Pharmaceutical Research, Vol. 5, No. 6, 1988

Report

Drug Transport from Thin Applications of Topical Dosage Forms: Development of Methodology

William J. Addicks,[1] Gordon L. Flynn,[1] Norman Weiner,[1,2] and Chia-Ming Chiang[1]

Received September 15, 1987; accepted January 24, 1988

There are presently no standards for *in vitro* research dealing with the release and delivery of drugs from semisolid dosage forms, largely because of inherent experimental difficulties. Among the problems, it has proven difficult to apply dosage forms to membranes mounted in *in vitro* diffusion cells in facsimile to the manner in which the dosage forms are applied clinically. In the present studies, methodology has been developed which allows films with thicknesses approaching clinical dimensions to be spread evenly over silicone rubber membranes. Using methyl *p*-aminobenzoate as a test permeant and gelled water and water/propylene glycol solvent systems as test vehicles, it has proven possible to spread films as thin as 75 μm, yielding highly reproducible delivery profiles. Using this application technique, it has been shown how the diffusive clearance of drug from films of fixed composition placed over a resistant membrane is dependent on the thickness of application. For a given medium and thickness of application, when the vehicle composition is enriched in propylene glycol, partitioning into the membrane is suppressed, resulting in a lessening of the absolute rate of delivery and, consequently, a prolongation of the period over which drug is released. Increasing the membrane's resistance, i.e., increasing the membrane's thickness, likewise slows down the absolute delivery rate, extending the effective period of total clearance of drug from the applied film.

KEY WORDS: topical; vehicles; thin applications; diffusion.

INTRODUCTION

Drugs intended for topical administration are incorporated into a variety of vehicles, each having unique drug delivery attributes. These vehicles include gelled solvent systems, emulsions, and hydrocarbon ointment bases. Although there has always been a strong sense that the manner of formulation has a profound influence on topical drug delivery, evaluating the degree to which vehicles affect mass transfer has been largely sidestepped by formulators due to a lack of established experimental methods for making such assessments. A major problem inherent in *in vitro* testing of such dosage forms is the difficulty attending application of the formulations as very thin layers of perhaps 20- to 40-μm thickness, as is done in the clinic (1,2). In many past cases in which semisolids have been studied at the bench, unrealistically thick layers of formulation have been employed. In other instances the amount of drug applied may have been controlled and limited but, generally, in circumstances in which the drug was placed on the skin as a solution in a volatile solvent. Such application leads to a thin film of solid drug on the surface of the skin, therefore generating information of questionable relevance to the performance of gels, creams, and ointments (3,4). Thus, a need for more realistic experimental designs for the study of semisolids is generally apparent. In particular, a reliable method for applying such vehicles in reasonable facsimile to the way they are spread clinically is needed. As a logical progression, one should first establish firm methodological and theoretical baselines using *in vitro* diffusion techniques with well-defined topical systems applied to membranes with well-characterized attributes (i.e., synthetic membranes) (1,5). For systems as gels and creams, this means studying the systems under occlusion so that the compositions remain invariant. Only after securing such baselines will it be possible to evaluate the far more complex issues of delivery from films undergoing dynamic compositional changes due to evaporative or other influences and deal with the vagaries of the skin membrane.

MATERIALS AND METHODS

Materials

Methyl *p*-aminobenzoate (Aldrich, Milwaukee, Wis.) was used as received. High-performance liquid chromatographic (HPLC)-grade methanol (Mallinckrodt, Paris, Ky.) was used for all assays. Deionized water was used for the assay, to prepare the gels, and in the receptor fluid in all permeation experiments. Unreinforced dimethylpolysiloxane sheets in thicknesses of 127 and 254 μm (Silastic sheeting, gift, Dow Corning, Midland, Mich.) were used as membranes. Propylene glycol (Fisher, Fair Lawn, N.J.) was used in the gels. Carbopol 940 (B. F. Goodrich, Cleveland,

[1] College of Pharmacy, University of Michigan, Ann Arbor, Michigan 48109.

