Measuring Barrier Function

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Plan of the Presentation

Introduction
Theoretical Background
Skin Stripping
Confocal Raman Spectroscopy
Opto-thermal Transient Emission Radiometry (OTTER)
Evaporimetry
Silicon Array Sensor
Summary & Conclusions
Introduction

Barrier membranes

Barrier membranes are everywhere.

The focus of this talk is focus is skin.
Introduction
Membrane barrier function

Barrier membranes are generally less than perfect, ie some stuff gets through.
In skin, pathways include:-

- Sweat glands
- Hair follicles
- Transdermal diffusion

I will only cover transdermal diffusion.
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Theoretical Background

Transdermal diffusion: Fick's Laws

Diffusion is an intermingling of molecules due to their random thermal motions. Diffusion is described by Fick's laws:

- Fick's 1\textsuperscript{st} Law: Time-independent steady-state diffusion
- Fick's 2\textsuperscript{nd} Law: Time-dependent non-steady-state diffusion

I will focus on steady-state diffusion, ie Fick's 1\textsuperscript{st} Law.
Theoretical Background

Molecular diffusion

![Diagram of molecular diffusion through a permeable membrane]

For individual molecules, all directions of migration are equally likely.

That means:-
The same percentage of molecules penetrate the barrier in each direction.

That means:-
More molecules migrate from high to low than from low to high.

That means:-
There is a net transport from high to low concentration.

But note:-
Pure diffusion is passive. There is no driving force.
SC hydration gradient and TEWL

The theoretical background involves the hydration gradient in the SC, indicating diffusion. For steady-state conditions, Fick’s first law (in one dimension) applies:

\[
J = -D_{SC} \frac{dc}{dz}
\]

Where
- \( J \) = Flux density (TEWL) \([\text{kgm}^2\text{s}^{-1}]\)
- \( D_{SC} \) = Mean diffusion coefficient \([\text{m}^2\text{s}^{-1}]\)
- \( c \) = Concentration \([\text{kgm}^{-3}]\)

For a linear hydration profile, this simplifies to:

\[
J = D_{SC} \frac{c_1 - c_2}{L_{SC}}
\]
Theoretical Background

Estimate of SC water barrier

Reasonable values for normal volar forearm SC might be:

<table>
<thead>
<tr>
<th>J</th>
<th>10</th>
<th>gm·m⁻²·h⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>c₁</td>
<td>700</td>
<td>kg·m⁻³</td>
</tr>
<tr>
<td>c₂</td>
<td>100</td>
<td>kg·m⁻³</td>
</tr>
<tr>
<td>L_SC</td>
<td>15</td>
<td>μm</td>
</tr>
</tbody>
</table>

These data are enough to give a rough estimate of $D_{SC} \sim 7 \times 10^{-14}$ m²·s⁻¹

and $\tau \approx \frac{L_{SC}^2}{D_{SC}} \sim$ 1 hour.

(For comparison, the self-diffusion coefficient for water @30°C is $D_{WW} \sim 2.7 \times 10^{-9}$ m²·s⁻¹ [1] & $\tau \sim$ 80ms).

**Theoretical Background**

Fick’s 1st Law & barrier property measurement

For steady-state diffusion, at any point within a membrane:

\[ J = -D \frac{dc}{dz} \]

Note that \( J \) is conserved (conservation of matter). Therefore, gradient changes are diffusion coefficient changes:

\[ \frac{dc}{dz} = \frac{-J}{D} \]

Flux density & concentration gradient can both be used to measure barrier function.
Theoretical Background

Barrier function measurement methods

From Fick's 1st Law, there are two equivalent approaches to measuring barrier function:

1. Measure flux density $J$, eg:-
   - Evaporimetry (restricted to water)
   - OTTER (Opto-thermal Transient Emission Radiometry)

2. Measure concentration gradient $dc/dz$, eg:-
   - Confocal Raman
   - OTTER (Opto-thermal Transient Emission Radiometry)

For this approach you'll need *Fick glasses* that let you see barrier properties while looking at concentration gradients.

Skin stripping and Franz cell procedures can also be used to characterise barrier function in combination with a variety of measurement methods. But these are not measurement methods by themselves.
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Skin Stripping
Tape stripping method

Tape stripping is a minimally invasive technique where adhesive tape is used to remove successive layers of SC, as illustrated on the left. The photo below shows what a tape looks like after a strip.

For each strip you can measure:-
- The quantity of SC removed
- The concentration of actives
- Transepidermal water loss (TEWL)
- Etc.

→ Mean thickness of SC removed
→ Penetration
→ Barrier property
Skin Stripping
The stripping process

A 5µm layer of SC is assumed to have been removed rapidly, to illustrate the stripping process.

Point 1: Before stripping
Steady-state surface hydration, hydration gradient & TEWL.

Point 2: Immediately after stripping
The surface hydration is elevated, therefore the vapour flux has increased. However, the hydration gradient is unchanged, therefore the TEWL is unchanged.

Point 3: After a new steady-state is reached
The surface hydration has decreased but remains above Point 1. The hydration gradient is now steeper than before, therefore the TEWL has increased. Vapour flux and TEWL are now equal again.

