IN VIVO SKIN STUDIES BY RAMAN SPECTROSCOPY

"It works"

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The Netherlands
Outline

- Information in Raman spectra of skin
- How to extract practical information
- Applications & validation
Information in Raman spectra of skin

It is all about:
Main chemical composition of the skin at different depths

Information in Raman spectra of skin, examples

- skin characterisation
  - water, nmf, lipids, carotenoids
  - typing, aging effects, ethnic effect, cultural effects
  - neutraceautical
- Penetration and penetration kinetics
  - glycerol, dmso, lactate, ethanol, caffeine, menthol, nicotinamide, uv absorbers, oils, retinol, carotenes
  - formulation optimization
  - transdermal drug delivery, various pain relief agents
  - Toxicological: pyrene, toluene, butoxyethanol
- metabolic processes
  - hydrolysis of esters
- effects of products
  - moisturization
  - pH modulation
- effects of environment / skin treatment
  - humidity, season, climate, solar exposure
  - bathing, washing
- medical (diseased/disfunctional skin)
  - Atopic dermatitis
How to extract practical information, concentration profiles
How to extract practical information, partition coefficients
How to extract practical information, partition coefficients

partition coefficient = $K = \frac{[\text{analyte}]_{\text{SC}}}{[\text{analyte}]_{\text{prod}}}$
How to extract practical information, kinetics of diffusion
How to extract practical information, absorption and flux calculations

Ingredient Concentration in mmol/gr keratin \(\rightarrow\) Absorbed amount in \(\mu g/cm^2\)

Area under the Curve

- 30 min application time
- 60 min application time
- 2 hr application time

Absorbed volume product
How to extract practical information, diffusion kinetics, Fick's 2nd law

Analysis of shape of the Curve

Theoretical:

\[
\frac{d([\text{analyte}])}{dt} = D \frac{d^2([\text{analyte}])}{dz^2}
\]

Simple solution 1:

\[
[\text{analyte}](z,t) = [\text{analyte}]_{z=0} \cdot \text{erf}\left(\frac{z}{\sqrt{4Dt}}\right)
\]

Simple solution 2:

\[
[\text{analyte}](z,t) = \frac{[\text{analyte}]_{z=0}}{A\sqrt{\pi Dt}} \cdot e^{-\frac{z^2}{4Dt}}
\]

analyte concentration in mmol/gr keratin  Diffusion Coefficient D
applications - caffeine diffusion constants

<table>
<thead>
<tr>
<th>Published</th>
<th>Caffeine Application</th>
<th>D (cm²/s)</th>
<th>Skin type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Southwell and Barry, JID</td>
<td>1 %, water ¹⁴C labels</td>
<td>4.5·10⁻¹⁰</td>
<td>Human in vitro</td>
</tr>
<tr>
<td>1983</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nalenz, Dissertation, Uni</td>
<td>?</td>
<td>1.7·10⁻¹⁰</td>
<td>Human in vitro</td>
</tr>
<tr>
<td>Basel, 2006</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hansen et al., Eur J Pharm.</td>
<td>1.25 %, water</td>
<td>3.9·10⁻¹¹</td>
<td>Human in vitro</td>
</tr>
<tr>
<td>And Biopharmac, 2008</td>
<td>D was found application</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>time dependent)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>River Diagnostics, 2009</td>
<td>1.8 %, water</td>
<td>8.28·10⁻¹²</td>
<td>Human in vivo</td>
</tr>
</tbody>
</table>

- D is very likely show large inter-individual variation
- D is a complicated parameter, as shown by the time dependence of D (by Hansen)
- Data on sampled skin all show higher, and sometimes much higher, D values. Excision of skin likely induces artefacts that influence the skin barrier as compared to in vivo skin.
- Best agreement found with the newest data.
### Caffeine Application and Absorption

<table>
<thead>
<tr>
<th>Published</th>
<th>Caffeine Application</th>
<th>Fluxes/Transfer</th>
<th>Skin type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Southwell and Barry, JID 1983</td>
<td>1%, water $^{14}$C labels</td>
<td>1.3 $\mu$g/cm$^2$/h</td>
<td>Human in vitro</td>
</tr>
<tr>
<td>Dias et al. 1999</td>
<td>3% in commercial formulation</td>
<td>15 - 150 $\mu$g/cm$^2$</td>
<td>Human in vitro</td>
</tr>
<tr>
<td>Pugh et al. 2004</td>
<td>4 $\mu$g/cm$^2$ in ethanol</td>
<td>0.48±0.16 $\mu$g/cm$^2$</td>
<td>Human in vitro</td>
</tr>
<tr>
<td>Van de Sandt et al. 2004</td>
<td>3% in commercial formulation</td>
<td>2.24±1.43 $\mu$g/cm$^2$/h</td>
<td>Human in vitro</td>
</tr>
<tr>
<td>Potard et al. 1999</td>
<td>3% in commercial formulation</td>
<td>2.3±2.0 $\mu$g/cm$^2$</td>
<td>Human in vitro</td>
</tr>
<tr>
<td>Nicoli et al. 2004</td>
<td>330 $\mu$g/cm$^2$</td>
<td>1 - 100 $\mu$g/cm$^2$</td>
<td>Rabbit ear in vitro</td>
</tr>
<tr>
<td>Hansen et al. 2008</td>
<td>1.25 % water</td>
<td>25.6 $\mu$g/cm$^2$/h</td>
<td>Human in vitro</td>
</tr>
<tr>
<td>River Diagnostics</td>
<td>1.8% in water (3.5 mg/cm$^2$)</td>
<td>25 $\mu$g/cm$^2$/h</td>
<td>Human in vivo</td>
</tr>
</tbody>
</table>
An In Vivo Confocal Raman Study of the Delivery of Trans-Retinol to the Skin