[2] To whom correspondence should be addressed.

0724-8741/88/0600-0377$06.00/0

Ohio) was used as the gelling agent. Diethylene oximide (morpholine) (Sigma) and sodium hydroxide (Fisher) were used as the neutralizing agents for gels prepared with and without propylene glycol, respectively.

Assay

Methyl *p*-aminobenzoate was assayed by HPLC, using UV detection at 285 nm. A typical chromatograph is shown in Fig. 1. The mobile phase consisted of methanol and water at the ratio of 45% methanol to 55% water (v/v). Quantitation was by peak height ratio to a known internal standard, ethyl *p*-aminobenzoate. Linear standard curves were obtained by first dissolving a known amount of the methyl ester in 1000 ml of water. Appropriate dilutions were made and 100-μl samples were injected onto the column. The retention time for methyl *p*-aminobenzoate was found to be approximately 5.25 min. Peak height ratios were plotted against concentration, and linear regressions of the data were performed.

Solubility Studies

An excess of ester was mixed with the solvent system under study and placed in a jacketed flask. The resulting suspension was stirred at 33°C for 3 days. Initial studies had indicated that equilibrium was achieved essentially within a day's time. Samples were collected on day 2 and day 3, the latter a check on the equilibrium condition. Samples were obtained via warmed, glass–wool-tipped pipets. The glass–wool wad removed all particulate matter as the samples were drawn. The samples were appropriately diluted with water and set aside for assay.

Preparation of Gels

Gels were prepared which consisted of methyl *p*-aminobenzoate dissolved in various propylene glycol/water solvent systems. The solvent systems used were water, 50% propylene glycol/50% water (v/v), and propylene glycol. For the water system, 1 g of Carbopol 940 was dispersed via constant stirring in 97 ml of a 1 mg/ml solution of methyl *p*-aminobenzoate. Gelling was effected upon neutralization of the dispersion by the addition of 2 ml of 10% sodium hydroxide. For the systems containing propylene glycol, 2 ml of morpholine was mixed with 97 ml of a drug solution, and 1 g of Carbopol 940 was dispersed via constant stirring. The mixture was then heated to 70°C to facilitate gelling. Drug concentrations in the gels were set so the test permeant was at a high enough thermodynamic activity to follow the permeation process easily. They were 22 mg/cm^3 for the propylene glycol gel, 6 mg/cm^3 for the 50% propylene glycol gel, and 1.2 mg/cm^3 for the water gel.

Application of Vehicle Films

The device used to obtain films of specified thickness is depicted in Fig. 2A. The apparatus consists of a stainless-steel membrane support screen, a circular template (i.d., .9 cm; o.d., 1.2 cm) constructed of brass or stainless steel, and a glass cover. The thickness of a template (75–1600 μm) determined the thickness of the application. Attempts to use templates less than 75 μm were unsuccessful because the material (stainless steel) was made so flexible that the applications could not be spread evenly. To apply a film to a membrane, the membrane is placed on the support screen, the template is placed on the membrane, and gel is placed into the well of the template. The gel is then troweled flush with the edge of the template with the aid of the straight edge of a square glass cover slide. The application thickness was verified by weighing the membrane before and after ap-

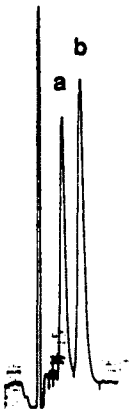

Fig. 1. Representative chromatograph of methyl *p*-aminobenzoate (a) and ethyl *p*-aminobenzoate internal standard (b) in phosphate buffer.

