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## 1. Introduction

The aim of the study was to assess the performance of a condenser-chamber TEWL instrument for barrier integrity testing. OECD Test Guideline 428 stipulates barrier integrity testing before permeation experiments are carried out. TEWL, electrical resistance and irritated water procedures are recognised for such tests.  
 In a comprehensive study using an open-chamber Tewameter, Netzlaff *et al.* found TEWL measurements to be of limited use for barrier integrity testing, being able to detect only severe damage in the samples they examined. Particular problems they identified included topically adhering water and the permeation of condensed water via capillary action through deliberately made pinholes in artificial and biological membranes. This study assesses the extent to which such problems are reduced when using a condenser-chamber TEWL instrument, which offers (i) rapid drying of topically adhering water in its controlled, low humidity microclimate and (ii) higher sensitivity.

## 2. Materials and Methods

Measurements were performed on artificial membranes (Sil-Tec from Technical Products Inc, USA and Teflon from Saarland University, Germany) and bio-membranes (excised human epidermis, excised human stratum corneum (SC) and snake sheddings from PentaPharm, Switzerland). Before the measurements, epidermis and SC samples were hydrated between wet filter paper sheets for 30 min, blotted dry, mounted on the Franz cell and left to acclimatise for 15 min. The system was then coupled to an AquaFlux measurement head and flux density time-series curves measured. Membrane damage was simulated by puncturing samples with a fine pin to produce perforations of ~50-100µm diameter.

TEWL was measured at room temperature using an AquaFlux AF200 instrument with a 9mm PermeGear Franz Diffusion Cell (PermeGear Inc, USA). A push-fit coupling between the TEWL measurement head and the Franz cell donor chamber was developed to give a reproducible, vapour-tight seal without the need to touch the membrane under test (Figure 1).



Figure 1: AquaFlux - Franz Cell Coupling

## 3. Results

### 3.1 Experiments with Sil-Tec membranes

Initial experiments used Sil-Tec membranes whose well controlled properties could be relied upon to verify the measurements. The controlled low-humidity microclimate within an AquaFlux measurement chamber offers a distinct advantage over conventional TEWL instruments, because any topical water evaporates quickly during the measurements. The measured flux curves clearly show the drying progress and therefore give quality control information for the tests. These points are illustrated in Figure 2, where three different curves are shown.

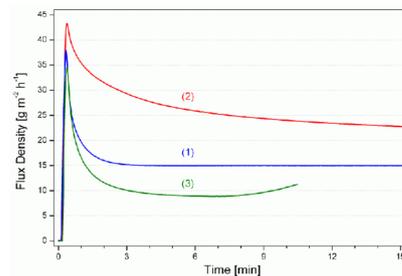


Figure 2: Franz-cell membrane tests using Sil-Tec membranes

Curve (1) shows rapid settling to a steady level. There was little donor-side moisture and the seal around the membrane was tight. Curve (2) settles more slowly as donor-side moisture evaporates. This causes the test to be prolonged, but the eventual result is valid. Curve (3) settles rapidly at first, then begins to rise again. This was found to be caused by a leaky seal around the membrane, resulting in a steadily increasing area of membrane contributing to the transport.

The validity of such measurements can be tested by correlating membrane diffusion resistance with membrane thickness, as illustrated in Figure 3.

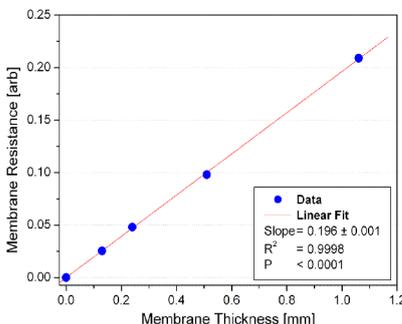


Figure 3: Diffusion Resistance Analysis for Sil-Tec membranes in the thickness range 0.13-1.06mm, showing an excellent linear correlation.

### 3.2 Experiments with Teflon Membranes

Similar measurements as published by Netzlaff *et al.* were performed on the same Teflon membranes, in order to assess condenser-chamber AquaFlux performance to quantify membrane damage. Netzlaff *et al.* were able to detect single punctures of ~1mm diameter. Additional punctures were found to have little effect on the measured TEWL.

AquaFlux measurements were performed with a number of smaller punctures of 50-100µm diameter. The measured flux density was found to increase with membrane damage as measured by the number of membrane punctures, as illustrated in Figure 4. The error bars relate to inconsistent puncture diameters rather than instrumental repeatability. Note that the sensitivity to membrane damage is highest at low damage.

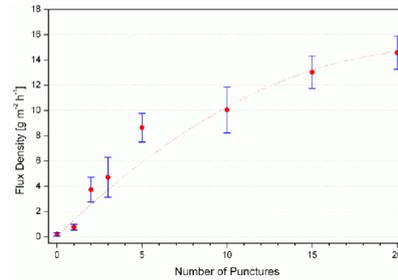


Figure 4: AquaFlux membrane damage measurements. Each point is the mean of membranes tested. The error bars are ± 1 standard deviation.

### 3.3 Experiments with SC

Measurements were performed on SC sheets from different donors before and after inflicting damage by means of a single puncture of 50-100µm diameter. The results are presented in Figure 5.

