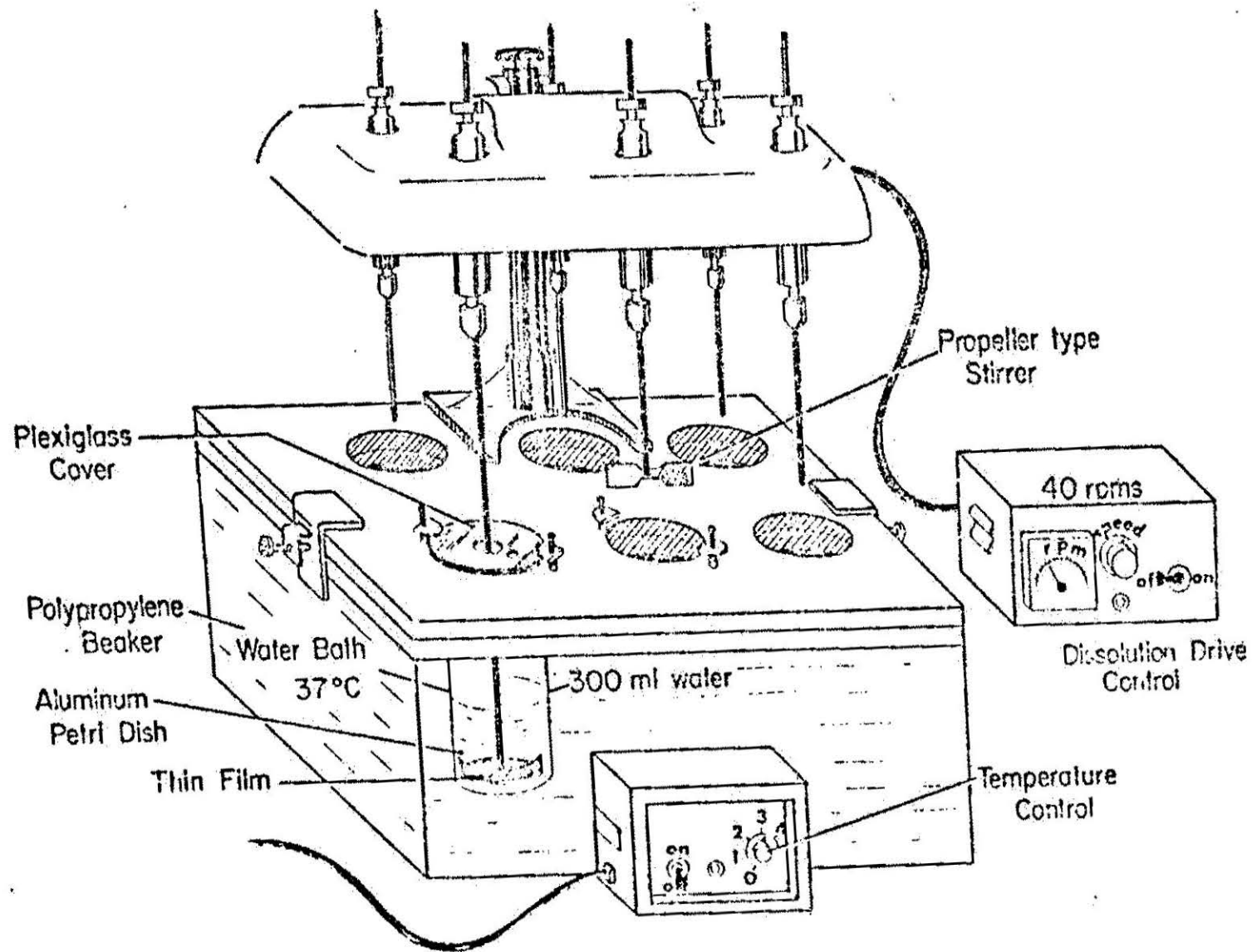


Figure 3a. Apparatus used for *In Vitro* release studies

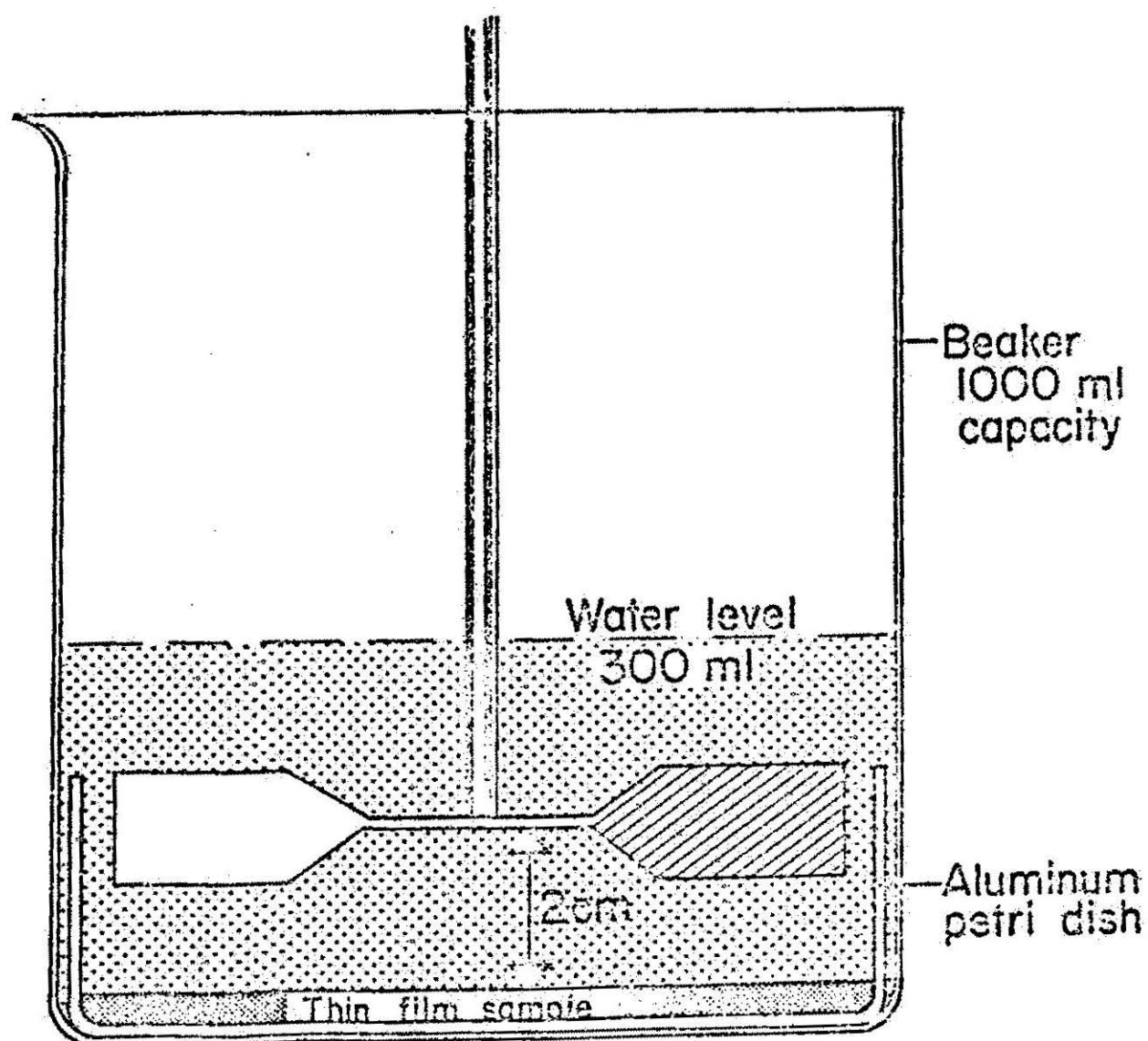


Figure 3b. Enlarged view of experimental beaker.

and they were replaced by 1 ml of distilled water to keep constant volume. Each sample was pipetted into a scintillation vial to which 10 ml of scintillation fluid²⁴ was added. These samples were then counted. The external standard ratio method was employed for calculating counting efficiency using a standard quench curve.

The release data were computed and analyzed by a computer²⁵ using a Fortran program (GRADER) written for this purpose. This program is given on the following page. Appropriate corrections were applied for the sample withdrawn and media added. All release studies were conducted in triplicate.

Results and Discussion

Solubility Studies

The solubility of triamcinolone acetonide in propylene glycol and in hexadecyl alcohol, at 22° and 37° is shown in Table VII. The solubility of triamcinolone acetonide in propylene glycol was found to be about eight times greater than its solubility in hexadecyl alcohol. The attainment of equilibrium solubility in 3 days was noted by the fact that there was no difference in absorbance between the samples analyzed at the end of 3 and 6 days.

²⁴Hydroscint, ICN Chemical and Radioisotope Div., Irvine, CA.

²⁵Burroughs B6700

Table VII

Solubility of Triamcinolone Acetonide in
Propylene Glycol and in Hexadecyl Alcohol

Solvent	Solubility ^a	
	22° \pm 0.5°	37° \pm 0.5°
	mg/ml	mg/ml
Propylene Glycol	8.04 \pm 0.19	12.09 \pm 0.18
Hexadecyl Alcohol	0.92 \pm 0.03	1.25 \pm 0.05

^aAll solubility values are expressed as mean \pm standard deviation of three determinations.

GRADER PROGRAM

```

L
100  % INPUT OF DATA
200  DIMENSION TIME(15),CPH(15),EFF(15),SQT(15),DPH(15),TDPH(15),C(15),
300  *CTDPH(15),PRL(15),TRL(15),Q(15)
400  READ(5,/)TAC,PDPH,AREA,VOL
500  READ(5,/)TIME,CPH,EFF
600  % WRITE HEADINGS
700  WRITE(6,1)
800  1 FORMAT(2X,'TIME (T)',3X,'SQT (T)',6X,'CPH',6X,'% EFF',
900  *7X,'DPH',7X,'TOTAL DPH',6X,'CORRECTED',2X
1000  *,',RELEASED',4X,'TOTAL AMOUNT',5X,'GX100')
1050  PRINT 2
1060  2 FORMAT(2X,'( MIN. )',5X,'TOTAL DPH',15X,'RELEASED(MG.)',2X
1080  *,',(MG/CMXCM)')
1300  % COMPUTE AND WRITE RESULTS
1400  C(1)=0
1500  DPH(1)=0
1600  DOSI=2,15
1700  SQT(1)=SQRT(TIME(1))
1800  DPH(1)=(CPH(1)/EFF(1))*100
1900  TDPH(1)=VOL*DPH(1)
2000  C(1)=C(1-1)+DPH(1-1)
2100  CTDPH(1)=TDPH(1)+C(1)
2200  PRL(1)=100*CTDPH(1)/PDPH
2300  TRL(1)=PRL(1)*TAC/100
2400  Q(1)=TRL(1)*100/AREA
2500  5 WRITE(6,25)TIME(1),SQT(1),CPH(1),EFF(1),
2600  *DPH(1),TDPH(1),CTDPH(1),PRL(1),TRL(1),Q(1)
2700  25 FORMAT(1X,I6,6X,F5.2,6X,F8.2,4X,F5.2,4X,F8.2,
2800  *4X,F10.2,5X,F10.2,4X,F5.2,
2900  *9X,F6.4,9X,F6.4)
3000  STOP
3100  END
#

```

NOTE: This program calculates the percent drug released and the total amount of drug released per unit area, based on the given CPM and percent efficiency of the liquid scintillation counter.

The hexadecyl alcohol was found to have strong absorption at 239 nm. This necessitated the use of the side of the absorption band. Actual measurements of steroid concentration were made at 265 nm after verifying compliance with Beer's law.

The effective partition coefficient of triamcinolone acetone between lanolin alcohol and water at 22° and 37° was found to be 5.99 ± 0.12 and 12.77 ± 0.15 , respectively. From this the apparent solubility of the steroid was calculated to be 0.13 ± 0.01 and 0.41 ± 0.01 mg/g, respectively, using equation 9. The attainment of equilibrium in 5 days was noted by running the partitioning experiment for a period of 8 days with daily sampling of the aqueous phase. No further reduction in radioactivity present in the aqueous phase was observed beyond a period of five days. The data as expressed above represent the mean \pm standard deviation of three determinations.

Density Determination

The densities of lanolin alcohol and ethyl cellulose were determined to be 0.98 and 1.38 g/ml, respectively, using the pycnometric method discussed earlier. The density values of propylene glycol (d 1.03) and hexadecyl alcohol (d 0.84) were supplied by their manufacturers.

Release Kinetics

Higuchi (36) has shown that Q versus $t^{\frac{1}{2}}$ relationship

is common to both homogeneous as well as granular matrix systems during the uni-directional leaching or extraction from a simple planar surface containing suspended drug (equations 3 and 4). These equations describe drug release as being linear with square root of time,

$$Q = k t^{\frac{1}{2}} \quad (\text{eq. 12})$$

where k is the release rate constant. In the case of a homogeneous matrix system,

$$k = \sqrt{D(2A - C_s) C_s} \quad (\text{eq. 13})$$

and in the case of a granular matrix system,

$$k = \sqrt{\frac{D\epsilon}{\tau} (2A - \epsilon C_s) C_s} \quad (\text{eq. 14})$$

These relationships have been confirmed experimentally by various workers using plastic and wax matrices (51-56). For both equations 3 and 4 to be obeyed, it is necessary that A be greater than C_s in the case of homogeneous matrix or ϵC_s in the case of granular matrix by a factor of three or four.

A first-order mechanism in which the release rate is proportional to the amount of drug left in the matrix might also be considered possible for the release of drugs from polymeric systems of this type (32,37). This can be shown as

$$\log (Q_{\infty} - Q) = \frac{-Kt}{2.303} + \log Q_{\infty} \quad (\text{eq. 15})$$

where Q_{∞} is the initial amount of drug present per unit area of the film, Q is the amount of drug present per unit area at time t , and K is the first-order rate constant.

In this study, the release data were analyzed to ascertain which release mechanism might be operative. The regression analysis was done by a regression program (PCHEM/REGG), using the triplicate data obtained for each composition of the film and concentration of the drug. In all these cases, the drug concentration was more than three times its solubility in the matrix based upon the solubility of the steroid in lanolin alcohol and the solvent-plasticizer. Q versus $t^{\frac{1}{2}}$ treatments of data for the release of triamcinolone acetonide from matrices containing propylene glycol and hexadecyl alcohol are shown in Tables VIII and IX, respectively. The corresponding first-order treatments of data are shown in Tables X and XI. Q versus $t^{\frac{1}{2}}$ plots of data are shown in Figures 4-7 and the corresponding first-order plots are as shown in Figures 8-11. As can be seen from Tables VIII and IX, the linear square root of time plots have high correlation coefficients. The observed lag times (time intercept extrapolated to $Q = 0$) were relatively small and the release rate constant, k , increased with increase in drug concentration for the same composition of the matrix as predicted by equations 3 and 4.

Table VIII

Q versus $t^{\frac{1}{2}}$ Treatments of Triamcinolone Acetonide Release Data from
Films Containing Propylene Glycol as Plasticizer at $37^{\circ} \pm 0.5^{\circ}$

Lanolin Alcohol:	Drug				
Ethyl Cellulose:	Concentration ^a	A	t_{lag}	$k \cdot 10^3^b$	Correlation
Propylene Glycol	%	mg/cm ³	min	mg cm ⁻² min ^{-$\frac{1}{2}$}	Coefficient
Ratio					r^b
8.0 : 1.5 : 0.5	0.50	5.12	24.05	0.405	0.992
8.0 : 1.5 : 0.5	0.99	10.24	4.70	1.056	0.995
8.0 : 1.5 : 0.5	1.96	20.47	0.35	1.650	0.994
8.0 : 1.5 : 0.5	2.91	30.71	6.26	2.849	0.994
8.5 : 1.0 : 0.5	0.50	5.04	17.14	0.448	0.997
8.5 : 1.0 : 0.5	0.99	10.07	-0.53	1.131	0.980
8.5 : 1.0 : 0.5	1.96	20.14	7.18	1.969	0.996
8.5 : 1.0 : 0.5	2.91	30.21	-0.07	2.384	0.979

^aBased on weight of drug per weight of dry film.

