In-vivo and in-vitro applications of closed-chamber TEWL measurements

R E Imhof [1, 2]

[1] Photophysics Research Centre, South Bank University, London SE1 0AA, UK.
[2] Biox Systems Ltd, Southwark Campus, 103 Borough Road, London SE1 0AA, UK.

Abstract

After a quarter-century of dominance, the open-chamber method of TEWL measurement is now challenged by new technologies using closed measurement chambers. Whilst the characteristics of the open-chamber method are well recognised, there is still uncertainty about the capabilities of the challengers.

This report provides the background to the oral presentation from the standpoint of measurement science: what are you trying to measure, how can you go about it, how do you interpret the data and what are the measurement errors. The main points from this analysis are:-

1. There are no direct methods for measuring TEWL.
2. TEWL is condensed water diffusing through the skin.
3. TEWL methods measure water evaporation from the skin surface.
4. The microclimate of uncovered skin is dominated by air movements.
5. All TEWL measurement chambers perturb the microclimate.
6. SC property change is slow compared with TEWL measurement times.
7. Wet-cup calibration methods are fundamentally flawed.
8. The droplet calibration method is traceable and has been independently verified.
9. Closed-chamber TEWL methods work.

The report also presents some comparative data, to illustrate the performance of closed-chamber instruments, and a description of a membrane integrity test for in-vitro measurements of percutaneous penetration.

Contents

1. Introduction

1.1 Conflict of Interest Statement

Before you get carried away by my presentation, I need to declare a potential conflict of interest. On the one hand, I am an academic researcher in measurement science applied to skin characterisation. My research led me to invent one of the featured closed-chamber TEWL measurement methods in an effort to overcome the limitations of the open-chamber method. On the other hand, I am also involved in the university spin-out company that is manufacturing a closed-chamber instrument based on this invention.

In this lecture, I wear my academic hat with a firm focus on fundamental measurement science. The aim is to provide a framework of understanding for TEWL measurements and thereby dispel some of the myths surrounding this topic. But if you get the impression that I regard the open-chamber method as the past and closed-chamber methods as the future, then you would be right. But that's an academic judgement.

1.2 History of TEWL Methods

The importance of transepidermal water loss (TEWL) as a measure of the skin barrier has been recognised for over half a century. Some early methods of measurement are described in [1, 2] and references therein. However, it was Nilsson's invention of the open-chamber method [3, 4] and its development into a commercial instrument (Evaporimeter, Servo Med AB, Sweden) that started the modern era of TEWL measurement. Today, open-chamber instruments are available from Cortex Technology, Denmark (DermaLab), and Courage & Khazaka GmbH, Germany (TewaMeter), but Servo Med appears to have ceased trading. The method is well established, having been used in innumerable scientific studies worldwide.

The open-chamber method suffers from a number of limitations. Chief of these is its vulnerability to disturbance from ambient air movements, which restricts its use to well-controlled laboratories, often with shielding boxes. Natural convection air movements from skin also restrict in-vivo measurements to horizontal surfaces. Guidelines have been developed for open-chamber instruments [5, 6] and these help to reduce measurement errors and achieve consistency among users.

Recently, a number of closed-chamber TEWL instruments have become available. Their history of development goes back further than that of the open-chamber method, even if non-electronic devices are disregarded. The earliest electronic closed-chamber device I could find was developed by Wallihan in 1964 [7] for measuring water loss from leaves in the parallel universe of botany. He used a cylindrical measurement chamber, closed at one end and equipped with a humidity sensor inside. The water vapour flux from a leaf in contact with the open end of the cylinder was determined from the time-rate of rise of humidity within the chamber. His chamber also had a tube connection for purging with dry air before or after a measurement, in order to remove excess water vapour. A similar device for TEWL measurement, but without the gas purging facility, was described by Miller *et al* in 1981 [8]. More recently, two commercial devices using this *unventilated-chamber* method were described by Tagami *et al* [9] (Model 4300, Nikkiso-Ysi Co Ltd, Japan) and Nuutinen *et al* [10] (VapoMeter, Delfin Technologies Ltd, Finland).

A different approach was taken by Imhof [11, 12], where a condenser was used to remove water vapour from the closed measurement chamber, thus enabling continuous flux measurements to be made without purging. A commercial instrument using this *condenser-chamber* measurement principle has since been developed [13] (AquaFlux, Biox Systems Ltd, England).

Whenever a new measurement method is introduced, questions of validity and relationship with established methods need to be answered. One approach to finding answers is to perform experimental comparisons, as in [14] and [15] for example. Conversely, a new method can challenge the traditional methods and thereby stimulate progress and understanding. Here I take the latter approach.

2. TEWL and Barrier Resistance

Here I attempt to define TEWL and how it relates to skin barrier function and evaporation rate. These fundamentals are useful for interpreting what the measurements are telling us. Note that, unless otherwise stated, the discussion and calculations assume normal healthy SC and normal volar forearm TEWL.

2.1 Definition of TEWL

General definitions of TEWL are not easy to find. According to Wikipedia [16], TEWL is:-

> ... the measurement of the quantity of water that passes from inside a body (animal or plant) through the epidermal layer (skin) to the surrounding atmosphere via diffusion and evaporation processes.

This is less than rigorous, but it does capture the essence. It may seem pedantic, but TEWL is neither a *measurement* nor a *quantity*. TEWL is a **flux density**, ie a quantity of water per unit area of skin per unit time. Evaporation into the surrounding atmosphere is not essential, since TEWL does not stop immediately when the skin is occluded, for example. However, evaporation into the surrounding atmosphere is necessary for the measurement of TEWL, because the measurement takes place in the air above the skin.

The above criticisms apart, this definition correctly states that (a) the water originates from inside a body, and (b) that it must pass through the skin by diffusion. The transport mechanism of diffusion excludes other forms of water loss such as perspiration - sensible or otherwise. The essential point is that TEWL is the flux of condensed water diffusing through the skin, from inside the body to the surface.

2.2 Definition of Flux Density

The terms flux and flux density are widely used to describe diverse physical quantities, including magnetic or electric fields, particle streams, etc. Within the present context, flux density $\boldsymbol{J}$ is defined as

$$J = \frac{Mass\ of\ Water}{Area \times Time} \tag{1}$$

This definition is equally valid for condensed water and water vapour. The *SI* units of ***J*** are $kg\,m^{-2}\,s^{-1}$ ie kilograms of water per square meter of skin per second. These units are useful for mathematical modelling, but they give numbers that are awkwardly small for practical use. For this reason, the practical units of $g\,m^{-2}\,h^{-1}$, ie grams of water per square meter of skin per hour are widely used. Also, whilst *flux density* is strictly correct, the term *flux* is often used interchangeably, when normalisation to unit area is either implied or not relevant in the context.

2.3 Diffusion Resistance

The main aim of TEWL measurement is to characterise the barrier property of the skin, which resides in its outermost layer, the stratum corneum (SC). The barrier is essential to life, because it protects us from dehydration and from the percutaneous absorption of xenobiotics.

TEWL provides a measure of the barrier property because the TEWL water has diffused through the barrier, from inside the body to the surface. For steady conditions, this diffusion process can adequately be described by the one-dimensional form of Fick's first law of diffusion [17, p626]

$$J = -D\frac{dc}{dz} \tag{2}$$

where

J	=	Flux density $\left(kg\,m^{-2}\,s^{-1}\right)$
D	=	Diffusion coefficient $\left(m^2\,s^{-1}\right)$
c	=	Concentration $\left(kg\,m^{-3}\right)$
z	=	Distance normal to the skin surface $\left(m\right)$

The barrier property of the SC is associated with the diffusion coefficient ***D*** and the thickness ***L*** of the SC. Of course, the SC is heterogeneous and both ***D*** and dc/dz change across its thickness. However, in the steady state, the flux density ***J*** is continuous throughout the thickness of the SC. Therefore, the product of ***D*** and dc/dz must be constant. For this reason, Eq.(2) can be written in the form

$$J = -\frac{\Delta c}{R_{SC}} \tag{3}$$

where Δc $\left(kg\,m^{-3}\right)$ is the concentration difference of condensed water across the SC and

$$R_{SC} = \frac{L}{\left\langle D_{SC}\right\rangle} \tag{4}$$

$\boldsymbol{R_{SC}}$ = SC diffusion resistance $\left(s\,m^{-1}\right)$

$\boldsymbol{L}$ = SC thickness (m)

$\langle D_{SC} \rangle$ = SC mean diffusion coefficient $\left(m^2\ s^{-1}\right)$

Eqs.(3) and (4) introduce a diffusion resistance $\boldsymbol{R_{SC}}$, in analogy with Ohm's law of electrical science [18]. $\boldsymbol{R_{SC}}$ is a convenient measure of SC barrier property, because it does not require detailed knowledge of $\boldsymbol{D}$ or $\boldsymbol{L}$. An equivalent definition of diffusion resistance can be used for characterising the diffusion of water vapour in air.

