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

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&

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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:-**

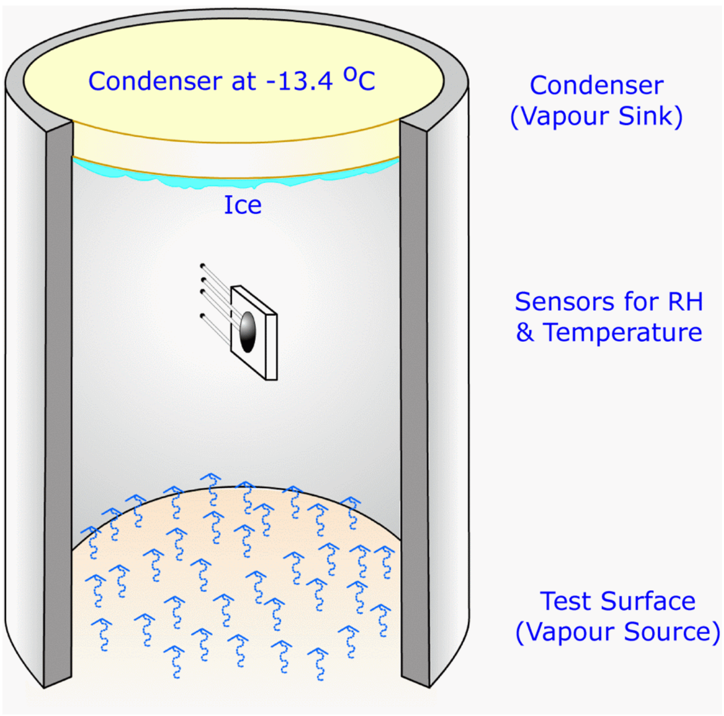
   From flux to membrane resistance

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.

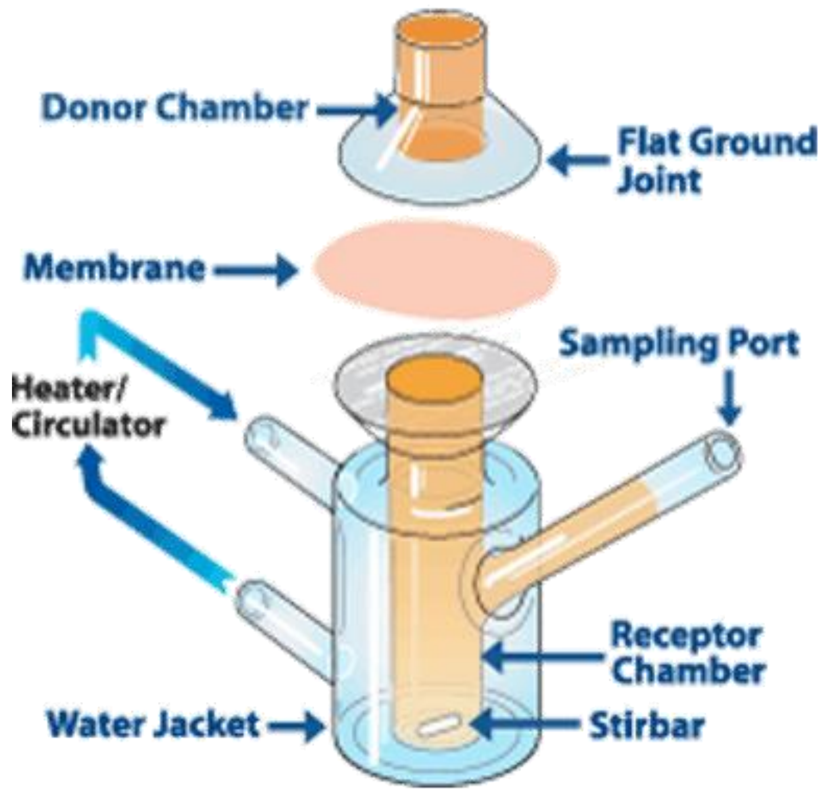
Controls the microclimate.