^bAll k and r values were computed from the regression line drawn from the data obtained by triplicate runs at each level by using the TEKTRONIX (Model 4005-1) graphics terminal.

Table IX

Q versus $t^{\frac{1}{2}}$ Treatments of Triamcinolone Acetonide Release Data from
Films Containing Hexadecyl Alcohol as Plasticizer at $37^{\circ} \pm 0.5^{\circ}$

Lanolin Alcohol:	Drug				
Ethyl Cellulose:	Concentration ^a	A	t_{lag}	$k \cdot 10^{3b}$	Correlation
Hexadecyl Alcohol	%	mg/cm ³	min	mg cm ⁻² min ^{-$\frac{1}{2}$}	Coefficient
Ratio					r^b
8.0 : 1.5 : 0.5	0.50	5.06	5.94	0.454	0.990
8.0 : 1.5 : 0.5	0.99	10.11	0.52	1.270	0.965
8.0 : 1.5 : 0.5	1.96	20.22	0.14	2.116	0.967
8.0 : 1.5 : 0.5	2.91	30.33	-0.07	2.926	0.932
8.5 : 1.0 : 0.5	0.50	4.98	-3.72	0.540	0.957
8.5 : 1.0 : 0.5	0.99	9.95	5.26	0.940	0.996
8.5 : 1.0 : 0.5	1.96	19.90	0.06	1.940	0.974
8.5 : 1.0 : 0.5	2.91	29.85	1.98	2.756	0.982

^aBased on weight of drug per weight of dry film.

^bAll k and r values were computed from the regression line drawn from the data obtained by triplicate runs at each level by using the TEKTRONIX (Model 4005-1) graphics terminal.

Table X

First-Order Treatments of Triamcinolone Acetonide Release Data from
Films Containing Propylene Glycol as Plasticizer at $37^{\circ} \pm 0.5^{\circ}$

Lanolin Alcohol:	Drug			
Ethyl Cellulose:	Concentration ^a	t_{lag}	$K \cdot 10^{3b}$	Correlation
Propylene Glycol	%	min	min^{-1}	Coefficient
Ratio				r^b
8.0 : 1.5 : 0.5	0.50	-54.51	0.639	0.990
8.0 : 1.5 : 0.5	0.99	-56.62	1.169	0.983
8.0 : 1.5 : 0.5	1.96	-195.74	0.696	0.978
8.0 : 1.5 : 0.5	2.91	-113.20	0.858	0.986
8.5 : 1.0 : 0.5	0.50	-65.47	0.764	0.996
8.5 : 1.0 : 0.5	0.99	-41.54	1.833	0.919
8.5 : 1.0 : 0.5	1.96	-92.23	0.929	0.995
8.5 : 1.0 : 0.5	2.91	-258.55	0.636	0.957

^aBased on weight of drug per weight of dry film.

^bAll k and r values were computed from the regression line drawn from the data obtained by triplicate runs at each level by using the TEKTRONIX (Model 4005-1) graphics terminal.

Table XI

First-Order Treatments of Triamcinolone Acetonide Release Data from
Films Containing Hexadecyl Alcohol as Plasticizer at $37^{\circ} \pm 0.5^{\circ}$

Lanolin Alcohol:	Drug			
Ethyl Cellulose:	Concentration ^a	t_{lag}	$K \cdot 10^{3b}$	Correlation
Hexadecyl Alcohol	%	min	min^{-1}	Coefficient
Ratio				r^b
8.0 : 1.5 : 0.5	0.50	-137.21	0.775	0.974
8.0 : 1.5 : 0.5	0.99	-7.65	2.342	0.993
8.0 : 1.5 : 0.5	1.96	-149.23	1.130	0.965
8.0 : 1.5 : 0.5	2.91	-243.97	0.882	0.929
8.5 : 1.0 : 0.5	0.50	-178.80	1.321	0.956
8.5 : 1.0 : 0.5	0.99	-121.66	0.847	0.990
8.5 : 1.0 : 0.5	1.96	-178.05	0.934	0.969
8.5 : 1.0 : 0.5	2.91	-172.92	0.796	0.967

^aBased on weight of drug per weight of dry film.

^bAll k and r values were computed from the regression line drawn from the data obtained by triplicate runs at each level by using the TEKTRONIX (Model 4005-1) graphics terminal.

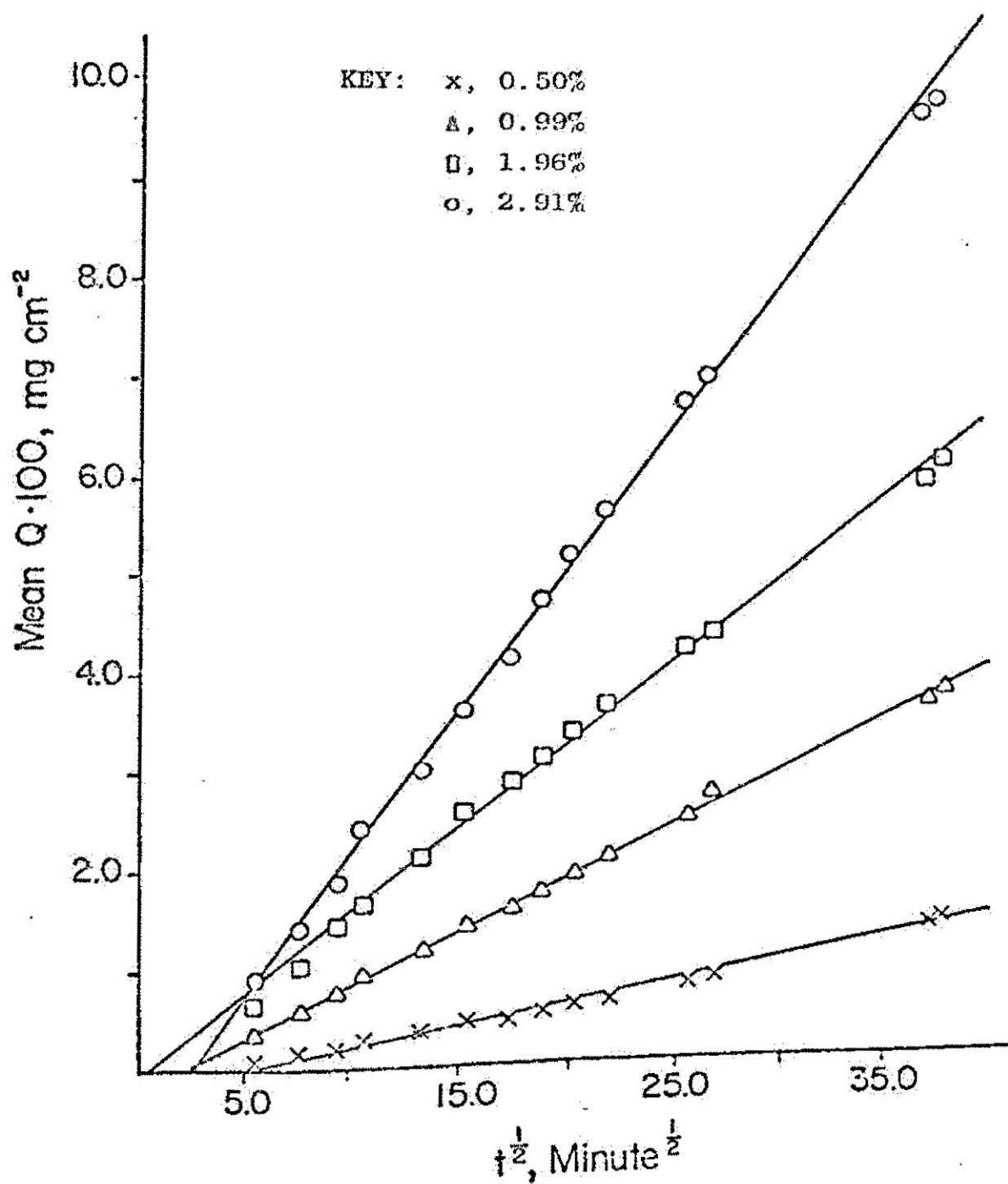


Figure 4. Drug release from films containing lanolin alcohol-ethyl cellulose-propylene glycol (8.0 : 1.5 : 0.5) at different concentrations of triamcinolone acetonide.

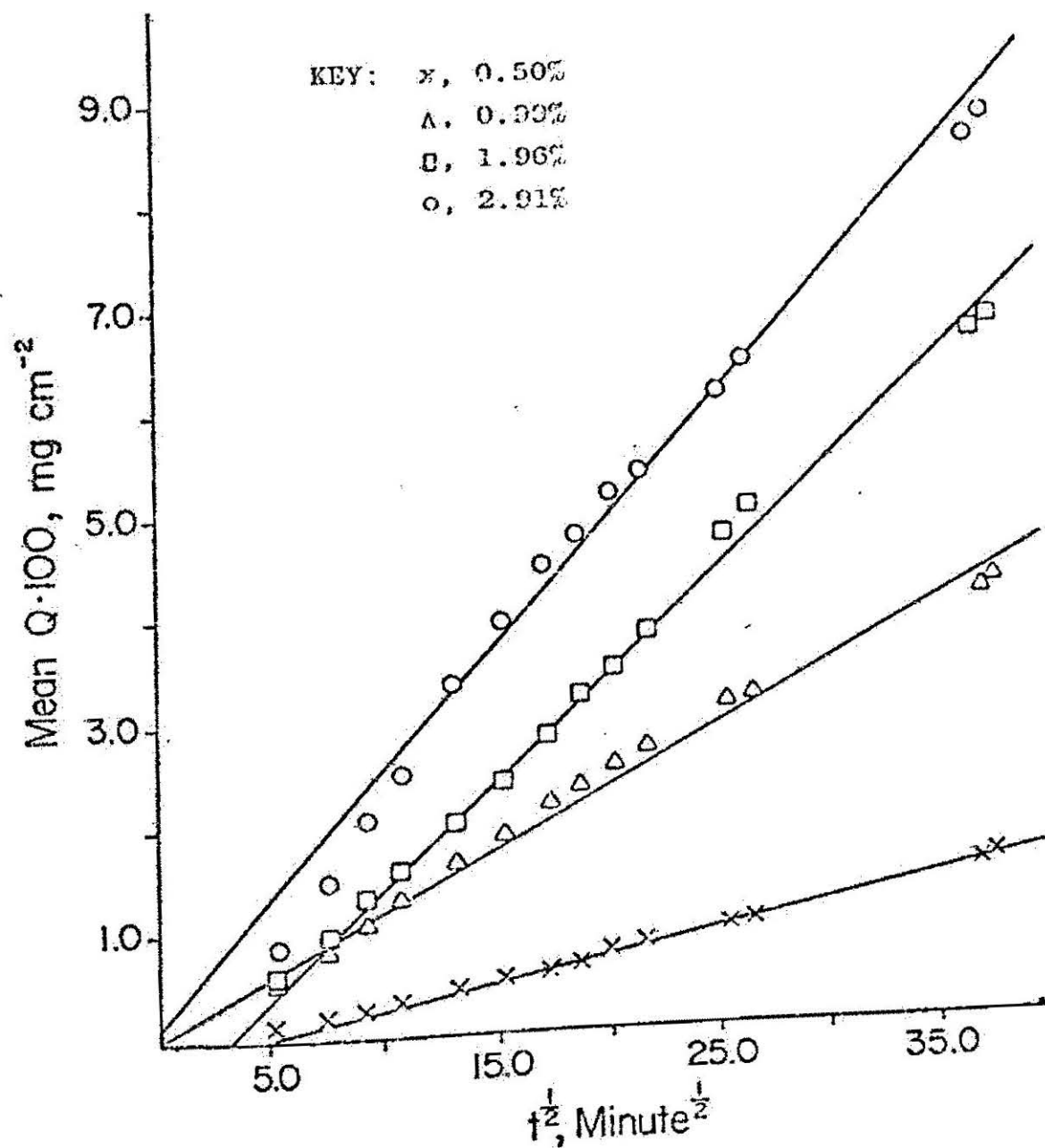


Figure 5. Drug release from films containing lanolin alcohol-ethyl cellulose-propylene glycol (8.5 : 1.0 : 0.5) at different concentrations of triamcinolone acetonide.

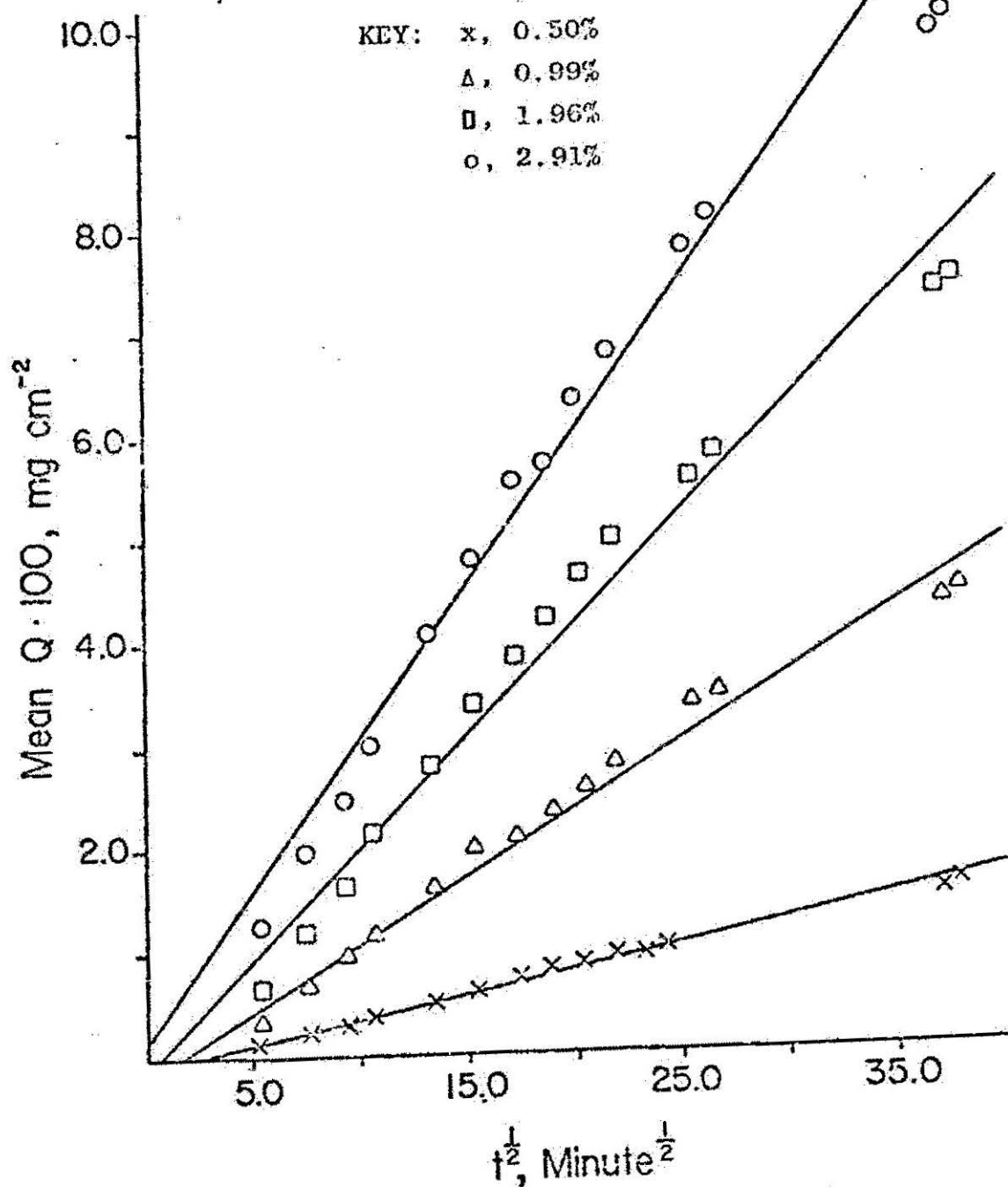


Figure 6. Drugs release from films containing lanolin alcohol-ethyl cellulose-hexadecyl alcohol (8.0 : 1.5 : 0.5) at different concentrations of triamcinolone acetonide.

