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Analysis of percutaneous permeation data: II. Evaluation of the lag time method

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Abstract

To evaluate the lag time method, the permeation of four drugs, propranolol (PR), triamcinolone acetonide (TA), physostigmine (PHY), and tetrahydroaminoacridine (THA), was studied across two skin membranes, hairless mouse skin (HMS), and human cadaver skin (HCS). The permeation studies were conducted with vertical Franz diffusion cell in the static mode, using an infinite dose technique. The resulting permeation profile for each individual drug was analyzed by the lag time method to estimate parameters such as the steady-state flux (J_{ss}), lag time (T_{lag}), diffusion coefficient (D) and skin/donor-phase partition coefficient (K_m). The diffusion coefficient (D) and skin/donor phase partition coefficient (K_m) were used to regenerate the entire permeation profile (pre-steady and steady states) using an equation based on Fick's laws of diffusion. The fit of the observed data to the regenerated curve was estimated by linear regression with the observed data as the independent variable. In experiments where steady state should have been achieved at the end of the experiment (experimental duration $> 3 \times T_{lag}$), R^2 was greater than 0.993, the slope was unity and the y-intercept zero for the regenerated profile vs the observed data regression (e.g., PR 72 h, TA with 2% Azone (AZ) pretreatment and 2% AZ as copenetrant, PHY, and THA experiments). For the PR 27 h experiment, TA control and TA with 2% propylene glycol (PG) pretreatment experiments, the slope was significantly greater than unity for linear regression of the regenerated profile vs the observed data. In these experiments, the experimental duration was less than $3 \times T_{lag}$ and steady state may not have been achieved, which could have resulted in inaccurate estimates of D and K_m and hence, the positive bias in the regenerated profiles. Overall, the lag time method was successful in the estimation of permeation parameters from

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Abbreviations/symbols: D, diffusion coefficient (cm^2/h); K_m, membrane (skin)-donor phase partition coefficient; C_s, saturation solubility of diffusant in the donor phase (mmol/ml); K_p, permeability coefficient (cm/h); h, thickness of the barrier (μm); t, time (h); J, flux of diffusant across the membrane (mmol/cm^2 per h); J_{ss}, flux of diffusant at steady state (mmol/cm^2 per h); T_{lag}, lag time of diffusion (h); A, cumulative amount of diffusant permeated through the membrane until time t (mmol/cm^2); PR, propranolol; TA, triamcinolone acetonide; PHY, physostigmine; THA, tetrahydroaminoacridine; HMS, hairless mouse skin; HCS, human cadaver skin; AZ, Azone; SC, serial correlation; MSC, model selection criterion; AIC, Akaike information criterion.

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which the entire profile could be regenerated for all the drugs studied. However, the lag time method may result in inaccurate estimates of D and K_m if steady state is not achieved as demonstrated by a positive bias (slope > 1) in the regenerated profile. The approach used in this study could be used to verify whether D and K_m obtained from a permeation experiment are precise, accurate and hence reliable.

Key words: Percutaneous permeation; Lag time method; Steady state; Diffusion; Propranolol; Triamcinolone acetonide; Physostigmine; Permeation enhancer; Tetrahydroaminoacridine; Azone; Skin

1. Introduction

To study the permeation of chemicals and drugs across skin, percutaneous permeation has been treated as a passive diffusion process across a homogeneous membrane and equations have been derived based on Fick's first and second laws of diffusion (Crank, 1975; Barry 1983; Durgard, 1983). The diffusion model describes the in vitro percutaneous permeation of drugs fairly well, despite the complex nature and structure of skin. The most commonly used method for analysis of percutaneous permeation data from an in vitro experiment is the lag time method (Barry, 1983; Durgard 1983). In the previous study, we illustrated with simulations that at least 3–4 lag times were required to achieve steady-state diffusion across the membrane (Shah, 1993). We demonstrated that the T_{lag} method of data analysis resulted in significant errors in J_{ss} and T_{lag}, if the experiment had not been conducted for a long enough time period to achieve steady state (Shah, 1993). The problem with the T_{lag} method becomes more acute for percutaneous permeation, since the lag time of diffusion across skin has been reported to range from a few minutes to several days due to the lower diffusion coefficient of drugs ($D = 10^{-6}$–10^{-13} cm^2/s) across skin (Barry, 1983; Martin et al., 1983). The long lag times suggest that in vitro percutaneous permeation should be conducted for a prolonged period of time to reach steady state. Prolonged permeation experiments are often impossible with skin due to model skin membranes being viable only for a short period of time in contrast to synthetic membranes.

