# Assessing topical drug bioavailability in the skin using Raman spectroscopy

04 November 2020

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# Session Description and Objectives

## Session description

The overall objective of this study was to test the hypotheses that spectroscopic (specifically, **Raman**) 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.

The specific aims here were to show that the loss of Raman signal with depth can be corrected using the attenuation of the amide I signal and determine the rate at which a **topically administered drug is cleared from the skin**.

## Learning objectives

**After this presentation, participants will be able to:**

1. Consider **Raman spectroscopy** as a tool for the evaluation of **skin pharmacokinetics**.
2. Consider **Raman spectroscopic** as a surrogate method to assess **bioequivalence/bioavailability of topical drug products**.
3. Understand how to use **Raman spectroscopic** to track **drug penetration and clearance to and from the skin**.

# Biography and Contact Information

- I am currently a **Postdoctoral Research Associate** at the **University of Bath** (U.K.). My reserch aims to assess the **skin pharmacokinetics** of topical drugs, and the bio(in)equivalence of topical drug products, using **Raman techniques**.
- Prior to that, I completed my **Ph.D.** in Pharmaceutical Sciences at the **University of Bath**. My doctoral thesis focused on the development of a **physiologically-based pharmacokinetic (PBPK) model for dermal absorption**.
- Graduated with a B.Sc. in Pharmacy from the Federal University of Pernambuco, Brazil.
- Graduated with a M.Sc. in Pharmaceutical Science from the Federal University of Pernambuco, Brazil.

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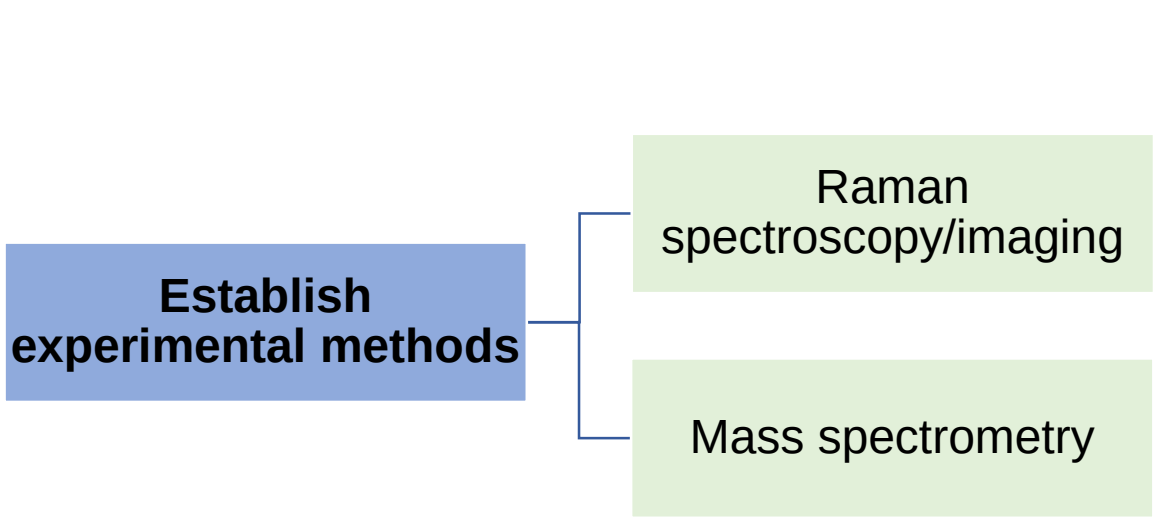
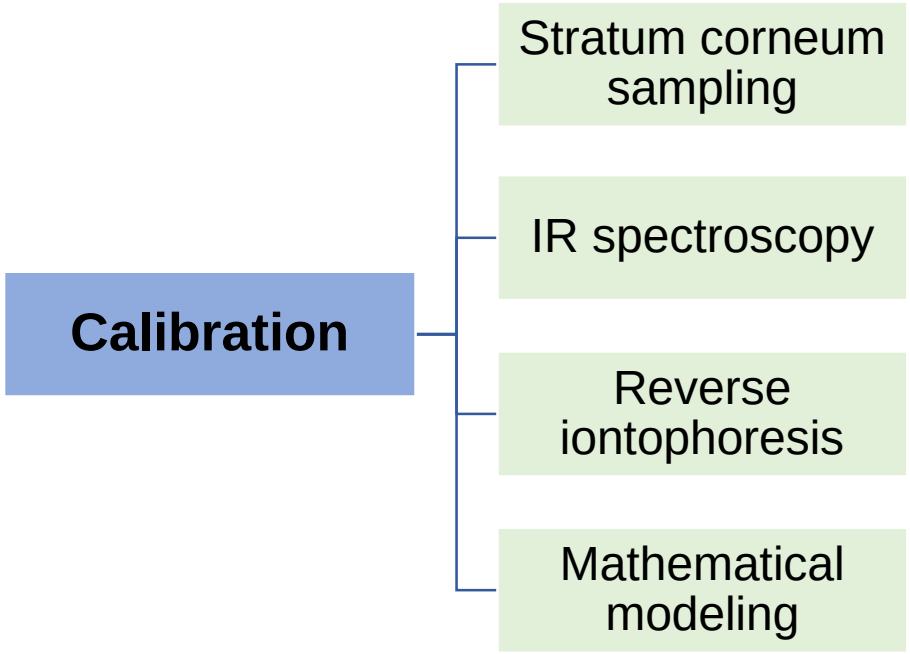
# Disclaimer