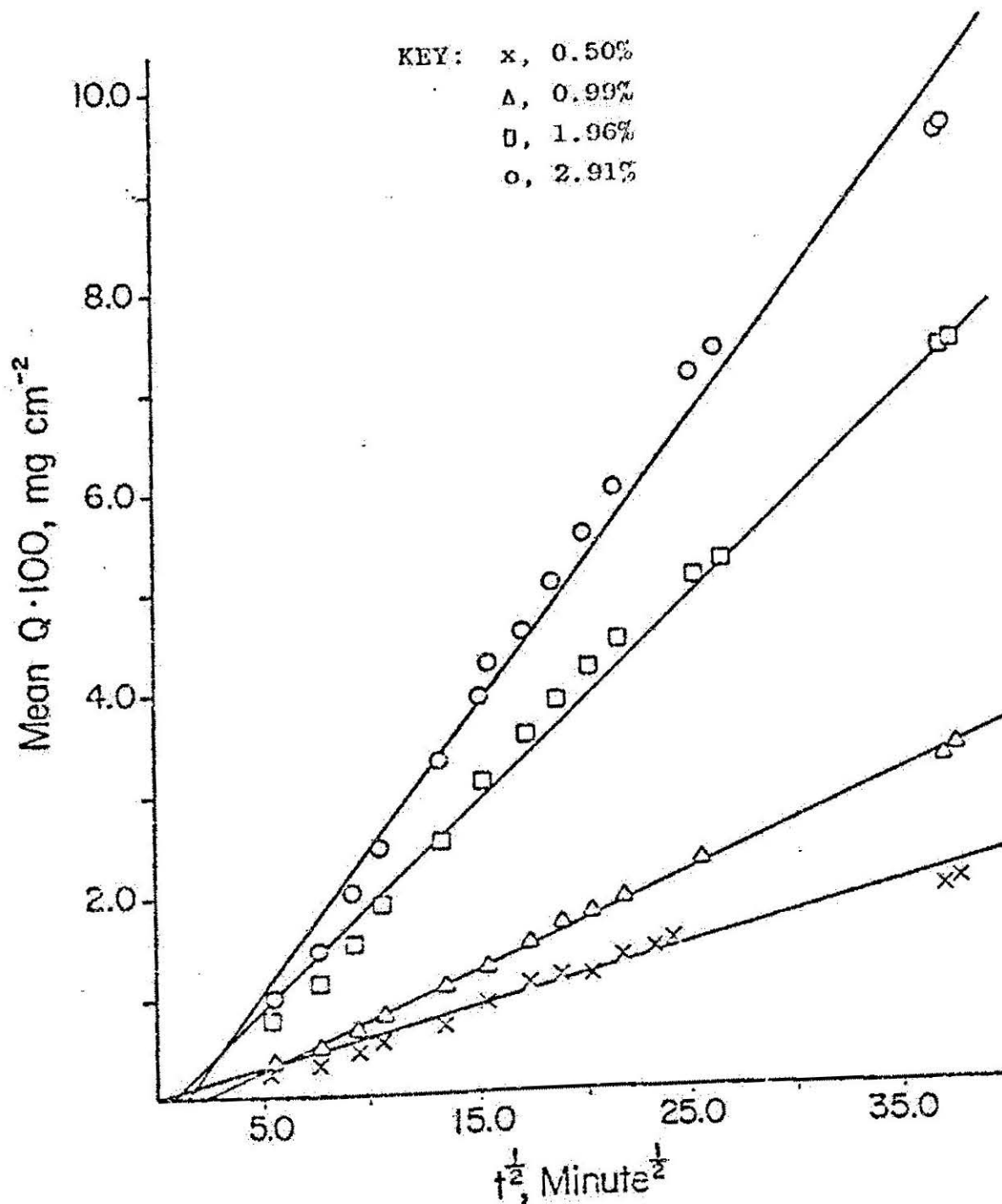


Figure 7. Drug release from films containing lanolin alcohol-ethyl cellulose-hexadecyl alcohol (8.5 : 1.0 : 0.5) at different concentrations of triamcinolone acetonide.

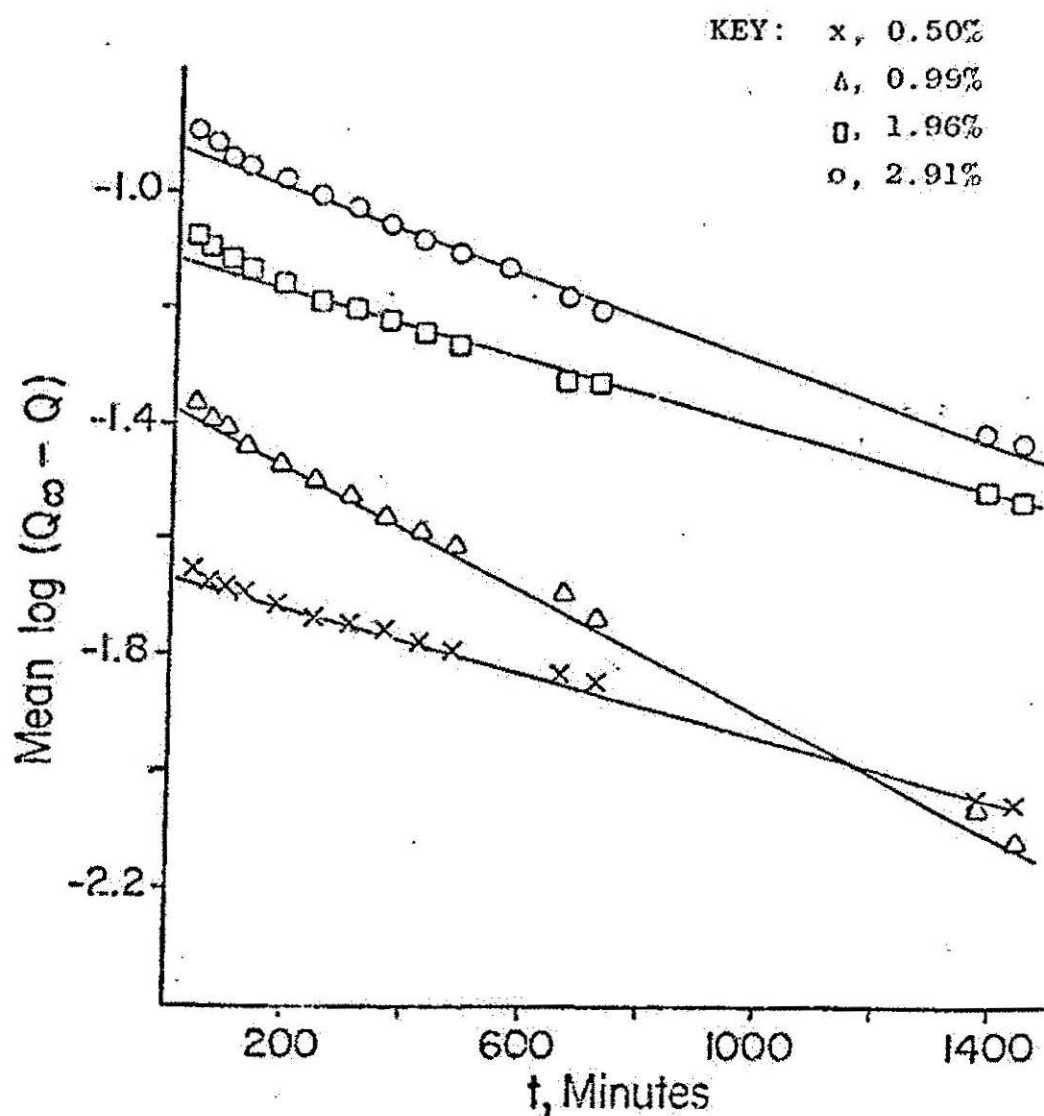


Figure 8. First-order plots of drug release from films containing lanolin alcohol-ethyl cellulose-propylene glycol (8.0 : 1.5 : 0.5) at different concentrations of triamcinolone acetonide.

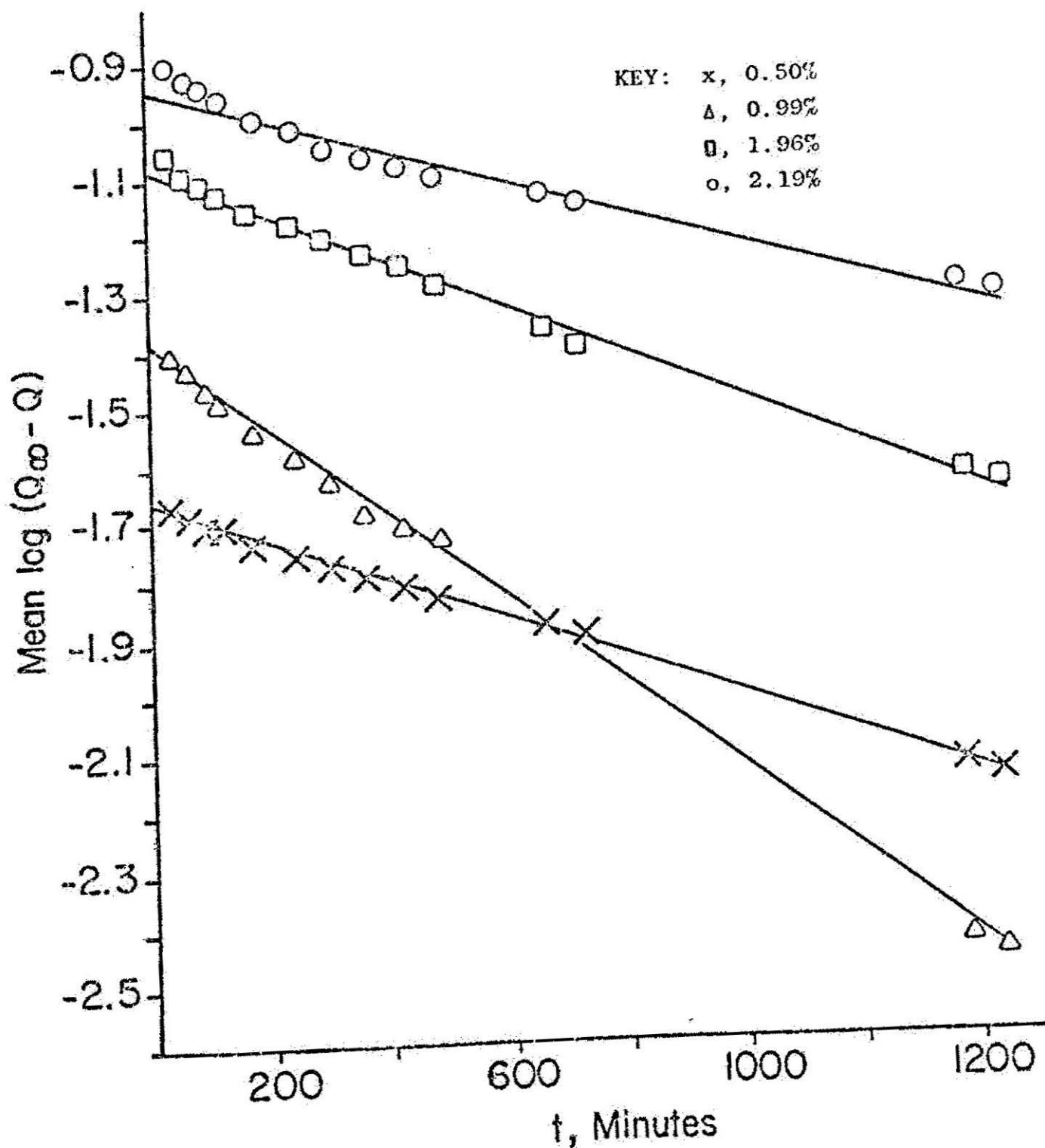


Figure 9. First-order plots of drug release from films containing lanolin alcohol-ethyl cellulose-propylene glycol (8.5 : 1.0 : 0.5) at different concentrations of triamcinolone acetonide.

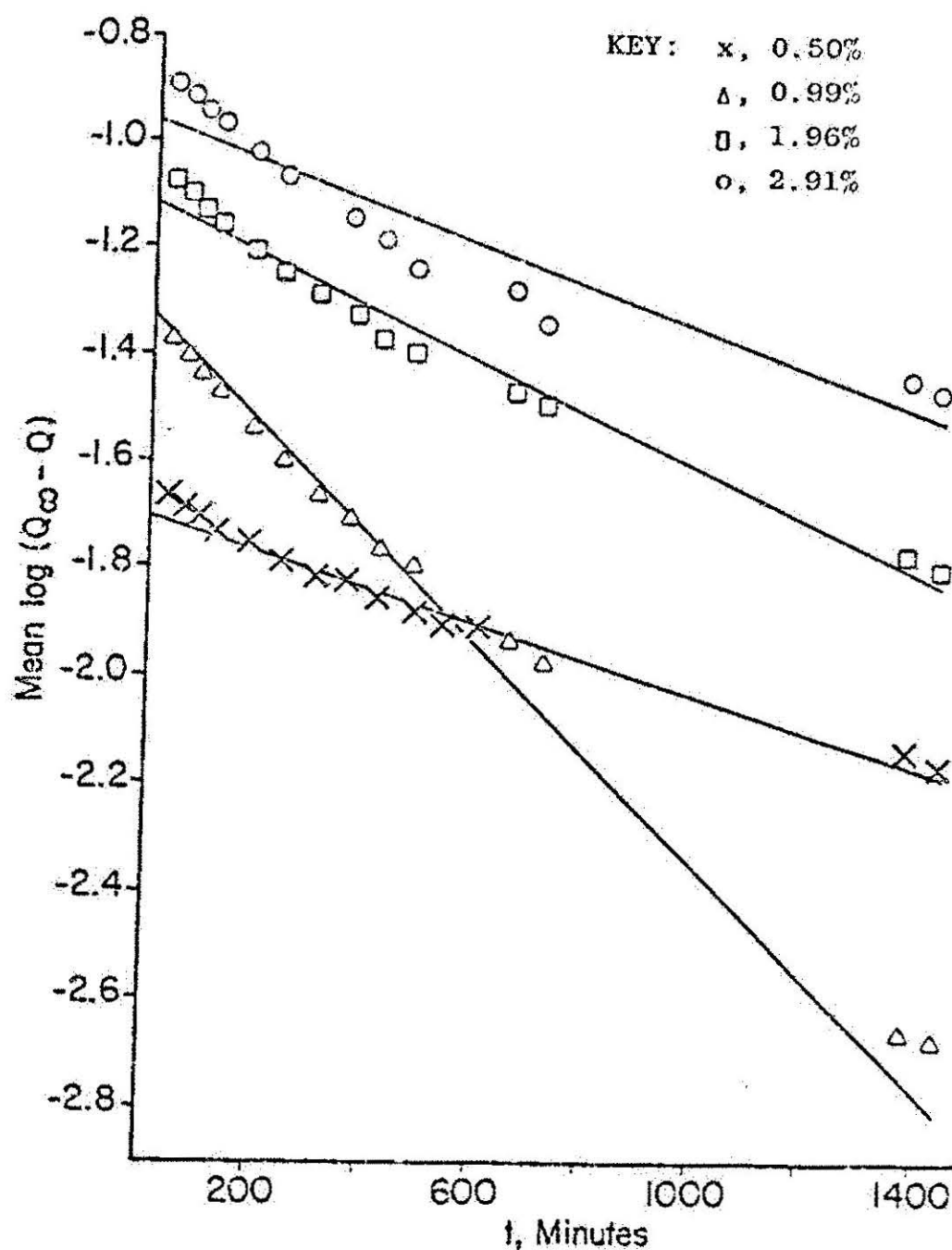


Figure 10. First-order plots of drug release from films containing lanolin alcohol-ethyl cellulose-hexadecyl alcohol (8.0 : 1.5 : 0.5) at different concentrations of triamcinolone acetonide.