The objective of the present study was to assess the reliability of the lag time method to estimate D and K_m accurately using experimentally obtained permeation data of four different drugs across two model skin membranes. We had previously studied permeation of propranolol (PR), triamcinolone acetonide (TA), physostigmine (PHY) and tetrahydroaminoacridine (THA) across hairless mouse skin (HMS) and human cadaver skin (HCS) (Chow et al., 1984; Lau, 1989; Tenjarla 1989; Lau, 1990). The permeation experiments were conducted using vertical Franz diffusion cells with an infinite dose technique as described below in section 2. The resulting permeation data were analyzed by the lag time method and the parameters so obtained were used in estimating the reliability of the method as described in section 3.

2. Materials and methods

2.1. Materials

Tritiated PR (specific activity = 22 Ci/mmol) and TA (specific activity = 37 Ci/mmol) were used as received from New England Nuclear (Boston, MA). PHY and THA-HCl were purchased from Sigma (St. Louis, MO) and Aldrich (Milwaukee, WI), respectively. THA-HCl was converted to THA with sodium bicarbonate. AZ was used as supplied by Nelson Research and Development Co. (Irvine, CA). Propylene glycol (PG), (MCB, Norwood, OH) was used as solvent for all the drugs applied to the membrane.

2.2. In vitro permeation study

Hairless mouse skin (HMS) was dissected from homozygous Hr/Hr hairless mice (HRS/J strain, age 2–4 months). Human cadaver skin (HCS) was obtained immediately after autopsy and stored

frozen at −20°C until the experiment. The HMS was used fresh while HCS was allowed to thaw at room temperature prior to the experiment.

Details of the in vitro permeation study have been published in an earlier paper (Chow et al., 1984). The skin sample was mounted between the donor and receptor chambers of the Franz diffusion cell (5 ml) with a 9 mm i.d. O-ring. The stratum corneum was exposed to ambient conditions, and the dermal side was in contact with saline-phosphate buffer (PBS, pH 7.4), simulating the physiological pH of the dermis, in the receptor chamber. The temperatures of the receptor compartment and the skin sample were maintained at 32°C for HMS and 37°C for HCS. The solution in the receptor compartment was stirred with a teflon-coated magnetic bar at 9000 rpm. Samples of 0.2 ml were withdrawn at various time intervals from the side arm and replaced with an equivalent volume of fresh PBS.

The permeabilities of PR and TA were studied across HMS while HCS was used as a barrier for permeation experiments of PHY and THA. The amounts of PR and TA permeated were estimated by liquid scintillation counting of the withdrawn sample in Aquasol-2. The diffusate samples containing PHY and THA were analyzed according to a previously published HPLC assay (Lau, 1990).

3. Theoretical treatment and data analysis

The lag time method is based on Fick's laws of diffusion, the basic assumption being that skin behaves as a homogeneous (simple one-phase) membrane. The apparent terminal linear (steady state) region was defined by at least four observed points with a minimum R^2 of 0.997, and the regression terminated when the inclusion of an additional point resulted in a decreased value of R^2. The apparent terminal linear portion of the observed permeation profile was fitted to the following equation:

$$A = J_{ss}(t - T_{lag}) \quad (1)$$

where,

$$T_{lag} = h^2/6D$$

and

$$J_{ss} = DK_mC_s/h \quad (2)$$

D was calculated from T_{lag}, taking the value of the thickness of fully hydrated stratum corneum to be 40 μm (Chow, 1984). From the values of J_{ss}, D, h, and the drug concentration in the donor solution (C_s), permeability coefficient (K_p) and skin/donor-phase partition coefficient (K_m) were calculated. The estimated parameters are listed in Table 1.

