Dermatopharmacokinetics: modelling, assessment and optimization

Richard H. Guy
University of Bath

"Innovations in Dermatological Sciences"
Centre for Dermal Research, Rutgers University
Piscataway, New Jersey, USA
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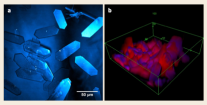


Acknowledgements: Begoña Delgado-Charro, Annette Bunge, Nota Zarmpi, Alice Maciel Tabosa, Pauline Vitry, Wing Sin Chiu, Hazel Garvie-Cook, Natalie Belsey, Dimitrios Tsikritsis, Vasundhara Tyagi, Priyanka Ghosh, Sam Raney, Markham Luke.
U.S. FDA awards 1-U01-FD004947 and 1-U01-FD006533.

1

Skin (or dermato-) pharmacokinetics

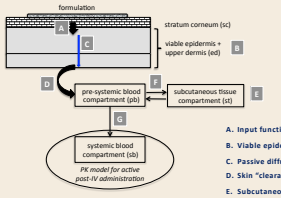
Objectives for a practical PK description of skin absorption:

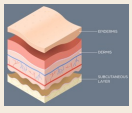
1. Incorporates a 'sensible' and relevant description of the "input" kinetics of the active from the applied formulation.
2. Realistically describes the 'clearance' of active from viable skin.
3. Permits estimation of the concentration of active in 'compartments of interest' in the skin (basal epidermis, appendages, etc.).
4. Enables systemic exposure to active (i.e., safety) to be assessed.
5. Is testable and validated.

William of Ockham (1287-1347): Occam's Razor - among competing hypotheses, the one with the fewest assumptions should be selected.

2

Pharmacokinetic model for skin absorption



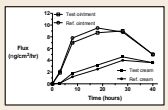


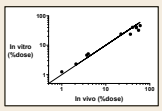
A. Input function of active from SC into viable skin.
B. Viable epidermis and upper dermis combined.
C. Passive diffusion from SC to dermal microcirculation… and more.
D. Skin "clearance" (diffusion, blood flow, 'extraction coefficient').
E. Subcutaneous tissue compartment.
F. Extent of distribution into subcutaneous tissue.
G. 'Elimination' of active from skin into blood.

3

Measuring and validating drug "input kinetics" *in vitro*

- In vitro skin penetration experiments
  - long history, substantial data resource, but…
  - no dermal microcirculation… clearance?
  - usually, not 'alive'… metabolism?
  - relevance of epidermal/dermal levels?
  - application technique(s) relevant to real-world use of products?





Franz, Lehmann, Raney, *Skin Pharmacol. Physiol.*, 2009, 2011

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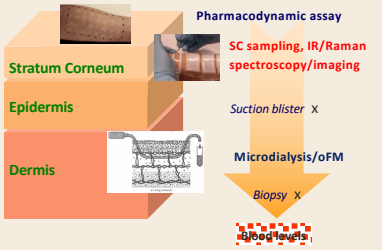
Assessing skin bioavailability *in vivo* in man



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Measuring and validating drug "input kinetics"

How can drug "input kinetics" into skin *in vivo* be measured, and the method validated?

A. **Hypothesize that drug quantification in stratum corneum provides useful information.**
B. **Test using transdermal drug delivery systems of well-characterised 'input'.**
C. **Additional opportunity to establish in vitro – in vivo correlations.**
D. **Proof-of-concept permits unknown "input kinetics" to be determined.**
E. **Example: scopolamine (buprenorphine, nicotine, lidocaine have also been studied).**

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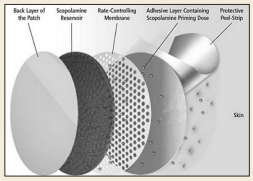
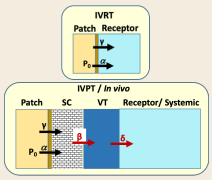
Transdermal scopolamine





Drug loading distributed between adhesive layer (~140 µg) and reservoir.

Delivery from a 2.5 cm² patch (across post-auricular skin) is about 1 mg over 3 days.

Pensado A *et al.* Mol Pharmaceut 2021; 18: 2714-2723

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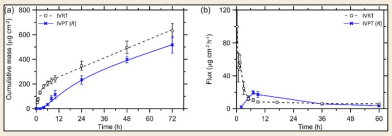
Scopolamine – in vitro release and skin penetration



High initial flux observed as 'priming' dose in adhesive is rapidly released.

Subsequently, slower, controlled delivery from the drug reservoir.

