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Modelling transepidermal water loss under steady-state and non-steady-state relative humidities

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Abstract

A gravimetric method was used for investigating the effect of relative humidity on transepidermal water loss (TEWL) across piglet stratum corneum. Measurements were made under both steady-state relative humidities (iso-RH%) and under constantly varying relative humidities (non-iso-RH%). It is shown that the TEWL-RH% relationships obtained using the two methods are qualitatively similar. The values obtained using the non-iso-RH% method were consistently higher than those determined with the iso-RH% method. Despite this bias, the non-iso-RH% method provides a rapid means for evaluating the effect of moisturisers on TEWL. Initial results suggest that urea markedly decreases TEWL but that pyrrolidone carboxylic acid has little effect.

Key words: Moisturiser; Modelling; Skin hydration; Skin permeability; Dry skin

1. Introduction

Healthy stratum corneum contains a minimum of 10% of water (Blank, 1952) under normal conditions. Disturbance of this equilibrium water content affects skin softness and flexibility (Blank, 1952, 1953; Park and Baddiel, 1972; Reiger and Deem, 1974; Van Duzee, 1978; Takahashi et al., 1981). Three factors interact to determine the equilibrium value: the transfer of water from the underlying tissue to the stratum corneum, the composition of the stratum corneum and the external environment (Scheuplein and Blank, 1971; Grice et al., 1971, 1972; Dugard 1983; Wu, 1983; Blank et al., 1984; Blank, 1985; Wilson and Maibach, 1989). With the latter, temperature, relative humidity and air flow are the most important factors affecting water loss from the skin (Anderson et al., 1973; Spencer et al., 1975; El-Shimi and Princen, 1978; Behl et al., 1980; Miller et al., 1981; Hilliard and Dorogi, 1989). In the presence of dermal pathology or as a result of excessive washing with harsh surfactants, the composition of the skin may be sufficiently altered to affect skin moisture content (Takenouchi et al., 1986; Agner and Serup, 1989; Horrobin 1989; Imokawa et al., 1989). In many such instances xerosis ensues. As a palliative measure skin hydrating agents or emollients are widely

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used in clinical practice (Hanifin, 1989). In a wider context, such agents are in general use as cosmetic agents.

Despite the availability of in vivo methods for studying such agents numerous in vitro techniques are still widely used when evaluating moisturising agents. In this report, an in vitro gravimetric method is used to elucidate further the state of hydration of stratum corneum at various relative humidities under steady-state (iso-RH%) and non-steady-state (non-iso-RH%) conditions and the potential for non-iso-RH% investigations into the mechanism of action of moisturising agents is probed.

2. Materials and methods

2.1. Preparation of piglet stratum corneum

Still-born piglets, either Large Whites, Landrace, or a cross of these, were obtained already