The views expressed in this presentation do not reflect the official policies of the U.S. Food & Drug Administration or the U.S. Department of Health & Human Services; nor does any mention of trade names, commercial practices, or organization imply endorsement by the United States Government.

# Background

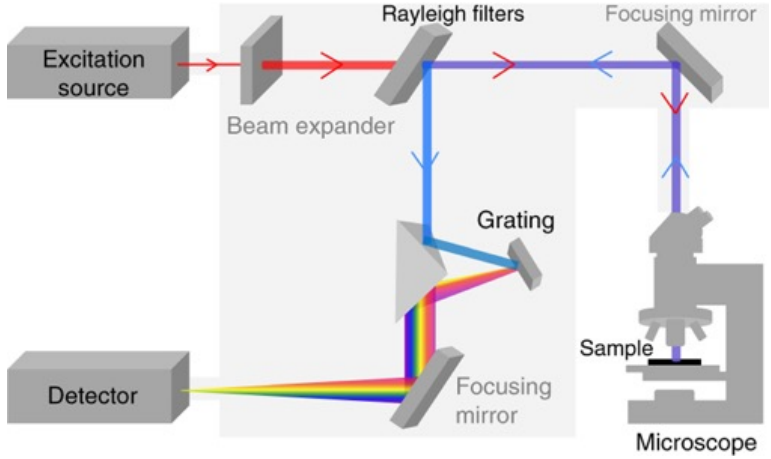
Therapeutic goal is to achieve drug concentrations at the site of action (viable epidermis, for example) after application of topical dermatological dosage forms that are high enough to produce the intended effect without producing adverse reactions.



# Central hypothesis

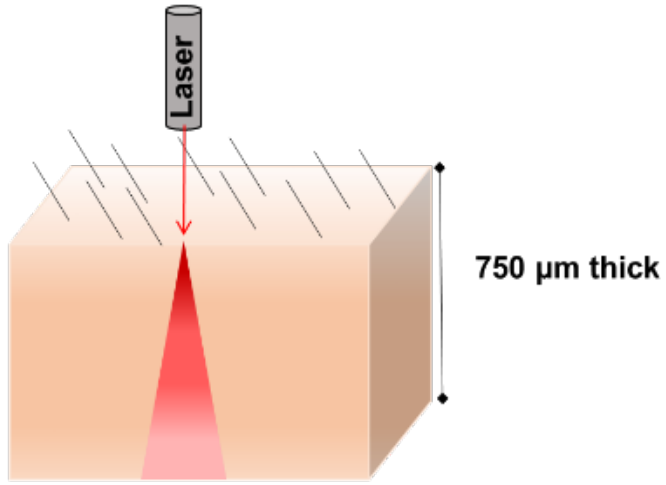
Spectroscopic (specifically, Raman) imaging offers a non-invasive, accurate, sensitive and reproducible method to determine the rate and extent to which a topically administered drug becomes available at or near its site of action below the SC.




