J. Pharm. Pharmac., 1977, **29**, 108

COMMUNICATIONS

A method for the measurement of diffusion constants suitable for studies of non-occluded skin

M. I. FOREMAN*, I. KELLY, G. A. LUKOWIECKI, *Department of Chemical Pharmacology, Scientific Development Group, Organon Laboratories Limited, Newhouse, Lanarkshire ML1 5SH, U.K.*

There is considerable current interest in the diffusion of therapeutic agents through skin (Brisson, 1974). Unfortunately there is a scarcity of information on this subject, and the number of experimental approaches to the problem are limited. Almost invariably, *in vitro* diffusion studies involving skin are carried out under conditions appropriate to the lag-time method (Daynes, 1920; Crank, 1956), whereby the skin sample is maintained between two solutions. Under these circumstances the skin is saturated with solvent, and in a clinical sense, the results obtained are, at best, only relevant to the behaviour of skin under rigorous occlusion.

To complement such studies it would be useful if diffusion could be measured by an *in vitro* experiment which relates more to the non-occluded skin situation. The simplest experiment which might be envisaged involves monitoring the appearance of a diffusant in an effectively infinite sink, which would approximate to the vascular system, following application of the diffusant to the skin surface which is left open to the air. To simplify the problem, the diffusant could be applied to the skin surface as an effectively infinitely thin film. Provided that this ideal can be approached in practice, problems of diffusion within the surface film, the nature of the film (whether amorphous or crystalline) and the dissolution step as the diffusant becomes incorporated into the membrane, may be neglected.

In this work we have investigated one possible approach to the above experimental situation using a synthetic membrane in the first instance. Diffusion constants for nandrolone and testosterone in a polydimethylsiloxane membrane (Dow-Corning RTV 615, without filler) have been determined by this technique and compared with those obtained by use of the lag-time experiment. The method appears to be a useful one, and will subsequently be applied to a study of steroid diffusion in human skin.

The experimental design is as shown in Fig. 1, the boundary conditions are inset. Radioactively labelled steroid was applied as a dilute methanol solution to the upper surface of the polymer sheet, using a fine hair brush, at time zero. At subsequent intervals, fresh solvent was passed into the lower chamber of the cell, the displaced solvent collected and the amount of radioactive material present estimated by liquid scintillation counting using a Unilux 11 counter. All measurements were carried out at 22 ± 2°.

To avoid the complexity of an analytical mathematical approach to the problem set by the boundary conditions of the above diffusion process, since no solution for this situation can be found in the literature, the ideal behaviour of the system was predicted by an elementary Monte-Carlo simulation using an Elliott 905 computer (King, 1951; Crank, 1956). In this manner a predicted plot of F_t, the fraction of the total applied dose passing through the membrane in the time interval 0 to t was obtained. The simulation was such that the mean particle path length was 0·02L where L is the membrane thickness. The validity of this type of approach was checked beforehand for the set of boundary conditions appropriate to the lag-time experiment, for which an analytical solution exists. The simulated behaviour in this case agrees very well with the more formal, mathematical prediction. For the experiment described above, the predicted F_t versus t curve is shown in Fig. 2.

It was found in practice that the ideal curve of Fig. 2 could be fitted to the experimental curves of Q_t versus t obtained under the conditions described above, where Q_t is the total amount of material passing through the membrane in the time interval 0 to t, by applying

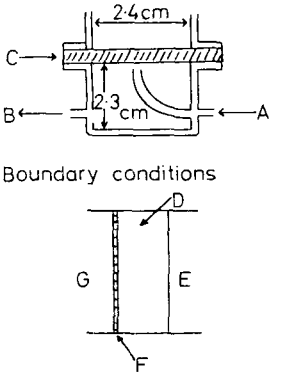

FIG. 1. Glass diffusion cell in cross-section. A—Solvent in, B—sink solution out, C—membrane, D—membrane thickness, L, E—sink concentration, C = 0, t ⩾ 0, F—diffusant film thickness l; L ≫ l, G—normal ambient atmosphere.

* Correspondence.

