FDA Workshop on Bioequivalence Testing of Topical Drug Products
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Correlation of physicochemical characteristics and *in vitro* permeation test (IVPT) results for acyclovir and metronidazole topical products

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Overview of where we started this study

How can we characterise semisolid products?

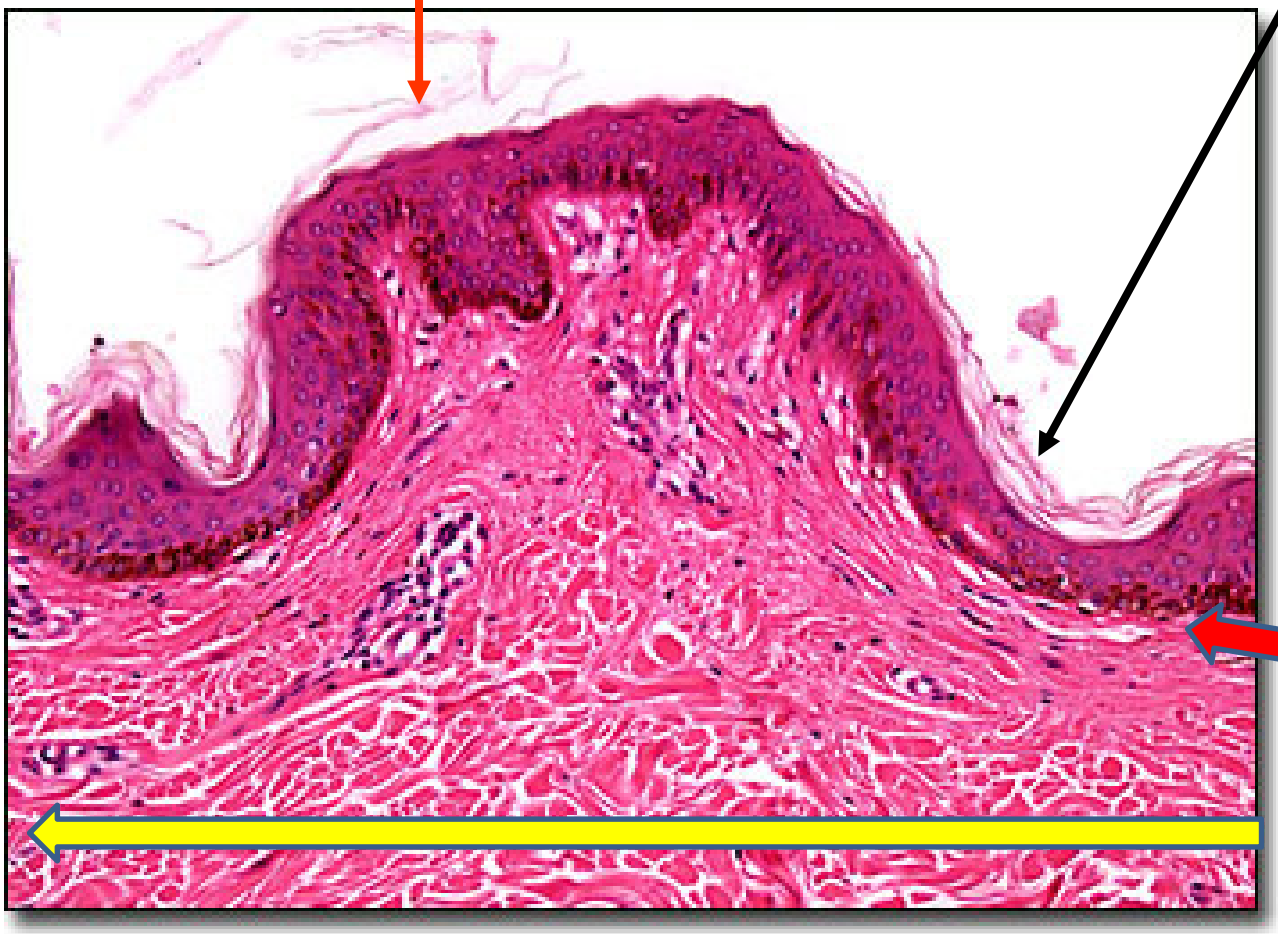
- Q1, Same components as the reference-listed drug;
- Q2, Same components in same concentration as the reference listed drug;
- Q3, Same arrangement of matter (microstructure) *(often assumed, but not always, with same components in same concentration)*

How do we define their quality?