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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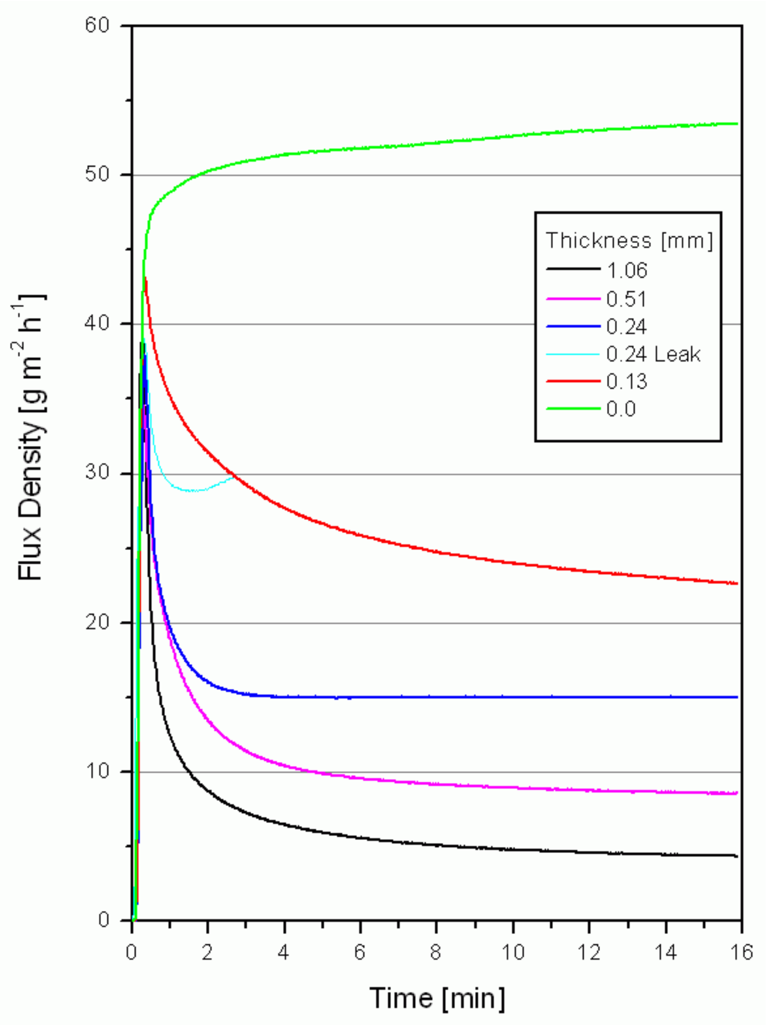
Artificial Membrane Measurements

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.

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

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

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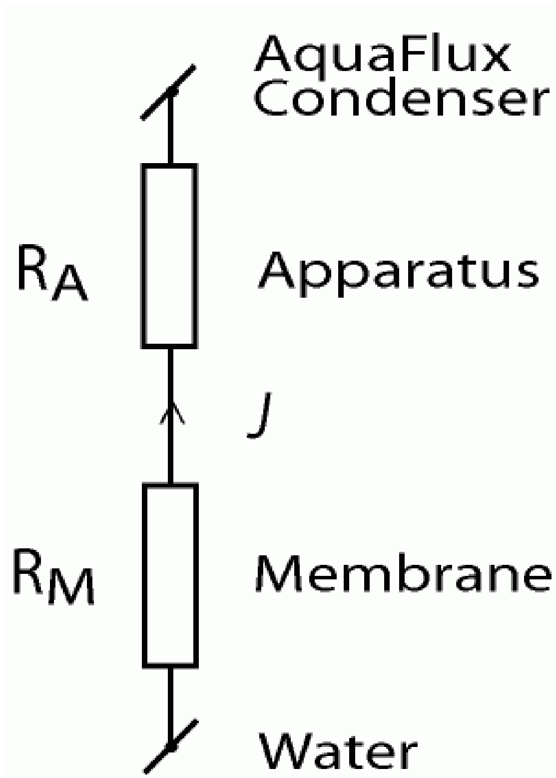
Artificial Membrane Analysis 1