# Research strategy

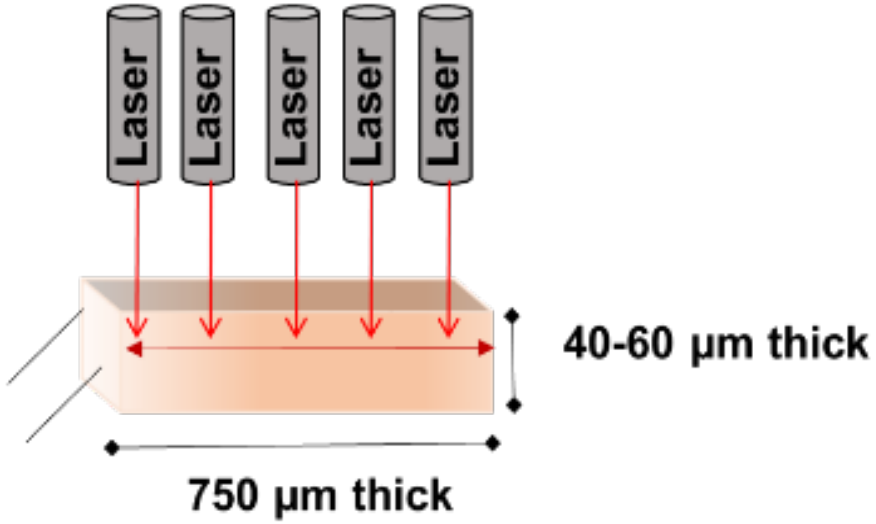
## How to correct loss of Raman signal with depth?

**“Top-down” experiments**



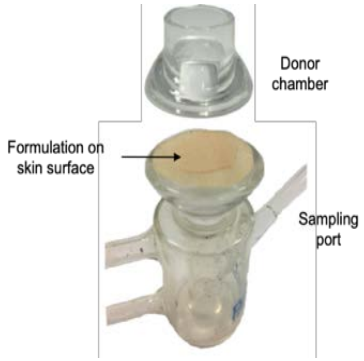

**Loss of signal within the tissue, which results in a weak backscattered signal from deeper layers. Correction of attenuation is therefore needed.**

**Cross-section experiments**




**Attenuation invariant across skin thickness**

# Experimental design




Application of the formulations on the porcine skin.

**"Top-down"** experiments

Uptake experiments

Clearance experiments

**Raman** spectra recorded from top to bottom of skin as a function of depth

**Cross-section** experiments

Skin is frozen & fixed at -25°C

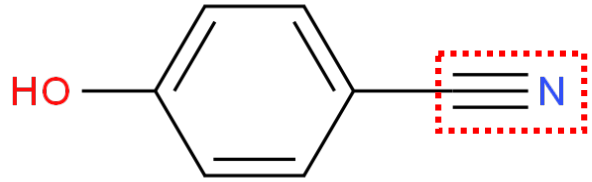
Skin is cut into 40-60 µm sections using cryostat

**Raman** spectra recorded from top to bottom of skin as a function of depth

# Experimental design

## Molecule of interest

Cyanophenol (CP) has strong -C≡N Raman signal (2230 $cm^{-1}$) in a frequency range where skin is spectroscopically 'transparent'.




## Cyanophenol formulations

1. Saturated solution (**170 mg $mL^{-1}$**) of CP in **50:50 v/v propylene glycol (PG)-water.**
2. 25% saturated solution (**42.5 mg $mL^{-1}$**) of CP in **50:50 v/v PG-water.**
3. Saturated solution (**17 mg $mL^{-1}$**) of CP in **10:90 v/v PG-water**.

# Experimental design

## Penetration experiments

Saturated CP formulations (300 µL) were applied to skin surface for 1 or 2 hr under occlusion.

Fully and 25% saturated 50:50 v/v PG-water formulations were similarly applied for 6 hr.

After each experiment, skin was cleaned, cut into small pieces, and CP disposition was assessed as functions of depth and time using Raman. Measurements (n = 6) were made either “top-down” or in “cross-section”.

## Clearance experiments

CP formulations (300 µL) were applied to skin surface for 6 hours under occlusion; skin surface was then cleaned.

