In Vitro Release of Propranolol Hydrochloride from Topical Vehicles

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Transdermal drug delivery is becoming increasingly important and for this reason it is clear that academia must ensure that current graduates are knowledgeable in all facets of topical drug administration. An *in vitro* diffusion cell experiment was designed to demonstrate the rate of release of propranolol hydrochloride (PHC) from three different topical vehicles: (*i*) an oil-in-water cream; (*ii*) a gel; and (*iii*) anointment. This experiment was performed by final-year students enroled in an undergraduate course on percutaneous absorption. *In vitro* release of PHC from the three bases to an aqueous receptor phase through silicone membrane was monitored spectrophotometrically at a wavelength of 290 nm. By monitoring and attempting to explain the numerous possible reasons for the different rates of drug release from the three vehicles, it was hoped that the students would gain a better understanding of the complexities of transdermal drug administration. Overall, the experiment would appear to be a good model for student investigation into factors affecting the release of drugs from topical formulations.

INTRODUCTION

There is currently a great deal of world-wide interest in the field of transdermal drug delivery and, consequently, broad classes of drugs are being evaluated for percutaneous absorption potential. The advantages of this mode of drug administration are numerous, the patient convenience and therapeutic optimization of using patch transdermal systems being major positive features. In response to this developing field, academia must ensure that current graduates are knowledgeable in all facets of topical drug adminis-

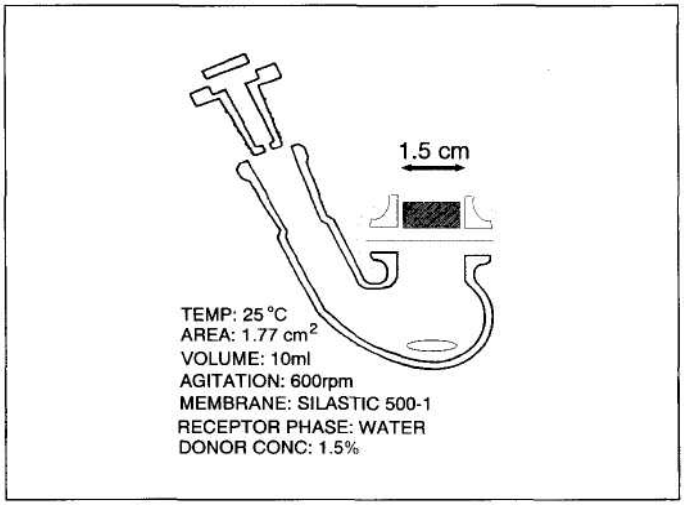

Fig. 1. Diagrammatic representation of the diffusion cell and the conditions of use.

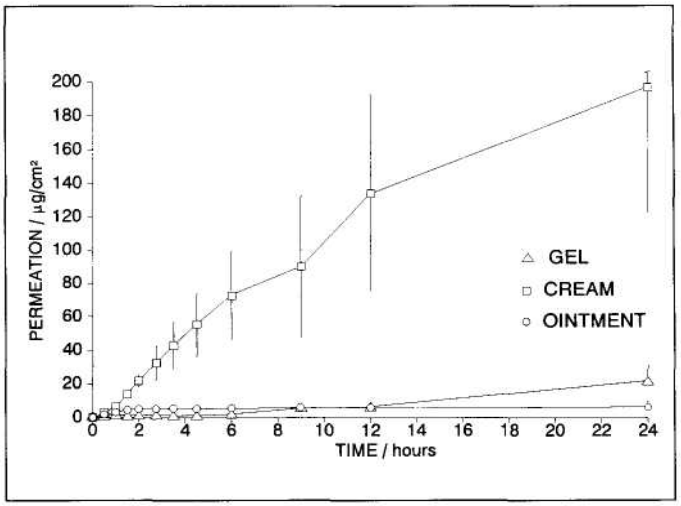

Fig. 2. Permeation of PHC through silicone membrane from cream, gel and ointment formulations.