Electrical Analogy - Diffusion Resistance



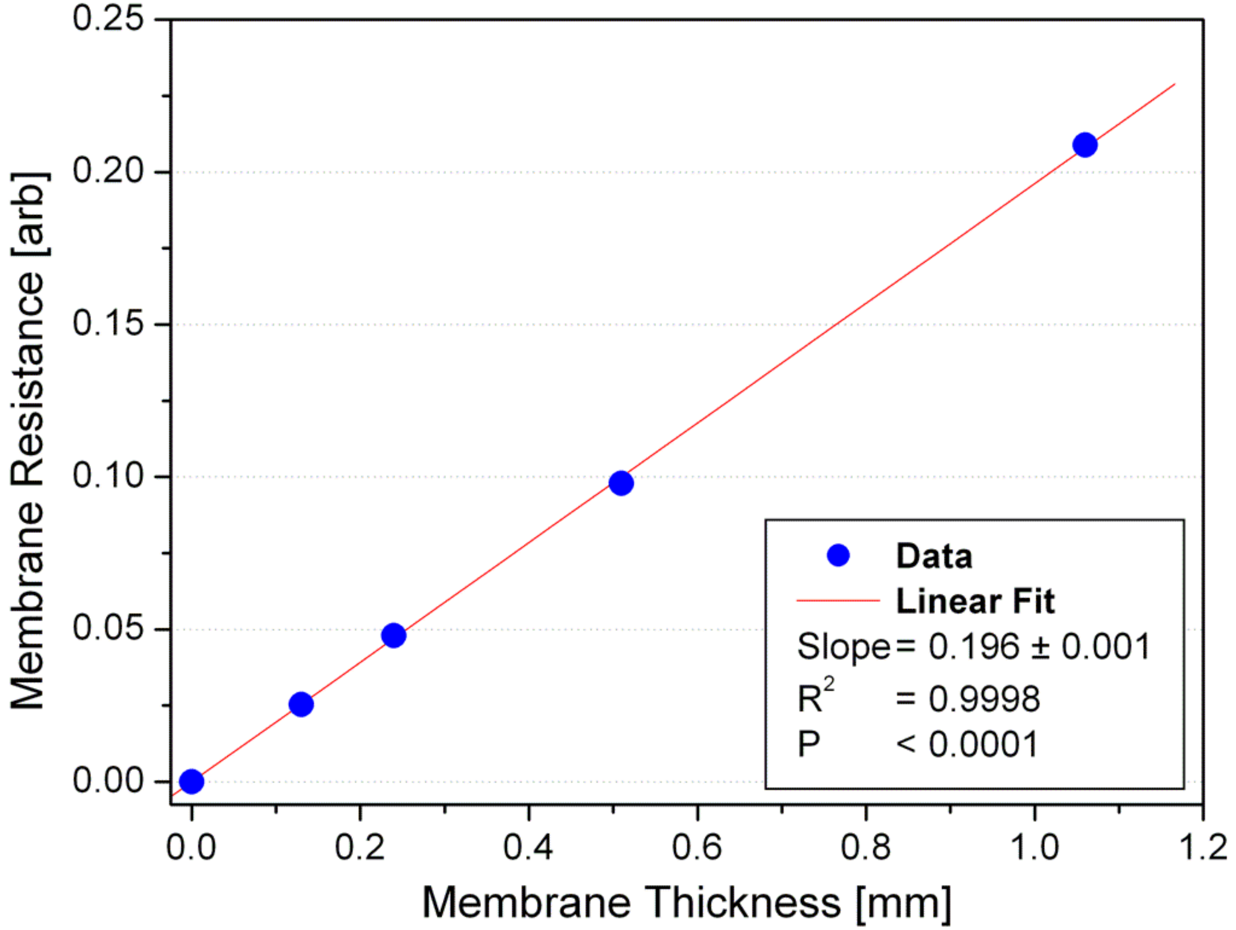
$$R_M \propto \frac{1}{J} - \frac{1}{J_0}$$

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.