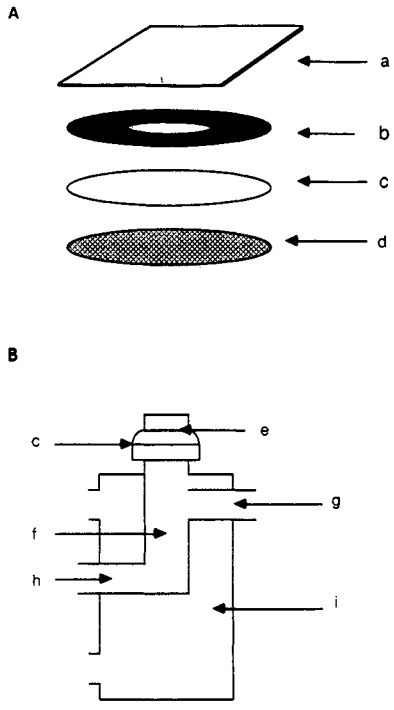

Fig. 2. Diagrammatic representations of the vehicle application apparatus (A) and flow-through cell (B). Components are as follows: (a) glass cover; (b) template; (c) membrane; (d) support screen; (e) cell cap; (f) receiver compartment; (g) solvent outlet; (h) solvent inlet; (i) water jacket.

plication and calculating the thickness from the template well volume using the gel density.

Permeation Studies

The flow-through cell (Crown Glass) is pictured in Fig. 2B. The 1.0-ml receiver compartment has an inlet and an outlet to allow for flow of receptor solvent. Details concerning the operation of this cell have been previously described (6). In all experiments, pH 7.0 phosphate buffer was used as the solvent. Experiments were carried out at 33 ± 1°C, nominally the surface temperature of the skin. A vehicle film was applied to a membrane, and the template, membrane, and support screen were mounted onto the diffusion cell. A rubber o-ring provided a seal between the support screen and the cell. A glass cover slide was then placed over the vehicle film to prevent evaporation of vehicle components, and the entire apparatus was clamped into place with the use of a cell cap and clamp. In that this paper represents initial efforts to develop and test the reproducibility of methodology for the application of thin films to membranes, it was desirable to eliminate as many variables as possible. If the surface of the application had been left uncovered, it is likely that the evaporation of vehicle components would have effected a continuous change in the partitioning of the drug between the vehicle and the membrane. Furthermore, as there are instances in which occlusive dressings are used clinically, it is relevant to study the case in which the application is occluded.

In order to eliminate variability in the results due to variable preparation time, 10 min was allowed to elapse between the application of the vehicle and the mounting of the membrane laminate within the cell. Prior to initiating a run, the diffusion cell was allowed to fill with solvent. After the membrane was clamped into place the progress of the solvent front through the outflow tubing was monitored, and "zero time" was defined as the point at which the initial solvent drop emerged from the tubing. Solvent flow rates ranged between 2 and 3 ml/hr. Samples were collected at preset intervals with an automatic fraction collector. The mass of drug collected during a particular interval was normalized by the total recovery of drug during the experiment, and the normalized mass was plotted versus time. It should be noted that, for a given formulation, the relative rate of release of a drug from a film application is essentially unaffected by the concentration chosen for the experiment as long as the film thickness is held constant. In these experiments the concentration of the permeant was adjusted to a given fractional solubility so that the absolute rate of release was consistent with an analytical sensitivity allowing each release study to be carried out over a reasonable time span. In general, the total drug recovery in these experiments was found to be of the order of 70% of the theoretically expected amount. Losses were presumed to be due to adsorption of drug to silica filler present in the membranes.

RESULTS AND DISCUSSION

Solubilities of methyl *p*-aminobenzoate in water, 50% propylene glycol, and propylene glycol were 2.3, 20.5, and 93 mg/ml, respectively. Its working concentrations in the test vehicles were set at appreciable fractions of these solubilities to assure that its permeation could be easily followed.

A typical set of cumulative mass versus time data is shown in Fig. 3. A high level of experiment-to-experiment reproducibility is evident from the data for the three separate runs, in terms of both the mass recovered at any given time and the total mass recovered over the course of an entire experiment. Overall drug release proceeds in a nonlinear fashion, a result which is indicative of non-steady-state kinetics.