Table 1
Percutaneous permeation parameters of various drugs obtained by analyses of the permeation profiles by the lag time method

Drug	Lag time method				
	J_{ss} (dpm/cm^2 per h)	T_{lag} (h)	K_p (cm/h)	D [a] (cm^2/h)	K_m
PR, 27 h	4.48×10^2	16.90	8.3×10^{-5}	1.63×10^{-7}	2.04
PR, 72 h	1.91×10^2	28.77	3.8×10^{-5}	0.93×10^{-7}	1.65
TA, control	5.63×10^2	2.23	2.2×10^{-5}	1.11×10^{-6}	0.08
TA, 2% PG pretreatment	6.39×10^3	3.94	2.6×10^{-4}	0.68×10^{-6}	1.53
TA, 2% AZ pretreatment	1.44×10^5	0.29	5.9×10^{-3}	9.20×10^{-6}	2.56
TA, 2% AZ copenetrant	0.82×10^5	3.91	3.4×10^{-3}	0.68×10^{-6}	19.71
PHY [b]	7.48 (μg/cm^2 per h)	22.16	5.0×10^{-5}	1.20×10^{-7}	1.66
THA [b]	42.31 (μg/cm^2 per h)	12.05	1.5×10^{-4}	2.21×10^{-7}	2.63

[a] For calculation of D, the thickness of the hydrated stratum corneum was assumed to be 40 μm.
[b] HCS, other studies used HMS.

To determine whether the estimated parameters, D and K_m were accurate and reflected the true permeation parameters (D and K_m) of the respective drugs, D and K_m obtained from J_{ss} and T_{lag} were used in Eq. 3 to regenerate the complete permeation profile (pre-steady and steady states). The amounts of drug permeated during various sampling time periods were calculated numerically using the values of D, K_m, C_s and h in Eq. 3. The equation was obtained by the Laplace transform technique on the appropriate differential equation (Shah, 1993) using the computer program, LAPLACE (Micromath Inc., UT):

$$A = \frac{DK_mC_s}{h}\left(t - \frac{h^2}{6D}\right) - \frac{2hK_mC_s}{\pi^2}\sum_{n=1}^{\infty}\frac{(-1)^n}{n^2}e^{(-Dn^2\pi^2t)} \quad (3)$$

The regenerated permeation profile was plotted and compared to the observed data in Fig. 1–8. To determine how closely the regenerated permeation profile correlates to that observed, a linear regression was conducted with observed values as the independent variable as shown below:

regenerated value

$$= \text{slope}(\text{observed value}) + y\text{-intercept} \quad (4)$$

The slope of the linear regression should be unity and the y-intercept zero, if the regenerated profile is identical to the observed profile ($R^2 = 1$). Therefore, the slope and y-intercept were evaluated statistically for equality to unity and zero, respectively, at $p = 0.05$, as shown in Table 2, using STATGRAPHICS Plus V.6 (Manugistics, Inc., MD). In addition, the R^2 between the observed and regenerated data was also calculated for each profile and listed in Table 2. Analysis of residuals was conducted to determine if the residuals were scattered randomly about zero as estimated by Serial Correlation (SC) using MINSQ (Draper and Smith, 1981; Micromath Inc., UT). In addition, the residual plots were constructed with time, and the regenerated values as independent variables, and evaluated visually for any systematic trend (Draper and Smith, 1981). This also enabled estimation of the model selection criterion (MSC) which is the Akaike

Table 2
Correlation of regenerated permeation profiles with observed permeation profiles by linear regression

