# Franz Cell Barrier Integrity Assessment using a Condenser-Chamber TEWL Instrument

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# Barrier Integrity Assessment

Barrier integrity assessment is an integral part of experimental protocols for in-vitro dermal absorption measurements.

**Aims:-**

1. **Assess performance of condenser-chamber instrument:-**

   Artificial membranes

   Snake skin sheddings

2. **Develop data analysis method:-**

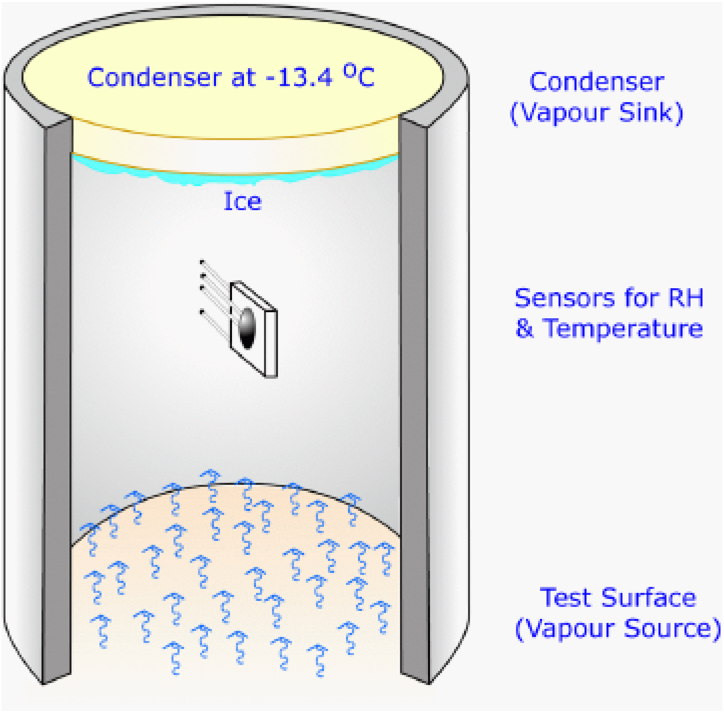
   From flux to membrane resistance

# Plan

1. Apparatus
2. Artificial membrane measurements
3. Artificial membrane analysis
4. Snake skin measurements
5. Snake skin analysis
6. Acknowledgements

# Apparatus 1

## Biox AquaFlux Condenser-chamber TEWL Method




### Closed-Chamber

Shields from ambient air movements.

### Condenser

Removes water vapour.

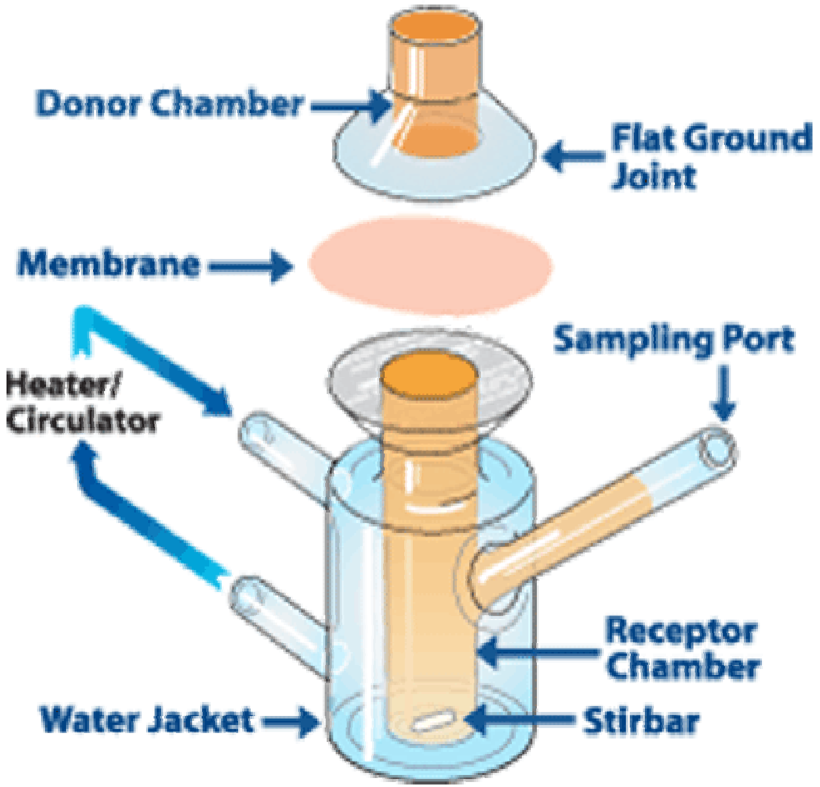
Controls the microclimate.