It should be pointed out that at the flow rates used in this study, sink conditions were suitably approximated. For instance, for the thinnest water gel, the fastest releasing system studied, about 40% release occurs over the first 100 min, with the concentration existing in the receiver during this 100-min period on avearge being <0.002 mg/ml. The drug concentration in the gel on the other side of the membrane after 100 min remains high at 0.72 mg/ml. Thus the drug in the gel is at all times over two orders of magnitude larger than in the receiver compartment.

Although the lag times for methyl p-aminobenzoate diffusing through the silicone rubber membranes of the thicknesses chosen for the study would be expected to be less than a minute, very long apparent lag times, of the order of 25–50 min, were actually observed. The adsorption of permeant to silica filler in the membrane undoubtedly had some influence here (1). Even more important is the fact that it took considerable time to transport the conents of the diffusion reservoir through the tubing leading to the fraction collector at the flow rate used in the study. Thus the mechanics of this diffusion cell make it difficult to produce diffusional profiles which instantaneously reflect the early diffusional time course. Improvements on the system to shorten this transport period are already being implemented. Nevertheless, except for a shift on the time axis associated with the transport of the receptor fluid to the fraction collector, the profiles of appearance of the drug as seen downstream are reasonably accurate reflections of the delivery of drug into the receiver and the cell does allow one to compare formulations.

Figure 4A shows the effect of increasing film thickness on the release of the test permeant from water gels through a

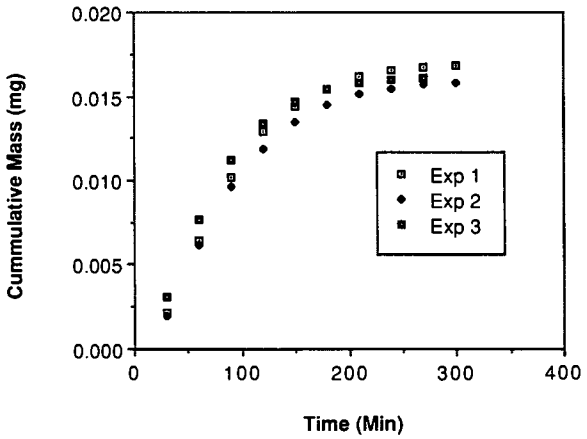

Fig. 3. Cumulative mass released through a 254-μm membrane versus time for methyl *p*-aminobenzoate formulated in 50% propylene glycol applied in a thickness of 75-μm.