As an example, we can use Eq.(3) to estimate the diffusion resistance of the SC barrier. Assume normal volar forearm skin, for which the following quantities are reasonable:-

$\boldsymbol{J}$ = $10\ g\,m^{-2}\,h^{-1}$ (=$2.8*10^{-6}\ kg\,m^{-2}s^{-1}$. Typical TEWL value)

$\boldsymbol{c_1}$ = $100\ kg\,m^{-3}$ (outer SC surface, interface with air, 10% hydration)

$\boldsymbol{c_2}$ = $800\ kg\,m^{-3}$ (inner SC surface, interface with the living epidermis)

Then,

$$R_{SC} = -\frac{\Delta c}{J} = \frac{c_2 - c_1}{J} = \frac{800-100}{2.8\times10^{-6}} = 2.5\times10^{8}\ sm^{-1} \qquad (5)$$

This number is not yet interesting. It provides a useful reference value for comparison with other diffusion processes, such as water vapour in air or membrane/water devices used for calibrating TEWL instruments. Read on.

2.4 TEWL Measurement

Interestingly, there are no methods for measuring TEWL directly. TEWL is defined as the flux of condensed water diffusing through the skin. TEWL methods measure the flux of water evaporating from the skin surface. If TEWL is the only source of water reaching the skin surface, and the skin surface remains dry, then the measured vapour flux is equal to TEWL. Otherwise it is not.

The vapour moves away from the skin by the mechanism of diffusion in the adjacent air. Vapour transport in moving air may be more important further away from the skin, but the air immediately adjacent to the skin is prevented from moving by viscous friction with the skin. For steady conditions, therefore this diffusion process can be described by Eq.(2), but using quantities related to water vapour in air, rather than condensed water in SC.

Note that there is some confusion in the literature about how to apply Fick's law to TEWL measurement. Humidity is a vapour property and cannot be used to characterise condensed water in the living epidermis, as in [1] for example. Water vapour, even at 100% RH, is more than four orders of magnitude less dense than condensed water. The relationship between the amount of condensed water in the SC and the relative humidity (RH) of the adjacent air is described by the sorption isotherm [19], as discussed further in Section 4.4. The approach I use here is to model the diffusion in the two regions separately, using continuity of flux to link them.

If the vapour flux from the skin surface is zero (and neglecting the temperature difference between skin and ambient air) then the humidity in the air adjacent to the skin is the same as the ambient humidity. If the vapour flux from the skin surface is finite, however, then the humidity next to the skin increases above ambient humidity. This creates a humidity gradient that is proportional to the vapour flux. Both open-chamber and condenser-chamber methods use this humidity gradient for measuring the flux.

2.5 Saturation Vapour Flux Density

Evaporation is an essential process in TEWL measurement, but what are the factors that determine the rate of evaporation from a surface? In general terms we know that temperature, humidity and air movement are involved. Clothes dry quicker when it's warm, dry and windy.

The minimum evaporation rate is zero. No water, no evaporation. But is there an upper limit to the evaporation rate? When the water vapour flux density is low and limited by TEWL, all the TEWL evaporates and the skin surface remains dry. The associated humidity gradient in the air adjacent to the skin is small and the humidity of the air immediately adjacent to the skin surface remains below saturation level. As the TEWL flux increases, so does the humidity immediately adjacent to the skin surface. A limit is approached as this humidity approaches the saturation point at 100% RH, when evaporation and re-condensation reach a dynamic balance. Under these conditions, free (ie unbound) water must be present on the skin surface. I call this the *saturation limit*, because it is the flux density at which the air immediately adjacent to the skin surface reaches the saturation point at 100% RH.

The saturation limit of water vapour flux is a microclimate property, not a skin property. When it's hot, dry and windy, the saturation flux density is high. Under these conditions, the perspiration water reaching your skin surface evaporates, keeping you dry and cool and comfortable. When it's muggy, the saturation flux density may be too low to keep up with your perspiration rate. Some perspiration water will then accumulate on the skin surface and you will be hot, wet and uncomfortable.

Different TEWL measurement chambers have different microclimates. Therefore they will have different saturation flux densities. This is what determines the maximum flux density that can be measured, ie the *measurement range* of the instrument.

3. Closed-chamber TEWL Methods

Here I describe two closed-chamber TEWL measurement methods, namely the unventilated-chamber and the condenser-chamber.

3.1 Unventilated-chamber Method

The main features of the unventilated-chamber method [7-10] are illustrated in Figure 1.

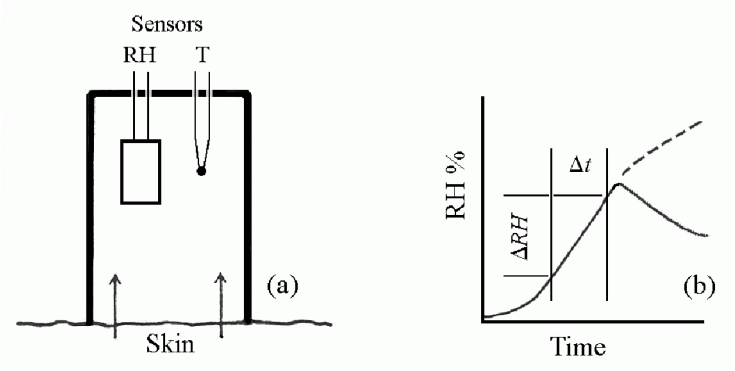

Figure 1: Schematic illustrations of the measurement head (a) and a typical signal (b) of the unventilated-chamber method.

The measurement chamber is in the form of a cylinder about the size of a thimble, as illustrated in Figure 1(a). One end of the cylinder is closed, the other end has a measurement orifice that can be placed in contact with the test surface - skin in this case. The chamber is equipped with sensors for relative humidity (RH) and temperature (T). Water vapour from the test surface collects in the chamber from which it cannot escape. This causes the humidity to rise with time, slowly at first but linearly thereafter, as illustrated in Figure 1(b). The flux density is calculated from the slope of the linearly rising part of the curve, indicated in Figure 1(b) by the intervals Δt and ΔRH. After the measurement is complete, the chamber needs to be lifted from the skin to allow the accumulated water vapour to escape (solid curve), otherwise the humidity would rise towards saturation level (dashed curve). This need to purge the measurement chamber of accumulated water vapour after every contact with the skin precludes the possibility of recording continuous flux density data.

3.2 Condenser-chamber Method

The main features of the condenser-chamber method [12-13] are illustrated in Figure 2.

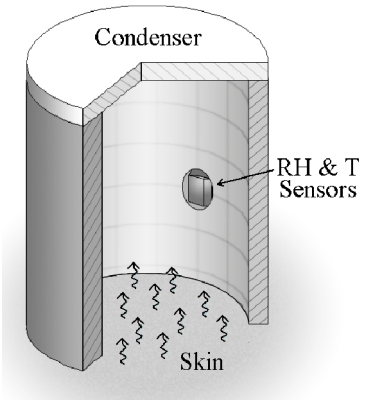

Figure 2: Schematic illustration of a condenser-chamber measurement head.

The measurement chamber shown in Figure 2 is in the form of a cylinder about the size of a thimble. Its dimensions are small enough for natural convection and other forms of air movement to be damped out by boundary losses, leaving diffusion as the only transport mechanism [20]. The chamber is closed at its upper end by an aluminium condenser that is maintained at a controlled temperature, several degrees below the freezing point of water. The

lower end of the chamber is open and acts as the measurement orifice that is placed into contact with the test surface.

The condenser has three distinct functions, as follows:-

1. It creates a humidity gradient that causes the incoming water vapour to diffuse away from the test surface. The humidity at the condenser is low and stable, determined by its temperature alone. The humidity at the test surface is higher, depending on the incoming water vapour flux.

2. It gets rid of the incoming water vapour by condensing it to ice. Condensation to ice occurs because the temperature of the condenser is below the frost point of water. This storage of water vapour in highly condensed form makes it possible to measure water vapour flux continuously for many hours.

3. It controls the microclimate humidity within the chamber independently of ambient humidity. The microclimate humidity depends only on the condenser temperature and the flux entering the chamber.

The humidity gradient described in point 1 above provides the means for measuring the flux density by the same diffusion-gradient method as in the open-chamber method [3, 4]. The gradient can be calculated from two humidity values at two spatially separated points. In the condenser-chamber method, one value is calculated from the readings of the RH and T sensors mounted in the chamber wall. The other value comes from the condenser, where the humidity can be calculated from its temperature without needing a second humidity sensor.

4. Microclimate

The microclimate in the air adjacent to the skin is intimately connected with TEWL and its measurement. One aspect is its effect on the evaporation of water from the skin surface. A quite separate aspect is its effect on the properties of the skin itself.