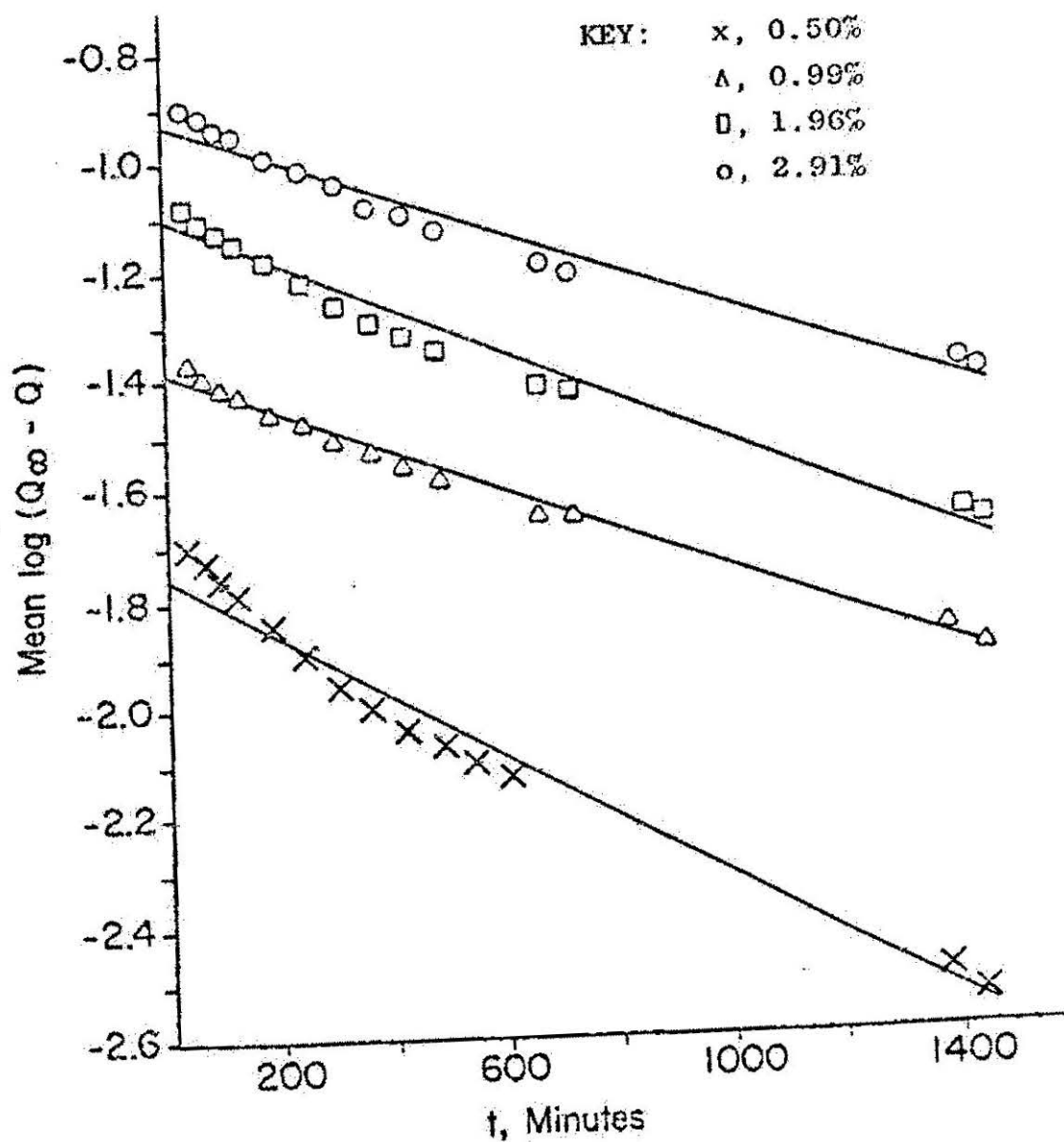


Figure 11. First-order plots of drug release from films containing lanolin alcohol-ethyl cellulose-hexadecyl alcohol (8.5 : 1.0 : 0.5) at different concentrations of triamcinolone acetonide.

On the other hand, relatively high correlation coefficients were also obtained for the first-order treatment of the release data. However, highly negative lag times and a lack of constancy in the first-order constants (Tables X and XI) strongly point against this mechanism. An initial curvature effect was also noticed in all first-order treatments of data as shown in Figures 8-11.

All the Q versus $t^{\frac{1}{2}}$ plots show the mean of three observations (Figures 4-7) because of the small standard deviations involved. The maximum amount of drug released varied from 7.6% to 46.0% of its solubility in distilled water at 37°, the solubility being 0.03 mg/ml. A good linear fit was observed in all cases with slight negative deviation at longer time intervals and at time intervals up to 60 minutes and for higher concentration of the drug. The deviation at higher time intervals might be due to the exhaustion of the drug in the suspension phase and due to the increase in diffusion distance for the drug in the film. The negative deviations observed up to 60 minutes at higher concentrations may be due to the surface drug present. The observed lag times never exceeded 1.7% of the total release period (24 hours).

Effect of Drug Concentration

The amount of drug present per unit volume of the matrix (A) was calculated from the density values of the materials in the matrix and is reported in Tables VIII

and IX. The effect of change in drug concentration on the release rate constant, k , was tested using these concentrations of triamcinolone acetonide for each composition of the film tested. In all cases, the k versus A plots were slightly more linear than k versus $A^{\frac{1}{2}}$ plots as shown in Table XII. While both equations 13 and 14 predict a linear relationship between k and $A^{\frac{1}{2}}$, the observed results could also be explained in terms of granular matrix alone, if initial porosity was assumed to be very small. Such an assumption appears to be reasonable since these matrices contain a plasticizer. Higuchi (36) has shown that in those instances where initial porosity is very small or where the fraction of the matrix volume occupied by the drug is relatively large, $\epsilon \approx K A$ and equation 14 reduces to

$$k = A \sqrt{\frac{Dk}{\tau} (2 - K C_s) C_s} \quad (\text{eq. 16})$$

where K is equal to the specific volume of the drug, if A is expressed in terms of grams per milliliter.

Effect of Change in Plasticizer

According to equation 13, one would expect the k value to decrease substantially upon changing the solvent-plasticizer from propylene glycol to hexadecyl alcohol in view of nearly eight-fold difference in the solubility of the drug in the vehicle. This would be a valid expectation if D , diffusion coefficient of the drug in the matrix, was

Table XII

Comparison of Correlation Coefficients from
 k versus A plots and k versus $A^{\frac{1}{2}}$ plots for
 films containing Propylene Glycol or
 Hexadecyl Alcohol as Plasticizer

Lanolin Alcohol-Ethyl Cellulose:	k vs A^a	k vs $A^{\frac{1}{2}}$
Plasticizer ratio	Correlation Coefficient	Correlation Coefficient
<u>Plasticizer: Propylene Glycol</u>		
8.0 : 1.5 : 0.5	0.993	0.965
8.5 : 1.0 : 0.5	0.999	0.960
<u>Plasticizer: Hexadecyl Alcohol</u>		
8.0 : 1.5 : 0.5	0.993	0.943
8.5 : 1.0 : 0.5	0.982	0.972

^a k values were obtained from Q vs $t^{\frac{1}{2}}$ plot of release data.

not altered significantly by change of solvent-plasticizer. Alternatively, in the same release medium, equation 14 would predict comparable k values for the two systems differing only in the nature of solvent-plasticizer, provided the volume, porosity, and tortuosity of the matrix are not significantly altered. This might well be the case for the systems studied, since the k values for the hexadecyl alcohol systems (Table IX) were found to be comparable to propylene glycol systems (Table VIII).

Effect of Vehicle Composition

Table XIII describes the effect of drug solubilization on drug release, by varying propylene glycol-lanolin alcohol concentrations. The steroid concentration (0.10% w/w) was chosen, in part, to permit this study to be conducted with films of sufficient integrity. The maximum release of drug was obtained in systems containing the drug in near-saturated solution (5% propylene glycol). The percent drug released decreased with systems containing no propylene glycol (major solubilizer) as well as with systems containing the solubilizer in excess of that required to dissolve the steroid completely (8 to 15% w/w propylene glycol). These vehicle effects are consistent with those reported for fluocinolone acetonide from propylene glycol-water gels (16).

Table XIII

Release of 0.10% w/w Triamcinolone Acetonide from
Matrices Containing Different Percentages
of Propylene Glycol after 6 Hours at 37°

Lanolin Alcohol-Ethyl Cellulose- Propylene Glycol Ratio	Amount ^a Released, %
8.5 : 1.5 : 0.0	37.11 ± 0.62
8.0 : 1.5 : 0.5	40.46 ± 1.12
7.7 : 1.5 : 0.8	32.38 ± 0.71
7.0 : 1.5 : 1.5	30.48 ± 0.29

^aAll values are expressed as (Mean ± S.D.) of three runs.

Chapter IV

IN VITRO PENETRATION STUDIES

After establishing the in vitro release kinetics of triamcinolone acetonide from the film-forming compositions of lanolin alcohol, the in vitro skin penetration profile of the steroid from these delivery systems was studied. An important goal of this study was to establish whether diffusion from the vehicle or diffusion across the skin was the rate-limiting step for the test drug, triamcinolone acetonide. The results of this study were also expected to provide greater insight into the factors that govern availability of triamcinolone acetonide from these matrix compositions.

In vitro penetration studies are subject to possible complications due to many variables, such as the vehicle effect on the permeability properties of the skin as discussed by Poulsen (15). Laboratory measurements of steady-state penetration rates and the experimental determination of the physical parameters affecting penetration rates provide valuable bench marks against which less well-controlled in vivo topical bioavailability studies can be compared.

Much of the definitive work being done today in the area of percutaneous absorption makes use of excised human skin as a model for its living counterpart. Recent publications by Scheuplein et al. (57), Ostrenga et al. (58, 59) and Newbold and Stoughton (60) demonstrate application of the in vitro technique to important problems in dermatopharmacology. Recently Chowhan et al. (61) have shown that for comparative evaluations of the formulation effects on percutaneous absorption, in vitro studies with animal skin may not provide information in agreement with in vitro human skin.

Determination of In Vitro Penetration Rate and Distribution of Steroid

Preparation of Skin. All the penetration experiments reported here utilize human abdominal skin obtained at autopsy. Immediately following incision, the skin was placed in a plastic bag and stored in a freezer for periods up to but not exceeding three months. This method of storage has been reported not to damage the skin (62). Before the experiment, the skin was allowed to thaw gradually to room temperature, following which the skin was placed on a smooth dissection board with the epidermal surface flat in contact with the board. All subcutaneous fat was completely removed by a scalpel. From each specimen (the skin of a single donor), 10 to 12 pieces of suitable sizes were cut.

Skin Cell. Each piece of skin was mounted in a special glass cell as illustrated in Figure 12. The skin cell used has been described in detail by Franz (62). The skin cell consisted of a lower glass chamber with a sampling port. A Teflon-coated magnetic bar placed at the bottom of the cell provided efficient mixing. The lower chamber was enclosed by a water jacket which allowed circulation of water at the selected temperature. The skin was placed in position on an o-ring between two ball joints of the top and bottom chambers, using a pinchtype, ground-joint clamp. The diffusion area was 2.01 cm^2 . The epidermal side of the skin was exposed to ambient conditions ($\approx 23^\circ$ and 40% relative humidity). A solution containing 0.9% sodium chloride and 0.01% thimersol was heated to expel dissolved gases and cooled to room temperature; 6.2 ml of this solution was pipetted into the skin cell bathing the dermal side. The sampling port was closed by a rubber closure and any air bubbles on the dermal side were carefully removed by slightly tilting the cell. Each cell was mounted on a magnetic stirrer. During each run, 12 cells were mounted on 12 magnetic stirrers fixed to a laboratory frame (48" x 48" x 18") as shown in Figure 13. The temperature of fluid in the lower chamber was maintained at $37^\circ \pm 0.5^\circ$ by circulating water from a constant temperature water circulator²⁶ through the jacket of each cell. By this

²⁶Haake Model-FE, VWR Scientific Inc., San Francisco, CA.

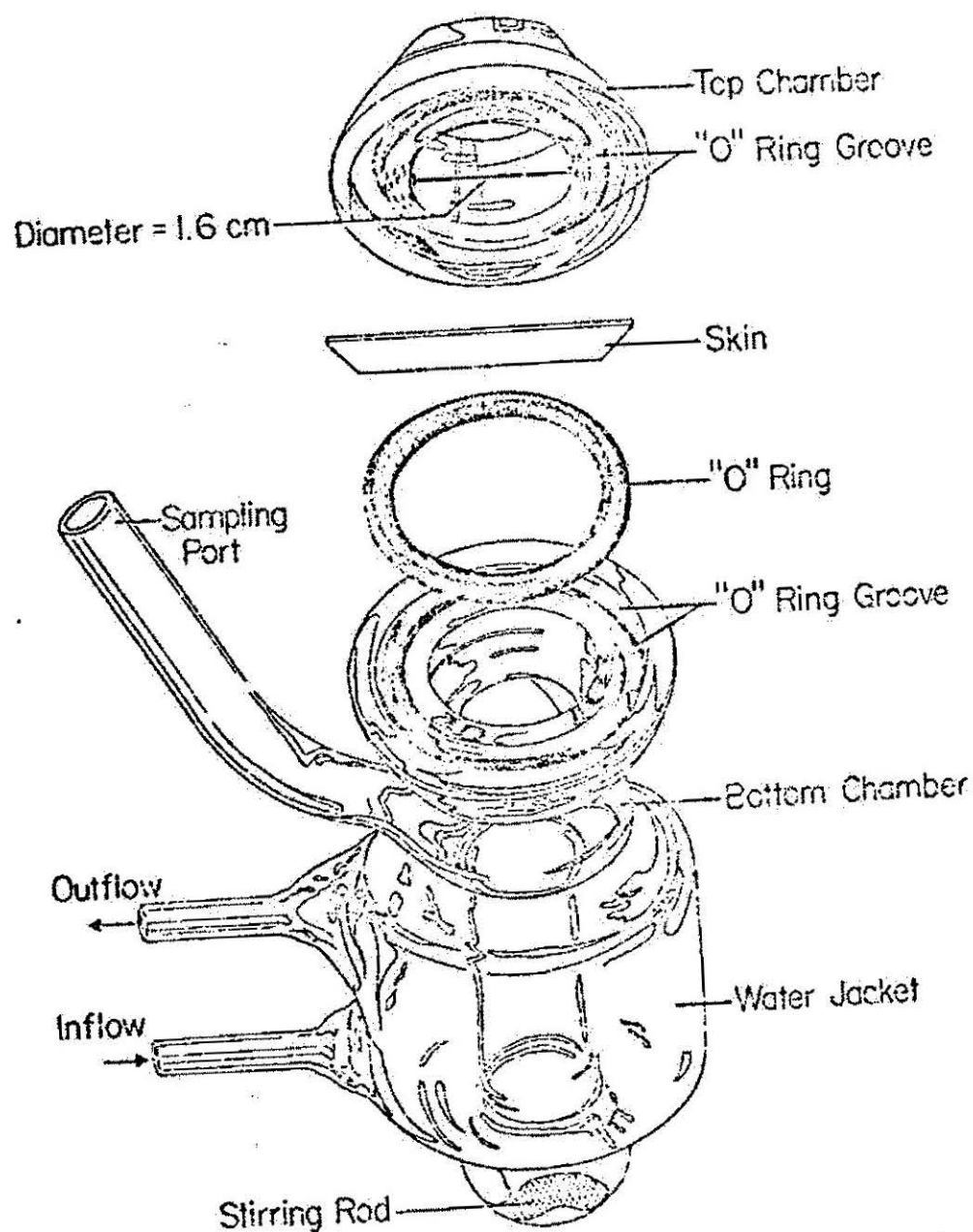


Figure 12. Diagrammatic representation of the diffusion cell used in penetration studies.