Drug	Regression parameters [a]			
	Slope (mean ± SE) (statistical result)	y-Intercept (statistical result)	P value for y-intercept	R^2
PR, 27 h	1.20 ± 0.04 (> 1)	−153 (0)	0.10	0.987
PR, 72 h	1.08 ± 0.05 (= 1)	−187 (0)	0.31	0.986
TA, control	1.13 ± 0.04 (> 1)	−555 (< 0)	0.001	0.982
TA, 2% PG pretreatment	1.06 ± 0.02 (> 1)	−1927 (< 0)	0.003	0.996
TA, 2% AZ pretreatment	0.97 ± 0.02 (= 1)	31777 (0)	0.19	0.993
TA, 2% AZ copenetrant	0.99 ± 0.01 (= 1)	13641 (> 0)	0.011	0.998
PHY [b]	1.00 ± 0.03 (= 1)	4 (0)	0.40	0.994
THA [b]	1.00 ± 0.01 (= 1)	8 (0)	0.51	0.999

[a] Equation for linear regression:

regenerated value = slope × (observed value) + y-intercept

If the regenerated values are accurately predicted by the parameters obtained by the lag time method, R^2 and the slope should be unity, and the y-intercept zero. The slope and the y-intercept calculated from the above regression were tested statistically for equality to unity and zero, respectively, at $p = 0.05$. The 95% confidence interval on the slope indicated its statistical equality to unity and if $p > 0.05$ the y-intercept was statistically zero.

[b] HCS, other studies used HMS.

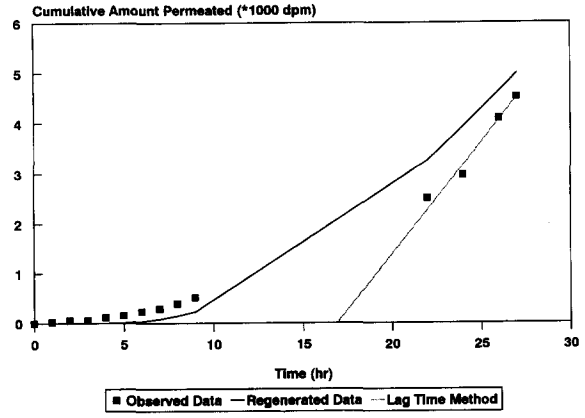

Fig. 1. Propranolol permeation profile (27 h experiment) across hairless mouse skin ($n = 4$).

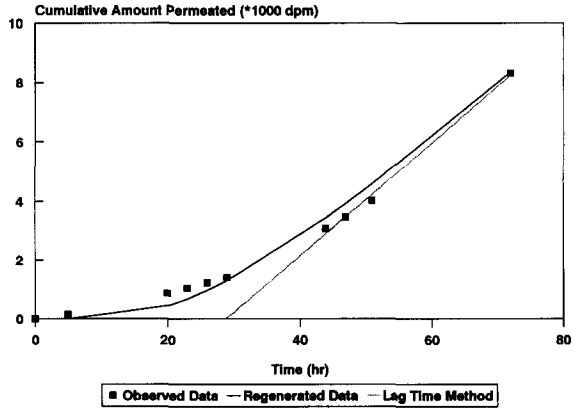

Fig. 2. Propranolol permeation profile (72 h experiment) across hairless mouse skin ($n = 4$).

information criterion (AIC) modified to eliminate the effects of magnitude and number of data points (Akaike, 1973, 1976; MINSQ user handbook). Higher values of MSC (MSC > 2) indicate a better fit of the data to the model (goodness of fit).