- Quality should be by design & testing
- However, semisolid dosage forms are complex systems that change in use
- A pharmacokinetic approach for topical products should relate to drug concentrations at the site of action (layers within the epidermis/dermis)
- Measuring epidermal and superficial dermal drug concentrations is presently a challenge
- We therefore use surrogate measures of product performance:
  - *In vivo* methods = microdialysis, dermal perfusion, tape stripping and imaging
  - *In vitro* permeation test (IVPT)
  - *In vitro testing* for product quality attributes by a comprehensive characterisation of Q3

Let us look at testing in terms of the skin morphology & sites of action

Sampling - stratum corneum stripping is potential method to assess skin permeation



Stratum corneum – main barrier – also potential target site

Various regions in viable epidermis & upper dermis = key target site

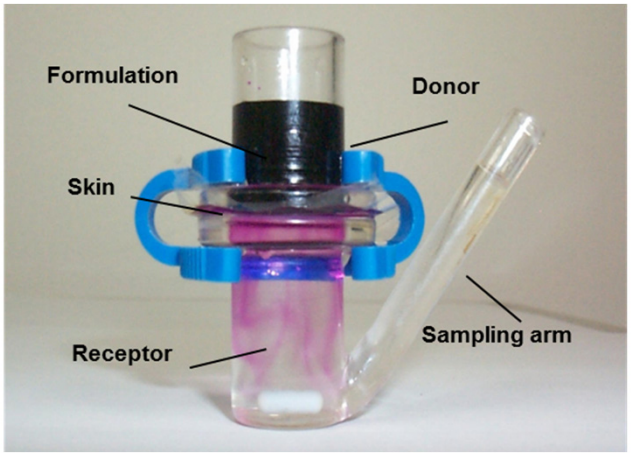
Epidermal membrane sampling site

Dermal sampling site for microdialysis and dermal microperfusion (*in vivo*) & *in vitro* dermatomed skin

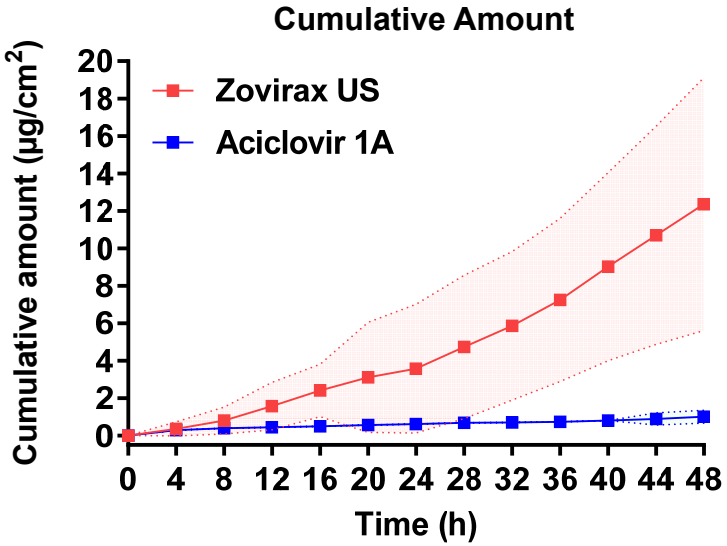
One focus is *In Vitro* Permeation Test (IVPT)