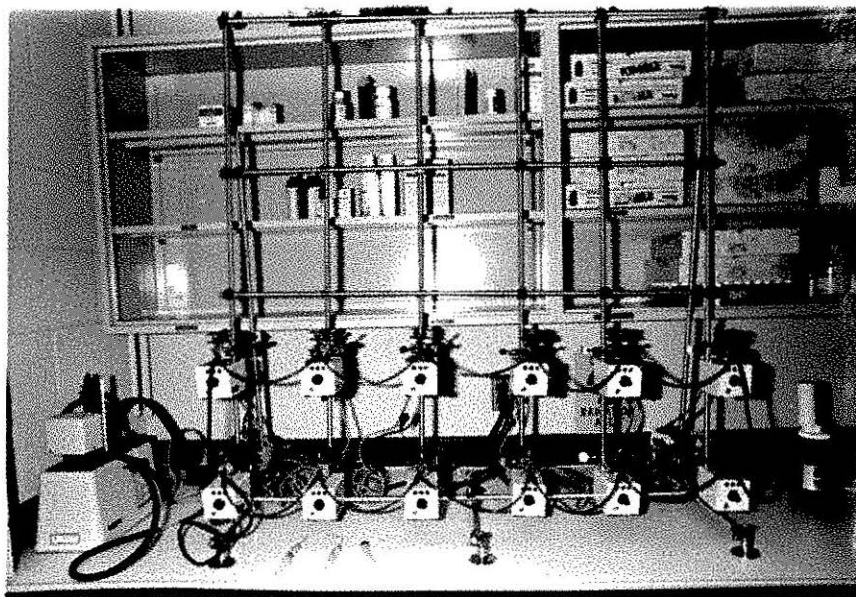


Figure 13. In vitro skin penetration assembly used in these studies.

arrangement, the epidermal side of the skin was exposed to ambient laboratory conditions while the dermal side was kept at $37^{\circ} \pm 0.5^{\circ}$ to simulate in vivo use conditions.

After mounting, each piece of skin was allowed to stand for 4 hours before beginning the experiment. This allowed some time for equilibration with respect to temperature and relative humidity of the surrounding environment.

Preparation of Steroid Solutions. Lanolin alcohol, ethyl cellulose, and propylene glycol or hexadecyl alcohol were weighed in required quantities to make 10-ml of 10% w/v solution in a 25-ml beaker. Five milliliters of isopropyl alcohol was added to this and the contents allowed to go into solution by gentle heating. The solution was cooled to room temperature and was transferred to a 10-ml volumetric flask. The required concentration of cold triamcinolone acetonide was added to this and allowed to go into solution. The quantity of steroid used was in addition to the 10% w/v concentration of film formers and plasticizer. This solution was then made up to volume using isopropyl alcohol. Two compositions which did not have any lanolin alcohol and ethyl cellulose were also tested. One contained 0.01% w/v of the steroid in isopropanol, and the other contained 0.01% w/v steroid and 1.5% w/v propylene glycol in isopropanol (henceforth referred to as compositions A and B respectively in the text).

Before each experiment, 0.5 ml of the prepared solution of desired composition was pipetted into a 2-ml beaker containing 5 μ Ci of ^3H -triamcinolone acetonide using a precision pipette. The beaker was covered and the solution was mixed thoroughly. Then 100 μ l of this radioactively labeled solution was pipetted onto the skin surface. This volume was found to be sufficient to spread across the entire exposed surface area of 2.01 cm^2 . The volatile isopropanol was allowed to escape in all cases.

At selected time intervals, following the addition of the solution to the epidermis, the receptor solution was completely removed through the sampling port using a disposable syringe with its needle attached to a thin flexible plastic tubing. This allowed for a quick and complete removal of the receptor solution and refilling with 6.2 ml of fresh saline solution. One milliliter of the removed receptor solution was then pipetted into a scintillation vial to which 10 ml of premade aqueous scintillation fluid²⁷ was added before counting.

The amount of quench due to the saline solution was determined using the external standard ratio method. The total amount of steroid penetrated at different time intervals was calculated using a Fortran program (LSC) written for this purpose which is shown in the following pages. The penetration studies were conducted for a period of 7 days.

²⁷ACSTM Amersham Corp., Arlington Heights, Ill.

LSC PROGRAM

```

L
100 FILE 4(KIND=DISK, FILETYPE=7, TITLE="CPM.")
130  Z THIS PROGRAM CALCULATES THE DPM BASED ON THE GIVEN
140  Z CPM AND PERCENT EFFICIENCY OF THE RADIOACTIVITY COUNTER.
150  Z IT THEN COMPUTES THE TOTAL DPM BASED ON THE VOLUME OF
160  Z SAMPLE UTILIZED FOR THE EXPERIMENT (0.2ML). THE TOTAL
170  Z DPM IS THEN CORRECTED TO ACCOUNT FOR THE
180  Z EXPERIMENTAL PROCEDURE. THE PERCENT DRUG RELEASED
190  Z AND THE TOTAL AMOUNT OF DRUG PENETRATING PER UNIT
200  Z AREA IS THUS CALCULATED USING STANDARD EQUATIONS.
210  Z NOTE THE FOLLOWING RECORDED MESSAGE FOR BETTER
220  Z UNDERSTANDING OF THE PROGRAM.
230  Z C = COUNTER
240  Z T = TIME IN MINUTES
250  Z D = AMOUNT OF DRUG PRESENT INITIALLY
260  Z A = AREA OF SKIN EXPOSED IN SQUARE CMS.
270  Z DPMCH = DPM FOR THE STUDY
280  Z R = PERCENT DRUG RELEASED
290  Z AP = TOTAL AMOUNT OF DRUG PENETRATED IN NANO GRAMS.
300  Z Q = AMOUNT PENETRATED PER UNIT AREA IN NANO GMS./SQ.CM.
310  Z EFF = EFFICIENCY
320  Z IDPM = TOTAL DISINTEGRATIONS PER MINUTE
330  Z CTDPM = CORRECTED TOTAL DISINTEGRATIONS PER MINUTE
340  Z CTDPA = CELL FOR STORAGE OF CTDPM IN PROGRAM.
350  Z DPM1 = DPM FOR FIRST DATA
360  Z DPM2 = DPM FOR SECOND DATA
370  Z DPM3 = DPM FOR THIRD DATA
380  Z CPM = COUNTS PER MINUTE
390  Z THE PROGRAM WORKS EXPECTEDLY ONLY WHEN THE FIRST
400  Z THREE DATAS ARE FED SEQUENTIALLY
410  Z NOTE : FEED A NEGATIVE TIME DATA TO INDICATE END.
420  Z PROGRAM PASSAGED BY RAJARAM.
430      REAL D, A, DPMCH, CPM, EFF, DPM, IDPM, CTDPM, DPM1
440      REAL DPM2, DPM3, CTDPA, R, AP, Q
450      PRINT//, '#####'
460      * '#####'
470      WRITE (6,123)
480 123 FORMAT (' TIME(MRS)      CPM      ZEFFICIENCY      DPM      ',
490      *      ' TOTAL DPM      CORR TOT DPM      ZPENETRATED      ',
500      *      ' TOTAL AMT.      GMS/SQ.CM. )' )
510      PRINT//, '#####'
520      * '#####'
530      C = 0
540      READ (4,150) D, DPMCH

```

```

550 150 FORMAT (16,F10.2)
560 A = 2.01
570 170 READ (4,180) T, CPM, EFF
580 180 FORMAT (13,F6.1,F5.2)
590 IF (T.EQ. 0) GO TO 520
600 C = C + 1
610 DPM = (CPM * 100)/EFF
620 TDPM = DPM * 6.2
630 IF (C.EQ. 1) GO TO 280
640 IF (C.EQ. 2) GO TO 310
650 IF (C.EQ. 3) GO TO 340
660 IF (C.EQ. 4) GO TO 370
670 IF (C.EQ. 4) GO TO 400
680 280 CTDP = TDPM
690 DPM1 = DPM
700 GO TO 420
710 310 CTDP = DPM1 + TDPM
720 DPM2 = DPM
730 GO TO 420
740 340 CTDP = DPM1 + DPM2 + TDPM
750 DPM3 = DPM
760 GO TO 420
770 370 CTDP = DPM1 + DPM2 + DPM3 + TDPM
780 CTDPA = CTDP
790 GO TO 420
800 400 CTDP = CTDPA + TDPM
810 CTDPA = CTDP
820 420 R = (CTDP * 100) / DPMCE
830 AP = (D * R) / 100
840 Q = AP/A
850 WRITE (5,490) T, CPM, EFF, DPM, TDPM, CTDP, R, AP, Q
860 490 FORMAT (3X,13,6X,F6.1,6X,F5.2,5X,F7.2,3X,F8.2,
870 * 6X,F9.2,7X,F7.4,7X,F5.2,9X,F10.4)
880 GO TO 170
890 520 STOP
900 END
#

```


The sampling was more frequent during the first 2 days than the following 5 days. All penetration studies were conducted in duplicate.

At the termination of a penetration experiment and after removing the receptor solution, 1 ml isopropanol was added to the epidermal surface in each cell and the cells were covered with cover slips for a period of 5 minutes. During that period the epidermal surface was washed of the remaining applied dose and the isopropanolic solution was transferred to a scintillation vial. A second washing was performed with 0.5 ml of isopropanol and this solution was also collected in the same vial. This procedure was repeated in all the 12 cells and 10 ml of a commercial organic scintillant²⁸ was added to the washings in each of the 12 vials. The activity in the vials was then counted. External standard ratio method was employed for calculating counting efficiency using a standard quench curve.

The skin was then removed from the cell and the circular portion of the skin that was in contact with the bathing fluid was cut out using a surgical scissor. The epidermis and dermis were easily separated by means of a forceps and collected separately in two scintillation vials. One milliliter of a tissue solubilizer²⁹ was

²⁸OCS, Amersham Corp., Arlington Heights, Ill.

²⁹NCS*, Amersham Corp., Arlington Heights, Ill.

pipetted into the vial containing epidermis and 2.5 ml into the vial containing dermis. Digestion of the tissue by the solubilizer to give a clear solution was accomplished by keeping the vials in an oven at $50^{\circ} \pm 1^{\circ}$ for a period of 4 hours. If the dermal tissues were not completely solubilized by this procedure, the contents of the vial were homogenized using a tissue homogenizer.³⁰ The homogenizer was washed with 0.5 ml of solubilizer and the wash was collected in the same vial and the vial contents digested for an additional one hour. Clear solutions could be obtained by this procedure. Then, to each of the vials 10 ml of the organic counting scintillant was added and the samples were counted. External standard ratio method was employed for applying quench correction. Standard quench curves were prepared by using fresh samples of dermis and epidermis having similar area as used in the penetration experiments and standard tritiated water of known radioactive concentration.

Results and Discussion

The penetration data obtained for the two compositions A and B were analyzed by plotting Q (amount penetrated per unit area) against time. All the plots were made by using mean Q values obtained from the duplicate runs using a graphics program [(Q024114) GRAPHICS]. Based

³⁰Tissuemizer^R (Model-SDT), Tekmar Co., Cincinnati, Ohio.

upon this plot, a regression analysis of the steady-state region of the penetration curve was carried out since equation 6 is applicable only in the steady-state region of penetration. The regression analysis was carried out using a regression program (PCHEM/REGG) and the regression line was extrapolated to time axis to establish the lag times as shown by the dotted lines in Figure 14. For these two compositions, A and B, penetration across the skin would be expected to be the rate-limiting step for the absorption of steroid. The composition B should have a near-saturated solution of the steroid in propylene glycol at room temperature after the evaporation of isopropanol based on the solubility of triamcinolone acetonide in propylene glycol. The penetration data obtained from the Q versus t plots are shown in Table XIV. The plot for composition B was curvilinear after the lag time period and seemed to fit into two steady-state regions. The existence of two steady-state regions suggested significant time-dependent changes in the interplay of forces governing the penetration of steroid across the skin. There is a notable variation in the concentration of the steroid in the donor phase during the extended period of study. For example, the concentration of the steroid in the donor phase dropped from 10 μg to 7.25 μg . About 56.4% of the drop occurred during first 67 hours of this study. Also, the simultaneous diffusion of some propylene glycol

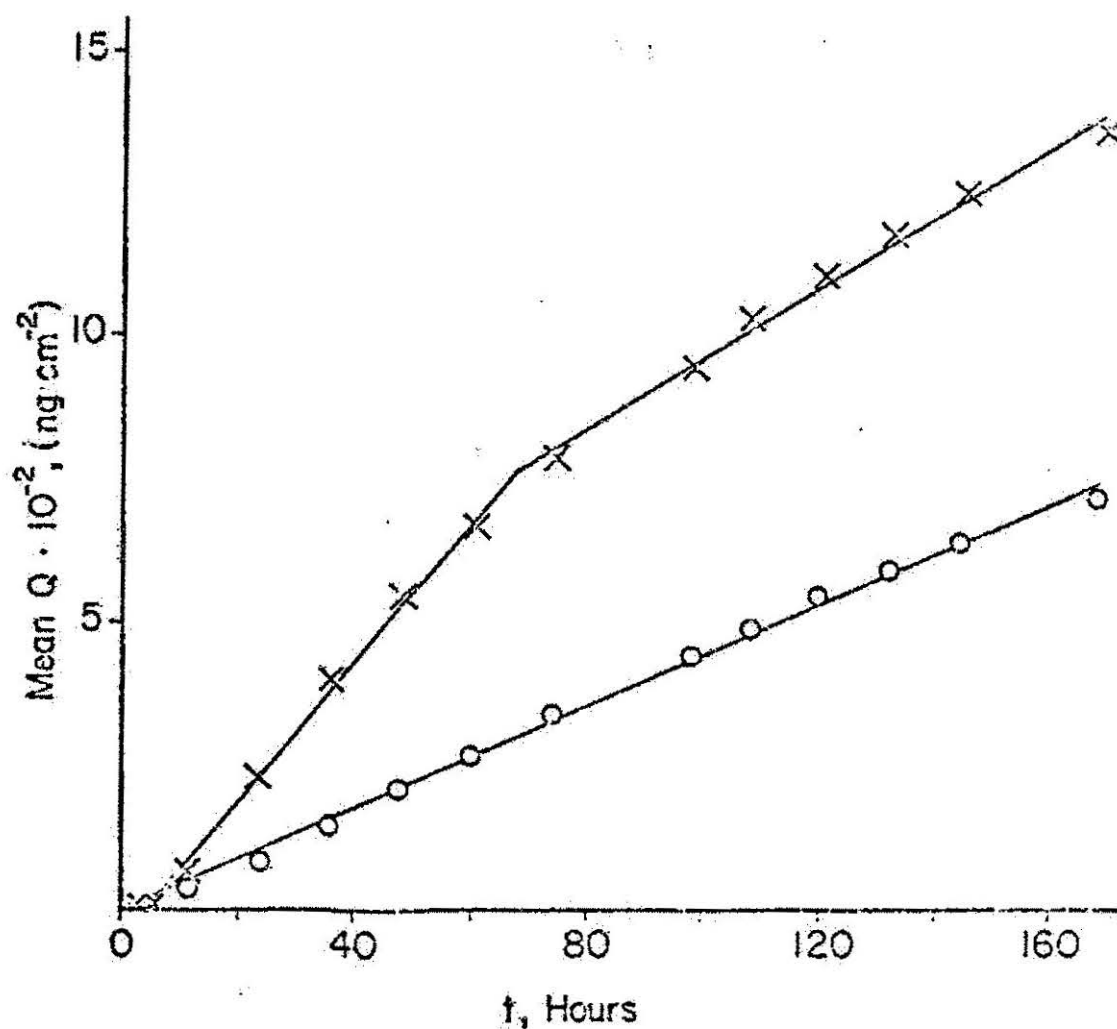


Figure 14. Q versus t plots of triamcinolone acetonide penetration from non occluded systems. Key: x, 0.01% solution in isopropanol containing 1.5% w/v of propylene glycol; o, 0.01% solution in isopropanol.