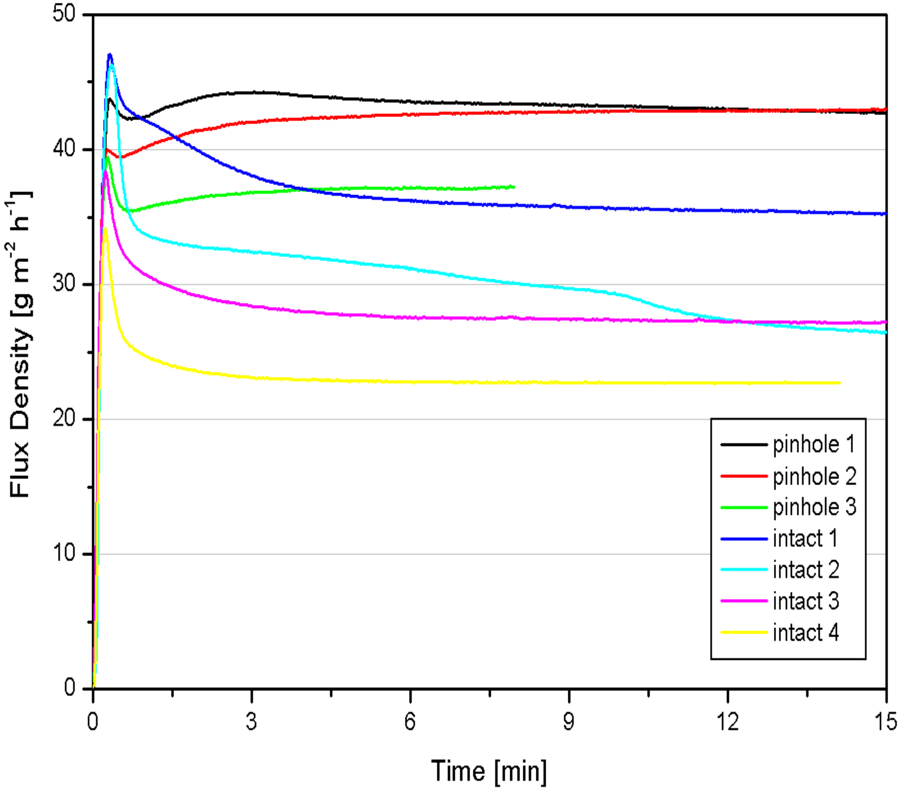
   Therefore mount with this surface facing the AquaFlux.

2. The inner surface is hydrophilic.

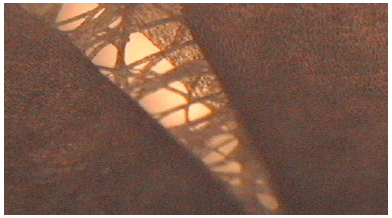
   Therefore mount with this surface in contact with the water.

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

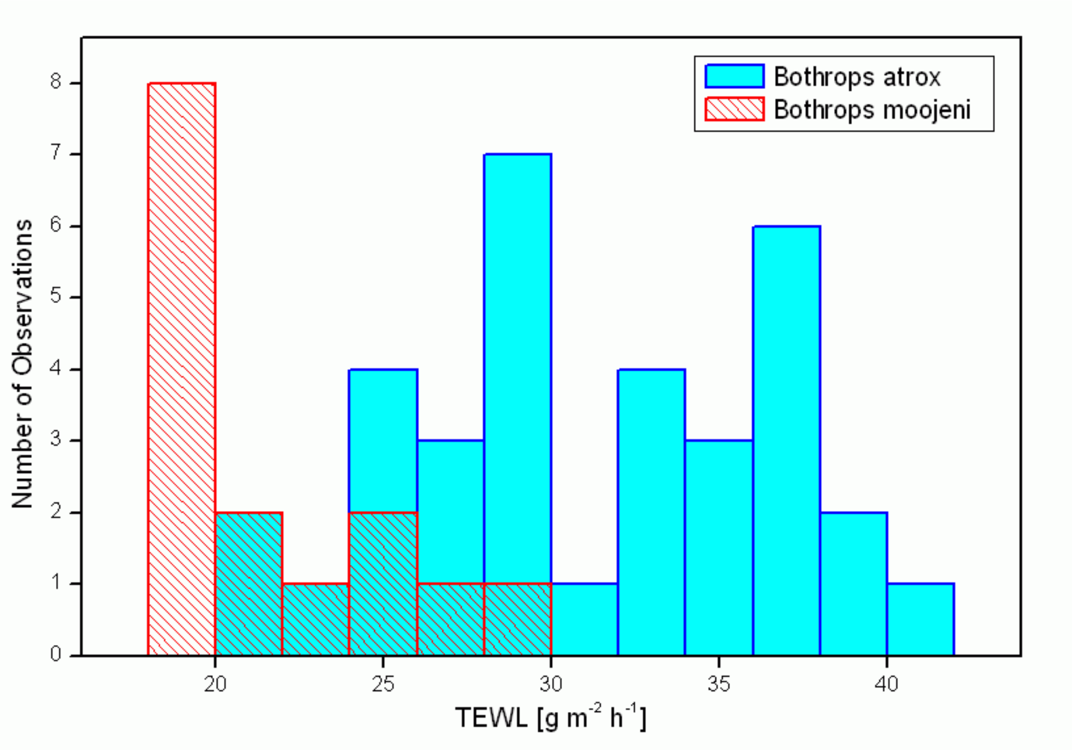
Sample Consistency

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.