The first step to understanding how microclimate affects TEWL and its measurement is to understand the microclimate itself. The main variables of microclimate are humidity, temperature and air movement. The microclimate of uncovered skin is different from the microclimate when clothed, or when a TEWL measurement chamber is placed on it. The microclimate is also different in different TEWL measurement chambers.

The calculations in this section use two TEWL measurements methods for illustration, namely the open-chamber and the condenser-chamber methods. The reason for this choice is that these are both steady-state methods where model calculations are straightforward. The unventilated-chamber method is more difficult to model, because it is a non-steady-state method. However, the discussion is general and the conclusions can be adapted straightforwardly to all TEWL measurement methods.

4.1 Microclimate of Uncovered Skin

Is there a natural microclimate next to uncovered skin? Nilsson [4] discussed the concept of a *zone of diffusion* adjacent to the skin, where the water vapour pressure gradient (I use vapour

density rather than vapour pressure, but the two quantities are related through the ideal gas law) is related to the evaporative flux from the skin surface. He claimed that his open-chamber method measures this gradient *with minimal influence on the microclimate surrounding the surface of the skin.*

The physical basis for this zone of diffusion can be found in fluid dynamics [17, pp349-450]. For uncovered skin, ambient air movements tend to dominate the microclimate, as illustrated schematically in Figure 3.

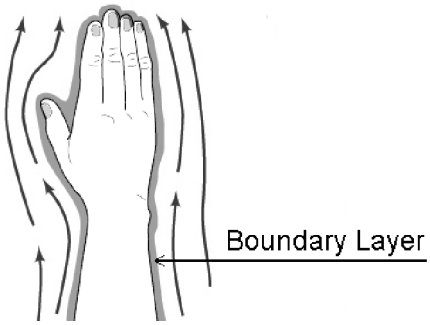

Figure 3: Schematic illustration of natural convection air-flow and associated boundary layer.

The skin surface is warmer than ambient laboratory air and this drives natural convection air movements, even when the laboratory air is otherwise perfectly still. The effects of air movements are difficult to predict, because air flow can be non-uniform, time-dependent, laminar, turbulent, etc. In the much simplified fluid dynamics model of natural convection, the flow is assumed to be laminar with a speed that varies from zero immediately next to a stationary surface to some speed some distance away. A further abstraction is to divide this graduated flow into just two regions, namely a boundary layer of perfectly still air next to the surface and a laminar flow region next to the boundary layer. Water vapour from TEWL would have to diffuse through the boundary layer before being removed by the moving air beyond. This boundary layer of fluid dynamics is Nilsson's zone of diffusion [18].

4.1.1 Boundary Layer Thickness

The thickness of the boundary layer depends on air flow, geometry of the object and temperature difference between the object and the air. Wheldon and Monteith [18] estimate that a typical value for the human body indoors is ~6mm, although values as large as 12mm are possible in very still conditions. These values are not stable and are perturbed by minor air movements, normal body movements and breathing. On the other hand, a boundary layer thickness in the range 10-30μm, as claimed in [21], would require supersonic air speeds!

Eq.(4) can be used to convert boundary layer thickness to diffusion resistance. In this case, you need to use the diffusion coefficient of water vapour in air, $D_{VA} \approx 2.42\times10^{-5}$ m^2s^{-1}. Using this value, the boundary layer thickness range 6-12mm works out to a diffusion resistance range ~250-500 sm^{-1}. Note that these values are very small compared with the barrier resistance of the SC, as estimated in Eq.(5).

4.1.2 Boundary Layer and Temperature

The boundary layer is also a zone of diffusion for heat loss by conduction from the skin surface. In the steady state, there is a temperature gradient across the boundary layer, with the air immediately adjacent to the SC surface at a higher temperature than that of the moving air

further away. The heat lost from the SC surface is supplied by the interior of the body. The SC is therefore at an intermediate temperature between that of the interior of the body and that of ambient air. This heat loss increases as the boundary layer thickness decreases - this is the physical basis of wind-chill. For still conditions typical of TEWL measurement, the temperature of the SC surface and the air in contact with it is typically 31°C. This is the value used in the model calculations of this report.

4.1.3 Boundary Layer and RH

The temperature gradient across the boundary layer has little effect on the diffusion of water vapour from the skin surface. However, the quantities RH and vapour pressure are both temperature-dependent. Therefore the RH immediately adjacent to the skin surface is generally different from ambient RH, depending on the local air temperature and the water vapour flux. This is why the skin surface RH values of Figure 4, for example, differ from ambient RH.

4.2 Microclimate within TEWL Measurement Chambers

The microclimate inside a TEWL measurement chamber is more stable than that of uncovered skin, because the chamber reduces the effects of external air movements. This is true even for the open-chamber, where the cylindrical wall gives some protection to the enclosed diffusion zone. Closed chambers provide a more stable environment for TEWL measurement, because they isolate the diffusion zone from the ambient air.

The microclimate conditions in the diffusion zone of a TEWL measurement chamber can be calculated from its diffusion resistance. For a typical open-chamber design (20mm chamber length, 10mm chamber diameter), the diffusion resistance works out to ~990 sm^{-1} [18]. The current geometry of condenser-chamber has a similar value, ~900 sm^{-1}. Note that these values are substantially larger than that of uncovered skin, but still very small compared with the barrier resistance of the SC, as estimated in Eq.(5). These values are used to calculate the microclimate humidity at the skin surface presented in Figure 4.

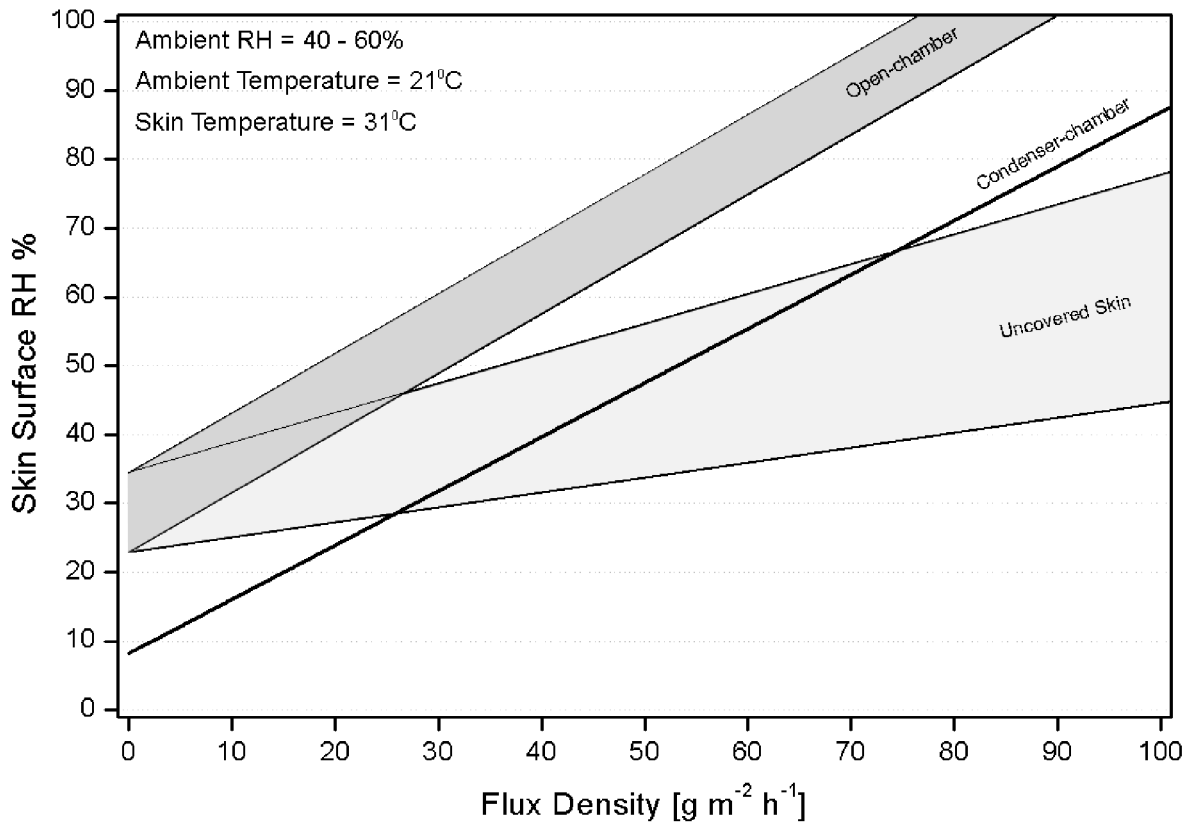

Figure 4: Microclimate RH at the skin surface for typical open-chamber and condenser chamber measurement heads, and for uncovered skin.

For uncovered skin and in the open chamber, the skin surface RH depends on ambient RH. This is indicated in the figure by shading, where the lower edges of the shaded areas correspond to 40% ambient RH and the upper edges to 60%. The skin surface RH in the condenser chamber is unaffected by ambient RH, because its microclimate is controlled by the condenser.