### Single RHT Sensor

Improves accuracy & sensitivity.

# Apparatus 2

## PermeGear Static Franz Cell



# Apparatus 3

## AquaFlux - Franz Cell Coupling

Leak-tight seals are essential for barrier integrity testing.

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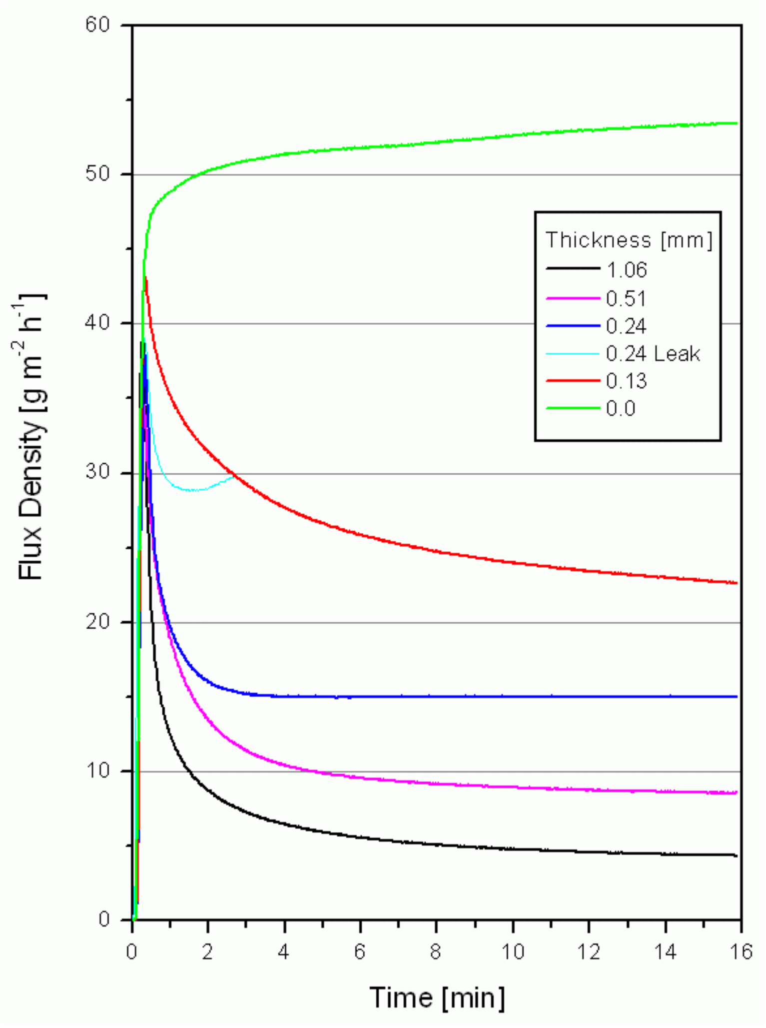
# Artificial Membrane Measurements