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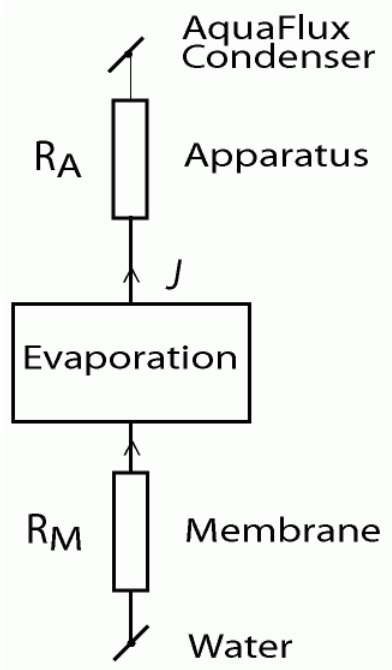
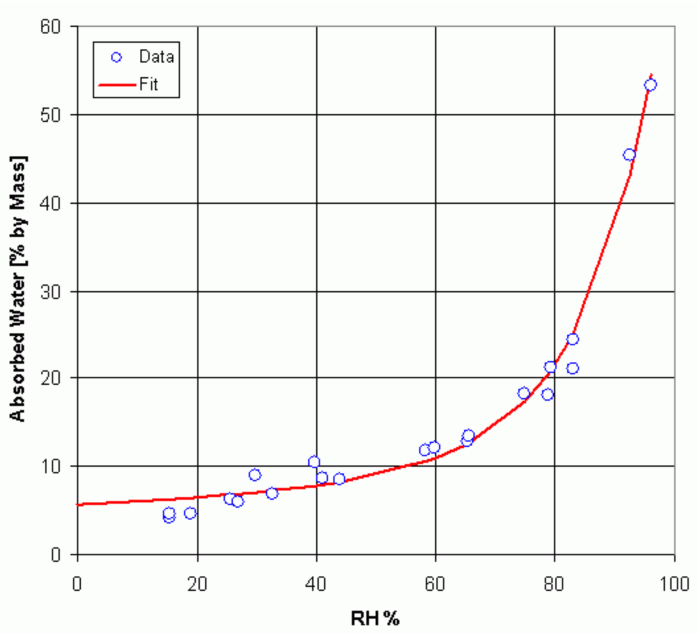
Snake Skin Analysis 1

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.

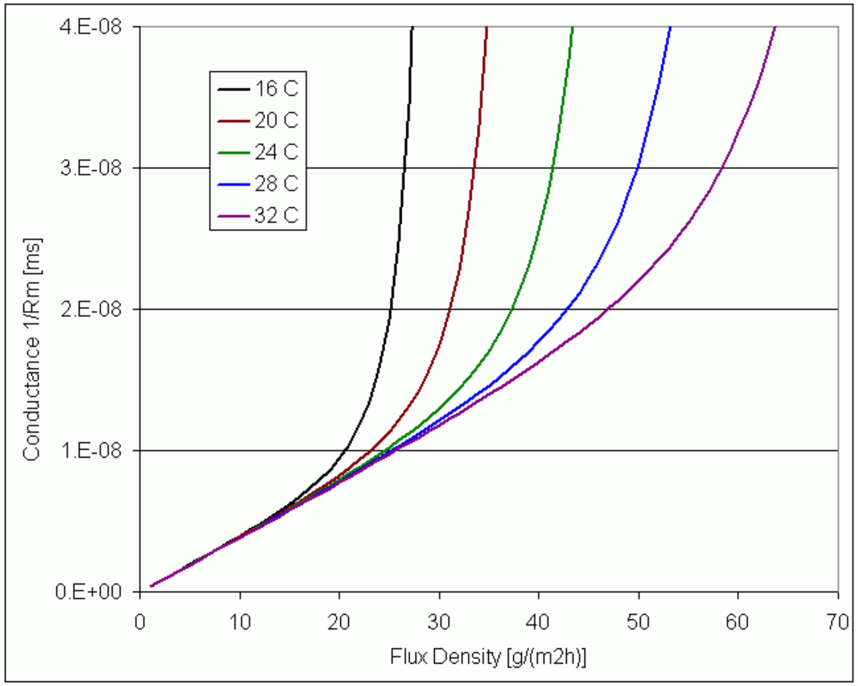
Sorption Isotherm for Human SC [1]



[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

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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!)