Table XIV
 Triamcinolone Acetonide (0.01% w/v)
 Penetration Data

Vehicle	Steady-State	Lag	Correlation Coefficient, r^b
	Penetration Rate ^a , $\text{ng cm}^{-2} \text{ hr}^{-1}$	Time, Hours	
Isopropanol ^c	4.52	1.84	0.996
1.5% w/v Propylene Glycol in Iso- propanol ^d	12.67	5.70	0.998

^a All steady-state values were computed from the regression line drawn from the data obtained by duplicate runs at each level by using TEKTRONIX (Model 4005-1) graphics terminal.

^b Reported r values are for the steady-state region of the penetration data.

^c Referred to as composition A in the text.

^d Referred to as composition B in the text.

into the skin could have caused changes in the thermodynamic activity of the drug in the donor phase or some alteration in the barrier properties of the skin. The value reported in Table XIV as penetration rate for this composition corresponds to the initial steady-state region (up to 67 hours of penetration experiment).

The distribution of the steroid at the end of 7 days is shown in Table XV for these two compositions. The epidermal content of the steroid was found to be more (44.3-55.0%) than the amount remaining on the surface of skin (15.9-20.0%) or the amount present in the dermis (2.5-7.0%). This would suggest for compositions A and B that the penetration of triamcinolone acetonide through the skin was the rate-limiting step. Alternatively these values could reflect relative solubilities rather than permeability differences. In view of the poor solubility of triamcinolone acetonide, the transfer of triamcinolone acetonide from the epidermis to the lower water-bearing tissues could be the slow step, resulting in the buildup of the steroid concentration in the epidermis.

Effect of Drug Concentration on the Penetration

In the matrices tested, the majority of drug was present as a suspension and the minimum concentration of the drug used was at least four times its solubility in the matrix. Q versus t plots (Figures 15 and 16) were made for the matrix compositions tested under the assumption

Table XV

Distribution of 0.01% w/v Triamcinolone Acetonide
7 Days After Application

Vehicle	Amount Penetrated, Percent	<u>Amount of Applied Dose Recovered,</u> <u>Percent</u>			Percent Accountability
		Isopropanol Wash of Epidermis	Epidermis	Dermis	
Isopropanol ^b	13.3 (15.1)	20.0 (18.1)	55.0 (54.3)	4.7 (1.9)	93.0 (89.4)
1.5% w/v Propylene Glycol in Isopropanol ^c	28.7 (26.3) ^a	18.5 (15.9)	45.3 (50.3)	2.5 (5.3)	95.0 (97.8)

^aThe numbers within parentheses represent the values obtained by duplicate run.

^bReferred to as composition A in the text.

^cReferred to as composition B in the text.

that the penetration through skin was rate-limiting. A summary of the data obtained from the steady-state penetration studies are shown in Tables XVI and XVII. The effect of increasing the concentration of the suspended drug in the same composition of the matrix containing propylene glycol is shown in Table XVI. The Table XVII shows corresponding data for matrices containing hexadecyl alcohol. As can be seen from these two tables, the increase in concentration of the suspended drug gave a corresponding increase in penetration rate. High correlation coefficients were obtained in the steady-state region of the penetration. There was no notable difference in penetration rate between matrices having propylene glycol- and hexadecyl alcohol. The corresponding increase in penetration rate with increases in concentration of the suspended drug was difficult to explain, if the penetration of the steroid through the skin was assumed to act as the rate-limiting step as was observed for compositions A and B. In theory, penetration should be equal from suspensions containing finely powdered drug (18) when the skin barrier is intact and acts as a rate-limiting barrier.

The increase in penetration with increases in concentration of the suspended drug suggested that the release of drug from the matrix might have acted as the rate-limiting step for the penetration through the skin and the skin acted as a perfect sink once the equilibration

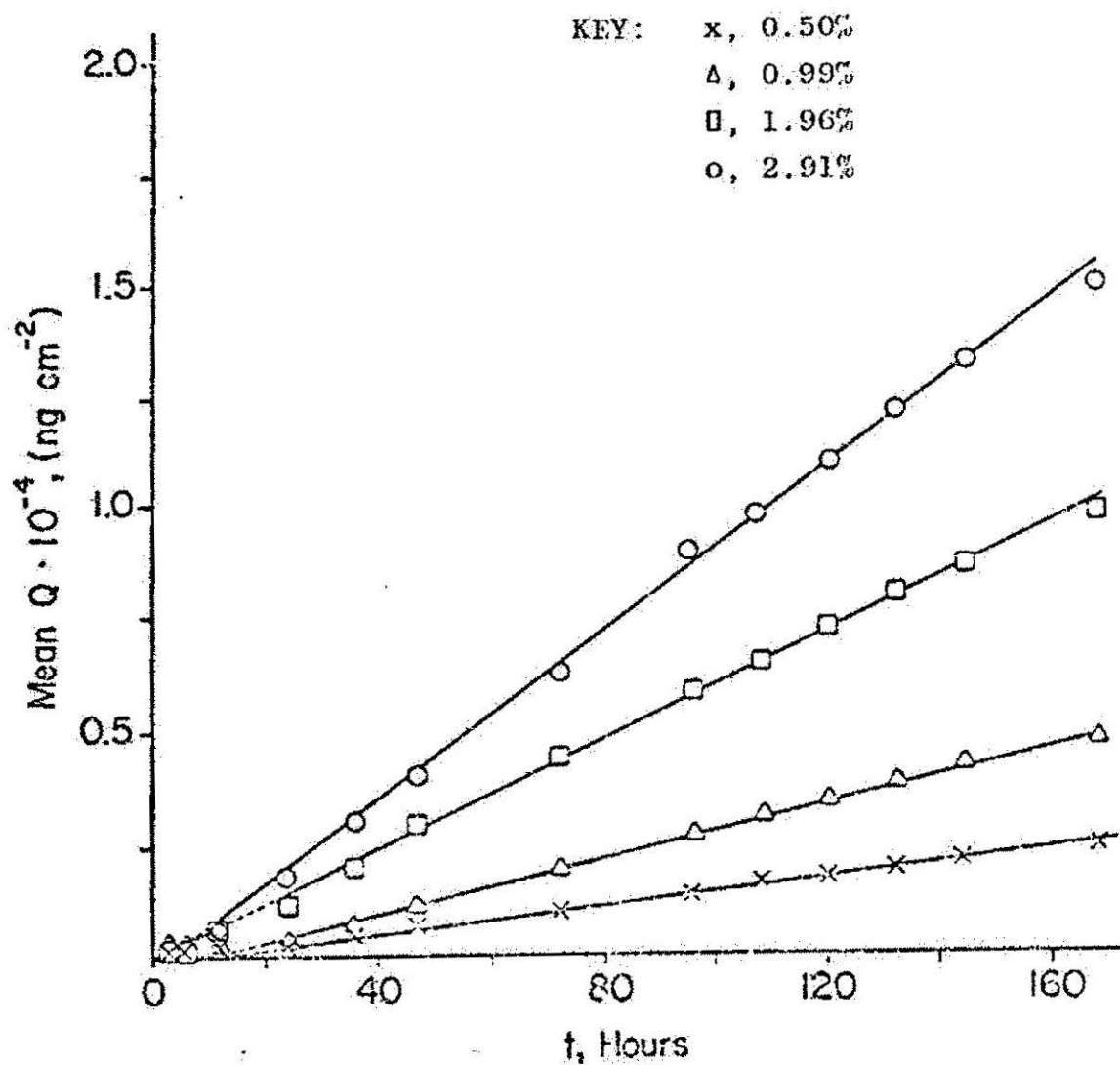


Figure 15. Q versus t plots of drug penetration from films containing lanolin alcohol-ethyl cellulose-propylene glycol (8.0 : 1.5 : 0.5) at different concentrations of triamcinolone acetonide.

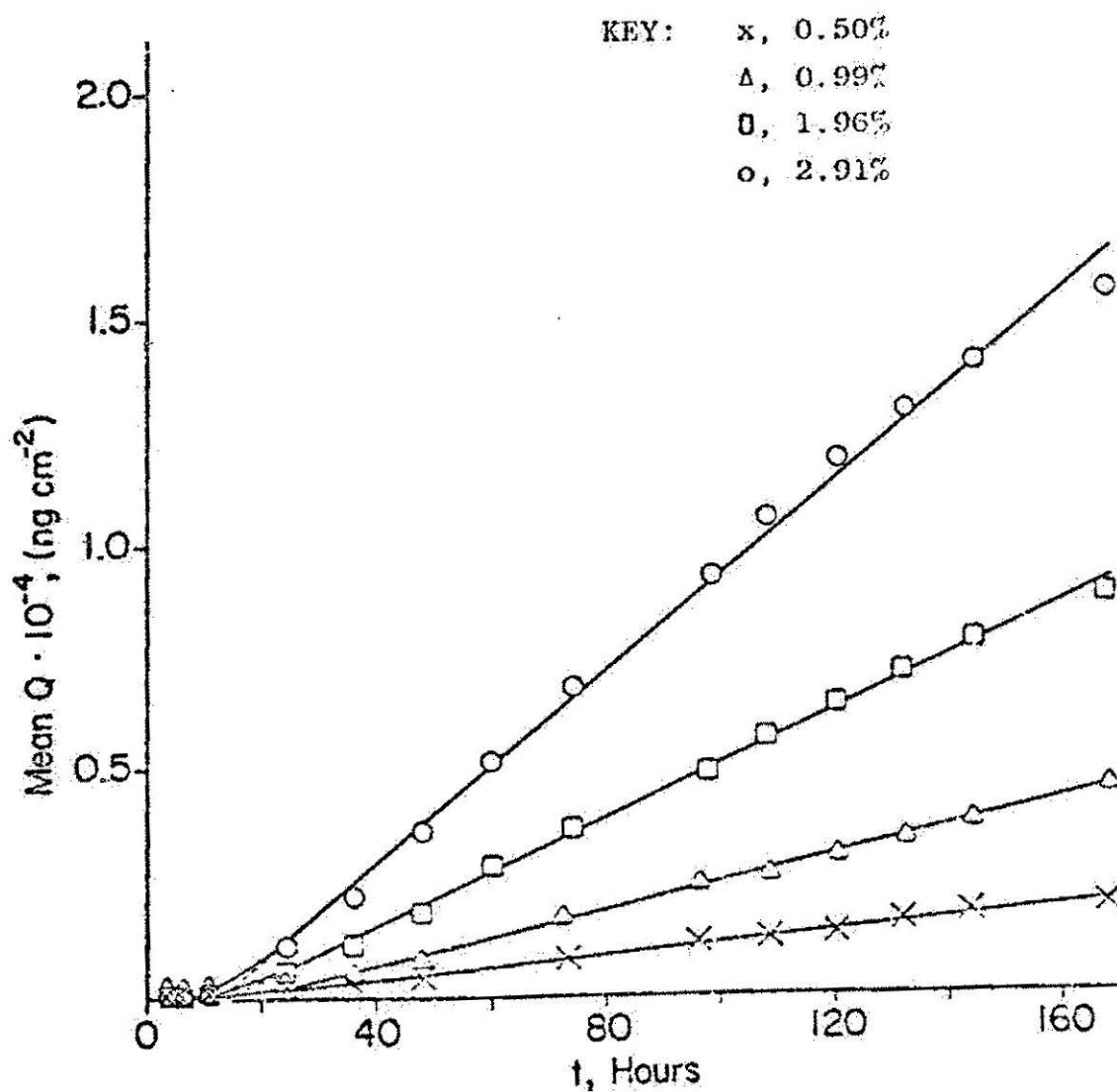


Figure 16. Q versus t plots of drug penetration from films containing lanolin alcohol-ethyl cellulose-hexadecyl alcohol (8.0 : 1.5 : 0.5) at different concentrations of triamcinolone acetonide.

Table XVI

Q versus t Treatments of Triamcinolone Acetonide Penetration
Data from Films Containing Propylene Glycol

Lanolin Alcohol:		Steady-State	Lag	
Ethyl Cellulose:	Drug Concentration,	Penetration	Time,	Correlation
Propylene Glycol	Percent ^a	Rate ^b ,	Hours	Coefficient,
Ratio		ng cm ⁻² hr ⁻¹		r ^c
8.0 : 1.5 : 0.5	0.50	15.13	7.04	0.999
8.0 : 1.5 : 0.5	0.99	32.14	13.43	0.999
8.0 : 1.5 : 0.5	1.96	61.55	2.39	0.999
8.0 : 1.5 : 0.5	2.91	95.19	5.45	0.999

^aBased on weight of drug per weight of dry film.

^bAll steady-state penetration rate values were computed from the regression line drawn from the data obtained by duplicate runs at each level by using TEKTRONIX (Model 4005-1) graphics terminal.

^cReported r values are for the steady-state region of the penetration data.

Table XVII

Q versus t Treatments of Triamcinolone Acetonide Penetration
Data from Films Containing Hexadecyl Alcohol.

Lanolin Alcohol:		Steady-State	Lag	
Ethyl Cellulose:	Drug Concentration,	Penetration	Time,	Correlation
Hexadecyl Alcohol	Percent ^a	Rate ^b ,	Hours	Coefficient,
Ratio		ng cm ⁻² hr ⁻¹		r ^c
8.0 : 1.5 : 0.5	0.50	12.86	13.21	0.999
8.0 : 1.5 : 0.5	0.99	30.60	20.50	0.999
8.0 : 1.5 : 0.5	1.96	60.32	16.00	0.999
8.0 : 1.5 : 0.5	2.91	106.11	11.81	0.997

^aBased on weight of drug per weight of dry film.

^bAll steady-state penetration rate values were computed from the regression line drawn from the data obtained by duplicate runs at each level by using TEKTRONIX (Model 4005-1) graphics terminal.