tration so that they will be adequately equipped to face the challenges that this field will pose in the future. In the final year of the pharmacy degree course presented at this institution, students are required to choose two elective courses which offer more detailed information on various specialized topics. One such course concerns percutaneous penetration and the exercise described here is one of the practical experiments specially designed for this course. A previously described experiment(1) concerned the *in vitro* release of salicylic acid from many different ointment bases and was recommended as an undergraduate practical experiment. It is a useful experiment, but we feel that the utilization of cream, ointment and gel vehicles and the use of a modern *in vitro* diffusion cell system is far more comprehensive in terms of understanding the mechanisms of drug release from semi-solid topical formulations. Furthermore, this standardized *in vitro* methodology appears to be the method which will be adopted by regulatory authorities in the future (2-4) and therefore it is essential for graduates to be familiar with this technology.

Beta-Blockers are required in small amounts in systemic circulation for therapeutic effect and undergo extensive first pass metabolism after oral administration. Hence, theoretically, these agents are prime candidates for transdermal delivery. An *in vitro* diffusion cell experiment was devised to assess the rate of release of a model β-blocker, propranolol hydrochloride (PHC), from three representative topical vehicles: (*i*) an oil-in-water cream; (*ii*) a glycero-gelatin gel; and (*iii*) a paraffin ointment. By monitoring the rate of drug release from the three vehicle types and attempting to explain this in terms of the physicochemical parameters governing the rate of release, it was hoped that the students would gain a better understanding of the complexities of transdermal administration.

METHODS

Ten-milliliter glass diffusion cells (Figure 1) were employed to assess the rate of drug release from each of three delivery vehicles(5). Aporous silicone membrane (Silastic type 500-1, 0.0127 cm thick, Dow Corning, USA) was used as the barrier membrane to separate the test formulation from the purified, degassed water receptor solvent which was agitated by teflon-coated star-head magnetic stirrers. The membrane was held firmly in position between the flange surfaces with the aid of Parafilm (American Can Co., USA), which created a water-tight seal and created a diffusion area of 1.77 cm^2.

PHC was incorporated into the three bases (Aqueous Cream BP. K-Y Jelly® (Johnson and Johnson, South Africa) and white soft paraffin) at a concentration of 1.5 percent (45 mg in 3 g base) by geometric trituration. The three bases were chosen for their different hydrophillic, hydrophobic and viscosity characteristics. Approximately 1 g of medicated formulation was then packed into each of three cell donor chambers, ensuring that there were no air bubbles between the formulation and donor surface of the silicone membrane. The donor chamber orifice was then covered with Parafilm to minimize evaporation of the formulation constituents during the diffusion process. In similar fashion three cells were packed with unmedicated formulation to act as controls for the spectrophotometric analysis. Ten milliliters of purified water was pipetted into each receptor cell containing a stirrer bar and the cells tilted to expel any air bubbles. The sampling side arm was then capped and the cell immersed in a thermostatically-controlled water bath maintained at 25°C such that the membrane was positioned just above the water level and each cell was positioned above a laboratory magnetic stirrer set at 600 rpm. Light opaque material was used to cover the cells and water bath as PHC is susceptible to photodegradation.

Sampling of the receptor solution was carried out at increasing intervals for the first 12 hours and a final sample was taken at 24 hours. Four milliliters of receptor solvent was removed from the cell at each sampling time using a micropipette and was transferred to the spectrophotometer cuvette. The absorbance of this solution was read against the blank with the instrument set at 290 nm. No chromatographic separation of the solutes in the donor phase was carried out and hence the need for inclusion of blank (unmedicated) diffusion cells so that delivery vehicle constituents that also dissolve in the receptor solvent and absorb UV light at 290 nm could be distinguished from PHC. The receptor solution was replaced with 4 ml purified water at each sampling time, thereby maintaining the receptor