## Vapour Flux Transmission through Sil-Tec Membranes




Comments:-

1. Leak-free coupling to membrane & TEWL instrument is essential.

2. Air-side must be dry for flux to indicate membrane resistance.

4. Flux settling time prolonged by air-side moisture. (eg red 0.13mm curve)

3. Flux curve shape indicates air-side leaks. (eg turquoise 0.24mm curve)

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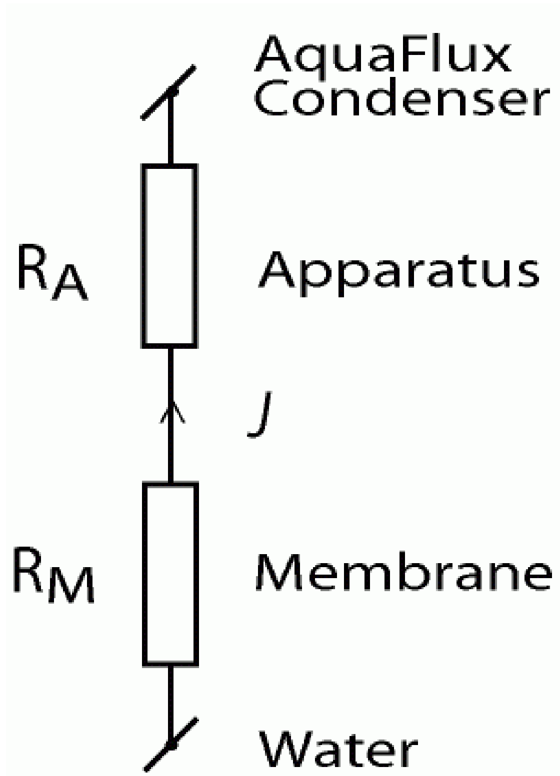
# Artificial Membrane Analysis 1