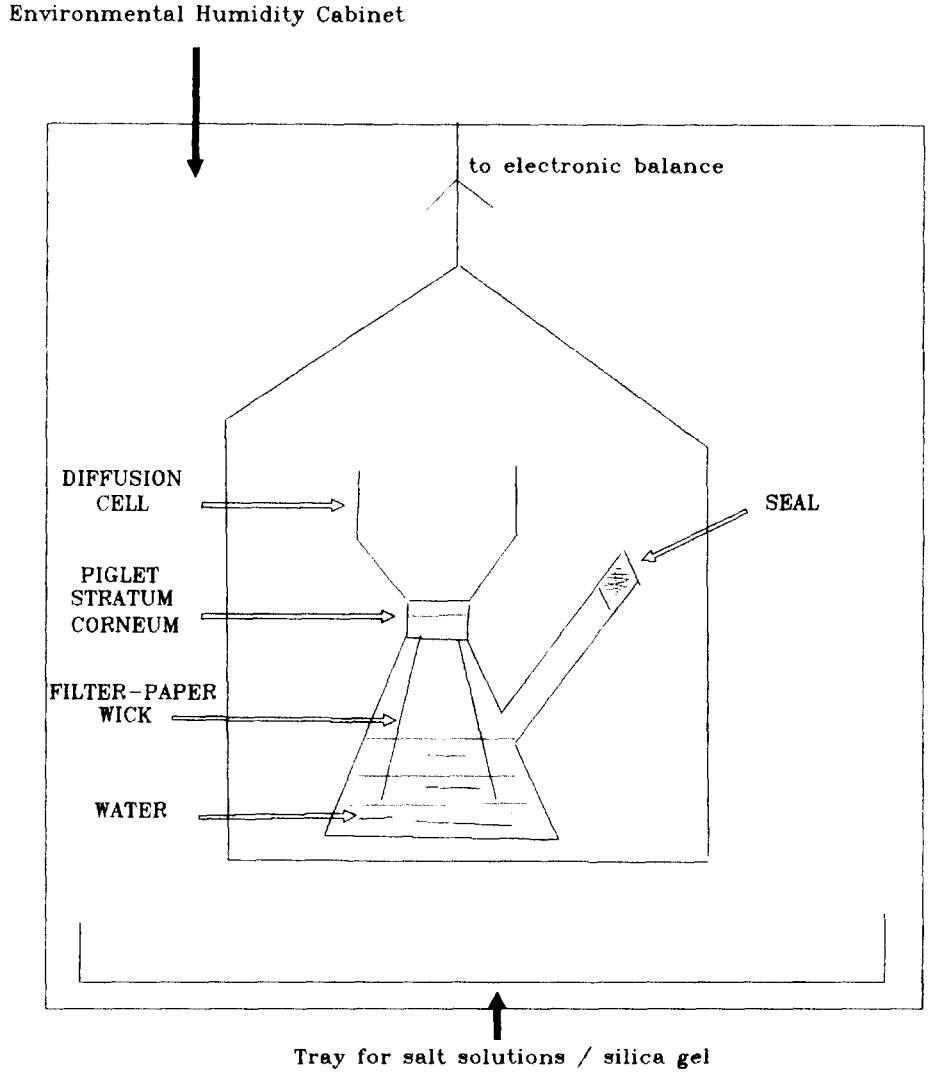

Fig. 1. Diffusion cell set-up for measurement of transepidermal water loss.

frozen, from Greenmount Agricultural College, Co. Antrim. The pigs were maintained at −20°C until required. When stratum corneum was to be removed, the piglets were defrosted overnight at room temperature. Standard animal clippers (Oster Golden A5, model 5-55E) were used to remove the coarse hairs. A closer shave was subsequently carried out by using disposable Bic® razors. To minimise damage to the stratum corneum at this stage, the close shave was carried out very carefully in the direction of hair-growth. The shaved piglet was then immersed in a water-bath at 60 ± 1°C for an initial period of 60 s. If necessary, the piglet was re-submerged for a further 30 s. The stratum corneum was then removed by peeling it away from the underlying layers in strips of approx. 2 cm × 4 cm, a size sufficient for one diffusion cell (Fig. 1). The isolated strips of stratum corneum were washed in deionised water, placed between layers of aluminium foil and stored at −20°C until required. Storage under these conditions was for periods of not longer than 4 months although a number of reports suggest that the process of freezing stratum corneum for up to 1 year has no significant effect on barrier function (Astley and Levine, 1976; Harrison et al., 1984).

2.2. Diffusion cell set-up

The stratum corneum, placed in deionised water at room temperature for 15 min prior to use, was mounted with the outer surface upwards, between the upper and lower compartments as shown (Fig. 1). The membrane was supported by a filter-paper wick (Whatman® no. 1) which ensured continuous contact between the water and the stratum corneum. The join between upper

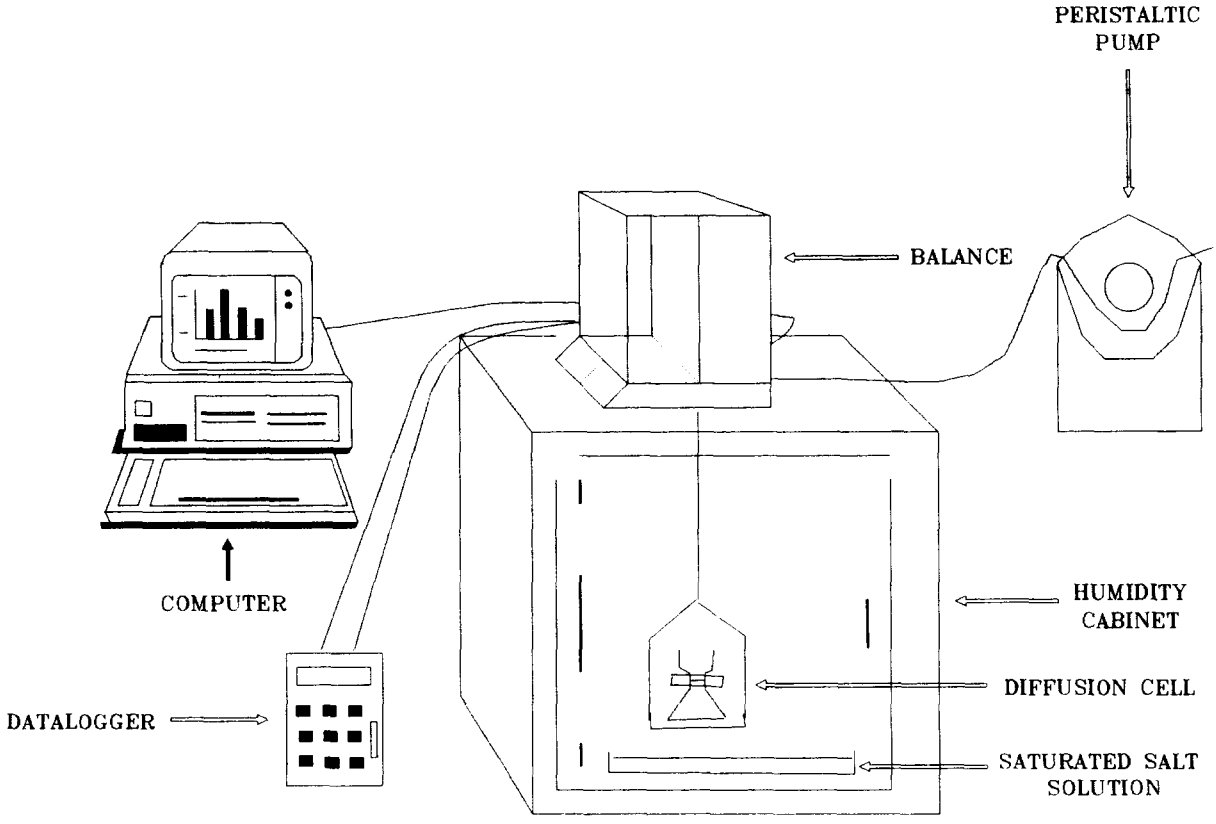

Fig. 2. In vitro set-up for measuring transepidermal water loss.