Pensado A *et al.* Mol Pharmaceut 2021; 18: 2714-2723

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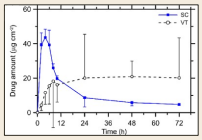
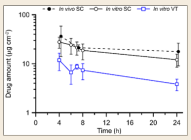
Scopolamine – SC sampling *in vitro*



SC uptake is initially substantial, reflecting flux measurements, then achieves a lower 'steady-state' level at longer times.

Amount in viable skin (VT) reaches steady-state level at ~12 hr that is then sustained over duration of patch use.



Skin disposition of drug tracked after patch removal.

Reasonable correlation between *in vitro* and *in vivo* measurements.

Pensado A *et al.* Mol Pharmaceut 2021; 18: 2714-2723

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Stratum corneum (SC) sampling *in vivo*

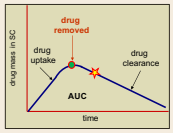
what if stratum corneum is not the target?

Measuring drug delivery rate from SC

- measure mass of drug in SC after period of clearance
- compare to mass of drug in SC at end of uptake

Calculate the average flux from the SC to deeper tissues:

$$\text{Average Flux} = \frac{(M_{Up} - M_{Clear})/A}{t_{Clear} - t_{Up}}$$



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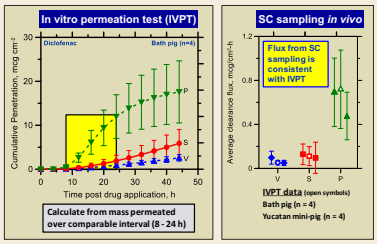
Stratum corneum (SC) sampling *in vivo*

Diclofenac: compare in vitro and in vivo delivery rates to skin



J_{input} from:

$$\frac{(M_{Up} - M_{Clear})/A}{t_{Clear} - t_{Up}}$$

Cordery SF *et al. Int J Pharm.* 529 (2017) 55-64.

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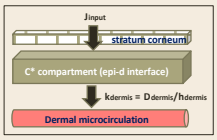
Higuchi's C* concept – concentration of active at its target



C* is effective concentration of active at site of action (e.g., basal cells of epidermis)

At steady-state:

Rate of active delivery (μg/cm²/h) to target = J_{input}

Rate of active depletion (μg/cm²/h) from target = k_{dermis} x C*

where $k_{dermis} = D_{dermis}/h_{dermis}$

D_{dermis} = diffusivity of active in dermis

h_{dermis} = distance from C* compartment to microcirculation

⇒ $J_{input} = k_{dermis}$ x C* and C* = J_{input}/k_{dermis}

Binding of active in SC and skin "1st-pass" is captured in J_{input}; binding in dermis by D_{dermis}.

Imanidis G et al., Pharm. Res. 11 (1994) 1035-1040. Mehta SC et al., J. Pharm. Sci. 86 (1997) 797-801.

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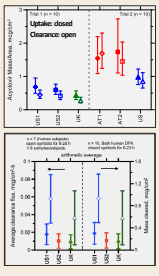
Higuchi's C* concept – acyclovir example



SC sampling *in vivo* in man after 6 h uptake and 17 h clearance.

J_{input} into epidermis deduced from:

$$\frac{(M_{Up} - M_{Clear})/A}{t_{Clear} - t_{Up}}$$

$J_{input} \cong 15$ ng/cm²/h

$D_{dermis} \cong 7 \times 10^{-3}$ cm²/h[a]

$h_{dermis} \cong 150 \times 10^{-4}$ cm[a]

⇒ C* = 30 ng/cm²

⇒ C* is below the target concentration deduced from animal studies[b]

[a] Krestos K et al., Inter. J. Pharmaceut. 346 (2008) 64–79.

[b] Mehta SC et al., J. Pharm. Sci. 86 (1997) 797-801.

Pensado A et al. (2019) Pharm. Res. 36:180

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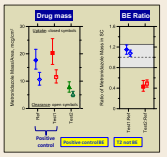
Topical bioavailability derived from SC sampling

- Both *in vivo* and *in vitro* methods showed that topical bioavailabilities of drugs from different products are not necessarily equivalent.
- Excellent *in vivo* : *in vitro* correlation for drug permeation observed.
- Therefore,
  - *in vitro* skin penetration measurements can be useful proxies for *in vivo* measurements, and
  - quantitative rates of active delivery to tissues beneath stratum corneum can be derived from tape-stripping.



de Araujo TP *et al.* Int J Pharm. 2018; 541: 167-172

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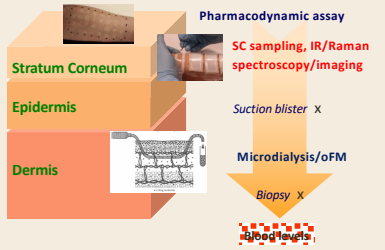
Assessing skin bioavailability *in vivo* in man



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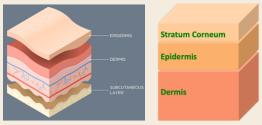
Skin pharmacokinetics

<u>Hypothesis:</u> Raman spectroscopy/imaging provides a non-invasive, accurate, sensitive and reproducible determination of a drug's local bioavailability in the skin.