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Measurement Science Unit, Unilever R&D, Colworth Science Park, Sharnbrook, Bedford, MK44 1LQ (P.D.A.P., M.M.); and River Diagnostics B.V., Rotterdam, The Netherlands (P.J.C., A.v.D.P., G.J.P.)

- **Solution 1:** 70 % ethanol, 30 % PG and 0.3 % trans-retinol
- **Solution 2:** 99.7 % MYRITOL®318 and 0.3 % trans-retinol
- monitor penetration for 0-6 hrs after application
applications - retinol delivery


![Graph showing retinol content over depth into the skin](image-url)
applications - retinol delivery


in PG/EtOH

in MYRITOL®318

(RETINOL 1 / Keratin) Normalised

(RETINOL 2 / Keratin) Normalised

Time after treatment (hours)

Depth into the skin (microns)
Validation – skin hydration

- Analysis of total water content of moisturized pigskin stratum corneum by Raman and independently by Karl Fischer titration
Table II: Water content of the stratum corneum determined by Karl Fischer method and confocal Raman microspectroscopy

<table>
<thead>
<tr>
<th>Relative humidity (%)</th>
<th>Karl Fischer</th>
<th>Confocal Raman</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Water content (% mass)</td>
<td>SD</td>
</tr>
<tr>
<td>0</td>
<td>7.7</td>
<td>0.80</td>
</tr>
<tr>
<td>11</td>
<td>8.9</td>
<td>0.75</td>
</tr>
<tr>
<td>32</td>
<td>11.0</td>
<td>0.91</td>
</tr>
<tr>
<td>75</td>
<td>13.8</td>
<td>0.98</td>
</tr>
<tr>
<td>100</td>
<td>24.4</td>
<td>0.94</td>
</tr>
</tbody>
</table>

SD, standard deviation. \( n = 3 \) for each relative humidity condition, the total number of samples was 15.
skin hydration: validation using pigskin and KF titration

\[ y = 1.067x \]
\[ R^2 = 0.9893 \]
skin hydration: swelling
skin hydration: swelling
The predicted swellings are indeed found with other methods:

- Good correlation between thicknesses and their changes upon moisturization as measured by confocal Raman and Optical Coherence Tomography (OCT)
- No direct correlation between capacitance readings and Raman results, from the paper by:
- Good correlation for thickness also for CVM (S. Bielfeldt et al., in preparation).
applications - skin hydration, syndet-bar vs. soap-bar


**Figure 7** Area under the water profile curve integrated over 0–20 μm for syndet and soap-based bar. n = 6. The total number of profiles from each product was 30.
information in Raman spectra of skin


It is all about:
Main chemical composition of the skin at different depths

Penetration of topically applied compounds

UV, climate
Filaggrin and atopic dermatitis (eczema)

organization of the keratin cytoskeleton

Drawing from A.V. Rawlings et al., JID 103 (1994)
Filaggrin and atopic dermatitis (eczema)

source for the NMF

Drawing from A.V. Rawlings et al., JID 103 (1994)
sensitization and skin barrier

intact barrier

FLG
sensitization and skin barrier

defective barrier

FLG

immune response

sensitization

AD
Loss-of-function mutations in the filaggrin gene lead to reduced levels of natural moisturizing factor in the stratum corneum

Sanja Kezic¹,², Patrick M. J. H. Kemperman²,³, Ellen Koster¹, Cindy de Jongh¹, Bing H. Thio², Linda E. Campbell³, Alan D. Irvine⁴,⁵ and W. H. Irwin McLean³, Gerwin J. Puppels⁶,⁷ and Peter J. Caspers⁶,⁷


- 149 volunteers + 10 AD patients
- screened for R501x, 2282del4, R2447x, S3247x
- included in study: 16 carriers and 23 wild-types
- in vivo Raman assessment of nmf (on forearm and thenar)
filaggrin - Raman study

Filaggrin and atopic dermatitis (eczema)

- early diagnosis & intervention
- more differentiated diagnosis
- more targeted intervention

...happy babies
Conclusions

Raman spectroscopy on skin

- validated in several areas
- more and more routinely used
- *It works!*
confocal Raman microspectroscopy

depth resolution: ~5 µm