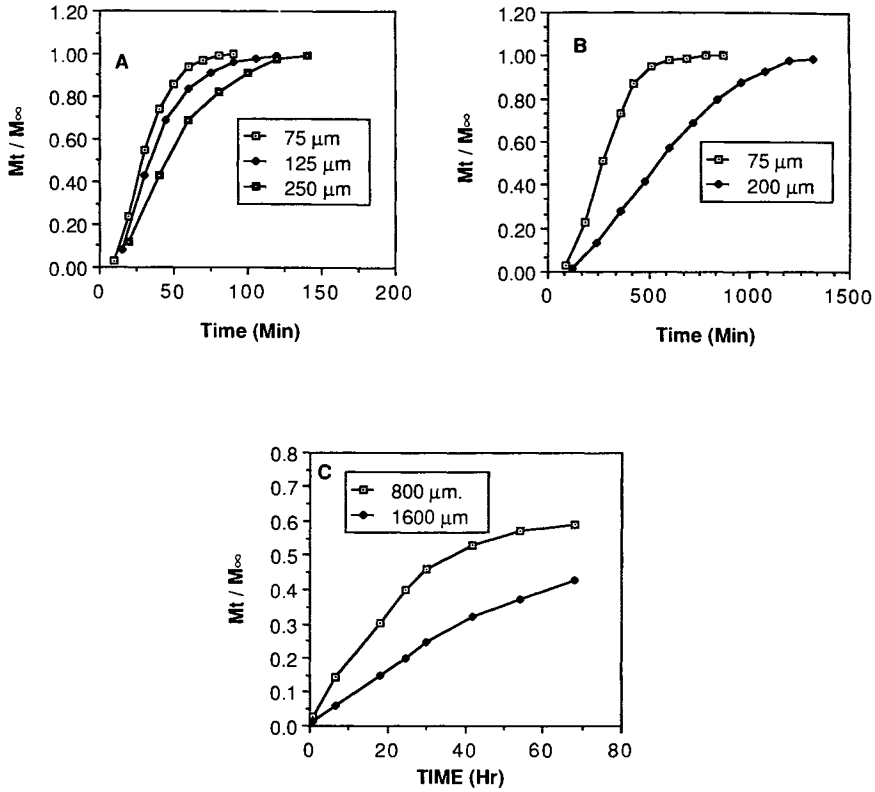

Fig. 4. Relative mass released versus time for methyl *p*-aminobenzoate formulated in water (A) and propylene glycol (B, C) gels applied in various thicknesses. Membrane thicknesses are 127 μm (A, C) and 254 μm (B). Each curve represents the average of three runs.

127-μm-thick membrane. As the thickness of the application increases, more total permeant is placed over a unit area of membrane, and the total contents of the film are released over a longer period of time in the manner initially predicted by Guy and Hadgraft (5). Similar results are observed for diffusion of methyl *p*-aminobenzoate from a propylene glycol gel through a 254-μm membrane (Fig. 4B). Similarly, Fig. 4C shows permeation patterns observed for propylene glycol gels applied in thicknesses of 800 and 1600 μm. It should be pointed out that whereas all other results were normalized by the total mass of drug recovered in the receiver compartment throughout the experiment, the results in Fig. 4C were normalized by the theoretical total mass of drug in the gel. The 1600-μm application yielded essentially a steady-state influx over a 30-hr period, a result which would be expected for the case in which the donor contains an infinite dose of drug. Here it can also be especially clearly seen that as the total drug load over the surface is increased, the duration of delivery of the drug is commensurately extended.

In Fig. 5, the effect of increasing the membrane thickness on delivery of the test permeant from 75-μm applications of a water gel is shown. An increase in the membrane thickness is evidenced in a shifting of the delivery profile on the time axis to a longer time. This reflects a slowdown in the absolute rate delivery and thus an increase in the time taken to clear drug diffusionally from a film of a given thickness. This general pattern is expected since the resistance of the membrane is proportional to its thickness.

The membrane/vehicle partition coefficient has been shown to play a key role in determining the rate at which a drug is delivered into and through the skin (7). Because it was desirable to investigate systems having a wide range of membrane/vehicle partition coefficients, several propylene glycol/water mixtures were used as solvent systems. It is expected that the partition coefficient between the gels and the membrane should change inversely in proportion to solubility changes and, based on the data for the three test formulations, solubility increases exponentially with increasing propylene glycol concentration. One of course has to as-

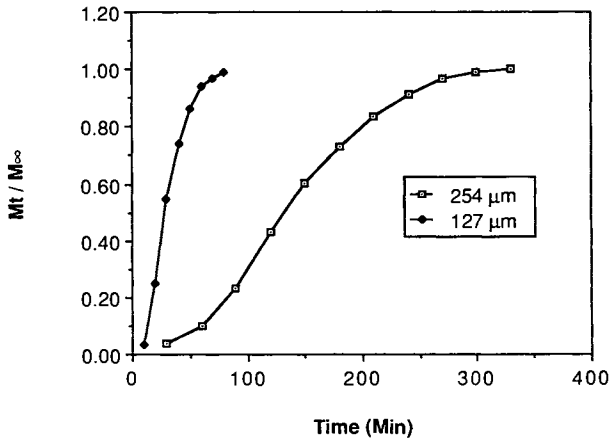

Fig. 5. Relative mass released through 127 and 254-μm membranes versus time for methyl *p*-aminobenzoate formulated in a water gel. The gel was applied in a thickness of 75 μm. Each curve represents the average of three runs.