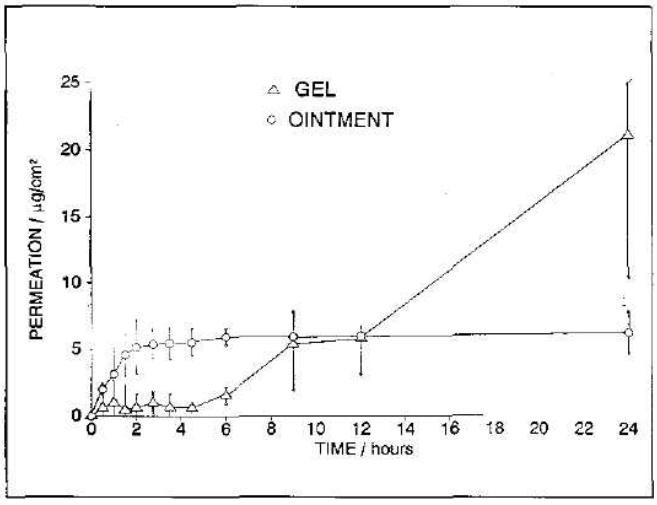

Fig. 3. Permeation of PHC through silicone membrane from gel and ointment formulations.

phase volume and near sink diffusion conditions.

A standard calibration curve for PHC in aqueous solution was constructed at 290 nm using drug concentrations between 0.5 (µg/ml and 25 µg/ml which was used to quantify the sample solutions(6). It was found that this spread of calibration concentrations adequately covered the range of sample absorbances obtained from the diffusion cells and generated an absorbance versus concentration line with a linear correlation coefficient of 0.9969.

RESULTS

The different rates of PHC delivery from the three vehicle types can clearly be seen from the graphs of permeation versus time. PHC delivery was much greater from the cream formulation (Figure 2) than from either the gel or ointment vehicles, which were similar in release potential. The steady-state flux rate of PHC released from the cream formulation calculated from the linear portion of the graph yields a delivery rate of 12.8 µg/cm^2.h. A decline in the flux rate from this formulation after 12 hours may indicate some matrix exhaustion of PHC from the vehicle.

Examination of the rates of PHC delivery from the gel and ointment vehicles (Figure 3) suggests that two different mechanisms of control may be active. The ointment formulation has an initially high release rate (2.8 µg/cm^2.h for the initial linear portion) which plateaus after approximately four hours. This pattern is similar to typical square-root-of-time kinetics described by Higuchi(7) for drug release from non-eroding matrices. This suggests that physical parameters in the donor vehicle are more influential in controlling PHC release from the ointment than are chemical factors. Removal of PHC from the vehicle/membrane interface creates a drug depletion zone which is only slowly replenished by further diffusion of PHC from the ointment core to the vehicle/membrane interface. This diffusion is hindered by the viscosity of the vehicle, hence the decline in PHC delivery rate with time.

The gel formulation demonstrates a long lag time (approximately four hours) before steady state diffusion is seen at a relatively low delivery rate of 1.1 µg/cm^2.h. This would suggest that chemical (dissolution and membrane partitioning) parameters are controlling the rate of delivery.

These delivery rates can be explained by examining Fick's law of diffusion. The flux is proportional to the partition coefficient, the diffusion coefficient and the donor concentration and inversely proportional to the thickness of the membrane. In this study the intrinsic diffusion coefficient, the membrane thickness and the donor concentration were constant. Hence, in the absence of physical mechanisms of the type described above for the ointment, the rate of delivery will be dictated by the partition coefficient between the donor formulation and the membrane.

PHC is highly water soluble (1:20) and therefore has a high affinity for the aqueous gel formulation. It will therefore only partition to a small extent into the less hydrophillic environment of the silicone membrane. Hence the long lag time and slow flux rates observed as these processes are concentration gradient dependent. Conversely it is proposed that PHC has a relatively greater affinity for the membrane than for the ointment vehicle and will therefore demonstrate higher initial partition and flux parameters from this vehicle (corroborated by the higher initial flux rates that have been observed).