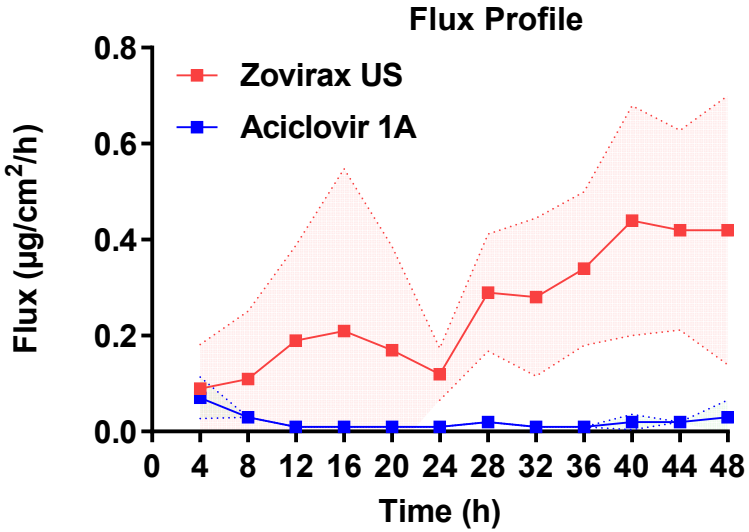
Sandwich stratum corneum, epidermis, dermatomed skin & full thickness skin in a static or flow through Franz diffusion cell

- Long history
- Robust
- Simple
- Precise
- Reproducible



Here, epidermal membranes used for 2 acyclovir products

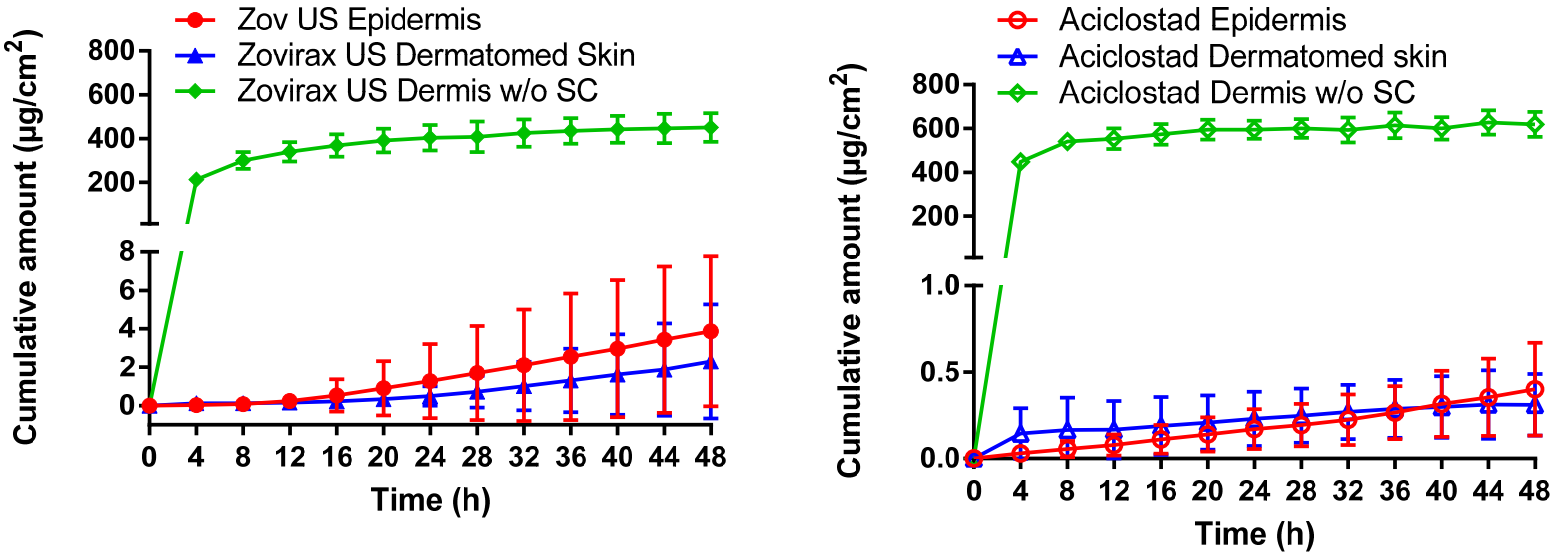




Data shown as mean ± 95% Confidence Interval (CI)
Each point is the mean of 9* (3 donors & 3 replicates per skin)