sume that the solubility of methyl *p*-aminobenzoate within the membrane will be unaffected by the solvent or solvent mix which is applied to the membrane to make the assumption that the partition coefficient will decrease exactly as the solubility increases. The effect of the partition coefficient is clearly shown in Figs. 6A and B. As the amount of propylene glycol in a given gel increases, the contents of the gel application are released over a longer time period. By putting the amount of drug delivered in fractional terms, i.e., M/M_∞, the relative amounts of drug delivered from gels of equal thickness become independent of the concentration per se. The actual or absolute rate of delivery is dependent on the prevailing thermodynamic activity in the vehicle, however. These two features of delivery combine such that the relative (fractional) rate of delivery is determined by the activity coefficient of the drug at isoactivity. Therefore, gels of fixed activity but having different solvent compositions release their total contents at different fractional rates dependent on the activity coefficient. Evidence for this behavior is observed in Fig. 6B, where the drug was formulated such that its initial thermodynamic activity within the gels was nearly equal (the drug concentrations were set at 29 and 22% of solubility in the propylene glycol and 50% propylene glycol gels, respectively). The diffusion patterns observed are dissimilar out of proportion to this slight difference in activity. The decrease in relative release rate as the propylene glycol concentration is raised is the result of the drug's increased affinity for the vehicle (decreased activity coefficient).

It was pointed out by Flynn *et al.* (1) that increasing the thickness of a solution gel film does not decrease the absolute release rate of a drug but increases the duration over which release occurs. In terms of total mass released over time, a thin film should initially release drug as a thick film. As the permeation process proceeds however, the flux is expected to fall off more rapidly for a thin film than for a thick one, as mass depletion in terms of the initial drug mass is slower for thick than for thin films. This effect can be seen in Fig. 7, which is a plot of the cumulative mass of drug released over time for a 75- and a 200-μm application. As would be expected for applications initially containing equal drug concentrations, both applications initially release drug at the same rate. However, the concentration of drug in the 75-μm application is exhausted more rapidly than that in the 200-μm application, and thus the 75-μm application reaches a plateau in the mass vs time plot more rapidly than its thicker counterpart.

In a recent review, Guy *et al.* (8) pointed out the need for an *in vitro* system for the measurement of topical bioavailability that is reproducible, reliable, relevant, and accessible. This is necessitated largely by the continual introduction of generic topical products into the marketplace. A standardized *in vitro* testing system would make it possible to compare directly a generic product's release characteristics with those of the brand name product and, thus, allow one to make conclusions regarding relative bioavailability. In this work such a reproducible method for the testing of topical dosage forms has been developed. The silicone rubber should predict the rank-order ability of topical dosage forms to deliver drugs through the intact skin, as the delivery is a function of the membrane/vehicle partition coefficient as the silicone rubber membrane, although a relatively low-resistance membrane, was still of a resistance high enough to exert control of permeation in every experiment. The permeation of intact skin should therefore evidence qualitatively identical patterns to those seen spread out over a far longer time frame. No shift from membrane