The cream formulation appears to present the ideal combination of solubility and physical diffusivity through the vehicle, yielding the highest PHC release rates. The cream is a lipophilic/hydrophillic mixture from which PHC appears to partition readily into the silicone matrix. Relatively facile diffusion through this vehicle of moderate viscosity appears to allow replenishment of the drug depletion layer as PHC is removed from the vehicle/membrane interface. Hence the observed high flux rate and only slight decline in delivery rate after 12 hours.

DISCUSSION

It is well documented that the delivery vehicle markedly affects the way in which the drug is released(8,9). For mass transfer to take place the drug must have some, and preferably greater, affinity for the membrane than for the vehicle, thereby maximizing the thermodynamic leaving potential. Differential drug delivery rates have been explained in terms of the partitioning of the drug between the formulation and the membrane, and in terms of the ease of diffusion of the drug in the delivery vehicle to replenish the depletion zone at the vehicle/membrane interface.

For a more in depth analysis of these permeation results, some calculation of the degree of ionization in each formulation is desirable. For this, the apparent pH of the aqueous formulations should be measured. Typical values we have measured are pH 6 for the gel and pH 7 for the cream. Knowing the pK_a value, the extent of ionization can be calculated using the Henderson equation. If one carries out this exercise, it is found that the ratio of ionized to unionized drug is 10 times greater in the gel formulation than in the cream. At face value it may be argued that only the unionized form of PHC is diffusing through the silicone membrane, hence the observed permeation rates. This theory is, however, incongruous with the rate of permeation observed from the anhydrous ointment formulation where there is low solubility of the PHC and negligible dissociation of this species would occur. Similarly, it is doubtful that the solubility of the unionized form of the permeant in the receptor phase would be great enough to produce the concentrations measured from the cream donor vehicle, the unionized species would have low affinity for the receptor phase and would not partition from the distal surface of the

membrane into the aqueous environment. Another point to consider is that, if the unionized drug form is the only-diffusing species, the hydrogen ions remaining in the donor phase would favour the formation of more ionized molecules. This should inhibit further permeation, but this phenomenon is not observed in either of the aqueous vehicle diffusion experiments. The ionized form of PHC must, therefore, also diffuse through this silastic medium. A more intensive spectrophotometric analysis of the receptor phase solution would be able to provide the ratio of ionized to unionized species; this determination could also be carried out by students if desirable.

We have found that it is sometimes necessary to lead students through the above discussions and we have devised a set of questions concerning this experiment which we find useful in this respect:

1. What is the total solubility of PHC in each vehicle?
2. Using the Henderson equation and the Pharmacopoeial pK_a value, calculate the masses of the ionized and non-ionized form of the drug present in the different formulations using their apparent pH values.
3. Which species permeates the membrane (charged or uncharged)?
4. What alternatives are theoretically possible?
5. How, using any analytical procedures, would you verify your proposal for the above?
6. Will the drug be in the same equilibrium state in each of the three formulations?
7. What effects do the physical characteristics of the vehicle have with regard to the rate of permeation?
8. What different mechanisms of release may be in operation?

CONCLUSIONS

This relatively simple experiment serves as an excellent undergraduate student project to augment the principles of transdermal drug delivery. The experiment is simple to perform. UV detection was purposefully chosen as most laboratories have spectrophotometric methods available. Although our newly designed permeation cells are described here, the experiment could be performed successfully using any of the commercially available diffusion cells or even the simplest of home made cells as described previously(1). Also, this exercise could be extended further to include the analysis of the three formulations.

In assessing and attempting to explain the different drug delivery rates in terms of physico-chemical diffusion principles, students gain a better understanding of the concepts involved in drug delivery from topical formulations. Students' perceptions of the usefulness of this experiment were ascertained over a two year period. Without exception, the student learning outcomes indicated that the experiment was a valuable aid to their comprehension of the sometimes difficult-to-understand phenomenon of transmembrane permeation.

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