In Vitro Permeation Test (IVPT) Studies

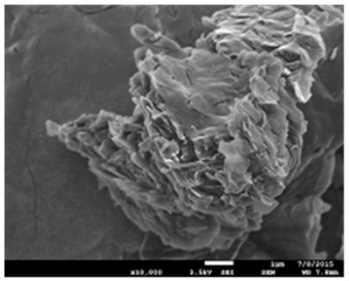
We found similar permeation profiles for 2 acyclovir products using human epidermal membranes & dermatomed skin; dermal membranes are very permeable!

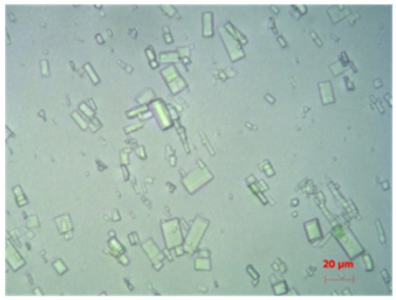


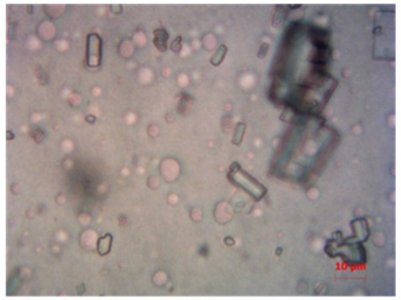
Data shown as mean ± 95% Confidence Interval (CI)
Each point is the mean of 9* (3 donors & 3 replicates per skin)

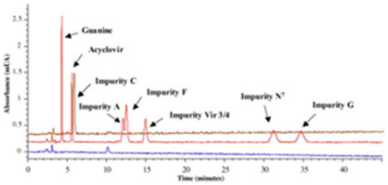
- Supports SC being main underlying barrier
- Suggests that either epidermal membranes or dermatomed skin could be used in acyclovir IVPT studies
- Skin barrier integrity is an important control component to get right.

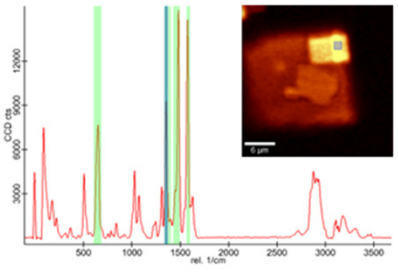
In vitro **testing for product quality** by an articulated battery of physicochemical tests - potential critical quality attributes, i.e. Q3