4. Results and discussion

The permeation profile was successfully regenerated for all the drugs under all studied conditions. The parameters obtained are listed in Table 1. There was fairly good correlation ($R^2 > 0.982$) between the regenerated profiles and the observed permeation profiles as seen from Fig. 1–8 and Table 2. This was also supported by the goodness of fit indicator, MSC, which was greater than 3.8 for all data except those of PR 27 h (MSC 2.51) and TA control (MSC 3.42). If the values of D and K_m are accurate, the regenerated curve should superimpose on the observed data points and the slope for regression should be unity and y-intercept zero as seen in the case of PR 72 h, TA, 2% AZ pretreatment and 2% AZ copenetrant, and PHY and THA permeation data. Despite good correlation ($R^2 > 0.982$), there was systematic deviation from the observed data in three of eight regenerated profiles (PR 27 h, TA control and TA, 2% PG pretreatment data). For PR 27 h, TA control and 2% PG pretreatment data, the slope was significantly greater than unity, indicating a trend of positive bias (slope > 1) in the regenerated values. The y-intercept was not significantly different from zero for five data sets but was negative for TA control, and TA, 2% PG pretreatment data, and positive for TA, 2% AZ copenetrant data. Although the y-intercept for TA, 2% AZ pretreatment data was highest (31 777), it was not significantly different from zero due to a large standard error of the estimate. However, the y-intercept was insignificant compared to the steady-state data which ranged from 1.12×10^5 to 1.63×10^6 dpm.

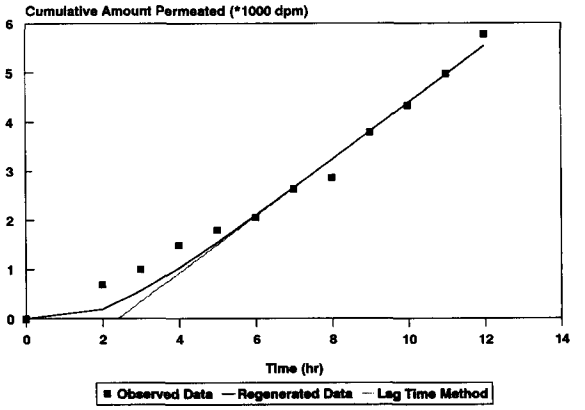

Fig. 3. Triamcinolone acetonide permeation profile (Control experiment) across hairless mouse skin ($n = 3$).

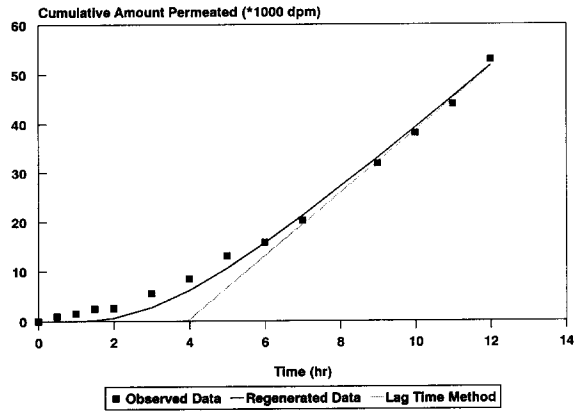

Fig. 4. Effect of 2% PG pretreatment on triamcinolone acetonide permeation across hairless mouse skin ($n = 2$).

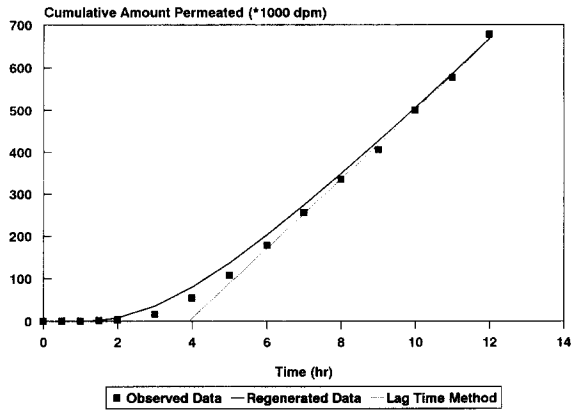

Fig. 6. Effect of 2% AZ copenetration on triamcinolone acetonide permeation across hairless mouse skin ($n = 3$).