## Electrical Analogy - Diffusion Resistance



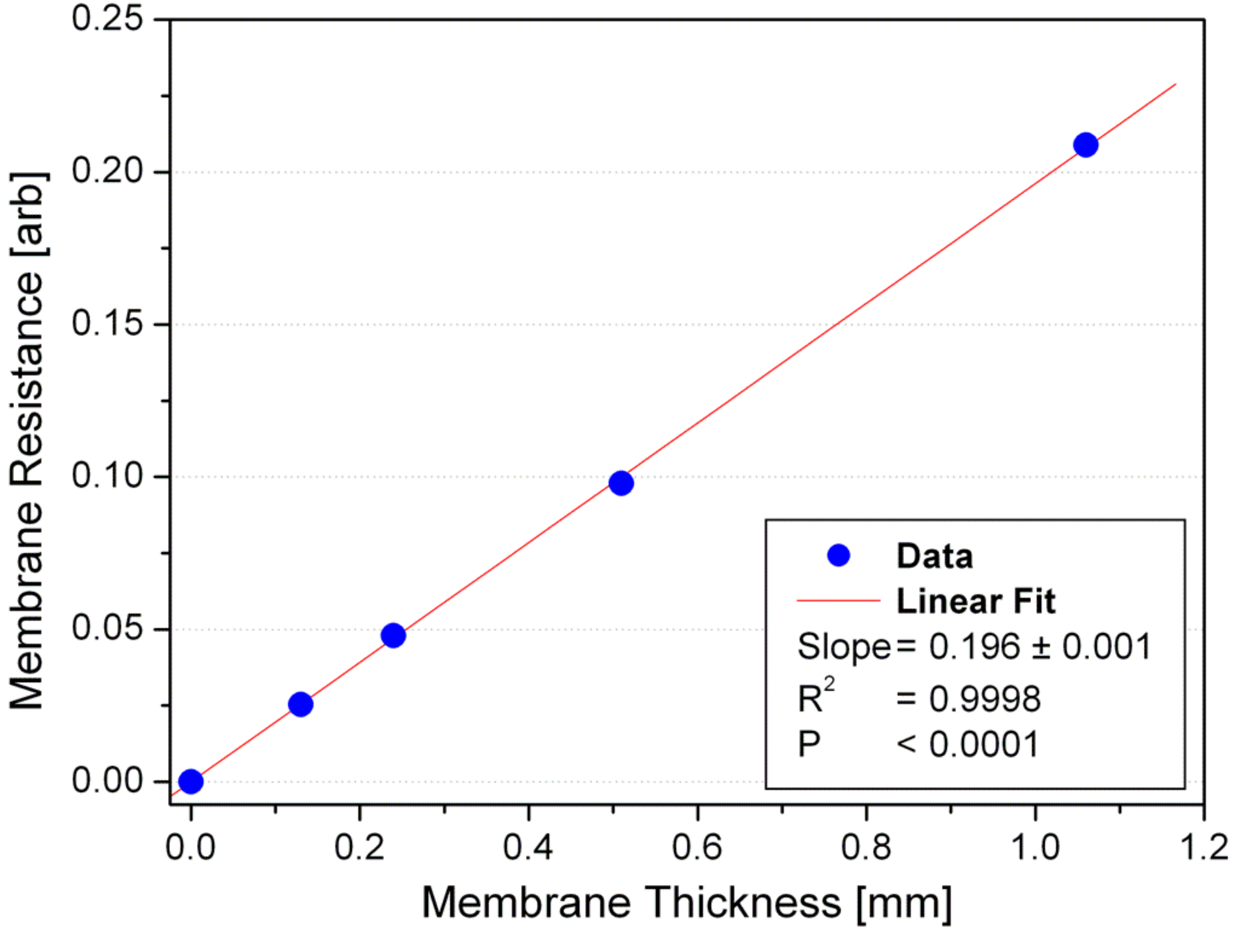

$$R_M \propto \frac{1}{J} - \frac{1}{J_0}$$

$\boldsymbol{J_0}$ is flux without membrane

NB: Sil-Tec is hydrophobic & water diffuses through it as vapour. Therefore, use a 100% RH boundary condition at the water/Sil-Tec interface.

# Artificial Membrane Analysis 2

## Diffusion Resistance Analysis



# Artificial Membrane Analysis 3

## Conclusions

1. Leak-free couplings to membrane & TEWL instrument are essential.

2. Air-side must be dry for flux to indicate membrane resistance.

3. Flux curve shape indicates air-side leaks.