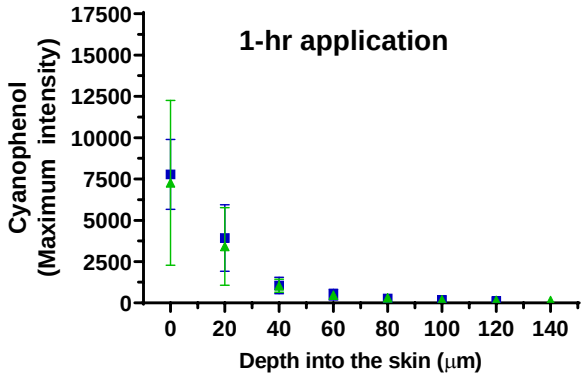
Tissue was cut into smaller pieces and mounted in a simple sample holder (unoccluded) permitting tissue hydration to be maintained while sequential, ‘top-down’ Raman measurements (n = 6) were recorded of CP clearance from skin over 6 hours.

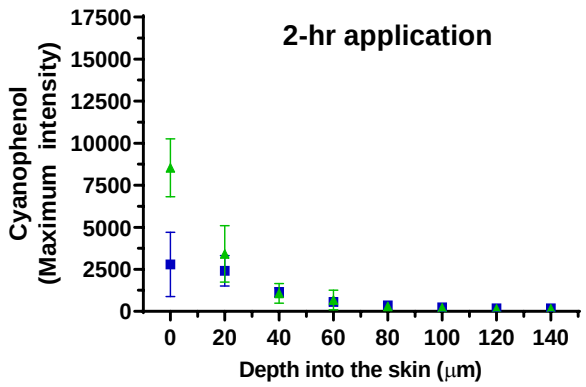
# Results – Penetration experiments

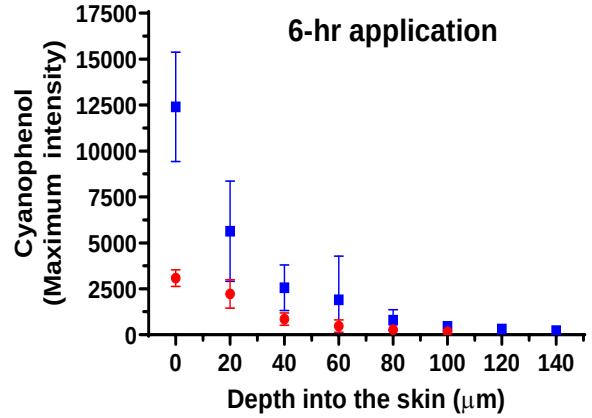
**Saturated CP (170 mg mL$^{-1}$) in 50:50 v/v PG-water** **25% saturated CP (42.5 mg mL$^{-1}$) in 50:50 v/v PG-water**