The higher diffusion resistance of the two measurement chambers compared with uncovered skin causes the skin surface RH within them to increase more rapidly with flux density than that of uncovered skin. In the open chamber, the skin surface RH is always higher than that of uncovered skin. In the condenser chamber, the skin surface RH starts at a lower value than that of uncovered skin and overlaps with it over a broad range of flux densities.

4.3 Microclimate and Saturation Flux Density

As the skin surface RH climbs towards 100%, the saturation limit of flux density is approached. The saturation characteristics for the three cases considered above are shown in Figure 5. These characteristics were calculated with the same parameter values as used Figure 4.

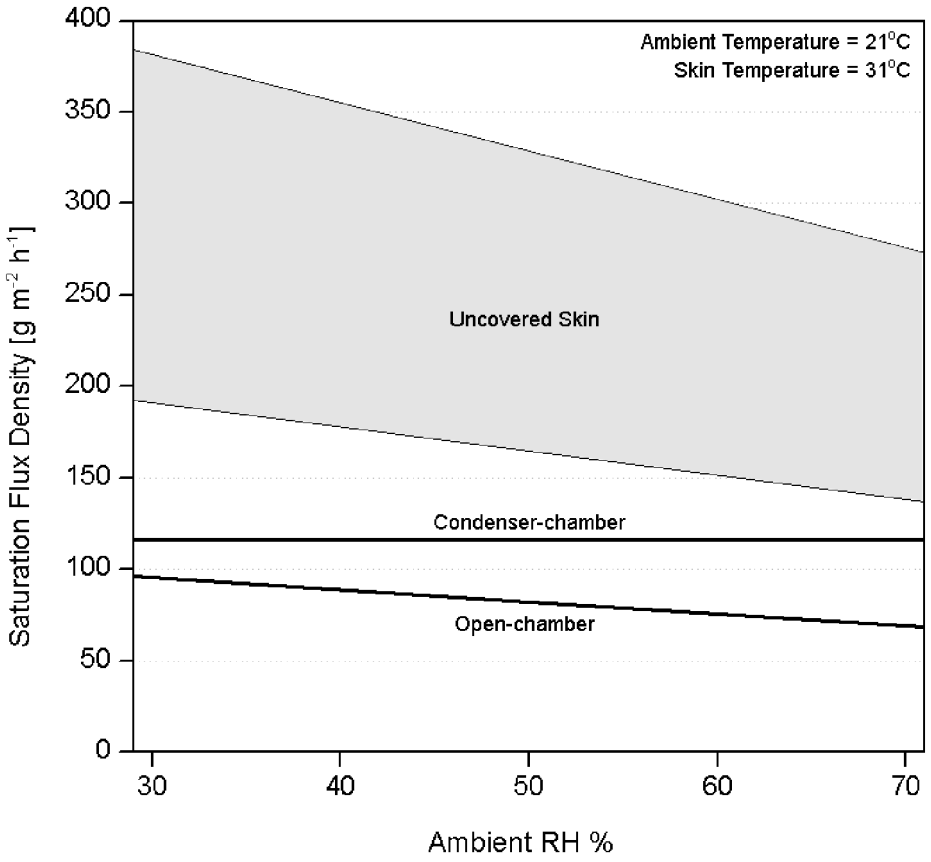

Figure 5: Saturation limits of flux density for uncovered skin, open-chamber and condenser-chamber measurement heads. This is the evaporation flux you would measure from a pure water surface at skin temperature. The shaded area for uncovered skin includes the boundary layer thickness range 6-12mm.

Figure 5 shows that still laboratory air can accommodate a higher skin surface evaporation rate than either of the two measurement chambers. That's good for acclimatisation prior to TEWL measurement. The condenser-chamber has a higher saturation limit than the open-chamber, partly because of its lower diffusion resistance but mainly because of the low humidity generated by the condenser. The condenser-chamber method is also unique in providing controlled evaporation conditions independently of ambient humidity. Note incidentally that the lines of Figure 5 do not extrapolate to zero flux at 100% ambient RH. This is because the air immediately adjacent to the skin is assumed to be at skin temperature, not at ambient temperature.

4.4 Microclimate and SC Properties

The microclimate also has an effect on the properties of the SC. The main interaction is between the RH adjacent to the skin surface and SC hydration. This interaction is described by the sorption and desorption isotherms, such as shown in Figure 6.

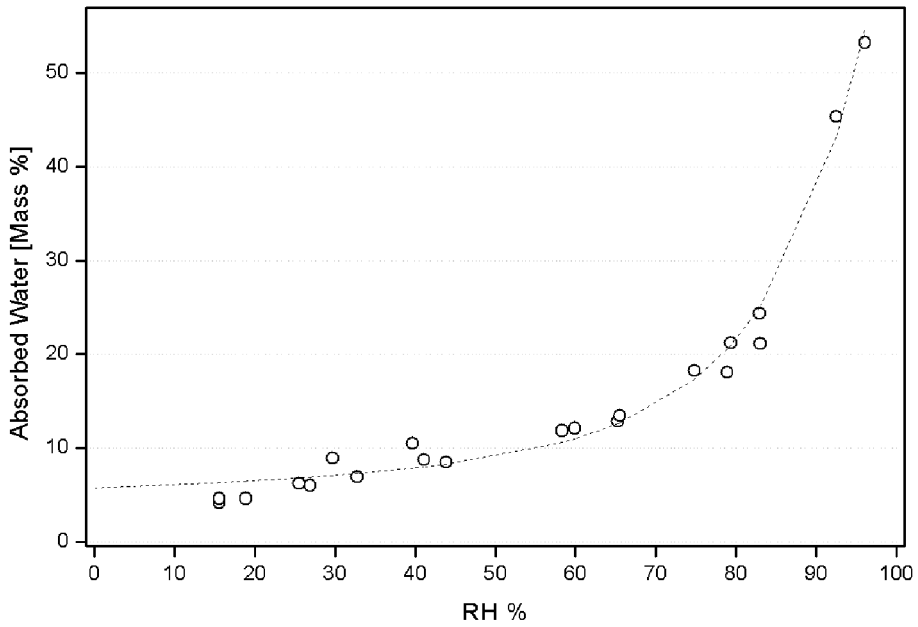

Figure 6: Sorption isotherm for excised SC, based on [19].

The data of Figure 6 were measured with excised SC [19]. They show that the equilibrium hydration is rather low, ~10% or less for RH values up to ~60%, but rises rapidly at RH values >80%.

4.5 SC Response Time to Microclimate Change

For in-vivo SC, the above microclimate effect needs to be superimposed onto hydrating effect from the living epidermis at its base. In this analysis, I am assuming that a sudden change of microclimate would have an immediate effect on the hydration of the SC surface, in accordance with the sorption isotherm. The resultant change of surface hydration would cause the concentration gradient in the layer below the surface to change, and this would affect the hydration of that layer. The next layer down would then react to the changes in the layer above and so on. Eventually, a new steady-state would be reached, with a new SC hydration profile and a new TEWL. To sum up, the model assumes that a sudden microclimate change at the SC surface would have an immediate effect on the SC surface, but the resultant change in the bulk of the SC would be more gradual, determined by the speed with which the surface change propagates through the SC by diffusion.

But how much time is needed to reach a new steady-state? One measure is the familiar acclimatisation time of TEWL measurement protocols. Part of this concerns perspiration, but TEWL values also need to adapt to a new environment, as illustrated in Figure 7. Acclimatisation times of 15-30 minutes are recommended [5, 6], but Figure 7 indicates that this may be an underestimate in this case.

Another measure of the time needed for the SC to adapt to a new environment comes from the work of Egawa *et al* [22]. They studied the effect of exposure of skin to a dry environment

and found that TEWL decreased significantly over a time period of 6 hours. Also, Ciortea *et al* [23] studied the effect of the low microclimate RH of a condenser-chamber TEWL instrument and found that the TEWL of volar forearm skin decreased at a rate of ~0.1% per minute of exposure.

