Calibration of Franz Cell Membrane Integrity Test by the TEWL Method

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1. Introduction

The aim of this work was to develop a calibration method for membrane integrity measurements by the TEWL method. OECD Test Guideline 428 stipulates barrier integrity testing before permeation experiments are carried out [1]. Tritiated water, electrical resistance and TEWL methods are recognised for such tests [2].

In both tritiated water and electrical resistance methods the membranes under test are fully saturated, in a state that may be compared with in-vivo skin that has been occluded for a prolonged period of time. TEWL methods are different because they measure the flux of water vapour in the air above the membrane. To be meaningful, the membrane under test needs to be dry on the donor side and wet on the acceptor site, a state that may be compared with normal in-vivo skin that has been acclimatised in ambient air. It is therefore tempting to use the same measure of TEWL to characterise in-vitro membrane integrity as is widely used to characterise in-vivo skin barrier function. However, to do so requires that the coupled system of Franz cell and TEWL instrument be calibrated.

2. TEWL and Barrier Function

The relationship between TEWL and barrier function is not straightforward, as was argued in detail in [3]. This is because TEWL measurement is indirect: TEWL is water diffusing through the membrane, TEWL instruments measure water vapour flux density in the air above the membrane. With an intact barrier, water vapour flux density is proportional to barrier permeability. An impermeable barrier would be characterised by a zero water vapour flux density. A barrier of low permeability would be characterised by a proportionately low water vapour flux density. This is the linear region of the calculated response curves [4] of Figure 1. In this region, all TEWL instruments should give the same readings, provided they are correctly calibrated.

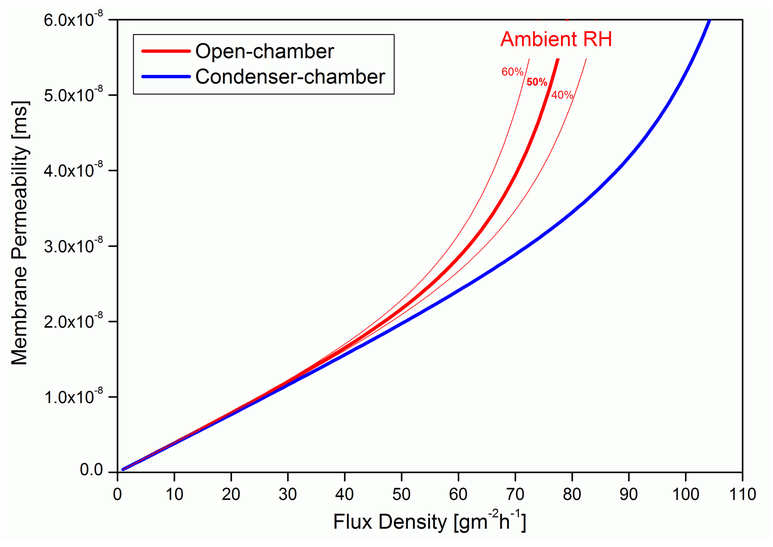

Figure 1: Model calculations of the relationship between flux density and membrane permeability for typical open-chamber and condenser-chamber instruments.

As membrane permeability increases, the curves in Figure 1 start to deviate from linearity. This happens as the humidity of the air immediately adjacent to the membrane surface approaches saturation at RH=100%. Eventually, the water vapour flux density asymptotically approaches a limiting value where the membrane no longer constrains water loss. This is the *saturation flux density* when the air immediately adjacent to the membrane surface is fully saturated at RH=100%. Saturation flux density is not a membrane barrier property. It depends on membrane temperature (31°C in these calculations), the design of the measurement head and how it is coupled to the membrane. For open chambers, the saturation flux density also depends on ambient RH.

3. Materials and Methods

TEWL was measured using an AquaFlux Model AF200 condenser-chamber instrument (Biox Systems Ltd, UK). Membrane integrity testing was performed by coupling its measurement head with the donor chamber of a Franz Diffusion Cell or flow cell using a purpose-designed push-fit coupling [5]. This method allows the whole exposed area of membrane to be tested in-situ (Figure 2).