^cReported r values are for the steady-state region of the penetration data.

had been attained. To further verify this possibility, Q versus $t^{\frac{1}{2}}$ plots of penetration data were made for all matrix compositions tested as shown in Figures 17 and 18. Q versus $t^{\frac{1}{2}}$ plots were made based on the conclusion that the release of triamcinolone acetonide from these matrices follows the diffusion-controlled matrix model which follows equation 4 discussed in the introductory chapter. High correlation coefficients were obtained in the steady-state region of penetration as shown in Tables XVIII and XIX. The lag times were found to be in the range of 18-27 hours.

As in the case of the release studies, the penetration rate for the matrices containing hexadecyl alcohol (Table XIX) was found to be comparable to matrices containing propylene glycol (Table XVIII), even though there is an eight-fold difference in the solubility of the drug in the matrix. This is consistent with the earlier conclusions from the release studies that the diffusion-controlled matrix model was 'granular' and not 'homogenous.'

The penetration rates obtained (Tables XVIII and XIX) were lower by an order of magnitude when compared with the release rate values obtained from the same matrices. This may be due to the fact that during the course of penetration studies, the matrix was not at 37° and not immersed into an aqueous system.

The effect of change in drug concentration on steady-state penetration rate was tested using the 'A' (amount of

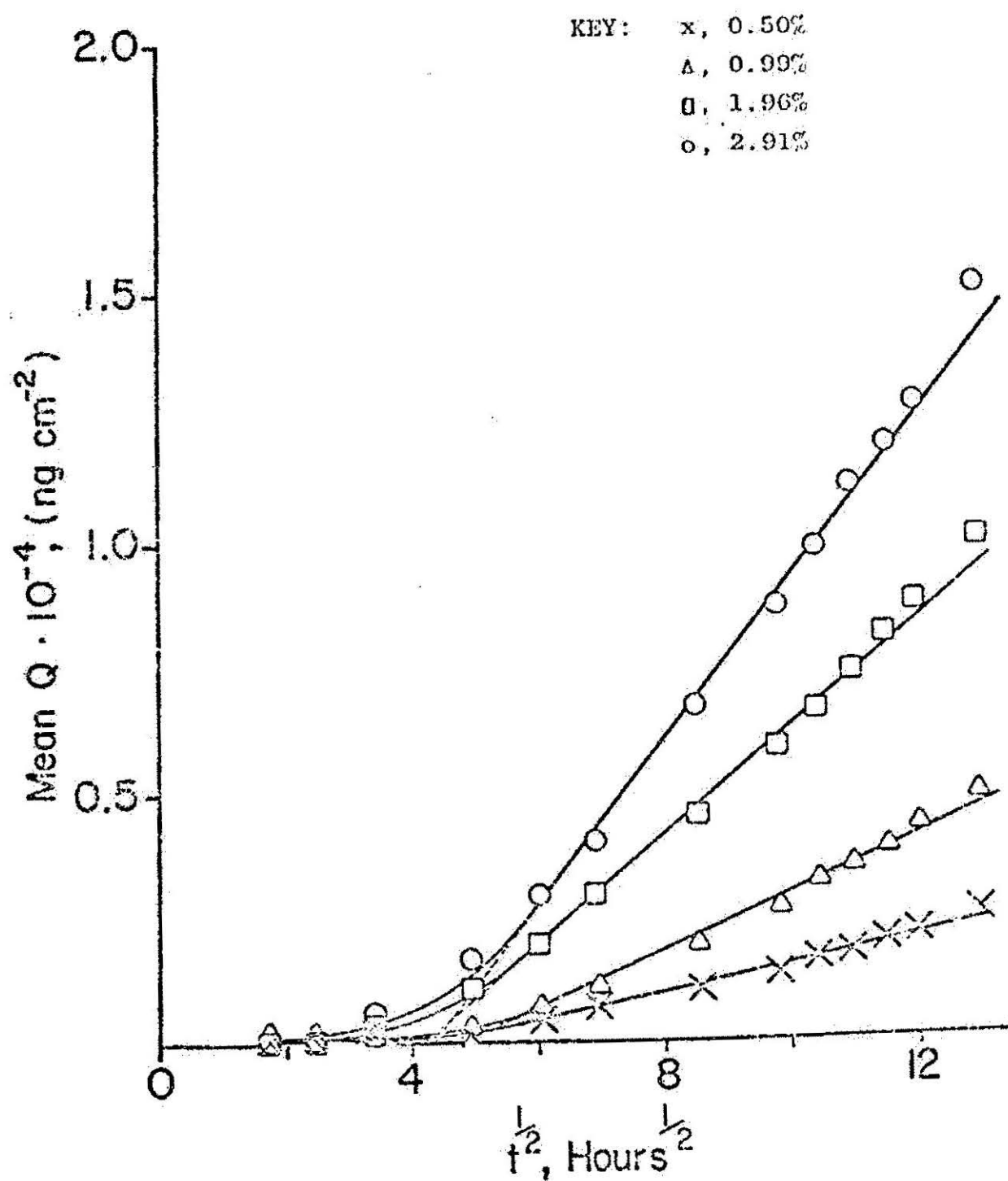


Figure 17. Q versus $t^{1/2}$ plots of drug penetration from films containing lanolin alcohol-ethyl cellulose-propylene glycol (8.0 : 1.5 : 0.5) at different concentrations of triamcinolone acetonide.

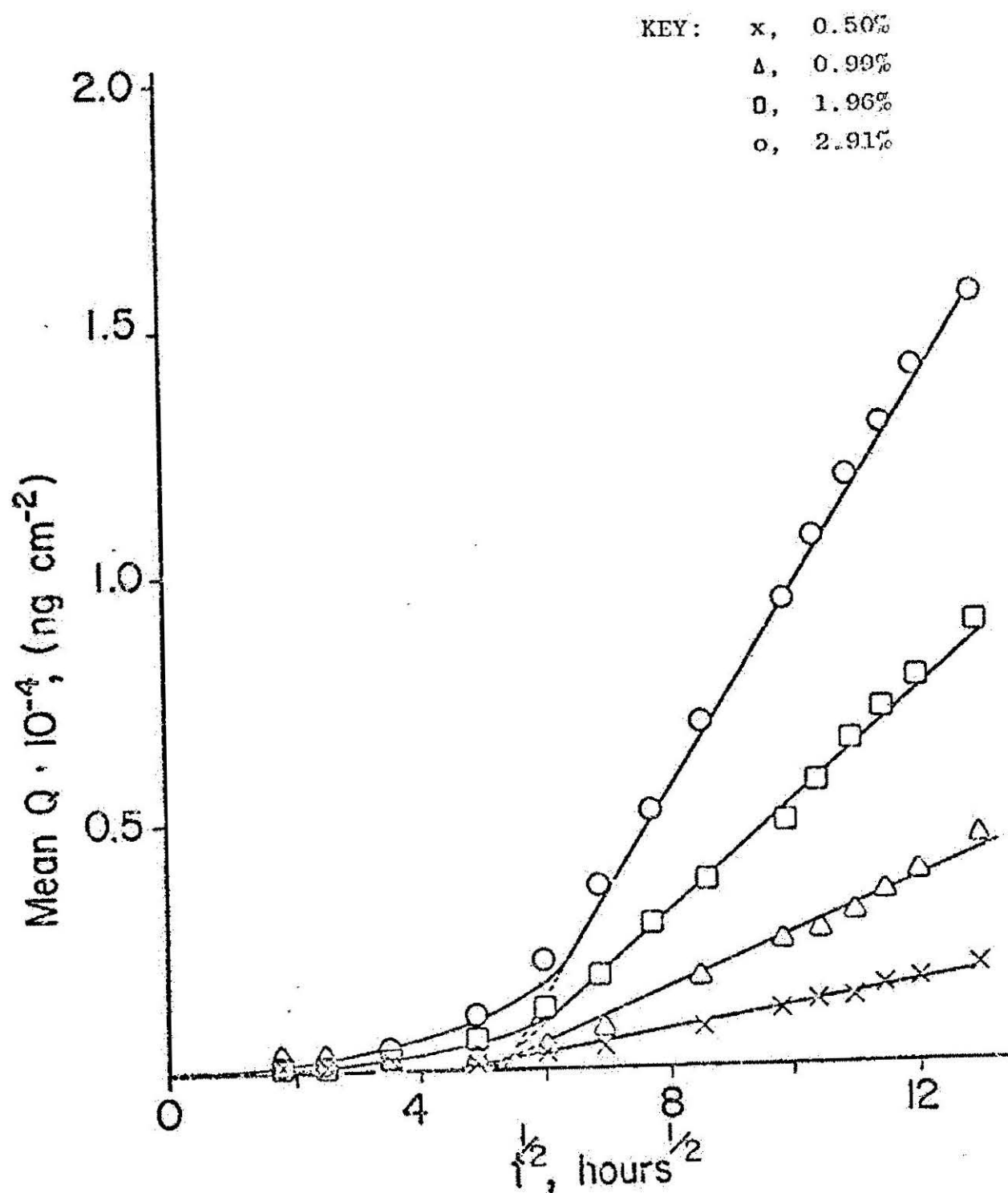


Figure 18. Q versus $t^{1/2}$ plots of drug penetration from films containing lanolin alcohol-ethyl cellulose-hexadecyl alcohol (8.0 : 1.5 : 0.5) at different concentrations of triamcinolone acetonide.

Table XVIII

Q versus $t^{\frac{1}{2}}$ Treatments of Triamcinolone Acetonide Penetration
Data from Films Containing Propylene Glycol

Lanolin Alcohol:		Steady-State	Lag	
Ethyl Cellulose:	Drug Concentration,	Penetration	Time,	Correlation
Propylene Glycol	Percent ^a	Rate ^b ,	Hours	Coefficient,
Ratio		ng cm ⁻² hr ^{-$\frac{1}{2}$}		r ^c
8.0 : 1.5 : 0.5	0.50	269.54	19.91	0.995
8.0 : 1.5 : 0.5	0.99	571.53	23.18	0.994
8.0 : 1.5 : 0.5	1.96	1099.39	17.79	0.997
8.0 : 1.5 : 0.5	2.91	1689.13	18.93	0.995

^aBased on weight of drug per weight of dry film.

^bAll k values were computed from the regression line drawn from the data obtained by duplicate runs at each level by using TEKTRONIX (Model 4005-1) graphics terminal.

^cReported r values are for the steady-state region of the penetration data.

Table XIX

Q versus $t^{\frac{1}{2}}$ Treatments of Triamcinolone Acetonide Penetration
Data from Films Containing Hexadecyl Alcohol

Lanolin Alcohol:		Steady-State	Lag	
Ethyl Cellulose:	Drug Concentration,	Penetration	Time,	Correlation
Hexadecyl Alcohol	Percent ^a	Rate ^b ,	Hours	Coefficient,
Ratio		ng cm ⁻² hr ^{-$\frac{1}{2}$}		r ^c
8.0 : 1.5 : 0.5	0.50	228.48	23.02	0.993
8.0 : 1.5 : 0.5	0.99	541.53	26.95	0.989
8.0 : 1.5 : 0.5	1.96	1079.11	25.13	0.993
8.0 : 1.5 : 0.5	2.91	1909.65	23.09	0.997

^aBased on weight of drug per weight of dry film.

^bAll k values were computed from the regression line drawn from the data obtained by duplicate runs at each level by using TEKTRONIX (model 4005-1) graphics terminal.

^cReported r values are for the steady-state region of the penetration data.

drug per unit volume of the matrix) values reported in Tables VIII and IX. For both the propylene glycol and hexadecyl alcohol containing matrices, there was better correlation between steady-state penetration rate (k) and A rather than between k and $A^{\frac{1}{2}}$ as in the case of release studies. The linear relationship between k and A is shown in Figures 19 and 20 for matrices containing propylene glycol and hexadecyl alcohol. These findings are also consistent with the results of the release studies reported earlier.

Distribution of triamcinolone acetonide at the end of 7 days is shown in Tables XX and XXI for all the concentrations studied from the two matrices containing propylene glycol and hexadecyl alcohol respectively. As can be seen, epidermal concentrations of the drug were low (1.9-6.7%) and slightly more than the dermal concentration (0.3-1.7%). The amount remaining in the applied dose was very high, contrary to the values obtained for the controls (the compositions A and B, Table XV). This distribution pattern of the steroid supported the view that the release from these film-matrices was the rate-limiting step.

Effect of Vehicle Composition

Table XXII describes the effect of drug solubility on drug penetration from the matrices containing lanolin alcohol. The variation in solubility was achieved by

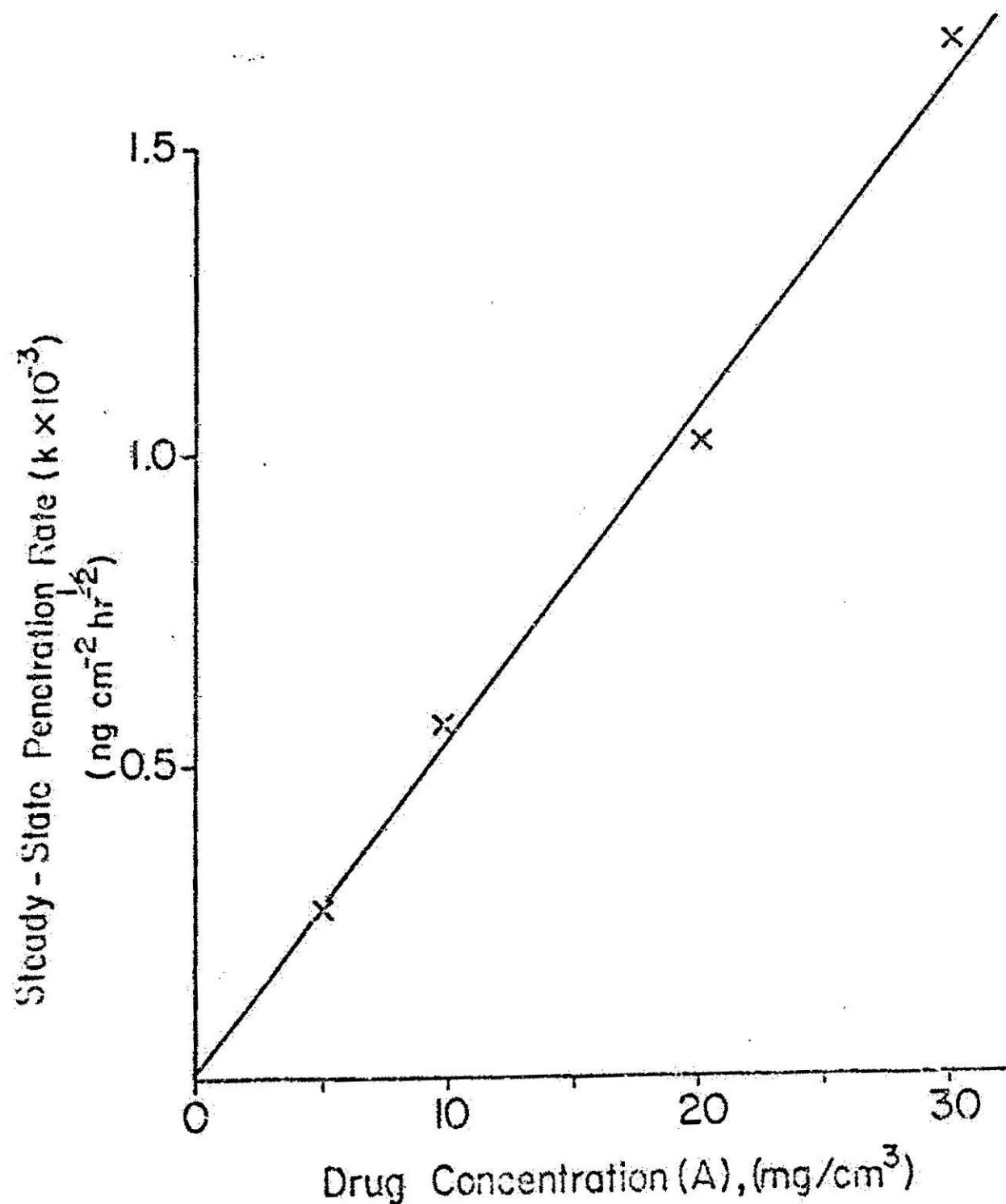


Figure 19. Effect of increasing the dispersed drug concentration in a matrix containing propylene glycol on the steady-state penetration rates obtained from Q versus t^2 plots.