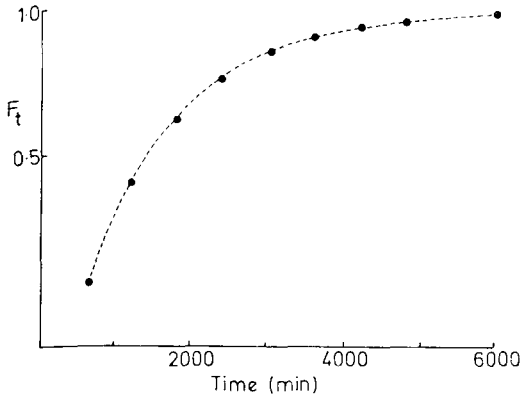

FIG. 2. Computer simulated plot of F_t versus t (dashed line). Circles indicate experimentally observed penetration of nandrolone through RTV 615 membrane at 22 ± 2°.

scaling factors S_F and S_t to the F_t and t axes respectively. The values of S_F and S_t for a particular case may be determined by a simple computer program in the following way. The program requires as input values of Q_t versus t, and an initial estimate of S_F, which is effectively the total dose of diffusant applied to the membrane. The data appropriate to the ideal curve is stored within the program in digitized form, with an appropriate interpolating sub-routine. The program initially computes a mean value of S_t corresponding to the input value of S_F and then employs an incremental step search routine to optimize both S_t and S_F in turn, such that the best least-squares fit is obtained between the scaled, interpolated, ideal curve and the experimental curve. The diffusion constant may then be evaluated from S_t by the following reasoning. The computer simulation of the diffusion process was set up such that each particle moved a distance of 0·02L in the ± X-direction in unit time, where L is the membrane thickness. The procedure described above identifies 'unit time' as being S_t units of real time, so that, using Einstein's relation (c.f. Glasstone, 1956), equation 1 applies.

$$D = \frac{(0{\cdot}02L)^2}{2S_t} \quad \ldots \quad \ldots \quad 1$$

Consequently, from the above experiment, both D and the applied dose (S_F) may in principle be determined.

The penetration of two steroids, nandrolone and testosterone, across polydimethylsiloxane sheet has been measured in the above manner. Replicate measurements were carried out in each case, and the computed least-squares fit between the observed and predicted curves was in all cases extremely good, one such fit is shown (closed circles) superimposed on the curve of Fig. 2. The calculated diffusion constants for each experimental run are shown in Table 1. From the tabulated data it is evident that the experiment shows an acceptable level of reproducibility, and the estimated parameters are realistic, insofar as there is no significant difference between the values observed for the two steroids. This is to be expected, at least for an inert polymer membrane such as that used here, since the two steroids have very similar molecular volumes.

Table 1. *Diffusion constants for nandrolone and testosterone in polydimethylsiloxane membranes at 22 ± 2°.*

Steroid	Run	Membrane thickness (cm)	Estimated diffusion constant ($cm^2\ s^{-1}$)
Nandrolone	1	0·0204	$6{\cdot}7 \times 10^{-8}$
	2	0·0176	$9{\cdot}3 \times 10^{-8}$
	3	0·0154	$7{\cdot}6 \times 10^{-8}$
	4	0·0202	$12{\cdot}7 \times 10^{-8}$
Testosterone	1	0·0185	$5{\cdot}6 \times 10^{-8}$
	2	0·0183	$8{\cdot}0 \times 10^{-8}$
	3	0·0167	$8{\cdot}7 \times 10^{-8}$

Mean values: Nandrolone $9{\cdot}1 \pm 1{\cdot}3 \times 10^{-8}\ cm^2 s^{-1}$
Testosterone $7{\cdot}4 \pm 1{\cdot}0 \times 10^{-8}\ cm^2\ s^{-1}$

As a further check on the validity of the method, the diffusion constant for nandrolone has also been determined for this system using the conventional method. The experimental procedure has been described in detail elsewhere (Foreman & Kelly, 1976). The value determined in this way for nandrolone was $11{\cdot}4 \pm 2{\cdot}0 \times 10^{-8}\ cm^2\ s^{-1}$ which is not significantly different from that obtained by the alternative method.

The simple experimental procedure outlined above for the measurement of diffusion constants under conditions which more closely resemble the non-occluded topical therapeutic condition yields values which are reproducible and consistent with alternative methods for the measurement of diffusion behaviour. It therefore becomes possible to study in more detail the effects of hydration of skin resulting from topical therapy under occlusion.

October 8, 1976

REFERENCES

BRISSON, P. (1974). *Can. med. Ass. J.*, **110**, 1182–1185.
CRANK, J. (1956). *The Mathematics of Diffusion*, p. 48, Oxford: Oxford University Press.
DAYNES, H. (1920). *Proc. R. Soc.*, A97, 286–307.
FOREMAN, M. I. & KELLY, I. (1976). *Br. J. Derm.*, **95**, 265–270.
GLASSTONE, S. (1956). *Textbook of Physical Chemistry*, p. 260, London: MacMillan.
KING, G. W. (1951). *Ind. Engng Chem.*, **43**, 2475–2478.