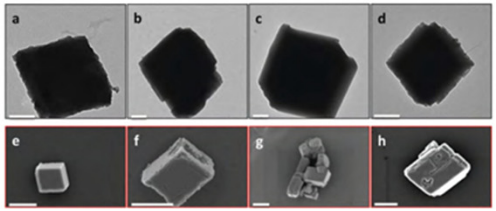


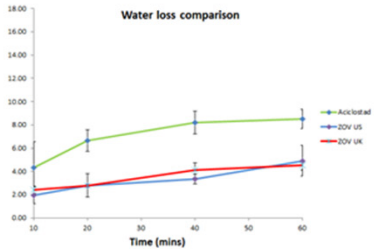


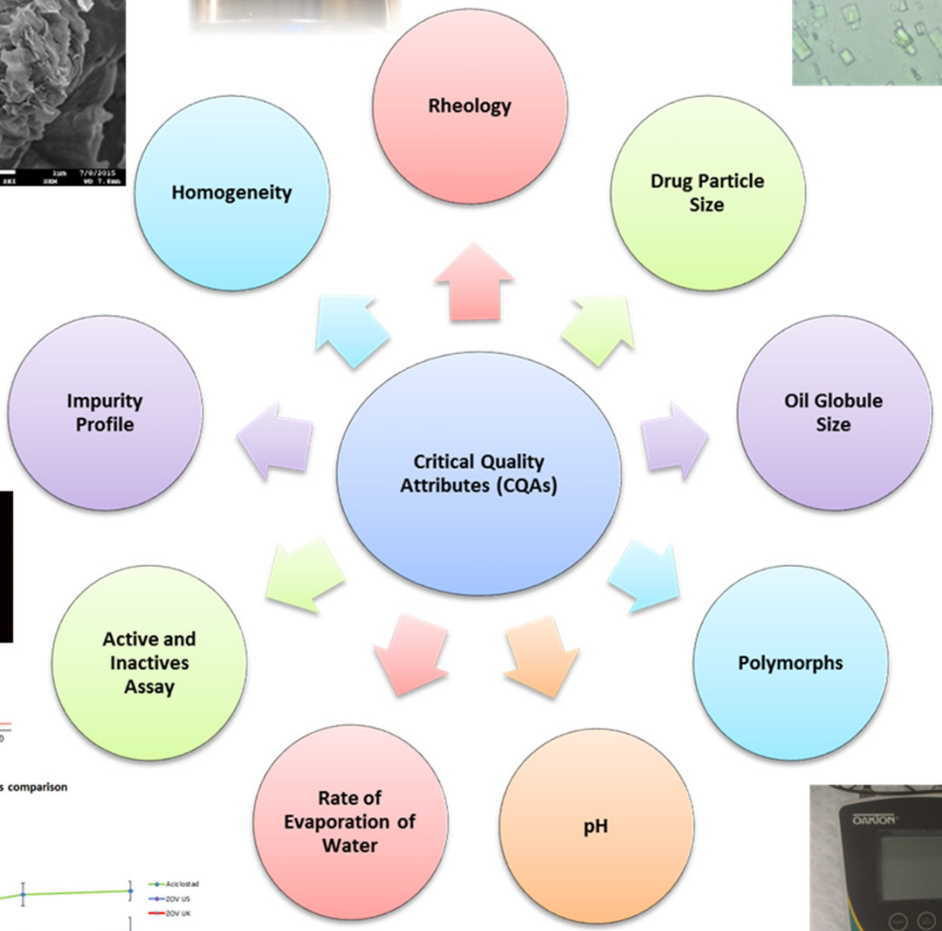












Rheology and tribology as particular critical quality attributes

In-use physics: Multiple scales of deformation

From rheology to tribology – applied to personal care & foods (micro-structured fluids)

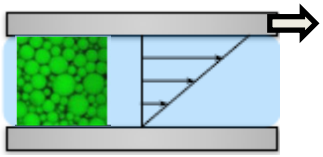
Packaged product

"In-use" product

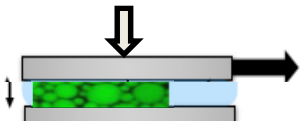
Film Thickness between shearing surfaces

> 100 μm | 100 – 5 μm | 10 – 0.1 μm | 1 – 0.001 μm

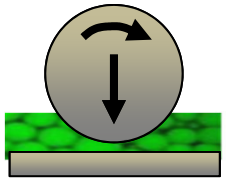
MACRO- | MICRO- | NANO-



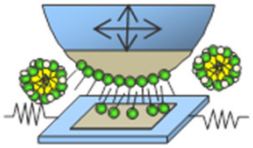
Rheology[1] (fixed gap)



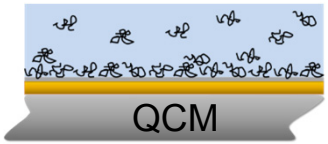
Narrow gap / GDR[2] (thin film)