Since the parameters used for regeneration are obtained by the lag time method from the terminal linear, apparent steady-state region, not unexpectedly, regeneration of the steady-state data was better, i.e., closer to the observed value, than the pre-steady-state data (Fig. 1–8). There was no systematic deviation of the pre-steady-state data from the model for five data sets. However, analysis of residuals by SC showed that for TA control, and TA, 2% AZ pretreatment and 2% AZ copenetration data ($SC > 1$), the residuals were not randomly distributed about zero. This implied that pre-steady-state data were equally well regenerated and predicted to the steady-state data without any systematic deviation (either positive or negative bias) from the model. The TA data encompass a broad range of amounts permeated (5×10^2–2×10^6 dpm), and hence, not surprisingly, lower amounts (with higher assay variability) in the pre-steady-state region exhibited small systematic deviation from the model in contrast to other drug permeation profiles. The residual plots confirmed the results obtained by the serial correlation test.

In an earlier publication (Shah, 1993), we demonstrated using simulations that $6 \times T_{lag}$ were required to achieve 100% J_{ss} and with $3 \times T_{lag}$, 97% J_{ss} was attained. If the experiment was

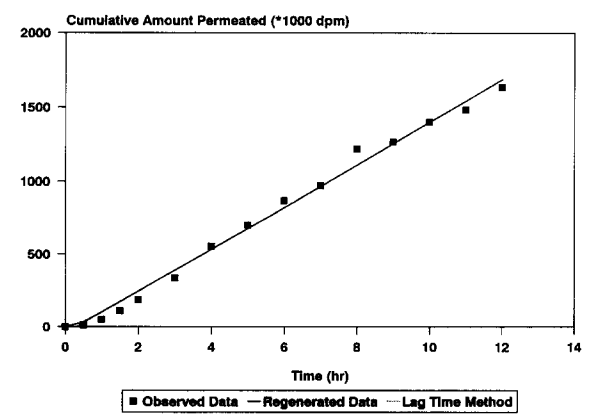

Fig. 5. Effect of 2% AZ pretreatment on triamcinolone acetonide permeation across hairless mouse skin ($n = 2$).

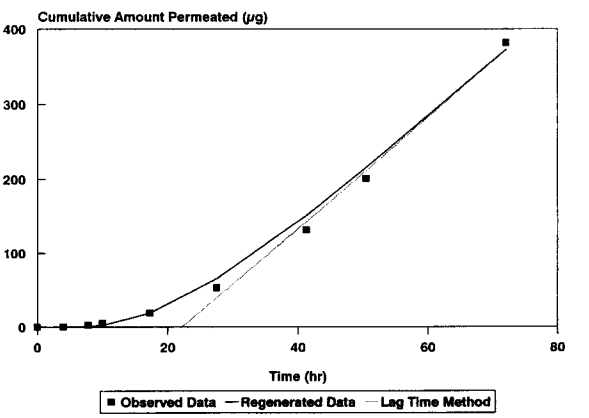

Fig. 7. Physostigmine permeation profile across human cadaver skin ($n = 5$).

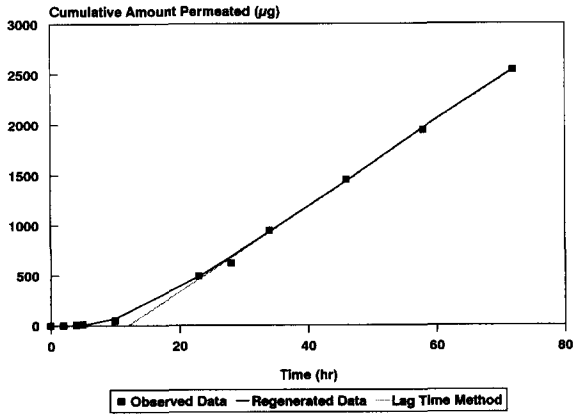

Fig. 8. Tetrahydroaminoacridine permeation profile across human cadaver skin ($n = 7$).