4. Flux settling time prolonged by air-side moisture.

5. Measurements verified by **Thickness** *vs* **Resistance** proportionality.

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# Snake Skin Measurements 1

## Typical Snake Skin

**Samples taken from:-**

Snakes 6 & 11: Bothrops atrox (F); Snake 8: Bothrops moojeni (M)

**Use samples from:-**

1. Near head
2. Centre body
3. Near Tail

# Snake Skin Measurements 2

## Typical Samples



1. The outer surface is is scaly & hydrophobic.

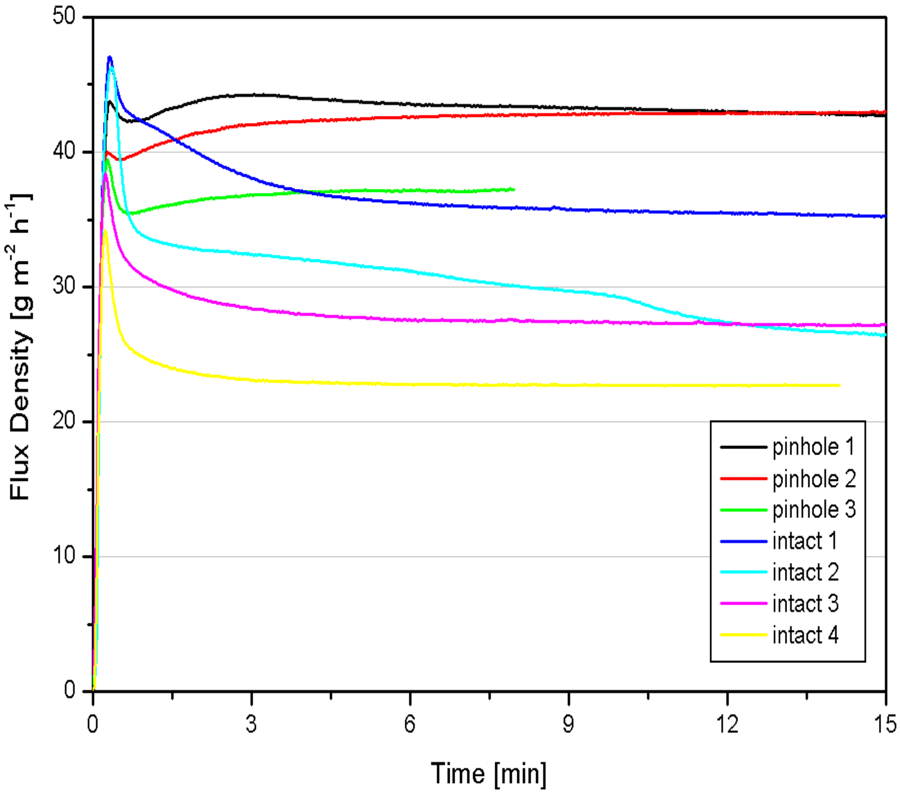
   Therefore mount with this surface facing the AquaFlux.