and lower compartments was sealed with silicon sealant (Silastic®), PTFE (Teflon®) tape and Parafilm®), which ensured a water-tight seal. 5 ml of deionised water were added to the receiver compartment via the side-arm using a 5 ml disposable syringe with filter straw. The side-arm was subsequently sealed using a water-tight seal as described above. The sealed cell was clamped and placed on a weigh pan positioned inside a humidity cabinet (LTE model 322/0650) maintained at 32 ± 1°C. The weigh pan was attached to the weighing mechanism of an Oertling balance (model NA264) placed outside the humidity cabinet as shown in Fig. 2.

The TEWL through the stratum corneum under various conditions was then monitored gravimetrically. An IBM computer (model XT) attached to the Oertling balance enabled continuous recording of changes in weight. A Psion organiser (model LZ64) with a humidity probe (R5SF) and temperature probe (THSF) attached and placed inside the humidity cabinet monitored the temperature and humidity conditions experienced by the stratum corneum sample under test (Fig. 2).

2.3. Steady state conditions

The diffusion cell was placed in a humidity cabinet at 32°C. Alteration of the ambient humidity was achieved by using various saturated salt solutions. Those used and the humidities they created are listed in Table 1. Different salt solutions were placed in a plastic salt tray every 24 h at the bottom of the humidity cabinet so that the humidity was increased by approx. 10% in that time span. Initially blue self-indicating silica gel (BDH Chemicals Ltd, Poole, U.K.) was used to create a low humidity. This atmosphere was maintained for 24 h after which time it was removed and replaced by lithium chloride (BDH Chemicals Ltd, Poole, U.K.) creating a humidity of 12 ± 3%. This humidity was maintained for 24 h and the substitution of salt solutions continued in the order listed in Table 1 until a humidity of 97 ± 3% was achieved and maintained for 24 h. All the salt solutions were heated to 32 ± 1.5°C in a water-bath placed outside the humidity cabinet prior to use. Temperature and humidity conditions were continuously monitored (at 5-min intervals) using a Psion organiser with a temperature and a humidity probe attached but placed inside the cabinet. Each experiment ran for approx. 240 h during which time the weight loss of the diffusion cell was recorded continuously by the IBM computer.

Table 1
Saturated salt solutions used during iso-RH% studies and the humidities they created

Saturated salt solution	RH ±3%
Lithium chloride	12
Potassium acetate	21
Magnesium chloride hexahydrate	32
Potassium carbonate	47
Sodium bromide	57
Sodium nitrite	66
Sodium chloride	78
Potassium chloride	90
Potassium sulphate	97

2.4. Non-steady-state humidity conditions

A piece of porcine stratum corneum was placed in a diffusion cell as described earlier. The cell with a diffusion surface area of about $1.9E-4m^2$ was placed on the weigh-pan extension of an Oertling balance in a humidity cabinet maintained at 32 ± 1.5°C. Peristaltic tubing was passed into the cabinet through a small hole in the roof. This orifice also provided the port of entry for the weigh-pan extension of the Oertling balance into the humidity cabinet. Tap water was maintained at 35 ± 1.5°C in a water-bath placed outside the humidity cabinet. Initially blue self-indicating silica gel, heated to 180°C for 1 h to drive off moisture prior to use, was placed in the bottom of the humidity cabinet to achieve a low relative humidity (typically 3.5 ± 2°C). Weight loss under these conditions was recorded on an IBM computer for a period of 48 h. After this time, using a peristaltic pump set at a low speed, the tap water was slowly pumped into the cabinet through the peristaltic tubing leading down to the plastic salt tray. Temperature and humidity conditions were continuously monitored (at 1-min

intervals) using a Psion organiser with a temperature and a humidity probe attached but placed inside the cabinet. As the ambient humidity inside the cabinet increased slowly, the weight loss of the diffusion cell was recorded continuously on an IBM computer. The results obtained for the non steady-state experiments were compared to those obtained for the steady-state to see if similar results were obtained.

2.5. Evaluation of moisturisers

The diffusion cell was set up as described for the non-steady-state state conditions and control data for untreated piglet stratum corneum were obtained by altering the humidity continuously as described for the non-iso-RH% experiments. This procedure was repeated to yield a second set of control data after a 24 h equilibration period at 3.5 ± 2 RH%. After a further 24 h equilibration period at 3.5 ± 2 RH%, 50 μl 10% w/w urea (Sigma, lot 108F-0354, catalogue no. U-1250) made up in HPLC water was applied to the stratum corneum and the humidity altered continuously as before. A subsequent 24 h equilibration period at 3.5 ± 2 RH% was followed by application of 50 μl 2% w/w pyrrolidone carboxylic acid (PCA) (Sigma, lot 84F-0015, catalogue no. P-0506) made up in HPLC water to the urea-treated stratum corneum. The piglet stratum corneum was again subjected to non-iso-RH% conditions and the results recorded.