**Saturated CP (17 mg mL$^{-1}$) in 10:90 v/v PG-water**

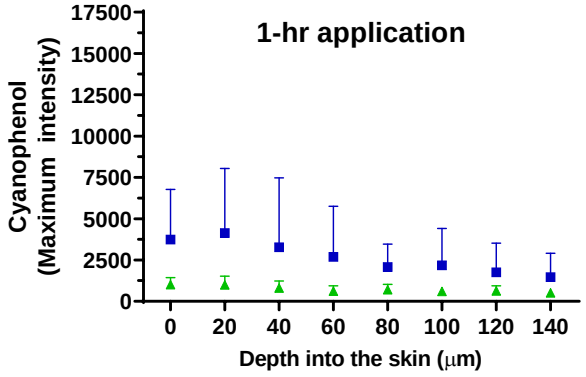
**"Top-down" experiments**

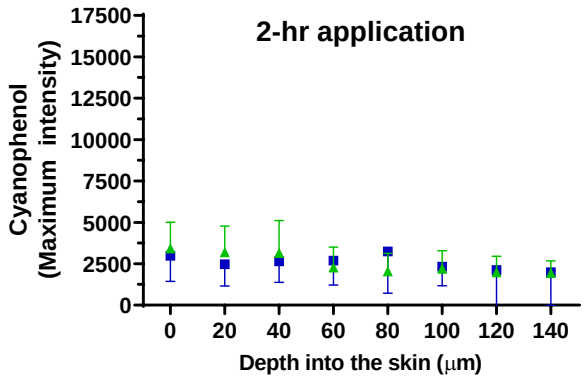


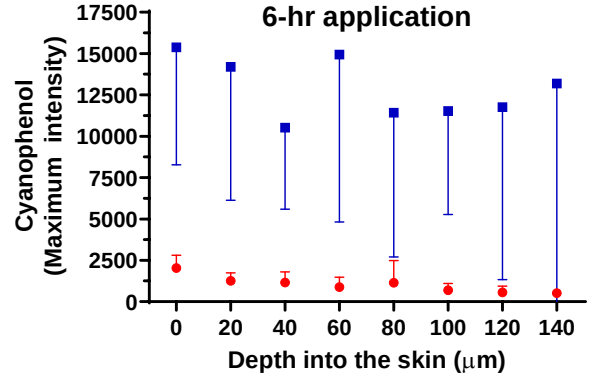




**Cross-section experiments**



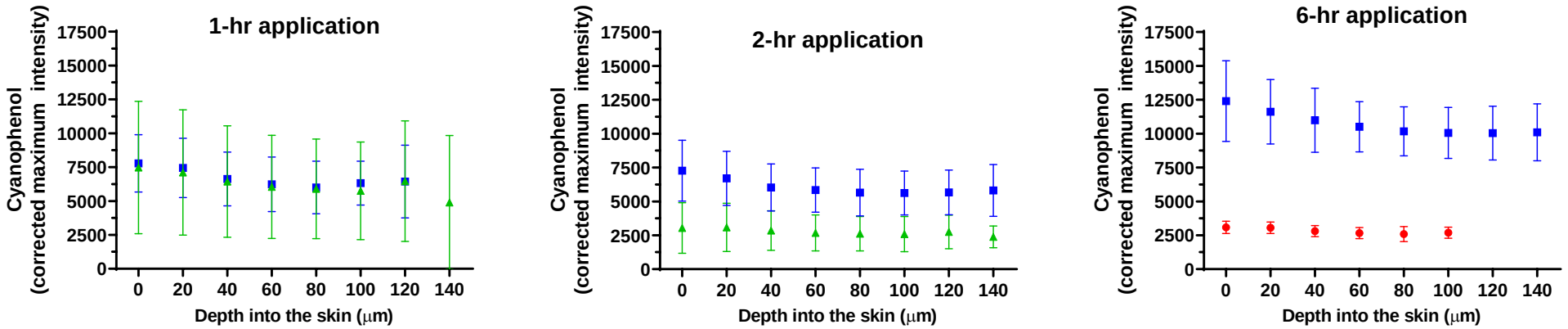



# Results – Penetration experiments

## Correction for the signal attenuation

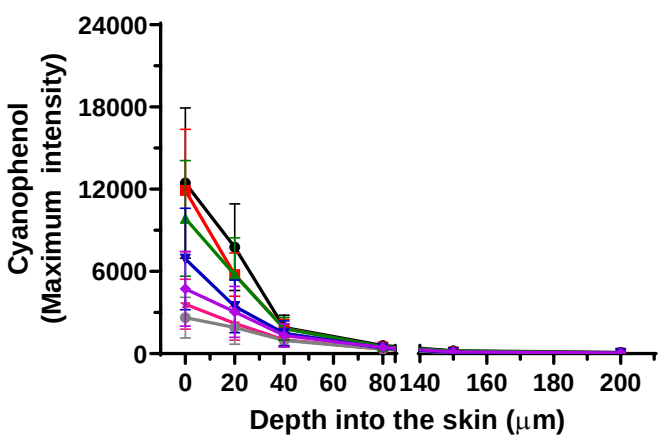
Attenuation with increasing depth of the CP signal in the “top-down” experiments was corrected using the Amide I intensity (from keratin) at 1650 $cm^{-1}$. The approximately constant CP concentration with depth is expected because there is no flux from the inner skin surface during uptake and the lag time for CP is ~0.5 h.