- Unambiguous Raman spectroscopic analysis of a drug in the skin despite the potential for significant, background signal interference.
- A robust approach to correct for drug signal attenuation as a function of increasing depth of measurement in the skin.
- Continuous, real-time Raman spectroscopy and/or imaging to provide a (semi-) quantitative measure of a drug's "input kinetics" into the viable epidermis ex vivo.
- Raman spectroscopy/imaging to characterise the epidermal bioavailability of a topically applied drug and distinguish correctly between formulations that are different.

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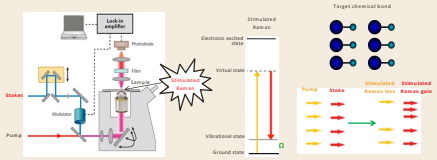
Coherent and Stimulated Raman Scattering



- Images the specific chemical bond of interest
- Stimulated excitation of coherent molecular vibration: $\omega_{pump} - \omega_{Stokes} = \omega_{vib}$
- SRS signal is linearly proportional to concentration of target molecule
- Information on penetration depth and pathways of multiple components of a formulation

A Zumbusch, GR Holtom, XS Xie, Phys Rev Lett 82, 4142-4145 (1999)

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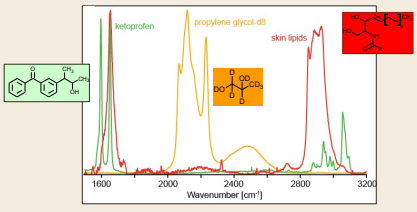
Raman spectra of key chemical species



SRS contrast is based on spontaneous Raman spectra, which are used to determine optimal excitation wavelengths: 1599 cm^{-1}, 2120 cm^{-1} and 2845 cm^{-1} report on ketoprofen, deuterated PG and skin lipids, respectively.

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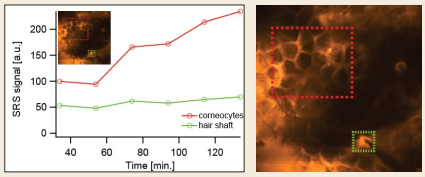
SRS analysis of skin penetration pathways



* Temporal profiles of PG-d8 at z = 6 µm from two distinct regions
* Intercellular penetration increases steadily with time
* Follicular transport already 'saturated' at 26 mins post-application

BG Saar, LR Contreras-Rojas, XS Xie, RH Guy, Molecular Pharmaceutics 8, 969-975 (2011)

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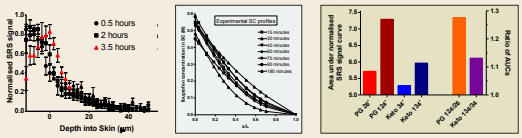
Depth-profiling analysis of skin penetration



* Qualitative agreement between SRS measurement of NSAID uptake into porcine skin *ex vivo* and human skin *in vivo* determined by stratum corneum tape-stripping.
* However, PG permeation is faster relative to that of NSAIDs…

N. Belsey et al., J. Control. Release 174 (2014) 37-42

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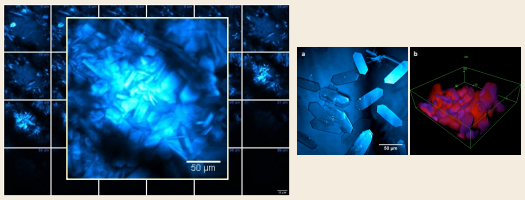
Metamorphosis of a formulation

Ibuprofen-d3 dissolved in propylene glycol at close to saturation



BG Saar et al., Molecular Pharmaceutics 8, 969-975 (2011); N. Belsey et al., J. Control. Release 174 (2014) 37-42

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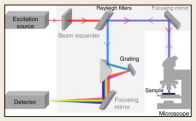
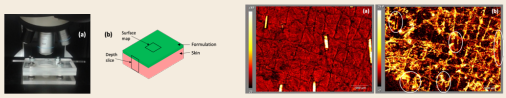
Confocal Raman spectroscopy



- Renishaw inVia Raman microscope working in reflection mode.
- Sample illuminated with a pre-calibrated 785 nm (100 mW) laser.
- *Ex vivo* abdominal pig skin on an aluminum support.
- A tool for probing the skin-(trans)dermal formulation interface.