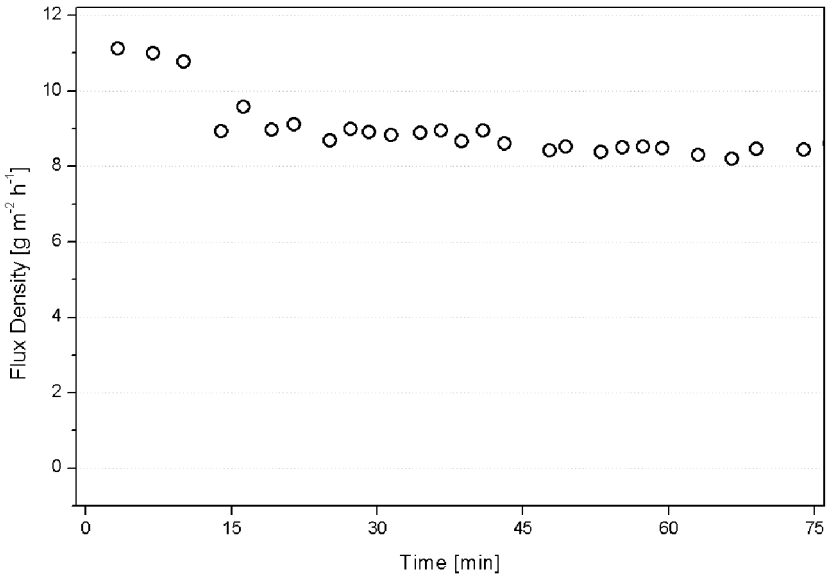

Figure 7: TEWL measurements of volar forearm skin during acclimatisation, measured with a condenser-chamber instrument.

The above observations of a decrease in TEWL caused by a decrease of skin surface RH contradict our model of TEWL based on Fick's law, as presented in Section 2.3. A decreased skin surface RH leads to a decreased skin surface hydration in accordance with the sorption isotherm. This leads to an increased concentration change across the SC and hence an increased TEWL, according to Eq.(3). However, this model ignores SC property changes with hydration. It is known that the diffusion coefficient and thickness of the SC change with hydration [24] and these effects are apparently more important in the above experiments.

Despite this limitation, the model of Section 2.3 can be used to get a rough estimate of the time taken for a surface hydration change to propagate through the SC. A general solution to Fick's second law of diffusion [17, Chapter 11] relates the penetration depth of a surface perturbation, $\boldsymbol{L}$, to a characteristic diffusion time τ though

$$L = \sqrt{\pi D \tau} \tag{6}$$

The new steady-state is approached asymptotically and it takes about three characteristic time periods for the process to finish.

Eq.(6) can easily be evaluated. For example, assuming a SC thickness of $L = 15\mu m$ and the same diffusion resistance as in Eq.(5), Eq.(6) works out to

$$\tau = \frac{LR_{SC}}{\pi} \approx \frac{15\times10^{-6}\times2.5\times10^{8}}{3.14} \approx 1200\,\text{sec} \tag{7}$$

This characteristic diffusion time of ~20 minutes is commensurate with the experimental data above. It can therefore be concluded, both from experimental evidence and from fundamental considerations, that the SC responds slowly to changes of microclimate. Therefore, it damps out fluctuations that are rapid compared with its characteristic diffusion time. Certainly, microclimate fluctuations on the timescale of seconds, as caused by air movements in uncovered skin and open chambers (see Figure 8) are too rapid to have more than a superficial effect on SC hydration.

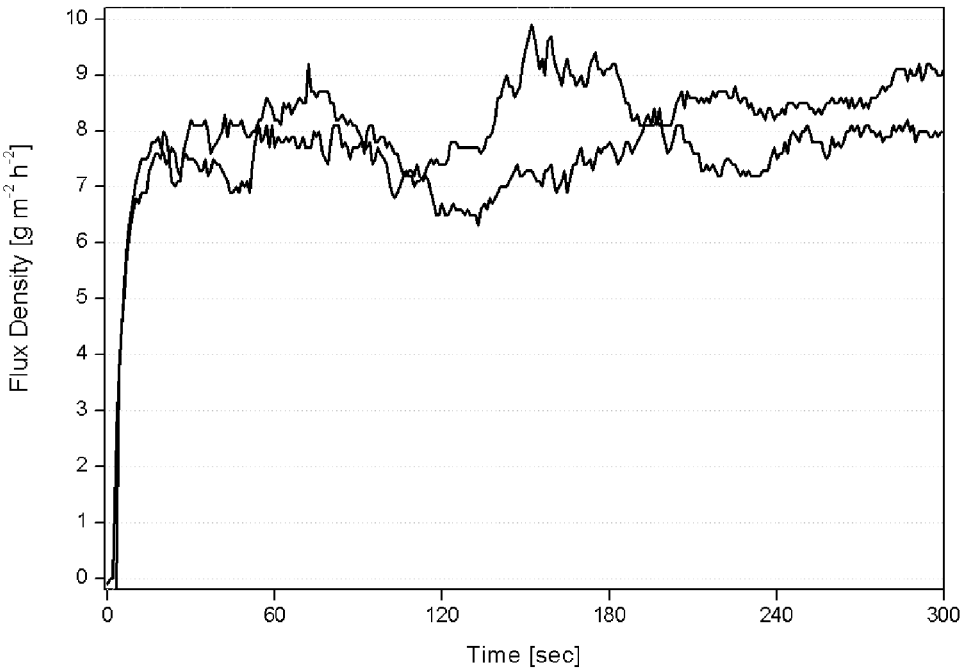

Figure 8: Fluctuations in open-chamber signals caused by air movements. These signals are typical for well-controlled laboratory conditions using procedures recommended by the manufacturer, but without a shielding box. Note that these are signal fluctuations, not TEWL fluctuations.

Therefore, such fluctuations can have little effect on TEWL, ie on the flux of condensed water through the SC. Their main effect is on the humidity distribution in the diffusion zone adjacent to the skin. This causes open-chamber signals to fluctuate, but the TEWL itself remains steady.

The characteristic response time of the SC is also long compared with a typical contact time required for TEWL measurement. Therefore, microclimate changes during TEWL measurement can have little effect on TEWL. Dark mutterings about the dire consequences of upsetting the boundary layer are off the mark. Yes, boundary layer disturbances affect open-chamber measurements through that hole in the top. But it's the signals that are affected, not the TEWL.

5. Calibration

An obvious source of disagreement among TEWL instruments is calibration, where arguments about the validity of different calibration methods continue. Here I describe two methods, the widely used wet-cup method and the new droplet method.

5.1 Wet-cup Method

The traditional method for calibrating TEWL instruments uses a *wet-cup* (membrane/water) method, as described in [5], for example. The water loss through the uncovered wet-cup membrane can be determined gravimetrically, from the weight loss of the cup with time. The water loss can also be measured by placing a TEWL measurement chamber in contact with the membrane. You calibrate a TEWL instrument by adjusting its reading to be the same as the gravimetrically determined water loss through the uncovered membrane. Right?

Wrong. You are not justified to assume that the flux entering the TEWL measurement chamber is the same as the gravimetrically determined flux through the uncovered membrane. It all comes down to diffusion resistance, as introduced in Section 2.3

There are similarities between the wet-cup and in-vivo skin in that both have a membrane to limit water loss. However, there is a vital difference. The lower surface of the SC is in contact with condensed water and the TEWL flux within the SC is condensed water. By contrast, the wet-cup membrane is separated from the water reservoir by an air gap and the flux within it is water vapour. In consequence, the diffusion resistances of the two membranes for the same flux density are very different.

The diffusion resistance of a wet-cup membrane itself is difficult to estimate, because you have to account for the diffusion resistance of the air above and below it. The approach I adopt here is to estimate the total diffusion resistance of (membrane + air), from the water surface into the ambient atmosphere. This *wet-cup resistance* is larger than the diffusion resistance of the wet-cup membrane itself, but this is unimportant in the present context.

With a flux density of $10\ g\,m^{-2}\,h^{-1}$ in ambient conditions of 21ºC and 50% RH, the wet-cup resistance $\boldsymbol{R_{WC}}$ works out as follows:-

$\boldsymbol{J}$ = $10\ g\,m^{-2}\,h^{-1}$ (=$2.8*10^{-6}\ kg\,m^{-2}s^{-1}$)

$\boldsymbol{c_1}$ = $0.0092\ kg\,m^{-3}$ (ambient air @ ~50% RH)

$\boldsymbol{c_2}$ = $0.018\ kg\,m^{-3}$ (air in contact with the water surface @ ~100% RH)

Therefore,

$$R_{WC} \approx \frac{0.018 - 0.0092}{2.8\times10^{-6}} = 3.1\times10^{3}\ sm^{-1} \qquad (7)$$

This wet-cup resistance is almost five orders of magnitude smaller than the diffusion resistance of the SC (see Section 2.3), but commensurate with the diffusion resistance of a TEWL measurement chamber (see Section 4.2).

You are now in a position to estimate the effect of placing a TEWL measurement chamber onto a membrane. If you add electrical resistance to a circuit, then the current drops. If you add diffusion resistance to a membrane, then the flux drops. In the case of in-vivo SC, the resistance change caused by placing a TEWL measurement chamber on it is negligible, <0.0004%. This is because the SC diffusion resistance is large compared with that of the measurement chamber. Different TEWL measurement chambers should therefore give the same reading of essentially unperturbed TEWL. In the case of the wet-cup, the resistance

change is significant, >30% in this example. Different TEWL measurement heads therefore give different readings, and these readings are different from the water loss through the uncovered membrane. This is why the wet-cup calibration method is flawed. Most emphatically, the wet cup is NOT a *constant water evaporation device*, as claimed in [5], for example.