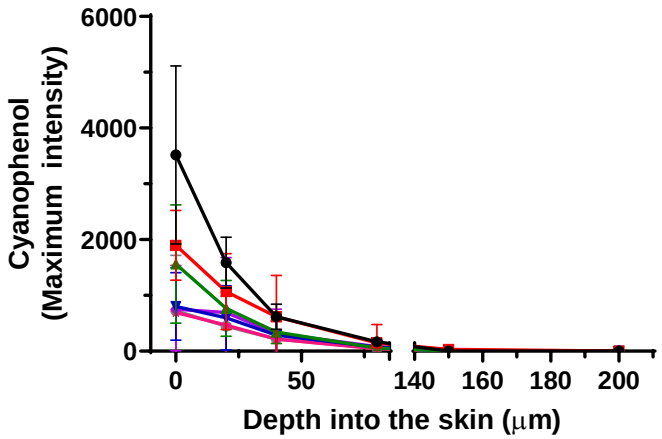


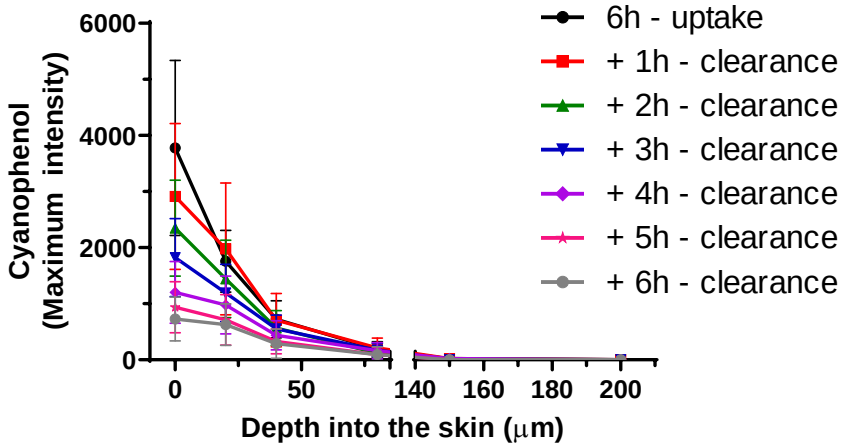

**Saturated CP (170 mg $mL^{-1}$) in 50:50 v/v PG-water** **25% saturated CP (42.5 mg $mL^{-1}$) in 50:50 v/v PG-water**

**Saturated CP (17 mg $mL^{-1}$) in 10:90 v/v PG-water**

# Results – Clearance experiments



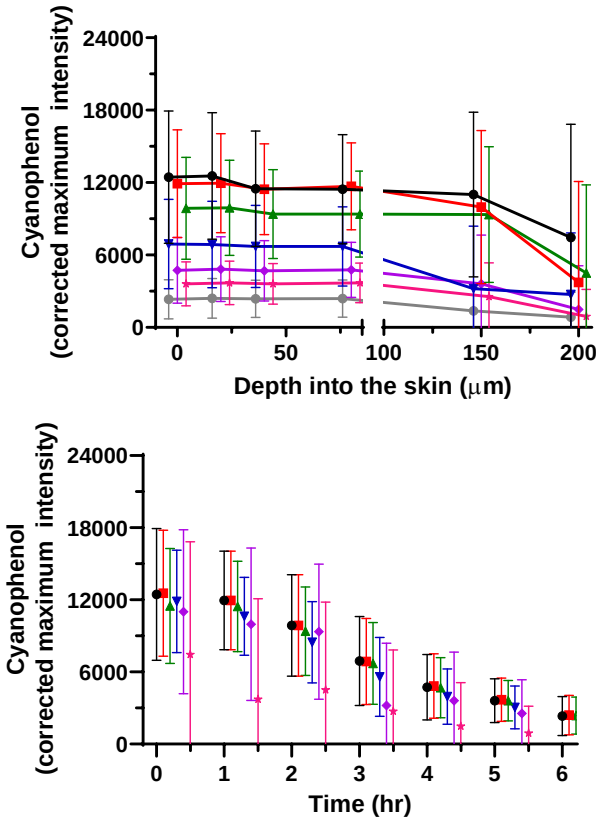

**Saturated CP (170 mg mL$^{-1}$) in 50:50 v/v PG-water**

**Saturated CP (17 mg mL$^{-1}$) in 10:90 v/v PG-water**

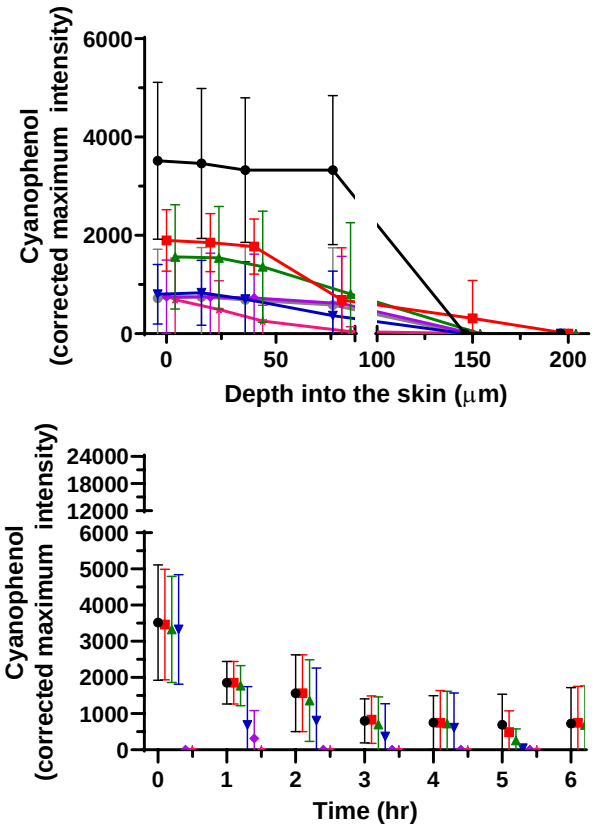
**25% saturated CP (42.5 mg mL$^{-1}$) in 50:50 v/v PG-water**

# Results – Clearance experiments

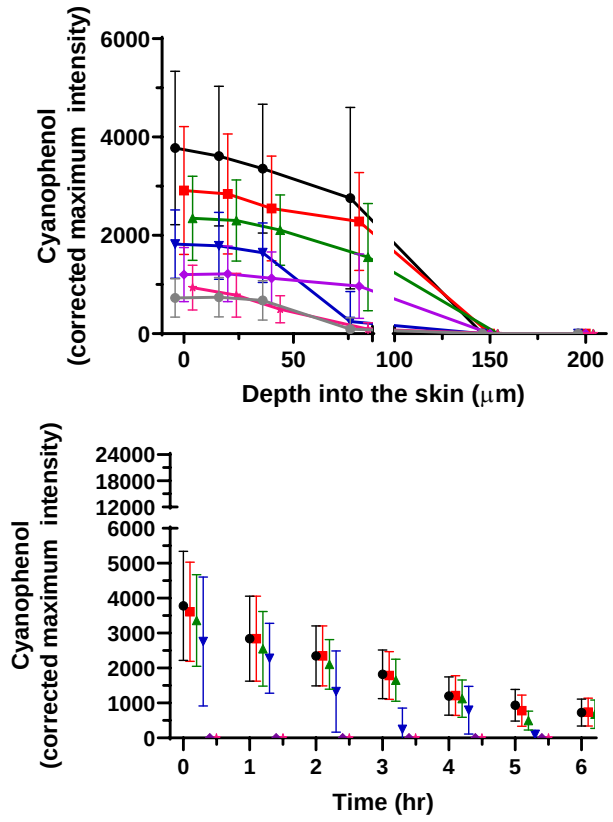
**170 mg/mL CP - 50:50 v/v PG-$H_2O$**

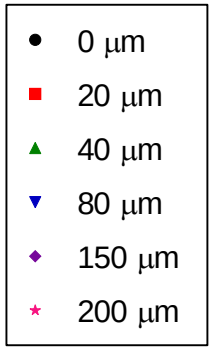



**17 mg/mL CP - 10:90 v/v PG-$H_2O$**




**42.5 mg/mL CP - 50:50 v/v PG-$H_2O$**



# Conclusions

- Raman spectroscopy can track drug penetration as a function of depth into skin (and beyond the stratum corneum).
- Raman spectroscopy can follow drug clearance from the skin from different formulations and to differentiate (in a semi-quantitative manner) between formulations.
- Artifacts due to signal attenuation by absorption/scattering of radiation can be mitigated by signal correction.

# Acknowledgements

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# Questions?

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