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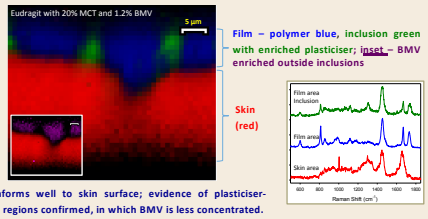
Raman mapping of the polymer film-skin interface
Acrylate polymer + hydrophobic plasticiser (MCT) + BMV



Film – polymer blue, inclusion green with enriched plasticiser; inset – BMV enriched outside inclusions

Skin (red)

Film conforms well to skin surface; evidence of plasticiser-enriched regions confirmed, in which BMV is less concentrated.

H. Garvie-Cook et al. J. Control. Release 212, 103-112 (2015)
H. Garvie-Cook et al. Molecular Pharmaceutics (2022) https://doi.org/10.1021/acs.molpharmaceut.2c00480

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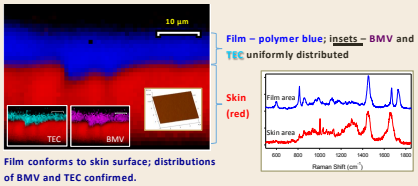
Raman mapping of the polymer film-skin interface
Acrylate polymer + plasticiser (TEC) + BMV



Film – polymer blue; insets – BMV and TEC uniformly distributed

Skin (red)

Film conforms to skin surface; distributions of BMV and TEC confirmed.

H. Garvie-Cook et al. Mol. Pharmaceut. 12, 751-757 (2015)
H. Garvie-Cook et al. Molecular Pharmaceutics (2022) https://doi.org/10.1021/acs.molpharmaceut.2c00480

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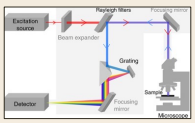
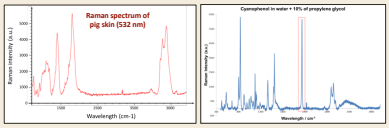
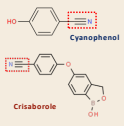
Confocal Raman spectroscopy



- Renishaw inVia Raman microscope working in reflection mode.
- Sample illuminated with a pre-calibrated 785 nm (100 mW) laser.
- *Ex vivo* abdominal pig skin on an aluminum support.
- **Crisaborole** and **cyanophenol** (CP) have strong **-C≡N** Raman signals in a frequency range where skin is spectroscopically 'transparent'.





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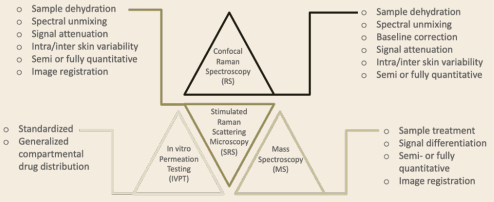
Raman spectroscopy and correlative approaches



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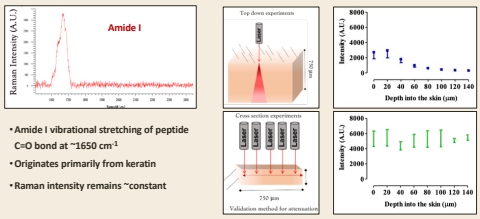
Confocal Raman spectroscopy – signal attenuation

Signal attenuation as a function of skin depth → weaker backscattered signal from deeper layers



- Amide I vibrational stretching of peptide C=O bond at ~1650 cm^{-1}
- Originates primarily from keratin
- Raman intensity remains ~constant

unpublished data

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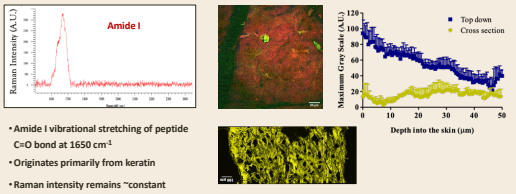
SRS – signal attenuation



- Amide I vibrational stretching of peptide C=O bond at 1650 cm^{-1}
- Originates primarily from keratin
- Raman intensity remains ~constant

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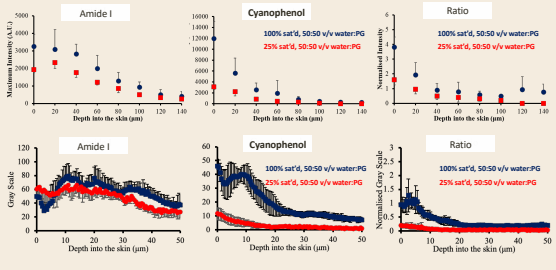
Confocal Raman spectroscopy and SRS – uptake into skin