Tribology[3] (fixed load)



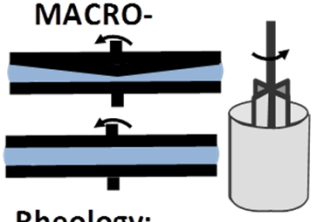
Micro & Nano Mechanics[4]



Adsorbed polymer films[5]

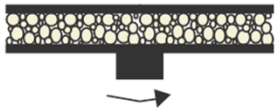
Apparatus

MACRO-



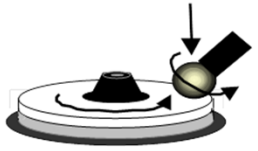
Rheology:
Cone-plate, large gap parallel plate, Vane-cup, etc.

MICRO-

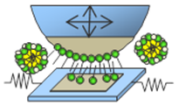


Micro-Rheology
Thin film / narrow gap parallel plate

NANO-



Soft-Tribology:
Sliding/rolling Ball-Disk on PDMS substrate



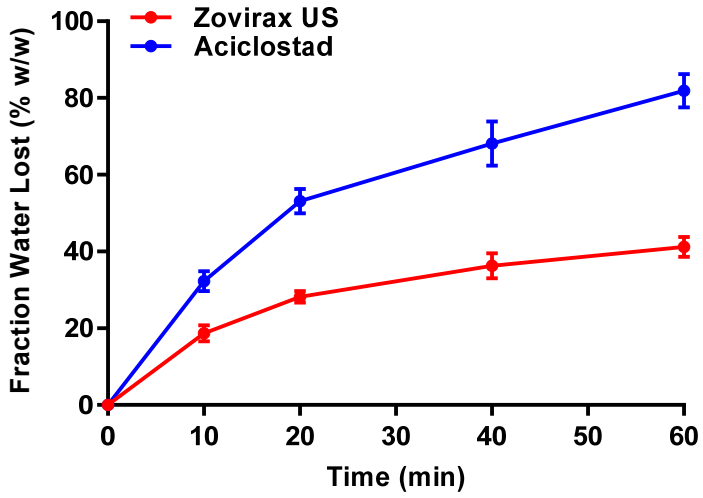
Nano-rheology, nano-tribology
AFM tip/colloid probe

Adaptation of slide courtesy of Prof. Jason Stokes, UQ

Let us now return to the Zovirax (US) and Aciclovir 1A products
What are the product differences that cause non-bioequivalence?

- Firstly, they differ in
  - ❖ Q1 (Qualitative – nature of ingredient) and
  - ❖ Q2 (Quantitative - amounts)
- Specific content differences
  - ❖ PG estimated by DSC-TGA data
  - ❖ Water content by Karl Fischer
- Product changes when applied to skin, described as product metamorphosis, may affect acyclovir bioavailability – especially as a result of evaporation
  - Slower evaporation for Zovirax due to presence of PG