5.2 Droplet Method

A recent project involving the UK National Physical Laboratory (NPL), several TEWL instrument manufacturers and TEWL users has come up with a new traceable method of calibration that can be used with TEWL methods capable of recording continuous flux time-series data (ie not with the unventilated-chamber method) [25, 26]. It uses a micro-syringe to dispense a droplet of water, typically 1mg, into a closed calibration cap. The cap is then sealed to the measurement chamber and a flux time-series recorded as the droplet evaporates. Figure 9 shows typical data for condenser-chamber and open-chamber instruments.

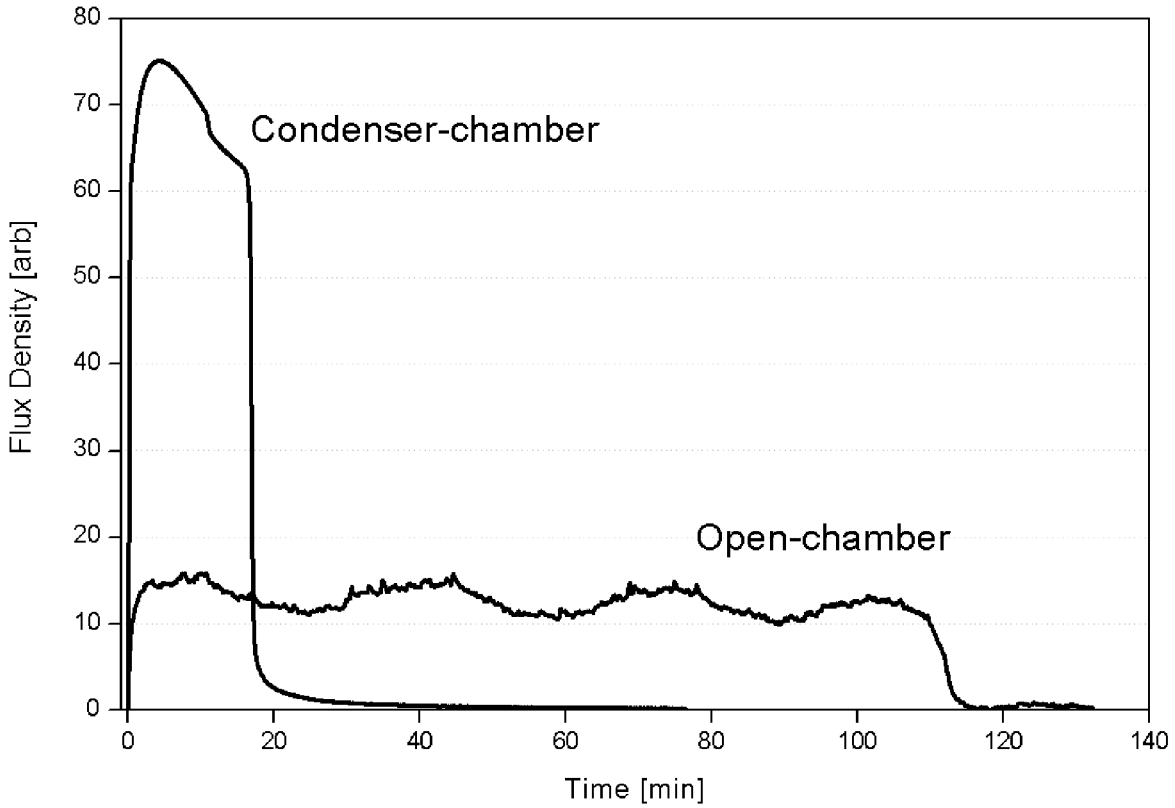

Figure 9: Flux time-series calibration curves recorded using 1mg water droplets. The calibration works by equating the area under the curve with the quantity of water dispensed. In this example, the areas for the two instruments are similar and their calibrations are therefore close.

The calibration works by equating the area under the flux curve with the quantity of water dispensed in the droplet. In the example shown in Figure 9, the areas under the two curves are similar, so their calibrations are close.

A noticeable difference between these two instruments is the rate of evaporation of the droplet, where the condenser-chamber instrument gives a high flux for a short time, whereas

the open-chamber instrument gives a lower flux for a longer time. The reason is the different saturation flux limits of the two instruments (see Section 4.2), which causes the water droplet to evaporate at different rates. Yet despite this, the two instruments will, if calibrated correctly, give the same TEWL readings on in-vivo skin, because the TEWL is determined by the SC barrier, not by the rate of evaporation.

The droplet method is straightforward to use and does not require any special facilities or a controlled environment beyond what the instrument itself requires. Independent research by the NPL has shown that this method brings TEWL measurements of different instruments closer together [25]. Let's hope!

6. TEWL Measurement

Having seen what TEWL instruments measure, we can go back to the question about how this relates to TEWL.

6.1 Conditions for TEWL Measurement

Here I examine the conditions that must be satisfied for TEWL instruments to measure TEWL. The relationship between what TEWL is and what TEWL instruments measure can be stated as follows:-

> TEWL is the flux of condensed water diffusing through the skin.
>
> TEWL instruments measure the flux of water vapour evaporating from the skin surface.

For these two quantities to be equal, the following conditions have to be met:-

(1) **TEWL must be the only source of water vapour.** Possible non-TEWL sources include the following:-

   (i) Perspiration. Perspiration is sporadic, induced by thermoregulation, emotions and other stimuli. Care needs to be taken to avoid it.

   (ii) Surface water (near-surface would be more accurate) from occlusion, insufficient acclimatisation, cosmetic products etc. Skin Surface Water Loss (SSWL) is additional to TEWL and therefore causes the readings to be elevated.

(2) **All must evaporate from the skin surface.** This requires the rate of supply of water from TEWL to be below the saturation limit of evaporation flux density. TEWL is a skin barrier property, but the saturation limit is a microclimate property in a given measurement head. With high TEWL, the skin surface humidity can approach the saturation point of 100% RH. Some transepidermal water would then remain as moisture on the skin surface and the readings would be too low.

For normal TEWL measurements under the right conditions, the water vapour flux is controlled by the SC barrier. All TEWL instruments should then give the same readings, if they are correctly calibrated. As the water vapour flux rises towards saturation levels, the readings become increasingly controlled by the chamber microclimate rather than the SC

barrier. Different instruments will then give different readings and these readings give information about the microclimate rather than the SC barrier.

6.2 TEWL Guidelines

A good starting point for meeting the conditions for TEWL measurement is to use appropriate protocols that include acclimatisation, with the TEWL guidelines [5, 6] providing some good advice. However, these guidelines apply to open-chamber instruments only. Table 1 presents a summary of their recommendations with comments on how these apply to the current closed-chamber VapoMeter (Delfin Technologies Ltd, Finland, unventilated-chamber) and AquaFlux (Biox Systems Ltd, condenser-chamber) instruments.

Table 1: Guidelines and Closed-chamber Instruments

Guideline Recommendations	Closed-chamber Applicability
Ambient Conditions *If climate room facilities are available, the ambient room temperature should be regulated to 20-22°C and the relative humidity to 40%* [5]. *Usually it is suggested to keep the temperature between 20 and 22±1°C and the relative humidity lower than 60%* [6].	These recommendations are valid for all measurement methods insofar as they provide conditions to avoid surface moisture and sweat gland activity. You cannot take the *bio* out of bioengineering.
Shielding Box *Perform all TEWL measurements within a large "open-top" box whenever possible* [5]. *Measurements should be carried out in a room with limited air circulation. A shielding box with an open top can be used if doubt exists whether undesirable air turbulence is present or not* [6].	Ambient air movements and turbulence have no effect on closed-chamber measurements.
Post-measurement Recovery *Allow the instrument to "zero" on its own (2-4 minutes), before attempting the next measurement* [5]. *An equilibrium time should be taken into consideration before the next measurement is started* [6].	**VapoMeter:** The humidity in the measurement chamber needs to return to ambient humidity before the next measurement can be started. This takes from 10 to 90 seconds, depending on prior flux. **AquaFlux:** No recovery time is necessary before starting the next measurement, because of the controlled microclimate. You can hop from site to site.
Holding the Probe *Do not hold the probe directly by hand. The probe should be handled with an insulating glove, or the calibration rubber stopper supplied with the equipment, or a burette clamp* [5]. *The measuring probe itself should not be touched before and during measurements and can be handled with the electrical wire, a coating or by wearing gloves* [6].	**VapoMeter:** TEWL values were found to increase significantly with probe temperature [14]. **AquaFlux**: You can hold the probe in whatever way is comfortable. A probe handle that you cannot hold in your hand - what will they think of next!
Contact Pressure *The contact pressure of the probe onto the skin should be kept low and constant* [5]. *... with a constant but light pressure. Measurements within one experiment should preferably be performed by the same operator* [6].	No measurable contact pressure effect has been found. This seems to be an engineering problem, not a skin property.
Measurement Surface Orientation *The measuring surface should be placed in a horizontal plane, and the probe applied parallel to this surface* [5]. *The measuring surface should be placed in a horizontal plane and the probe should be applied perpendicularly to this surface* [6].	Closed chambers do not suffer the same "chimney effect" interference from convection air movements as open-chamber instruments do. They can therefore be used with all surface orientations.