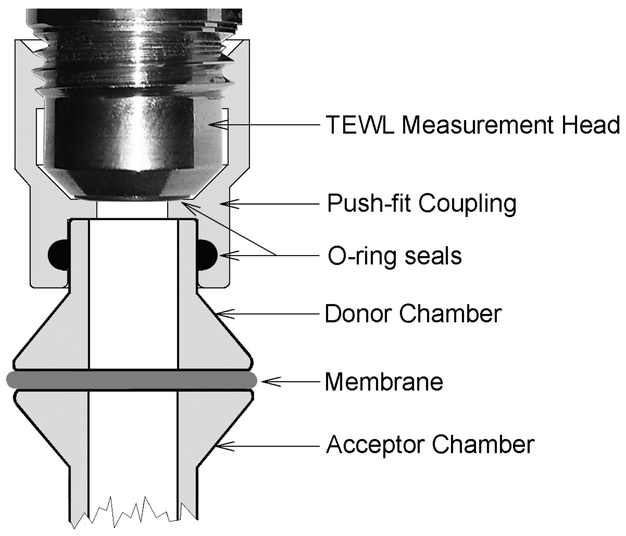

Figure 2: TEWL Measurement Head coupled to a glass Franz cell for membrane integrity testing.

The method of membrane integrity testing is as follows:-

1. Assemble the Franz cell & membrane.
2. Fill the acceptor chamber.
3. Ensure good contact between acceptor fluid and membrane.
4. Warm the assembly to working temperature.
5. Acclimatise the membrane. Use a fan to reduce the high humidity of the stagnant air trapped within the donor chamber.
6. When ready, couple the AquaFlux instrument to the donor chamber and record water vapour flux v time curve until the flux stabilises.
7. The final steady flux density is the required membrane integrity indicator.

The recorded flux v time curves are also useful for quality control. Fault conditions such as leakage or insufficient acclimatisation can be recognised.

4. Cap Factor

The calibration method described here is aimed at the low-flux linear region of the response curves of Figure 1. The instrument itself is assumed already to be calibrated by the droplet method [3], but this is valid only for the standard measurement cap shown in Figure 3 (left).

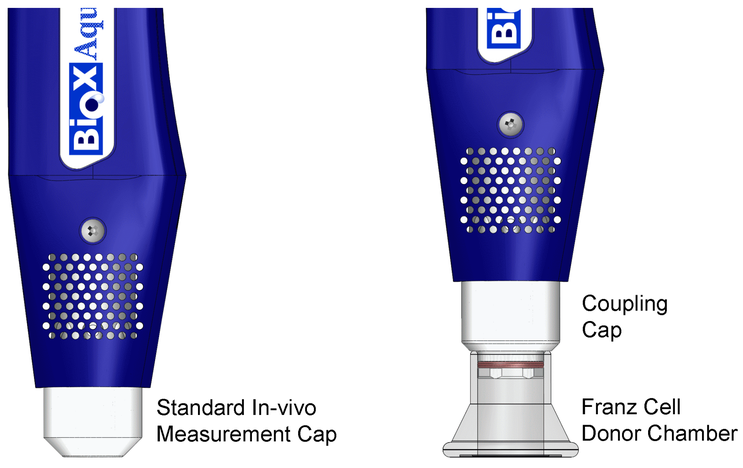

Figure 3: AquaFlux instrument equipped with a standard in-vivo measurement cap (left) and coupled to a 15mm Franz cell donor chamber (right). The cap-factor calibration method compares same-site readings on in-vivo skin.

For membrane integrity testing, the standard measurement cap is replaced by a coupling cap and Franz cell donor chamber shown in Figure 3 (right). The geometry of the two configurations is clearly different and the instrumental response will therefore also be different. But since the instrument is already calibrated for the standard configuration on the left, it only remains to apply a correction factor (*cap factor*) to the configuration on the right to bring the readings into agreement.

5. Calibration Method

The *cap factor* calibration method uses intact and well-acclimatised in-vivo volar forearm skin as a calibration source. The permeability of this source is low, well within the linear range of the characteristic curves of Figure 1. It is also reasonably uniform over areas typically used in Franz cells. In practice, several test areas are marked and subsequently measured with the two configurations depicted in Figure 3. The cap factor is then calculated as the ratio of the mean readings recorded with the two configurations.

6. Results

Typical results for two designs of Franz cell are summarised in the table below. Eight readings were averaged for each configuration.

	Standard Cap	Franz Cell 1	Franz Cell 2	Units
Mean Reading	9.20	19.37	25.45	$gm^{-2}h^{-1}$
StDev	0.45	1.05	0.96	$gm^{-2}h^{-1}$
CV	4.9	5.4	3.8	%
Cap Factor	-	**2.11**	**2.77**	-

With these Franz cells, the readings are larger than those with the standard measurement cap. This is because the area of membrane under test is larger than the orifice area of the standard measurement cap.

7. Conclusions

The above cap factor calibration works for TEWL values that correspond to the low to medium permeabilities of reasonably intact skin barriers. However, the in-vivo in-vitro correspondence does not apply to highly compromised skin barriers where the flux depends more on the rate of evaporation from the membrane surface than the rate of diffusion through the membrane.

References

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