

Raman spectroscopic assessment of topical drug bioavailability in the skin

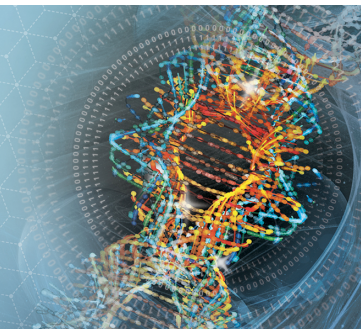
M. A. Maciel Tabosa¹, P. Vitry¹, N.A.Belsey², D. Tsikritsis², T.J. Woodman¹, A.L. Bunge³, M.B. Delgado-Charro¹, R.H. Guy¹

¹University of Bath, U.K.; ²National Physical Laboratory, U.K., ³Colorado School of Mines, U.S.A.

CONTACT INFORMATION: m.a.macieltabosa@bath.ac.uk

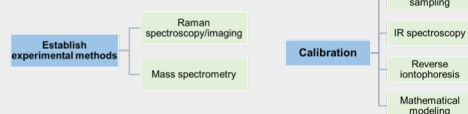


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PURPOSE

- Quantitative evaluation of a **topically applied drug's bioavailability** (BA) at its site of action in the skin represents an unmet scientific challenge.
- Most dermatological drug targets are located in the epidermis/upper dermis, below the stratum corneum (SC = principal barrier to drug permeation).
- While a few alternatives to clinical assessment exist, identification and validation of surrogate approaches to evaluate of local BA represents a work-in-progress.



OBJECTIVE

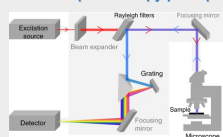
To test the hypothesis that spectroscopic (specifically, Raman) imaging offers a non-invasive, **accurate sensitive and reproducible method to determine the rate and extent** to which a topically administered drug becomes available at or near its site of action below the SC.

METHODS

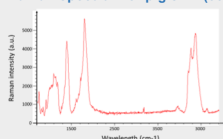
RAMAN SPECTROSCOPY

- 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.

Raman spectroscopy principle

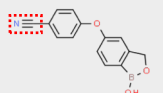


Raman spectrum of pig skin (532 nm)

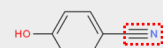


MOLECULES OF INTEREST

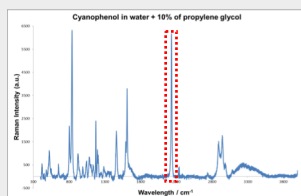
Crisaborole and **cyanophenol** (CP) have strong -C≡N Raman signals in a frequency range where skin is spectroscopically 'transparent'.



Crisaborole



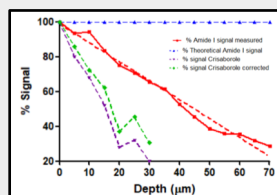
Cyanophenol



RESULTS

CORRECTION FOR SIGNAL ATTENUATION

Signal attenuation from drug as a function of depth into skin is corrected using the Amide I signal at 1650 cm⁻¹ (either the actual data points – solid red curve – or the linear fit – dashed red line).

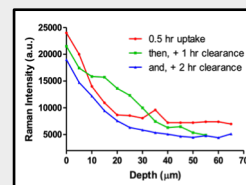


Example of **crisaborole** signal correction using the Amide I 'normalization'.

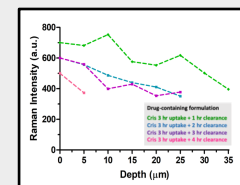
Drug applied for 3 hr, formulation removed and spectra acquired 3 hr later.

TRACKING DRUG CLEARANCE FROM SC

- CP (17.5 mg/mL) in 10:90 v/v propylene glycol (PG)-water.
- After 0.5 hr application, skin surface cleaned and Raman measurements recorded.
- CP clearance from skin determined at 1 and 2 hr following removal of formulation.
- Crisaborole (5.9 mg/mL) in 70:30 v/v propylene glycol (PG)-water.
- After 3 hr application, skin surface cleaned and Raman profile was recorded at 1, 2, 3 and 4 hr following removal of formulation ('clearance').



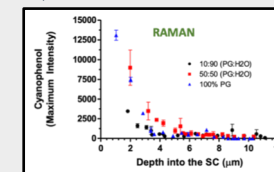
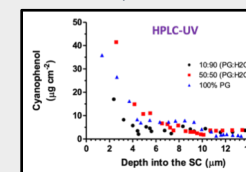
Cyanophenol Raman signal during 'uptake' (0.5 hr) and 'clearance' (1 and 2 hr).



Crisaborole (Cris) Raman signal during 'clearance' (1, 2, 3 and 4 hr).

COMPARING FORMULATIONS

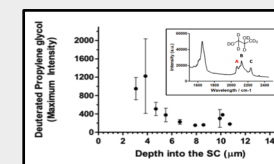
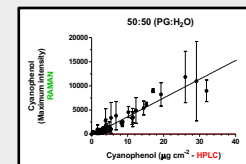
Three formulations of 4-CP (saturated solutions in pure PG, 50:50 v/v PG/ water and 10:90 v/v PG/ water) were applied to porcine skin *ex vivo* for 1 hour and uptake into the SC was assessed by tape-stripping and by Raman spectroscopy (spectra from three different positions of the skin were taken).



Data points are the average maximum intensities ± standard deviation, n = 3.

Overlap between the results was self-evident, with high correlations between Raman signal intensity and CP measured in the SC (illustrated for the 50:50 formulation below).

Data have also been acquired, for example, tracking an excipient penetrating into skin (shown for deuterated propylene glycol below).



Data points are the average maximum intensities ± standard deviation, n = 3.

CONCLUSIONS

Raman spectroscopy is able to:

- [1] track drug penetration as a function of depth into the skin (and beyond the SC), and
- [2] track drug clearance from the SC.

Furthermore, the approach has the potential to compare drug delivery into skin from different formulations, and to follow the penetration of certain formulation excipients.

Artifacts due to signal attenuation by absorption/scattering of radiation can be mitigated by normalization, and correlation with complementary techniques (e.g., SC sampling) may offer the opportunity for at least semi-quantification of drug bioavailability using the Raman approach.

REFERENCES

- [1] Saar, B.G., et al. Imaging drug delivery to skin with stimulated Raman scattering microscopy. *Molecular Pharmaceutics* 8 (2011) 969-975.
- [2] Chiu, W.S., et al. Molecular diffusion in the human nail measured by stimulated Raman scattering microscopy. *Proc. Natl. Acad. Sci.* 112 (2015) 7725-7730.
- [3] Belsey, N.A., et al. Evaluation of drug delivery to intact and porated skin by coherent Raman scattering and fluorescence microscopies. *J. Control. Release* 174 (2014) 37-42.

ACKNOWLEDGMENTS

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