2. The inner surface is hydrophilic.

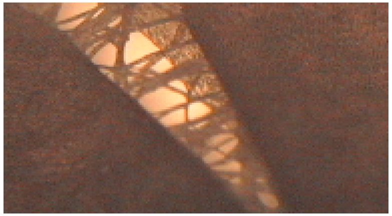
   Therefore mount with this surface in contact with the water.

# Snake Skin Measurements 3

## Centre Body Samples




**Large Pinholes**



**Small Pinholes**

**Intact**

Pinholes can be identified from elevated TEWL.

Flux curve shape can be used to detect fault conditions

# Snake Skin Measurements 4

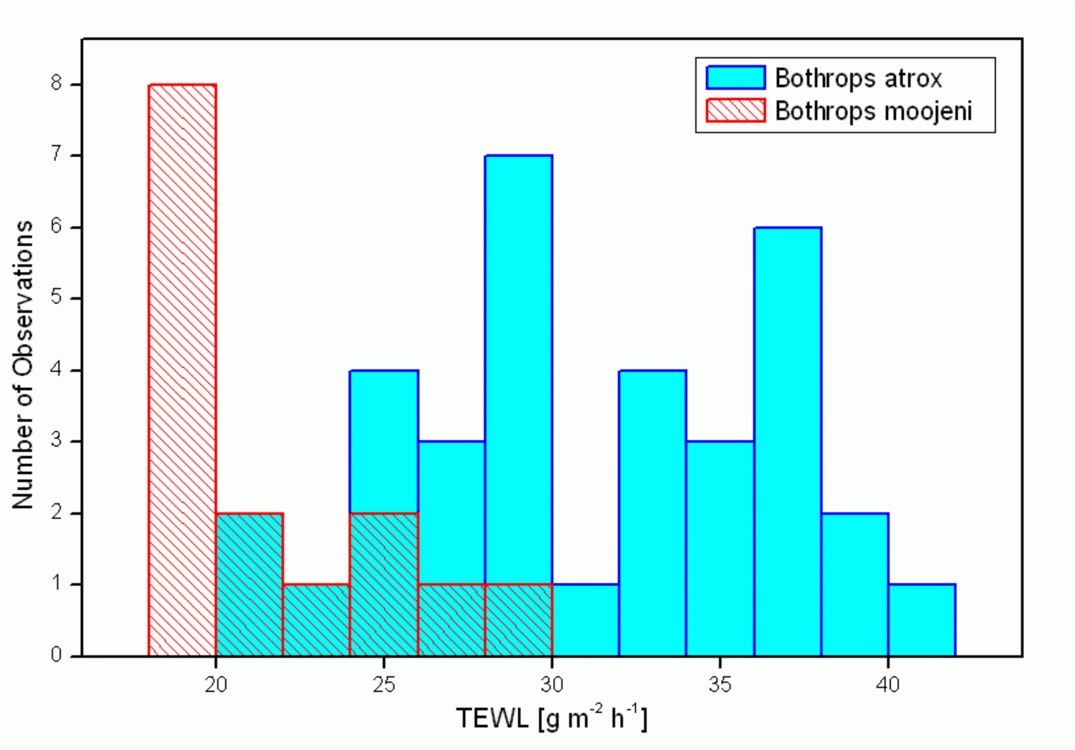
## Sample Consistency

| Sample | Quantity | Tail | Middle | Head | All Parts |
| --- | --- | --- | --- | --- | --- |
| **Snake 6** Bothrops atrox | <J> $[gm^{-2}h^{-1}]$ | 29.9 | 30.2 | 37.7 | **32.0** |
| | CV (N) | 19% (6) | 13% (7) | 7.7% (4) | **16% (17)** |
| | | | | | |
| **Snake 8** Bothrops moojeni | <J> $[gm^{-2}h^{-1}]$ | 23.5 | 20.3 | 20.7 | **21.5** |
| | CV (N) | 18% (5) | 7.4% (6) | 18% (4) | **15% (15)** |
| | | | | | |
| **Snake 11** Bothrops atrox | <J> $[gm^{-2}h^{-1}]$ | 34.0 | 32.1 | 26.9 | **30.2** |
| | CV (N) | 9.7% (5) | 26% (5) | 14% (7) | **20% (17)** |
| | | | | | |
| **All three** | <J> $[gm^{-2}h^{-1}]$ | **29.2** | **27.4** | **28.1** | **28.2** |
| | CV (N) | **21% (16)** | **25% (18)** | **26% (15)** | **23% (49)** |

**<J>** = Mean TEWL
**CV** = Coefficient of Variation
**(N)** = Number of Samples Measured

# Snake Skin Measurements 5

## Species Analysis




**Bothrops atrox:** Mean TEWL = 31.2 $gm^{-2}h^{-1}$ (34 Samples)

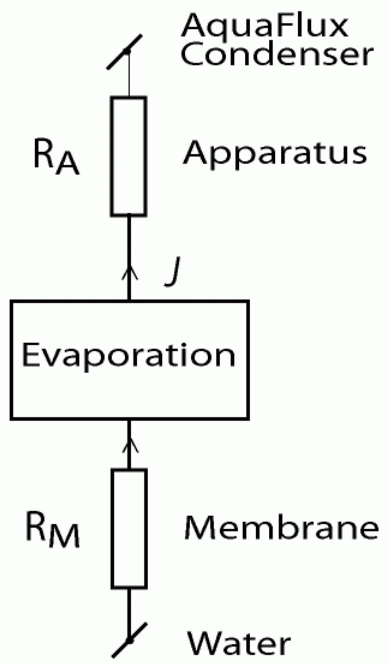
**Bothrops moojeni:** Mean TEWL = 21.5 $gm^{-2}h^{-1}$ (15 Samples)

ANOVA test gives $p = 7.6 \times 10^{-8}$, ie the means are significantly different.