The entire procedure was repeated using a different piece of piglet stratum corneum and in this instance the PCA solution was applied to the membrane first followed by application of urea to the PCA-treated stratum corneum. Both experiments were repeated using different pieces of piglet stratum corneum.

3. Results and discussion

3.1. Effect of relative humidity under steady-state state conditions

Fig. 3 illustrates a typical profile for the rate of water loss across porcine stratum corneum at

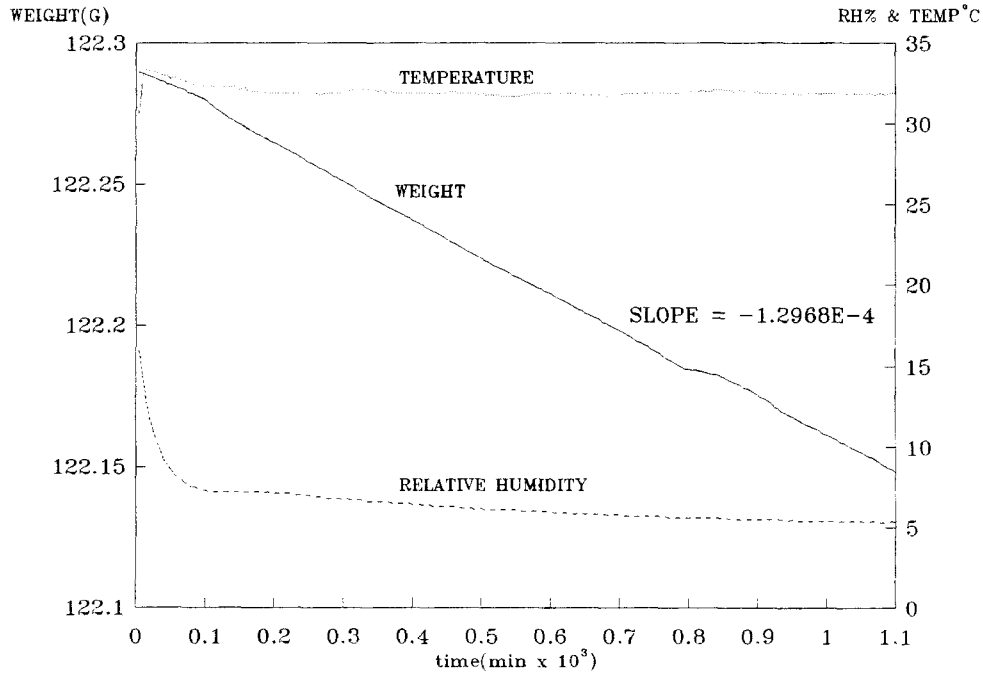

Fig. 3. Typical profile for gravimetric water loss across piglet stratum corneum at 5 RH% and 32°C.

Table 2
Water transfer across porcine stratum corneum at 32 and 37°C and 3.5 ± 2 RH%

Replicate	Slope (g/min)	Temperature (°C)	TEWL (g/m^2 per h)
1	$-1.30E-4$	32	41.23
2	$-1.35E-4$	32	43.04
3	$-1.56E-4$	32	49.66
4	$-2.94E-4$	37	93.42
5	$-2.90E-4$	37	92.18
6	$-2.67E-4$	37	84.90

32°C and 5 RH%. It can be seen that an initial non-steady state is soon followed by equilibration with respect to both humidity and temperature. During steady-state conditions the rate of transepidermal loss is constant as demonstrated by the constant slope and as predicted by Fick's first law of diffusion.

Table 2 shows the variability in the steady-state rates of TEWL using samples of stratum corneum from the same piglet at two different temperatures. It is obvious that temperature exerts an important effect on TEWL as previously reported by Grice et al. (1972).

Subsequent work consisted of maintaining constant temperature and systematically altering relative humidity (RH%) in the environmental cabinet to better define the relationship between TEWL and RH%. Grice et al. (1972) showed that in human subjects, the relationship between TEWL and relative humidity was apparently parabolic with maximum rate of loss in the range 20–40 RH%. At 2.6, 25, 49 and 79 RH% the TEWL were reported to have mean values of 3.6, 5.8, 4.7 and 3.0 g/m^2 per h, respectively. Blank et al. (1984), however, reported a monotonic decrease in water flux with an increase in relative humidity. Using piglet stratum corneum, the present study showed that TEWL decreased monotonically with increase in relative humidity (Fig. 4). The triplicate results using stratum corneum from three different piglets showed that the effect of relative humidity on TEWL can be satisfactorily described by an exponential relationship $TEWL = a\ \exp(b(RH\%))$ where a and b are constants. Logarithmic transformation and linear regression produce the linear relationship:

$$\ln(TEWL) = 3.78 - 0.0229RH\%$$

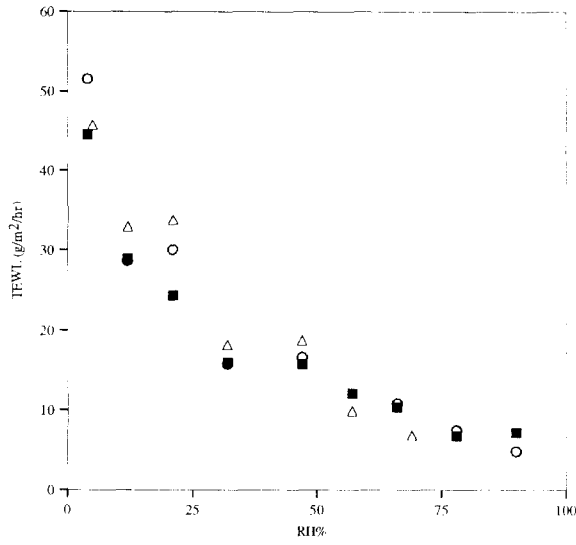

Fig. 4. Relationship between transepidermal water loss (TEWL) and RH% for piglet stratum corneum at 32°C under steady-state conditions; (△) TEWL for replicate 1, (■) TEWL for replicate 2 and (○) TEWL for replicate 3.

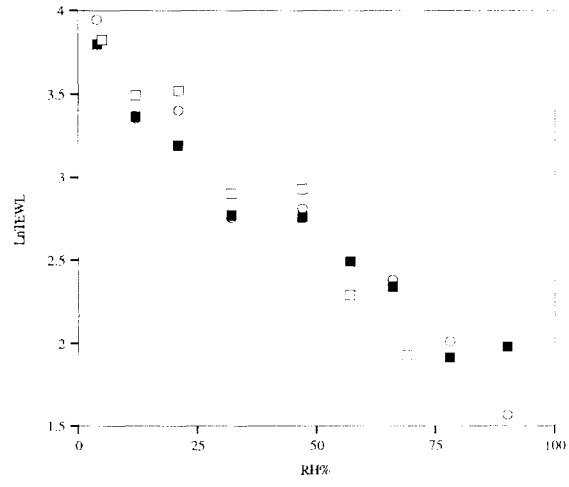

Fig. 5. Relationship between ln(TEWL) and RH% for piglet stratum corneum at 32°C and under steady-state conditions; (□) ln(TEWL) for replicate 1, (■) ln(TEWL) for replicate 2 and (○) ln(TEWL) for replicate 3.

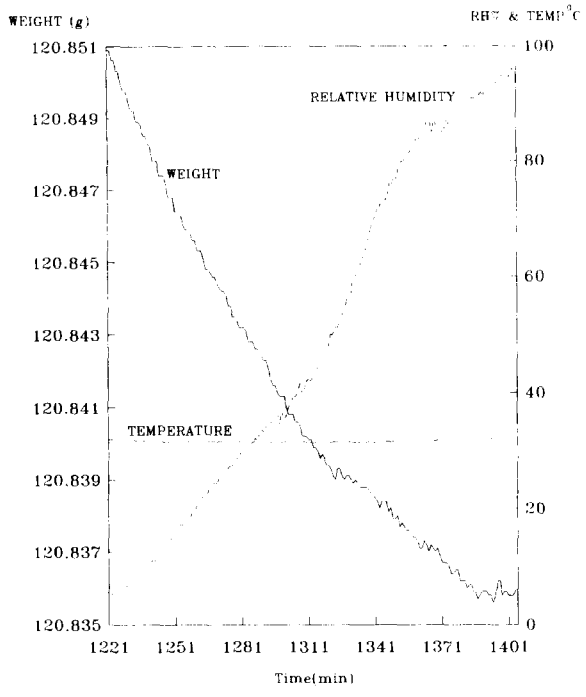

Fig. 6. Typical gravimetric water loss through piglet stratum corneum at 32°C under non-iso-RH% conditions.

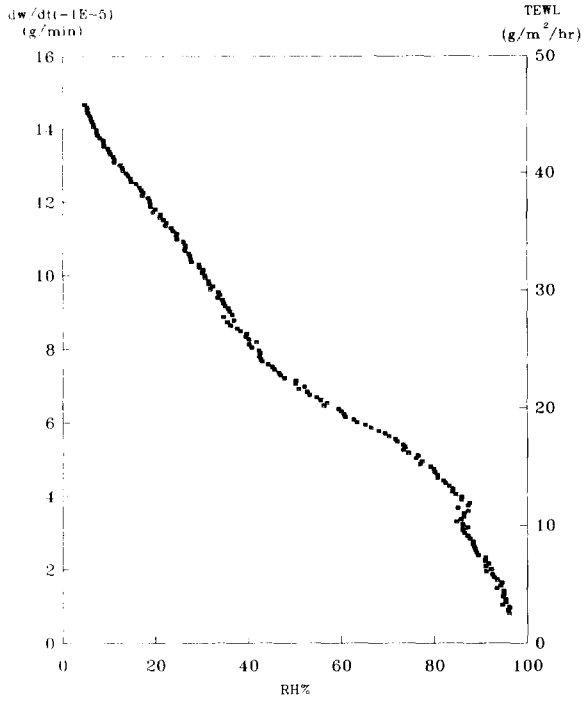

Fig. 7. Typical profile for dw/dt (g/min) and TEWL (g/m^2 per h) against RH% for piglet stratum corneum under non-steady-state conditions.

with a squared correlation coefficient of $r^2 = 93.9\%$ and with a mean square error (MSE) of 0.031 (Fig. 5).