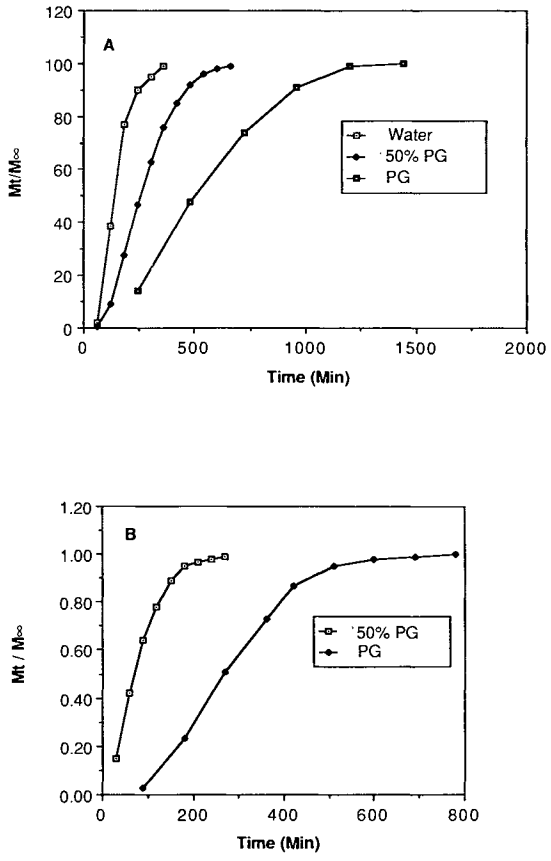

Fig. 6. Relative mass released through a 254-μm membrane versus time for methyl *p*-aminobenzoate formulated in various propylene glycol/water gel systems. The gels were applied in thicknesses of 200 μm (A) and 75 μm (B). Each curve represents the average of three runs.

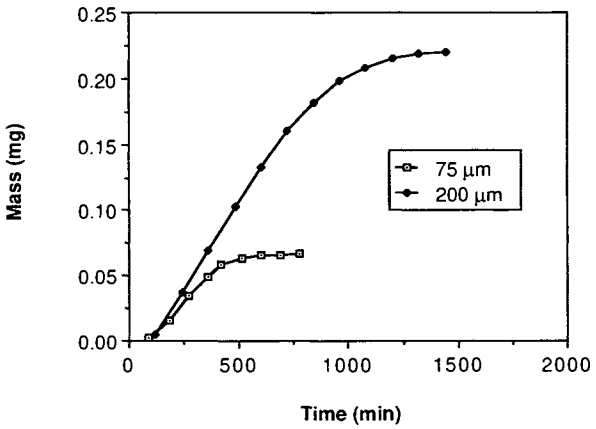

Fig. 7. Cumulative mass released through a 254-μm membrane versus time for methyl *p*-aminobenzoate formulated in a propylene glycol gel system. The gel was applied in thicknesses of 75 and 200 μm. Each curve represents the average of three runs.

control would be involved. Importantly, we have been able to demonstrate that one can apply a finite dose of a topical formulation to skin in thicknesses approaching true usage situations. The methodology properly sorts out partitioning, film thickness, and membrane thickness effects. However, slow transport of the diffusion-cell receiver contents to the collecting chambers in the fraction collector displaces the temporal profiles toward longer times, with loss of important early detail. Adsorptive influences were also encountered. Further refinements of the methodology are being considered to eliminate these undesirable influences.

ACKNOWLEDGMENTS

The splendid cooperation of the Dow–Corning technical staff, who supplied the membranes of study, is recognized. This study was supported by Grants AI/GM22303 and DA04061 from the National Institutes of Health. William J. Addicks wishes to thank the United States Pharmacopeial Convention, Inc., for their support in awarding him a fellowship.

REFERENCES

1. G. Flynn, E. Topp, and G. Amidon. *Topics in Pharmaceutical Sciences 1985*, Elsevier, Amsterdam, 1985, pp. 313–328.
2. J. Ayres and F. Lindstrom. *J. Pharm Sci.* **51**:654–662 (1977).
3. T. Franz. *J. Invest. Derm.* **64**:190–195 (1975).
4. B. Mollgaard and A. Hoelgaard. *Int. J. Pharm.* **15**:184–197 (1983).
5. R. Guy and J. Hadgraft. *Int. J. Pharm.* 6:321–332 (1980).
6. W. Addicks, G. Flynn, and N. Weiner. *Pharm. Res.* 4:337–341 (1987).
7. B. Poulsen, E. Young, V. Coquilla, and M. Katz. *J. Pharm Sci.* 57:928–933 (1968).
8. R. H. Guy, A. H. Guy, H. I. Maibach, and V. P. Shah. *Pharm Res.* 3:253–262 (1986).