# Plan

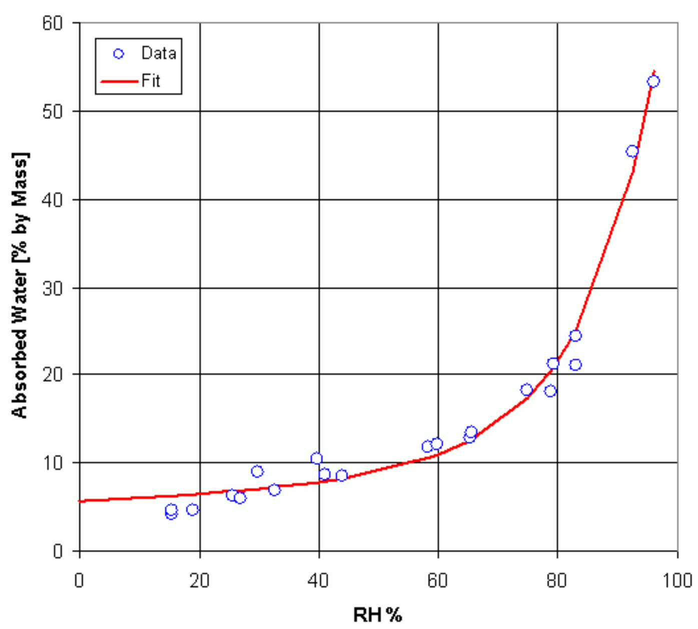
# Snake Skin Analysis 1

## Electrical Analogy with Surface Evaporation




NB: Snake skin is permeated with water. Evaporation on the air-side is characterised by a sorption isotherm. Use human SC isotherm (right) for rough initial analysis.

### Sorption Isotherm for Human SC [1]

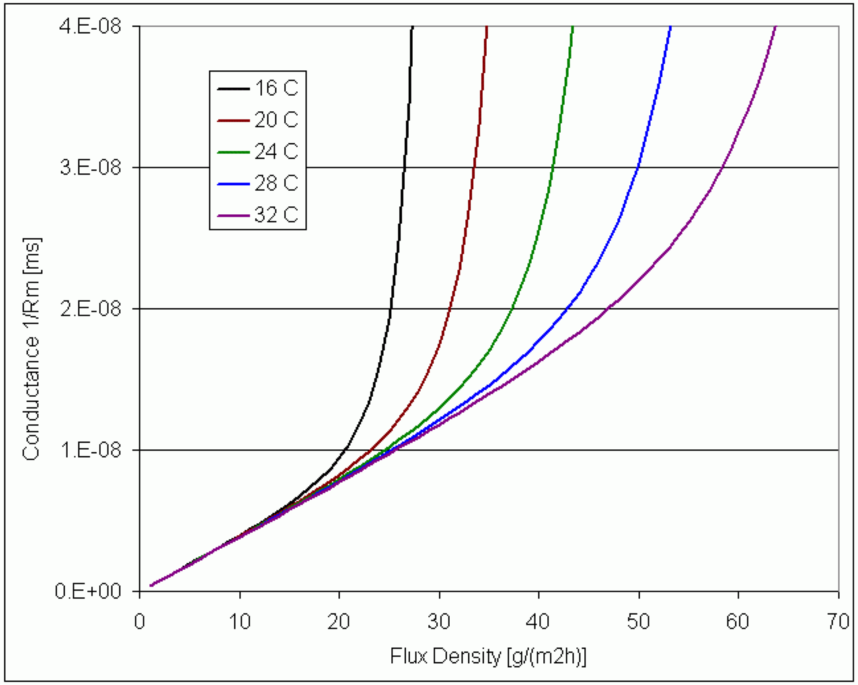




[1] J-L Lévêque: Water-Keratin Interactions In Bioengineering of the Skin: Water and the Stratum Corneum, (Editors: P Elsner, E Berardesca & HI Maibach), 2, 13-22 CRC Press, Boca Raton 1994.

# Snake Skin Analysis 2

## Relationship between TEWL & Membrane Resistance




High membrane resistance is independent of temperature because the flux is controlled by the membrane.

# Snake Skin Analysis 3

## Conclusions

1. TEWL variability is typically ~25% CV.

2. Pinholes can be identified from elevated TEWL.

3. Flux curve shape can be used to detect fault conditions.

4. TEWL characterisation with leak-free coupling is reliable.

5. High membrane resistance is independent of temperature.

6. Low membrane resistance depends on temperature.

7. Snake skin sheddings have potential as bio-membranes in transdermal diffusion measurements.

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# Acknowledgements

Rainer Voegeli (Pentapharm)

Supply of snake sheddings & scientific input.

Gary Grove - (Cyberderm)

Advice on how to handle snake sheddings.

(Including what they smell like when they're off!)