7. Examples of TEWL Measurements

Here I show some examples to illustrate the capabilities and limitations of closed-chamber TEWL measurements. The featured closed-chamber instruments are the unventilated-chamber VapoMeter (Delfin Technologies Ltd, Finland) and the condenser-chamber AquaFlux (Biox Systems Ltd). The focus is on instrument comparisons, because these studies give an impression of the performance you can expect from a given instrument.

7.1 VapoMeter vs TewaMeter

A recent validation study [14] reported a number of parallel in-vivo measurements, comparing an unventilated-chamber VapoMeter Model SWL-2 with an open-chamber TewaMeter Model TM210. Many aspects of performance were compared on the inner sides of the forearms of 16 healthy female volunteers aged 22-31 years.

Here I want to focus on just one small corner of this comprehensive study, namely the baseline TEWL measurements on four sites of the volar forearms, left and right, of the 16 volunteers, reproduced in Table 2 below. The numbers in the grey areas were calculated for this report and do not appear in [14]. Note that the rules of Gaussian statistics were used to calculate averages of standard deviations.

Table 2: Baseline TEWL Data from [14]. The grey areas are calculated from the published data.

	Zone	TewaMeter			VapoMeter		
		Mean TEWL $gm^{-2}h^{-1}$	**StDev** $gm^{-2}h^{-1}$	**CV %**	**Mean TEWL** $gm^{-2}h^{-1}$	**StDev** $gm^{-2}h^{-1}$	**CV %**
Left:-	1	14.2	2.7	19	8	3	38
	2	13.1	2.3	18	8	3	38
	3	12.5	2.4	19	7	3	43
	4	14.5	3.2	22	7	4	57
Right:-	1	14.1	3.1	22	8	3	38
	2	12.9	1.8	14	7	2	29
	3	12.2	2.2	18	7	2	29
	4	15.1	2.7	18	6	4	67
Averages:-	**all**	**13.6**	**2.6**	**19.1**	**7.3**	**3.1**	**42.0**

Each mean TEWL value is an average over 16 volunteers. The average TEWL over all sites of all volunteers, ie 128 measurements, works out to 13.6 $gm^{-2}h^{-1}$ for the TewaMeter, almost double the value of 7.3 $gm^{-2}h^{-1}$ measured with the VapoMeter. This looks like a calibration problem, given that both manufacturers use variants of the flawed wet-cup method for calibration (see Section 5.1). The error is ~ ±30% from the mean.

Another point of difference is in the standard deviations, where you would expect similar values for the two instruments, if the main cause of the scatter they represent is skin-related. However, such a comparison only makes sense if you make allowance for the calibration difference, either by normalising the data or by comparing Coefficients of Variation (CV) rather than standard deviations. Table 2 shows that the average coefficient of variation of the TewaMeter data, CV=19.1% is considerably smaller than the equivalent value for the

VapoMeter data, CV=42%. The difference is unlikely to be skin-related, given that the measurements were conducted in parallel. More likely is that the two instruments have different repeatability characteristics. This is confirmed by the more broadly-based conclusion of the study that *the TewaMeter is able to detect significantly smaller differences than the VapoMeter* [14].

7.2 VapoMeter vs AquaFlux

The protocol of this study [27] was designed to assess measurement uncertainties associated with rapid TEWL measurement. To this end, skin-related uncertainties were reduced by confining the study to a single test area of a single volunteer in a single session. The measurements were performed on seven test sites marked on the left volar forearm of an elderly volunteer (REI), as illustrated in Figure 10.

Figure 10: Test sites 1 - 7 on the left volar forearm.

The TEWL of the 7 sites was measured in rapid sequence by means of a condenser-chamber AquaFlux Model AF103 for a total of 12 repeats, by moving the probe from site to site as rapidly as possible, without any recovery delays (site-hopping). The ambient temperature and relative humidity during the test were 23.8 °C and 45%. The skin was acclimatised to these conditions for about one hour before the start of the measurements.

Equivalent measurements were also performed with an unventilated-chamber VapoMeter. A site-hopping protocol is not possible with this instrument, because the water vapour captured during a measurement needs to be allowed to escape before the next measurement can be started. Instead, the contact with the test surface was confined to the measurement phase only, and the next measurement was initiated as quickly as possible after completion of the ventilation phase of the previous measurement. The DelWin software was used to minimise data display delays. This protocol accords with the manufacturer's recommendations in the instruction manual.

The results are presented in Figure 11 and Table 3.

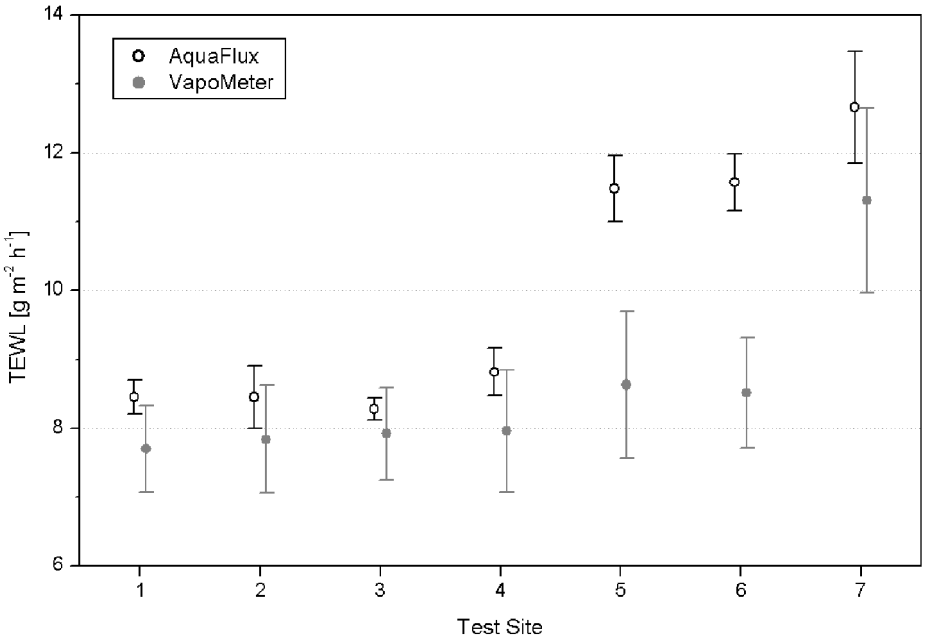

Figure 11: In-vivo comparison between TEWL measurements using condenser-chamber AquaFlux (black open circles) and unventilated-chamber VapoMeter (grey filled circles) instruments. The error bars are ±1 Standard Deviation.

In-vivo Comparison of Performance						
	AquaFlux			VapoMeter		
Zone	Mean TEWL $gm^{-2}h^{-1}$	StDev $gm^{-2}h^{-1}$	CV %	Mean TEWL $gm^{-2}h^{-1}$	StDev $gm^{-2}h^{-1}$	CV %
1	8.5	0.25	3.0	7.7	0.63	8.2
2	8.5	0.46	5.4	7.8	0.78	9.9
3	8.3	0.16	2.0	7.9	0.67	8.5
4	8.8	0.35	4.0	8.0	0.89	11.2
5	11.5	0.48	4.2	8.6	1.06	12.3
6	11.6	0.41	3.6	8.5	0.80	9.4
7	12.7	0.81	6.4	11.3	1.34	11.9
Averages:-	**9.96**	**0.46**	**4.6**	**8.55**	**0.91**	**10.6**

Five features are apparent in this comparison:-

1. The calibration of the two instruments are closer than in [14] ~ ±8% from the mean.

2. The TEWL values measured with the two instruments correlate quite well, with a Pearson correlation coefficient of R = 0.82.

3. The mean measurement repeat-time was found to be 64 seconds for the AquaFlux site-hopping protocol and 38 seconds for the equivalent rapid measurement protocol of the VapoMeter. A more recent study using the faster AF200 AquaFlux model found a repeat-time of 48 seconds.

4. The uncertainties of the VapoMeter readings are significantly smaller than those found in study [14], as described in Section 7.1.1. This confirms that fewer skin-related uncertainties contributed to the present study.

5. The uncertainties of the VapoMeter readings are significantly larger than those of the AquaFlux. The difference is unlikely to be skin-related in this case, given that the measurements were conducted on the same sites, in sequence, during the same session. More likely is that the two instruments have different repeatability characteristics.