conducted for a shorter duration than three lag times, D would be overestimated and K_m underestimated significantly (Shah, 1993). In the present study, the duration of the experiment was almost equal to or greater than three lag times except for the PR 27 h experiment ($< 2 \times T_{lag}$). Futhermore, we find a strong positive bias in the regenerated profile of the PR 27 h experiment with a slope of 1.2. In contrast, in the THA and TA with 2% AZ pretreatment experiments, the experimental duration was at least $6 \times T_{lag}$. In both cases, there was excellent correlation between the regenerated and observed profiles ($R^2 > 0.999$ and slope = 1 for THA and 0.97 for TA, 2% AZ pretreatment). Thus, these results indicate that the lag time method is very reliable in accurately estimating the effective parameters D and K_m, if the experiment is conducted for at least three lag times. The effective parameters D and K_m so obtained reliably regenerated the complete pre-steady-state and steady-state permeation profile.

It is common practice to commence permeation studies with an unknown compound on an in vitro skin model with no prior knowledge of the physicochemical properties of the compound. As a result, the first few experiments may not reveal whether a steady state is achieved and if the estimated D and K_m are precise. Sometimes, the viability of skin precludes lengthy experiments to obtain steady-state data. In these scenarios, the approach and the methodology we have used in this study can be used to verify the precision and accuracy of estimation of the primary permeation parameters, D and K_m. First, a pilot experiment must be conducted of sufficiently long duration to estimate the D and K_m using Eq. 1 and 2. Then, the entire profile can be regenerated using Eq. 3 with the estimated values of D, K_m and known values of C_s and h, using a numerical method. A significant linear regression (slope = 1 and y-intercept = 0) between the regenerated and observed permeation profiles would indicate that the experimental duration was sufficient to have achieved steady-state diffusion and the estimated D and K_m are precise and accurate and hence true values. In addition, a residual plot and serial correlation test may indicate if there is a systematic deviation of the regenerated data from those observed.

Two basic assumptions in the aforementioned method should be noted; firstly, skin is treated as a single-phase (homogeneous) membrane, and secondly, the permeability characteristics of the skin do not alter during the experiment. Treating skin as a homogeneous membrane may not explain the permeation properties of all compounds under all circumstances. In addition, the presence of permeation enhancers and/or solvents may alter the permeability characteristics of the skin during the experiment. Even changes in the hydration level of the skin are known to alter its permeability characteristics during the experiment (Barry, 1983, 1987). However, in spite of these assumptions, we have been successful in evaluating the lag time method and applying it to four permeants with or without enhancer across two model skin barriers, which is indicative of the robustness of the method.

5. Effect of permeation enhancers

Pretreatment of HMS with 2% PG increased the permeability of TA which was due to an increase in K_m, the skin/PG partition coefficient. Therefore, PG appeared to enhance permeation by a purely solvent action. In contrast, pretreatment of skin with 2% AZ significantly

increased both the D and K_m of TA. The permeability of TA co-applied with 2% AZ was increased significantly due to an increase in K_m only, as D was unchanged. This dichotomy of results indicates that a finite time is required for AZ to alter the barrier properties of the skin to increase the D of the permeant. Therefore, pretreatment with the same amount of AZ (2%) resulted in significantly greater enhancement of flux by primarily increasing D and a small increase in K_m.

When Az was co-applied with TA, since AZ itself penetrated the skin, the permeability characteristics of the skin were modified. As TA penetrated through the skin, it experienced the ever-changing effects of the moving AZ front and resulted in increasing values of D and K_m of TA. Thus, in this scenario, a single set of D and K_m was inadequate to describe the overall permeation profile of TA. The values of D and K_m obtained in the study were apparent values that would depend upon the permeation rate of AZ through the skin relative to that of TA, and the differential effects of the solvent (PG) on the permeation of AZ and of TA. PG pretreatment increased K_m by purely solvent and hydration effects, which increased the affinity of the stratum corneum for TA (Barry, 1987). In contrast, AZ pretreatment increased D in addition to K_m, indicating a change in the structural properties of stratum corneum resulting in increased diffusivity of the permeant.

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