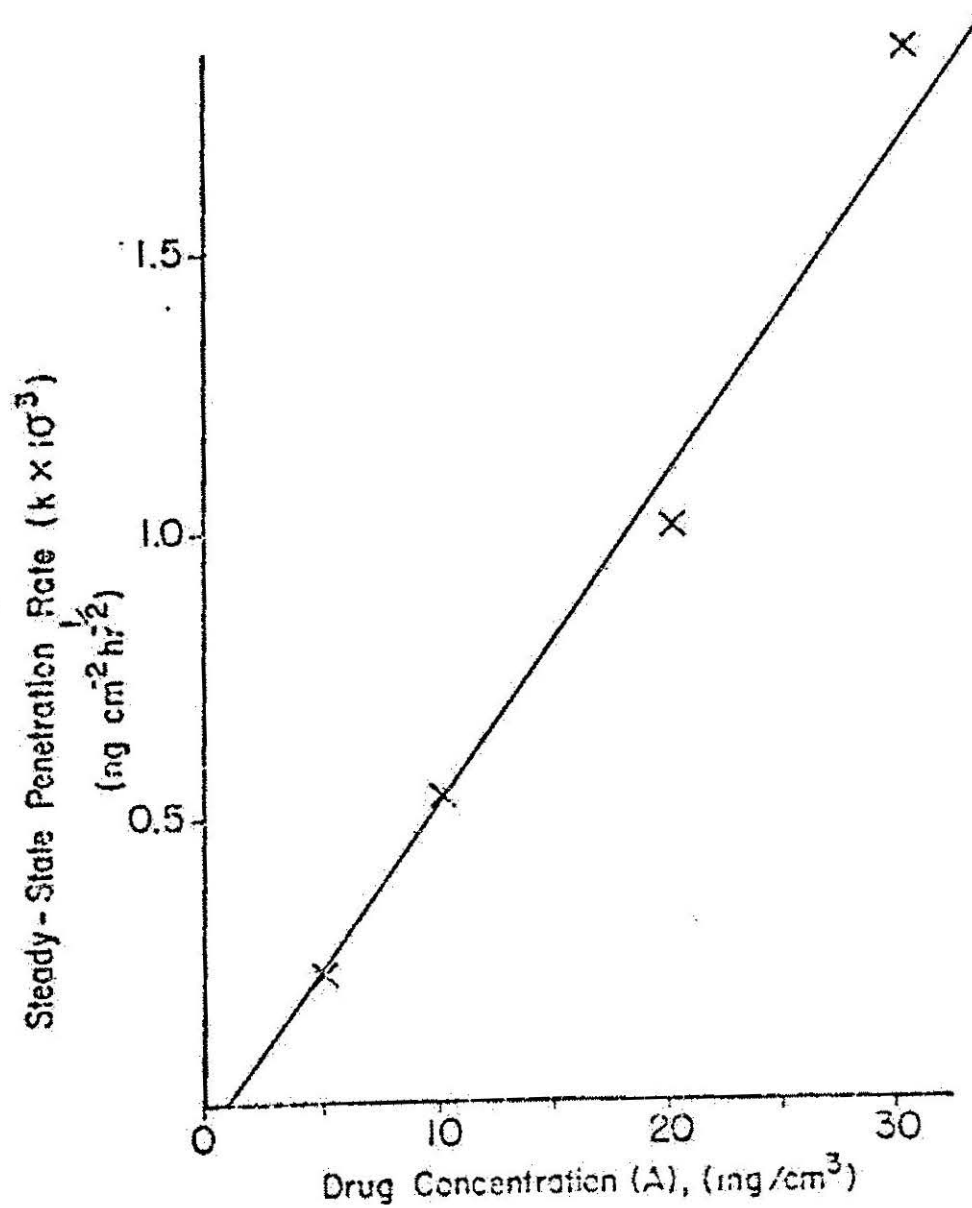


Figure 20. Effect of increasing the dispersed drug concentration in a matrix containing hexadecyl alcohol on the steady-state penetration rates obtained from Q versus $t^{1/2}$ plots.

Table XX

Distribution of Triamcinolone Acetonide 7 Days After
Application from Films Containing Propylene Glycol

Lanolin Alcohol: Ethyl Cellulose: Propylene Glycol Ratio ^a	Drug Concentra- tion, Percent ^b	Amount Penetrated, Percent	<u>Amount of Applied Dose Recovered,</u> <u>Percent</u>			
			Isopropanol Wash of Epidermis	Epidermis	Dermis	Percent Accountability
8.0 : 1.5 : 0.5	0.50	9.0 (10.1) ^c	68.6 (67.6)	3.1 (3.6)	0.5 (1.7)	81.2 (83.5)
8.0 : 1.5 : 0.5	0.99	9.1 (10.5)	64.1 (65.2)	3.1 (4.1)	1.4 (2.5)	77.7 (82.3)
8.0 : 1.5 : 0.5	1.96	10.5 (9.4)	73.6 (71.3)	4.5 (3.2)	0.6 (0.5)	89.3 (82.4)
8.0 : 1.5 : 0.5	2.91	9.9 (10.3)	67.1 (68.2)	6.7 (3.4)	0.6 (0.3)	84.3 (82.2)

^aAll solutions were applied as 10% w/v solution of film formers in isopropyl alcohol.

^bBased on weight of drug per weight of dry film.

^cThe numbers within parentheses represent the values obtained by duplicate run.

Table XXI

Distribution of Triamcinolone Acetonide 7 Days After
Application from Films Containing Hexadecyl Alcohol

Lanolin Alcohol: Ethyl Cellulose: Propylene Glycol Ratio ^a	Drug Concentra- tion, Percent ^b	Amount Penetrated, Percent	<u>Amount of Applied Dose Recovered,</u> <u>Percent</u>			
			Isopropanol Wash of Epidermis	Epidermis	Dermis	Percent Accountability
8.0 : 1.5 : 0.5	0.50	8.5 (7.3) ^c	58.6 (70.7)	1.9 (1.7)	1.9 (0.6)	71.1 (80.3)
8.0 : 1.5 : 0.5	0.99	9.8 (8.3)	67.7 (72.9)	3.1 (3.0)	1.2 (0.8)	81.8 (85.0)
8.0 : 1.5 : 0.5	1.96	6.2 (11.6)	72.1 (74.9)	6.5 (3.0)	0.7 (1.6)	85.5 (91.1)
8.0 : 1.5 : 0.5	2.91	8.2 (12.7)	73.7 (77.3)	2.8 (2.5)	1.1 (1.0)	85.8 (93.5)

^aAll solutions were applied as 10% w/v solution of film formers in isopropyl alcohol.

^bBased on weight of drug per weight of dry film.

^cThe numbers within parentheses represent the values obtained by duplicate run.

Table XXII

In Vitro Penetration of 0.10% w/w Triamcinolone
from Matrices Containing Different
Percentages of Propylene Glycol and
Lanolin Alcohol at 48 and 120 Hours
After Application

Lanolin Alcohol: Ethyl Cellulose: Propylene Glycol Ratio	<u>Amount Penetrated,</u> <u>Percent</u> ^a	
	48 hours	120 Hours
8.5 : 1.5 : 0.0	0.2	1.0
8.2 : 1.5 : 0.3	0.3	1.4
8.0 : 1.5 : 0.5	0.4	1.6
7.7 : 1.5 : 0.8	0.5	2.5
7.5 : 1.5 : 1.0	0.7	2.7
7.0 : 1.5 : 1.5	1.0	4.3
6.5 : 1.5 : 2.0	5.2	12.0
6.0 : 1.5 : 2.5	5.0	10.8

^aAll values are expressed as the mean of duplicate runs.

changing the percentage of propylene glycol in the matrix between 0 to 25% w/w with a corresponding decrease in the percentage of lanolin alcohol. The ethyl cellulose concentration was kept constant at 15% w/w. The integrity of the propylene glycol-containing films determined the upper limit of 25% w/w propylene glycol. The steroid concentration of 0.10% w/w was chosen, in part, to permit the study over a wide spectrum of solubility ranging from nearly complete suspension to dilute solution.

The reduced penetration of the steroid from film compositions containing 0 to 10% w/w propylene glycol could be attributed to insufficient propylene glycol being present to dissolve all of the triamcinolone acetonide. This would cause the diffusion of the drug into the skin to be dissolution rate-limited causing a reduction in release rate which in turn would be reflected as reduction in the amount of drug penetration. The diffusion gradients formed within the permeating fluid in the pores and channels of the matrix or in the matrix itself could also be a contributing factor. A similar release and penetration pattern has been reported by Poulsen (15) for fluocinolone acetonide and fluocinonide.

As seen from Table XXII, the maximum penetration was obtained from a system containing 20% w/w of propylene glycol rather than the film containing 15% w/w of propylene glycol though the latter constituted a near-saturated

solution. In this respect the results are at variance from the results of the release studies where the maximum release was obtained from the saturated solution. The amount of steroid penetrated decreased at 25% w/w propylene glycol concentration.

This can be explained since at 20 and 25% w/w propylene glycol concentrations the vehicle is probably behaving more as a homogeneous system like an ointment with the drug in solution rather than as a granular matrix. Such a transition makes the situation more complex and definitive explanation more difficult. In such circumstances, the rate of release is increasing and approaching the rate of penetration and perhaps surpassing it as the propylene glycol concentration is gradually increased to 20% w/w and above. The drop in amount penetrated at 25% w/w propylene glycol appears to be due to the drop in thermodynamic activity associated with the dilution of the drug.

The distribution of triamcinolone acetonide at the end of 7 days from the matrices is shown in Table XXIII. The epidermal concentration was much lower than the concentration remaining in the vehicle-matrix. These observations are comparable to data reported earlier for varying amounts of suspended drug in the same film vehicle (Tables XX and XXI).

Table XXIII

Distribution of 0.10% w/w Triamcinolone Acetonide 7 Days After
Application from Films Containing Differing Concentrations
of Propylene Glycol and Lanolin Alcohol

Lanolin Alcohol:	Amount	Amount of Applied Dose Recovered,			
		Percent			
Ethyl Cellulose:	Penetrated,	Isopropanol		Percent	
Propylene Glycol	Percent	Wash of	Epidermis	Dermis	Accounta-
Ratio ^a		Epidermis			bility
8.5 : 1.5 : 0.0	1.5 (1.8) ^b	63.1 (76.2)	3.1 (2.8)	1.4 (0.8)	69.1 (81.6)
8.2 : 1.5 : 0.3	2.2 (2.6)	65.7 (75.6)	2.9 (4.2)	1.3 (1.7)	72.1 (84.1)
8.0 : 1.5 : 0.5	2.6 (2.5)	85.0 (84.1)	5.6 (7.2)	1.2 (1.8)	94.4 (95.6)
7.7 : 1.5 : 0.8	2.8 (5.4)	82.9 (85.0)	7.0 (8.0)	1.2 (2.0)	93.9 (98.4)
7.5 : 1.5 : 1.0	4.1 (4.7)	79.0 (80.2)	7.0 (6.5)	2.6 (2.9)	92.7 (94.3)
7.0 : 1.5 : 1.5	6.9 (6.1)	69.9 (80.8)	7.5 (6.6)	2.8 (3.2)	85.1 (96.7)
6.5 : 1.5 : 2.0	16.7 (12.8)	63.0 (56.9)	6.4 (7.5)	1.5 (2.3)	87.6 (79.5)
6.0 : 1.5 : 2.5	14.4 (11.2)	65.0 (64.2)	5.1 (6.5)	0.7 (0.8)	85.2 (82.7)

^aAll solutions were applied as 10% w/v solution of film formers in isopropyl alcohol.

^bThe numbers within parentheses represent the values obtained by duplicate run.

Chapter V

CONCLUDING REMARKS

A preliminary screening of the film-forming potential of various high molecular weight alcohols such as cetyl alcohol, stearyl alcohol and lanolin alcohol revealed that lanolin alcohol formed thin films without the addition of other adjuvants. Cetyl alcohol and stearyl alcohol did not form thin films that could be isolated. The integrity and tacky nature of lanolin alcohol films could be altered by the addition of small quantities of ethyl cellulose and propylene glycol or hexadecyl alcohol. The composition with 15% w/w ethyl cellulose and 7.5 to 10% w/w propylene glycol produced the best films. Further increases in proportion of propylene glycol up to 25% w/w with corresponding decreases in lanolin alcohol concentration did not substantially alter the modulus of elasticity of the films although a gradual reduction in the overall film quality was apparent. This reported film-forming capability of lanolin alcohol is significant and appears to have remained unrecognized in spite of the widespread use of the material in a variety of cosmetics. Lanolin alcohol is a mixture of many compounds. Additional work must be done to identify

the principal film-forming component(s). Further exploration of the pharmaceutical and cosmetic application of this property is warranted.

The evaluation of the occlusive capability of the selected films revealed that these films could reduce the normal transepidermal moisture loss substantially. The occlusive capability appeared to decrease with increasing concentrations of solvent-plasticizer and decreasing concentrations of lanolin alcohol, and appeared to increase with increasing concentrations of ethyl cellulose and decreasing concentrations of lanolin alcohol in the film.

The in vitro release of triamcinolone acetonide from selected film compositions of lanolin alcohol with propylene glycol or hexadecyl alcohol as solvent-plasticizer suggested that the drug release followed a diffusion-controlled granular matrix model proposed by Higuchi (36) when the majority of the drug was present as a suspension in the concentrations studied. A linear relationship was observed between drug concentration and release rate. The studies on the effect of solubilization of the drug by increasing the propylene glycol concentration in the matrix revealed that maximum release was obtained from a system having the drug as a near-saturated solution. The reduced release from matrices containing excess solubilizer can be attributed to the reduced thermodynamic activity of the drug. The equations for release from vehicles where the drug is in

highly suspended state or when the drug is in solution are well defined. However, these physical-chemical models appear to be inadequate in explaining observed release profiles in the narrow region where the drug concentrations are slightly above the saturation solubility in the vehicle. In this respect, this study confirms the experience of other investigators in this area, and it points to a very fruitful area for further research.

The in vitro penetration data obtained from the same matrix-forming compositions as in the case of the release studies seemed to indicate that release from the matrix rather than penetration through the skin acted as the rate-limiting step for the absorption of triamcinolone acetate from these formulations. The overall penetration data were consistent with the release data with one exception. The maximum penetration was obtained from a system containing a slightly higher concentration of propylene glycol than required to make a saturated solution. This may have resulted from a fundamental change in the nature of the matrix system associated with the changes in the propylene glycol concentration.

The film delivery system investigated in this study is interesting since the release from the matrix is the rate-controlling step. This feature may not be desirable for drugs with low aqueous solubilities and/or poor penetration properties across intact skin. On the other hand, the slow release property may be highly desirable for drugs

with narrow therapeutic indices intended to be used in conditions such as psoriasis. The rate-controlling feature of the delivery system could be attributed to the granular nature of the matrix. What is the contribution of film thickness to the controlled nature of drug delivery? Will the release-rate control be still operative during clinical situations where the film thickness might be 5- to 10-fold less than those studied in vitro? What is the role of the demonstrated occlusive property of the film in facilitating drug penetration? Additional in vitro and in vivo studies may help answer some of these questions.

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