3.2. Non-iso-RH% study

Isovariate studies are time consuming and it is often desirable to carry out such studies under non-steady-state conditions. For example, non-isothermal studies are often used to obtain rapid estimates of activation energies in single experiments (Li Wan Po et al., 1983). To investigate whether non-iso-RH% studies gave the same information as those obtained under iso-RH% con-

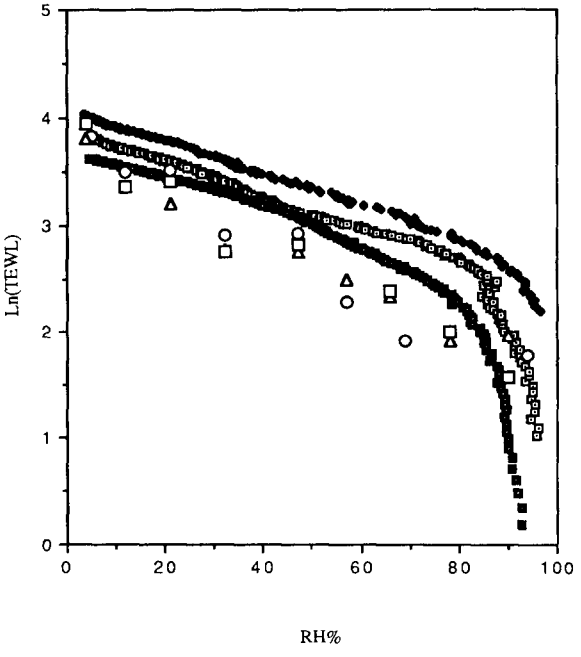

Fig. 8. Relationship between ln(TEWL) and RH% for piglet stratum corneum under both steady-state and non-steady-state conditions; (△, □, ○) steady-state data for three replicates using three different pieces of piglet stratum corneum and (⊡, ♦, ■) non-steady-state data for three replicates using three different pieces of piglet stratum corneum.

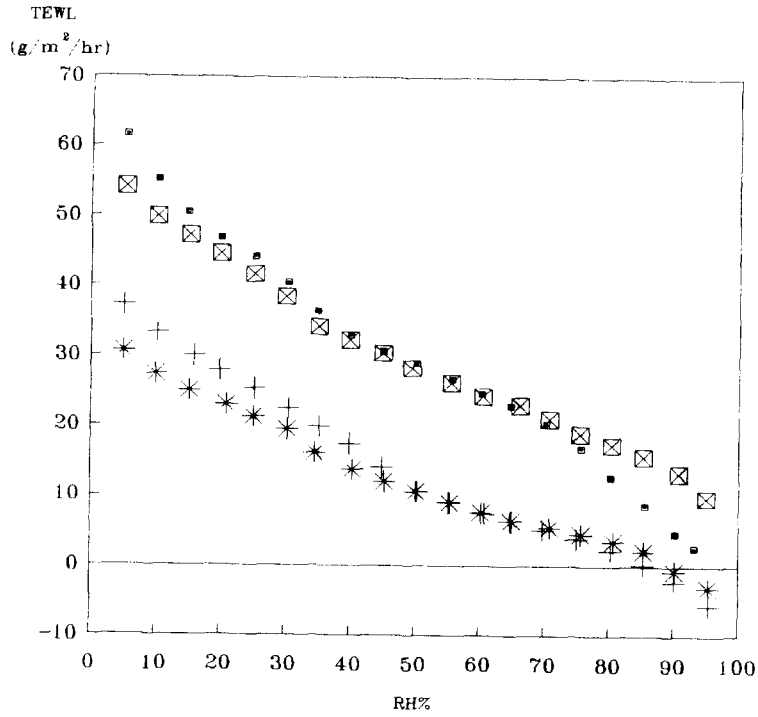

Fig. 9. Effect of application of urea followed by PCA on transepidermal water loss (TEWL) across piglet stratum corneum under non-iso-RH% conditions; (⊠, ■) control data, (+) application of urea and (*) application of PCA to urea-treated stratum corneum.