An in-vitro comparison is also described in [27], to eliminate skin-related uncertainties and quantify instrumental repeatability characteristics.

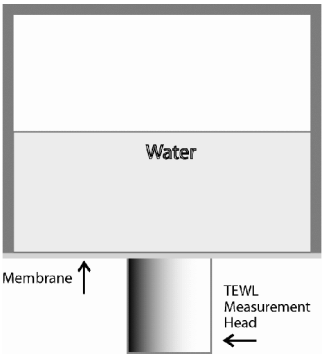

Figure 12: Schematic representation of an in-vitro water vapour flux source, where water is in contact with a membrane that is impermeable to condensed water, but permeable to water vapour.

It used an upside-down wet-cup flux source, shown schematically in Figure 12. This design improves flux stability by eliminating the air gap between the water surface and the membrane. The use of Sil-Tec membranes ensured that the source has similar properties as conventional wet-cups described in Section 5.1. Sil-Tec membranes are hydrophobic and impervious to condensed water. Therefore, only water vapour can diffuse through these membranes and their diffusion resistance is similarly low for a given flux as estimated in Section 5.1.

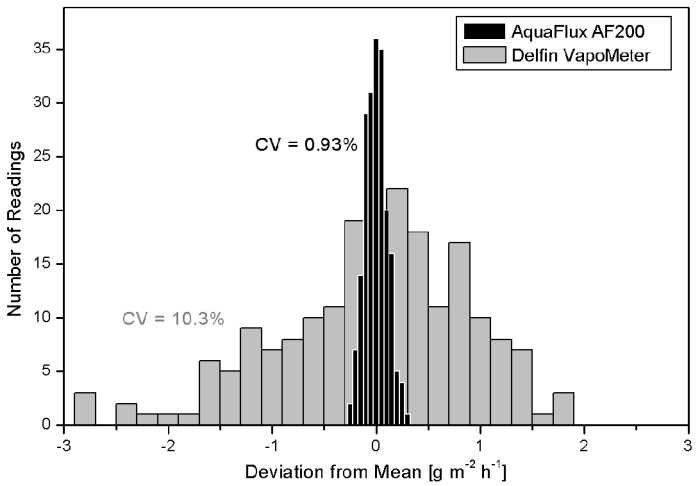

Figure 13: In-vitro repeatability comparison between a VapoMeter and an AquaFlux Model AF200, using the water vapour flux source shown in Figure 12.

The main finding from 200 repeat measurements with this source for each instrument was that the in-vitro repeatability of the AquaFlux was CV~1%, as shown in Figure 13. The VapoMeter was found to be characterised by a CV of ~10%, whether in-vivo or in-vitro. This

indicates that the observed VapoMeter in-vivo measurement uncertainties were attributable to instrumental factors rather than to the skin in this case.

7.3 AquaFlux Membrane Integrity Testing

An application where the condenser-chamber method has a particular advantage is membrane integrity testing for in-vitro permeation studies using Franz diffusion cells. OECD Guideline 428 stipulates barrier integrity testing before permeation experiments are carried out [28]. TEWL, electrical resistance and tritiated water procedures are recognised for such tests [29].

In a comprehensive study using a TewaMeter, Netzlaff *et al* [30] found that TEWL measurements appear to be of limited use as a barrier integrity test, being able to detect only severe damage in the samples they tested. Part of the problem was associated with topically adhering water. In this respect Netzlaff's measurement conditions did not comply with the conditions needed for TEWL measurement, as stated in Rule 1 of Section 6: TEWL must be the only source of water vapour. Another part of the problem was associated with deliberately made pinholes in the membranes, where water was found to permeate through in condensed form.

The controlled microclimate within a condenser-chamber instrument offers a distinct advantage over conventional TEWL instruments for such measurements. The low humidity causes topical water to dry off quickly during the measurements. You therefore do not need to include a drying phase into such test protocols. Furthermore, the measured flux curves clearly show the drying progress and therefore 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. These points are illustrated in Figure 14.

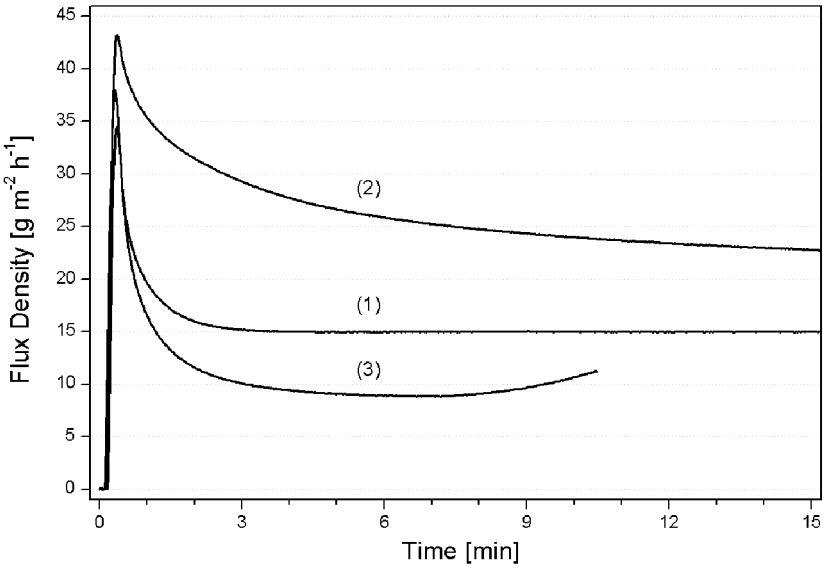

Figure 14: Franz-cell membrane tests using Sil-Tec membranes.

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 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 15.

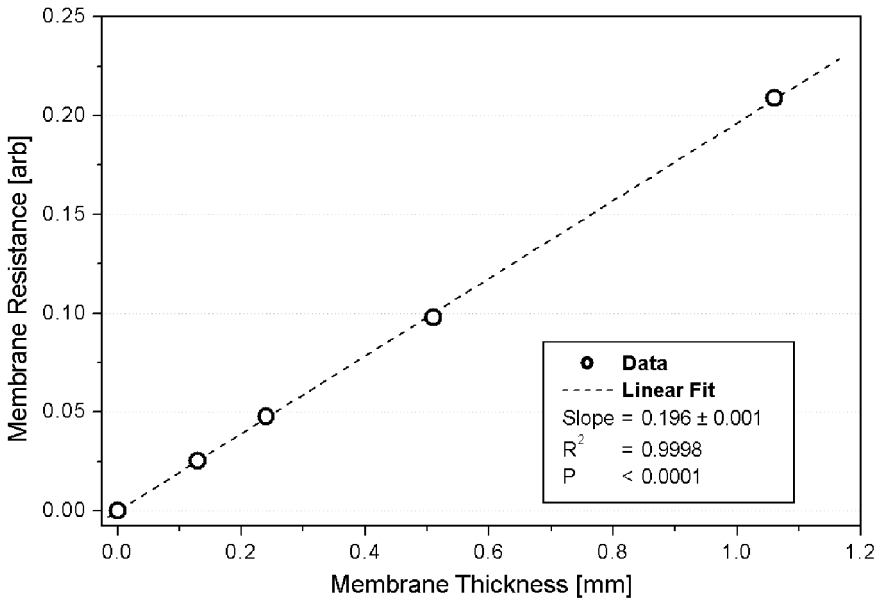

Figure 15: Correlation of membrane thickness with diffusion resistance. Sil-Tec membranes of thickness 0.13-1.06mm were used in this test.

The method was also tested with snake sheddings [31] where it was found to be sensitive enough to find a species-difference.

8. Summary And Conclusion

The main points from this analysis are:-

1. There are no direct methods for measuring TEWL.
2. TEWL is condensed water diffusing through the skin.
3. TEWL methods measure water evaporation from the skin surface.
4. The microclimate of uncovered skin is dominated by air movements.
5. All TEWL measurement chambers perturb the microclimate.
6. SC property change is slow compared with TEWL measurement times.
7. Wet-cup calibration methods are fundamentally flawed.
8. The droplet calibration method is traceable and has been independently verified.
9. Closed-chamber TEWL methods work.

For normal TEWL measurements under the right conditions, the water vapour flux is controlled by the SC barrier and all TEWL instruments should give the same readings, if they are correctly calibrated. As the water vapour flux rises towards saturation levels, the readings become increasingly controlled by the chamber microclimate rather than the SC barrier.

Different instruments will then give different readings and these readings give information about the microclimate rather than the SC barrier.

The open-chamber method has been around for a long time, so the newer closed-chamber instruments have something to prove. But to describe the open-chamber method as the *gold standard* of TEWL measurement is unjustified. Being around for a long time does not make you right, as grumpy old men know only too well. It is also an unfortunate metaphor, given that the world abandoned the gold standard (*that barbarous relic* according to John Maynard Keynes) before the open-chamber method was invented!

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