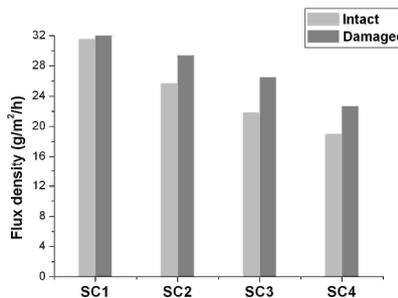


Figure 5: AquaFlux TEWL measurements on intact and damaged SC samples from different donors.

The effect of membrane damage is clearly visible in SC samples 2-4, where the intact membrane permeability is low. Sample 1 has a higher intact permeability and shows little effect from the additional damage.

### 3.4 Experiments with Epidermis

Similar experiments were performed with epidermis sheets. The results presented in Figure 6 demonstrate the capability of the AquaFlux to differentiate between intact and damaged membranes.

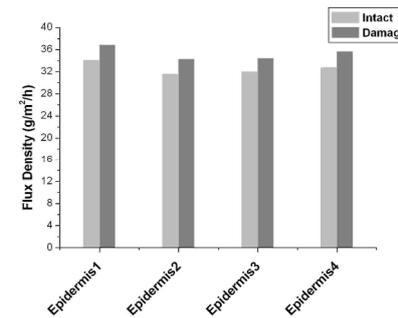


Figure 6: AquaFlux TEWL measurements on intact and damaged epidermis samples from different donors.

### 3.5 Snake Skin Measurements

Apart from SC and epidermis, measurements were also performed on snake skin sheddings from animals of two different species (Bothrops atrox and Bothrops moojeni). The results shown in Figure 7 indicate a clear species difference.

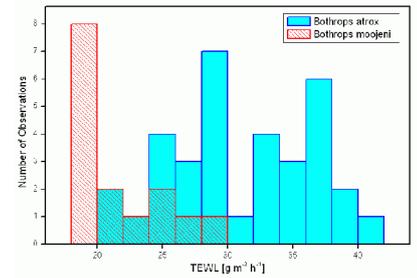


Figure 7: Snake Skin Species Analysis.

Bothrops atrox: Mean TEWL = 31.2 gm<sup>2</sup>h<sup>-1</sup> (34 Samples)

Bothrops moojeni: Mean TEWL = 21.5 gm<sup>2</sup>h<sup>-1</sup> (15 Samples)

ANOVA test gives  $p = 7.6 \cdot 10^{-4}$ , ie the means are significantly different.

## 4. Repeatability of Measurements

Repeatability is an important instrumental attribute in membrane integrity testing, because it determines the extent to which small differences in readings are meaningful in terms of membrane permeability. The repeatability of the AquaFlux was assessed by performing multiple measurements on the same sample under otherwise similar conditions. Both SC and epidermis samples were used, with each sample tested 9 times. The Franz cell was uncoupled from the instrument for typically one minute between repeat measurements.

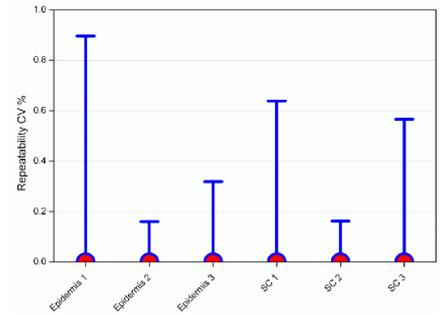


Figure 8: Coefficient of Variation CV% for nine repeat measurements on three epidermis and three SC samples.

The Coefficient of Variation was found to be less than 1% for all the samples tested. This corresponds to a standard deviation of less than 0.3 g m<sup>2</sup> h<sup>-1</sup>. Therefore, the changes of flux density recorded in Figure 5 are undoubtedly caused by sample damage and not by random fluctuations.

## 5. Conclusions

The main points arising from this study are:-

- Experiments with Sil-Tec membranes of known thickness show that AquaFlux measurements correlate linearly with membrane diffusion resistance (ie 1/permeability), with a correlation coefficient close to unity ( $P < 0.0001$ ).
- Experiments with Teflon membranes show that AquaFlux measurements can detect membrane damage, with highest sensitivity for samples of lowest permeability. These findings are confirmed by the measurements on epidermis and SC.
- Experiments with snake sheddings show that AquaFlux measurements are sufficiently sensitive to detect a difference in samples from two different species.
- Experiments with repeat measurements on epidermis and SC under otherwise similar conditions show that AquaFlux measurements are repeatable to better than 1% Coefficient of Variation.

In practical membrane integrity testing, the AquaFlux offers distinct advantages over conventional TEWL measurements:-

- Its controlled microclimate produces consistent measurement conditions irrespective of ambient humidity.
- The low chamber humidity causes topically adhering water to dry off quickly during measurements, thus reducing reliance on drying prior to measurement.
- The recorded water vapour flux curves clearly show the drying progress and give quality control information for the tests.
- The software can be set to terminate the test automatically when the quality criteria are met, thus ensuring that the tests are neither prematurely terminated nor are run for longer than necessary.

## Acknowledgements

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