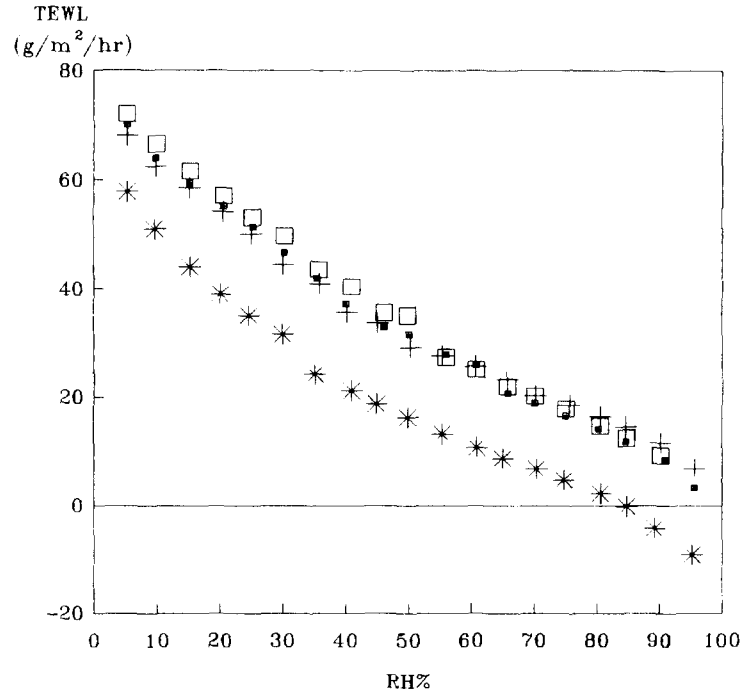

Fig. 10. Effect of application of PCA followed by urea on transepidermal water loss (TEWL) across piglet stratum corneum under non-iso-RH% conditions; (□, ■) control data, (+) application of PCA and (*) application of urea to PCA-treated stratum corneum.

ditions, TEWL was measured under non-steady-state RH%.

Fig. 6 shows a typical weight (g), RH% and temperature (°C)-time (min) profile. Temperature is constant throughout the experiment as seen by the flat profile while humidity increases with time. It is worth recalling that in the experimental set-up (Fig. 1) the relative humidity being measured is that of the environmental cabinet. One side of the skin is in contact with water and can hence be regarded as being exposed to 100 RH% throughout the experiment. TEWL is clearly seen to decrease with increasing RH%.

Continuous increases in the RH% results in continuous decreases in the concentration gradient. As the stratum corneum becomes hydrated, the thickness of the membrane increases as does the diffusion coefficient. These opposing forces influence the overall result obtained. The weight (w) vs time (t) profile can be approximated by a polynomial equation using least-squares fit. For the profile shown in Fig. 6 this gives the equation:

$$w = 122 - 0.00105t + (3.7E - 7)t^2$$

where weight is in g and time in min. The relative humidity-time profile can be similarly approximated to give the equation:

$$\mathrm{RH\%} = -687 + 0.561t$$

Differentiation of the equation relating weight to time and substituting for t using the equation for time and relative humidity yields an equation relating dw/dt and RH%:

$$dw/dt = 0.00014 - (1.29E - 6)\mathrm{RH\%}$$

where dw/dt represents the instantaneous rate of water loss or TEWL in g per min. TEWL is normalised to give the weight loss in g/m^2 per h so that the relationship becomes:

$$\mathrm{TEWL} = 44.8 - 0.416\mathrm{RH\%}$$

This is shown in Fig. 7.

Therefore, using these two equations, one is able to construct the TEWL or dw/dt vs RH% curve. Logarithmic transformation and linear regression of triplicate results using stratum corneum from three different piglets produce the linear relationship:

$$\ln(\mathrm{TEWL}) = 3.95 - 0.0165\mathrm{RH\%}$$

with a squared correlation coefficient of $r^2 = 84.7\%$ and with a mean square error of 0.024. Fig. 8 illustrates the ln(TEWL) values observed using both steady-state ($n = 3$) and non-steady-state ($n = 3$) relative humidities. It is seen that the non-steady-state relative humidity data are consistently higher than the steady-state data. One possible explanation is that since the humidity probe estimates the ambient RH% it is likely to underestimate the humidity gradient across the stratum corneum. The bias is likely to be higher under non-steady-state than under steady-state RH% conditions. The qualitative agreement in TEWL values obtained using steady-state RH% and non-iso-RH% conditions is, however, good (Fig. 8).

To illustrate the use of the non-iso-RH% method for studying the effect of urea and pyrrolidone carboxylic acid (PCA) on TEWL, the TEWL/RH% profile was constructed using a sample of piglet stratum corneum; the experiment was then repeated after applying urea onto the same sample of skin and then again after applying PCA. The combined results are shown in Fig. 9. It is obvious that urea produces a marked decrease in TEWL at all relative humidities. PCA did not further depress TEWL except at the lower RH%. Reversing the order of application of urea and PCA, so that PCA was applied first, confirmed the marked decrease in TEWL produced by urea and the relative lack of effect produced by PCA (Fig. 10). These results are obviously preliminary but nonetheless provide support for the value of the non-iso-RH% method proposed.

4. References

Agner, T. and Serup, J., Seasonal variations of skin resistance to irritants. *Br. J. Dermatol.*, 121 (1989) 323–328.

Anderson, R.L., Cassidy, J.M., Hansen, J.R. and Yellin, W., Hydration of stratum corneum. *Biopolymers*, 12 (1973) 2789–2802.

Astley, J.P. and Levine, M., Effect of dimethyl sulfoxide on permeability of human skin in vitro. *J. Pharm. Sci.*, 65 (1976) 210–215.