There is an important lesson here. Wait for steady-state conditions before measuring hydration & TEWL.
Skin Stripping

Transient & steady-state SC surface hydration

This shows calculated transient & steady-state SC surface hydration with 0, 1, 2, ... microns of SC removed. The excess hydration immediately after a strip is subsequently lost by evaporation from the SC surface (=Skin Surface Water Loss, SSWL).
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Confocal Raman Spectroscopy

Raman spectroscopy

Raman spectra relate to molecular vibrations.

Google Image Search: Private communication.
Confocal Raman Spectroscopy

Raman scattering

Raman spectra enable specific chemical species to be identified & studied.

Diagram adapted from PJ Matts, private communication.
Confocal Raman Spectroscopy

Confocal method

With the confocal method you can measure concentration depth profiles.

Left diagram:- Adapted from http://www.tcd.ie/Physics/Optoelectronics/spg/research/plasmon.php. Right diagram:- PJ Matts, private communication
Confocal Raman Spectroscopy

Hardware for in-vivo measurement

The skin is in contact with a glass plate.

Photos from River Diagnostics sales literature
Confocal Raman Spectroscopy

Hydration depth profiles for untreated skin in-vivo

For steady-state TEWL,
\[
\frac{dc}{dz} = -\frac{J}{D}
\]

Steep gradients in the SC mean low \(D\), ie barrier function.

Shallow gradients in the VE mean high \(D\), ie mobile water.

But what about the negative near-surface gradients? These imply water diffusing in from the skin surface. The likely causes are:-

1. Non-steady-state (occlusion)
2. Non-planar SC surface
3. Instrumental effect (spacial resolution)

Data from River Diagnostics
Confocal Raman Spectroscopy

Hydration depth profiles for over-hydrated skin in-vivo

Volar forearm sites hydrated with wet towel for 2 hours. Remove towel, pat dry & measure over a 4 hour period.

NB:- Except for the 1st & the last, these are NOT steady-state profiles.

Figure from PJ Matts, private communication
Confocal Raman Spectroscopy

Depth profiles for chemicals other than water

NMF concentration peaks in the SC. Either $J = D = 0$ or NMF is diffusing away from a source, in both directions.

Figure from PJ Matts, private communication
Confocal Raman Spectroscopy

Niacinamide penetration

Comparison of confocal Raman depth profile with tape strip (HPLC) measurements.
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Opto-thermal Transient Emission Radiometry (OTTER)

Measurement principle

Non-contacting optical technique.
Chemical selectivity in both excitation & emission radiations.
Depth-dependent information from the shape of the heat radiation transients.
Opto-thermal Transient Emission Radiometry (OTTER)

Apparatus using fibre-optic measurement head

Can be used on most body sites.
Measurement diameter ~1mm, 20-30sec per point.
2.94μm excitation wavelength. Absorption depth in water is ~800nm.
Opto-thermal Transient Emission Radiometry (OTTER)

Data analysis to calculate depth-dependent information

Analyse for:-

- Concentration depth profiles
- Mean concentration
- Surface concentration
- Concentration gradient
Opto-thermal Transient Emission Radiometry (OTTER)

In-vivo penetration of solvents – measurement of flux density

Surface disappearance measurements following ~30 minute solvent application.

Opto-thermal Transient Emission Radiometry (OTTER)

Glycerol depth profiles in nail

Apply glycerol for 5 minutes

Wipe off excess

Measure with 9.5µm emission wavelength
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Evaporimetry

Evaporimeters can be used to measure TEWL

TEWL is (liquid) water flux inside the SC.
Evaporimeters measure water vapour flux in the adjacent air.
In the steady-state, flux is conserved, ie vapour flux = TEWL.

- Liquid Water
- Water Vapour
Evaporimetry

But only in steady-state diffusion conditions

In non-steady-state conditions, vapour flux = TEWL + SSWL (+sweat)
Evaporimetry

Typical hardware

From left to right:-

Ventilated-chamber (Skinsos)
Unventilated-chamber (Delfin)
Open-chamber (Courage & Khazaka)
Condenser-chamber (Biox)
Evaporimetry

Measurement example: - TEWL changes during stripping

TEWL increases as more SC layers are removed. Remember to wait after stripping while the SC acclimatises to a new steady-state.

The reciprocal (1/TEWL) is often found to decrease ~linearly with the cumulative thickness of SC removed. The intercept with the horizontal axis gives the thickness of the intact SC [2].

Non-linear (1/TEWL) plots are sometimes observed.


Evaporimetry

Measurement example: - Occlusion recovery

Occlude with Silgel wound dressing & measure subsequent flux & SSWL.

From the straight-line fit, only 17±1.6% of baseline TEWL was recovered as SSWL.
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**Silicon Array Sensor**

Summary & Conclusions
Silicon Array Sensor

Fingerprint sensor technology

Pioneered by Lévêque and co-workers [1,2].
Electrical capacitance measurement principle.
Similar to Corneometer, but >70000 of them!

Silicon Array Sensor
Solvent depth profiles

Expose volar forearm skin for 5 minutes, then wipe, strip & measure.
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Summary & Conclusions
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• Barrier function is complex, depending on skin, chemical, formulation, method of exposure, etc, etc.

• The optical technologies are expensive but powerful, yielding new insights.

• Skin stripping is cheap, direct and useful.

• Silicon array sensors have promise, especially for studying heterogeneity.

• There is a need for standards, cross-validation & calibration.

• There is a need to develop theoretical models based on reliable experimental data.