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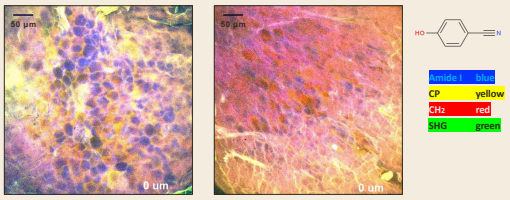
SRS – uptake into skin from different formulations



100% sat'd, 50:50 v/v water:PG

25% sat'd, 50:50 v/v water:PG

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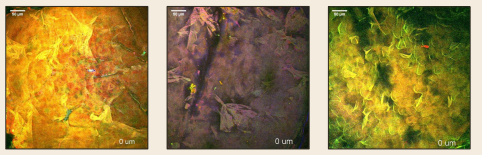
SRS – uptake into skin as a function of time



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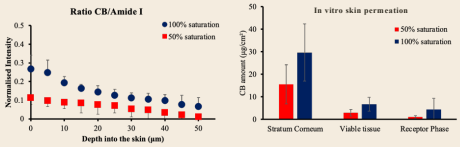
Confocal Raman spectroscopy – crisaborole uptake into skin

Crisaborole formulated in propylene carbonate (PC)

300 µL of 50 and 100% saturated Crisaborole in PC, applied for 24 hours to porcine skin (n = 5)



Ratio of areas under normalised intensity vs. depth profiles and of drug amounts taken up into skin in vitro are both proportional to degree of saturation of drug in tested formulations (i.e., 2).

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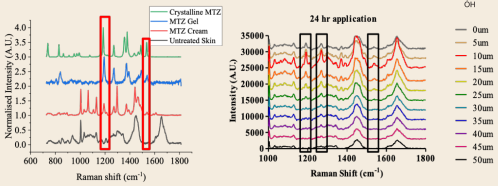
Topical bioavailability of metronidazole (MTZ)



Raman spectra normalised based on maximum intensity in each spectrum.

C=N at ~1200 cm⁻¹; C=C at ~1525 cm⁻¹.

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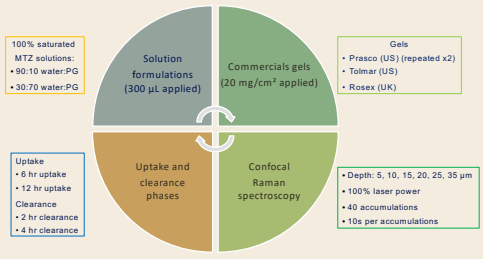
Metronidazole confocal Raman study



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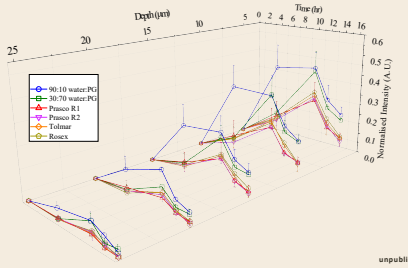
MTZ Normalised profiles – all formulations



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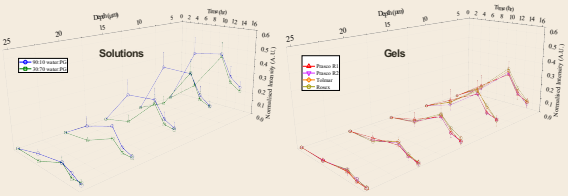
MTZ Normalised profiles – solutions vs. gels comparison



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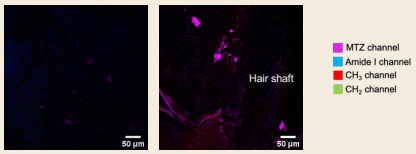
SRS – visualisation of MTZ on skin surface… metamorphosis



- Following application in formulations with high water content, MTZ precipitation or crystallization is observed on skin surface and around the base of hair follicles.
- Potentially significant with respect to formulation microstructure and to impact on drug delivery.

unpublished data

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Conclusions

- Results to-date demonstrate that Raman spectroscopy and imaging can provide non-invasive, sensitive, and reproducible determination of the rate and extent at which a topically administered drug becomes available at its site of action in the skin.
- Experimental methods, combining Raman spectroscopic tools with complementary calibration techniques have enabled this hypothesis to be established *ex vivo*.
- The ultimate goal is to provide evidence that the routine, facile and non-invasive measurement of drug pharmacokinetics in the skin *in vivo* is achievable and has considerable potential for application in regulatory science and decision-making.

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