Behl, C.R., Flynn, G.L., Kurihara, T., Harper, N., Smith, W., Hugichi, W.I., Ho, N.F.H. and Pierson, C.L., Hydration

and percutaneous absorption: I. Influence of hydration on alkanol permeation through hairless mouse skin. *J. Invest. Dermatol.*, 75 (1980) 346–352.

Blank, I.H., Effect of hydration on the permeability of the skin. In Bronaugh, R.C. and Maibach, H.I. (Eds), *Dermatology: Vol. 6, Percutaneous Absorption. Mechanisms – Methodology – Drug Delivery*, Dekker, New York, 1985, pp. 97–107.

Blank, I.H., Factors which influence the water content of the stratum corneum. *J. Invest. Dermatol.*, 18 (1952) 433–439.

Blank, I.H., Further observations on factors which influence the water content of the stratum corneum. *J. Invest. Dermatol.*, 21 (1953) 259–271.

Blank, I.H., Moloney, J., Emslie, A.G., Simon, I. and Apt, C., The diffusion of water across the stratum corneum as a function of its water content. *J. Invest. Dermatol.*, 82 (1984) 188–194.

Dugard, P.H., Skin permeability theory in relation to measurements of pecutaneous absorption in toxicology. In Marzulli, F.N. and Maibach, H.I. (Eds), *Dermatotoxicology*, 2nd Edn, Hemisphere, Washington, 1983, pp. 91–116.

El-Shimi, A.F. and Princen, H.M., Water vapour sorption and desorption behaviour of some keratins. *Colloid Polym. Sci.*, 256 (1978) 105–114.

Grice, K., Sattar, H. and Baker, H., The effect of ambient humidity on transepidermal water loss. *J. Invest. Dermatol.*, 58 (1972) 343–346.

Grice, K., Sattar, H., Sharratt, M. and Baker, H., Skin temperature and transepidermal water loss. *J. Invest. Dermatol.*, 57 (1971) 108–110.

Hanifin, J.M., Recognizing and managing clinical problems in atopic dermatitis. *Allergy Proc.*, 10 (1989) 397–402.

Harrison, S.M., Barry, B.W. and Dugard, P.H., Effects of freezing on human skin permeability. *J. Pharm. Pharmacol.*, 36 (1984) 261–262.

Hilliard, P.R. and Dorogi, P.L., Investigation of temperature and water activity effects on pig skin in vitro. *J. Soc. Cosmet. Chem.*, 40 (1989) 1–20.

Horrobin, D.F., Essential fatty acids in clinical dematology. *J. Am. Acad. Dermatol.*, 20 (1989) 1045–1053.

Imokawa, G., Akasaki, S., Minematsu, Y. and Kawai, M., Importance of intercellular lipids in water-retention properties of the stratum corneum: induction and recovery study of surfactant dry skin. *Arch. Dermatol. Res.*, 281 (1989) 45–51.

Li Wan Po, A., Elias, A.N. and Irwin, W.J., Non-isothermal and non-isopH kinetics in formulation studies. *Acta Pharm. Suec.*, 20 (1983) 277–286.

Miller, D.L., Brown, A.M. and Artz, E.J., Indirect measures of transepidermal water loss. In Marks, R. and Payne, P.A. (Eds), *Bioengineering and the Skin*, MTP Press, Lancaster, 1981, pp. 161–171.

Park, A.C. and Baddiel, C.B., Rheology of stratum corneum: II. A physiochemical investigation of factors influencing the water content of the corneum. *J. Soc. Cosmet. Chem.*, 23 (1972) 13–21.

Reiger, M.M. and Deem, D.E., Skin moisturizers: I. Methods for measuring water regain mechanical properties and transepidermal moisture loss of stratum corneum. *J. Soc. Cosmet. Chem.*, 25 (1974) 239–252.

Scheuplein, R.J. and Blank, I.H., Permeability of the skin – Review. *Physiol. Rev.*, 51 (1971) 702–747.

Spencer, T.S. Linamen, C.E., Akers, W.A. and Jones, H.E., Temperature dependance of water content of stratum corneum. *Br. J. Dermatol.*, 93 (1975) 159–164.

Takahashi, M., Kawasali, K., Tanaka, M., Ohta, S. and Tsuda, Y., The mechanisms of stratum corneum plasticization with water. In Marks, R. and Payne, P.A. (Eds), *Bioengineering and the Skin*, MTP Press, Lancaster, 1981, pp. 67–75.

Takenouchi, M., Suzuki, H. and Tagami, H., Hydration characteristics of pathologic stratum corneum – evaluation of bound water. *J. Invest. Dermatol.*, 87 (1986) 574–576.

Van Duzee, B.F., The influence of water content, chemical treatment and temperature on the rheological properties of stratum corneum. *J. Invest. Dermatol.*, 71 (1978) 140–144.

Wilson, D.R. and Maibach, H., Transepidermal water loss: A review. In Leveque, J.-L. (Ed.), *Cutaneous Investigation in Health and Disease: Noninvasive Methods and Instrumentation*, Dekker, New York, 1989, pp. 113–133.

Wu, M.-S., Water diffusivity and water concentration profile in human stratum corneum from transepidermal water loss measurements. *J. Soc. Cosmet. Chem.*, 34 (1983) 191–196.