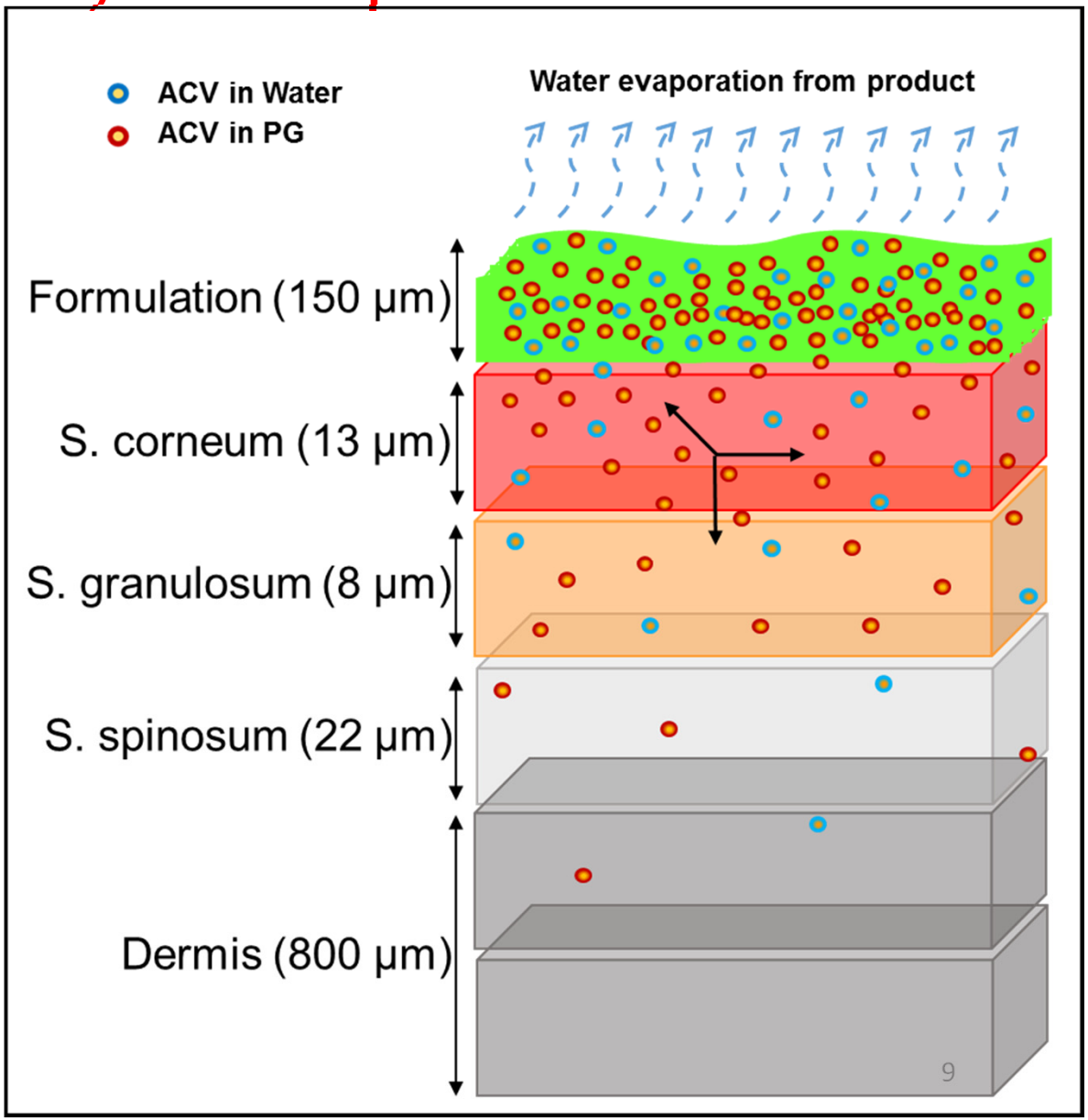
Ingredient Name	Zovirax (U.S.)	Aciclovir 1A Pharma (Austria)
Acylovir concentration	5% w/w	5% w/w
Propylene glycol (PG)	**40% w/w**	**15% w/w** [*1]
Water Content	**≈ 1/3 w/w**	**≈ 2/3 w/w**
Other Ingredients:	Cetostearyl alcohol Mineral oil Poloxamer 407 Sodium lauryl sulfate Water White petrolatum	White Vaseline Viscous paraffin Glycerol monostearate Polyoxyethylene stearate Dimethicone Purified water



[*1] Trottet, L., H. Owen, P. Holme, J. Heylings, I. P. Collin, A. P. Breen, M. N. Siyad, R. S. Nandra and A. F. Davis (2005). "Are all aciclovir cream formulations bioequivalent?" Int J Pharm 304(1-2): 63-71.

Excipients interact directly with the stratum corneum (SC) can impact on IVPT

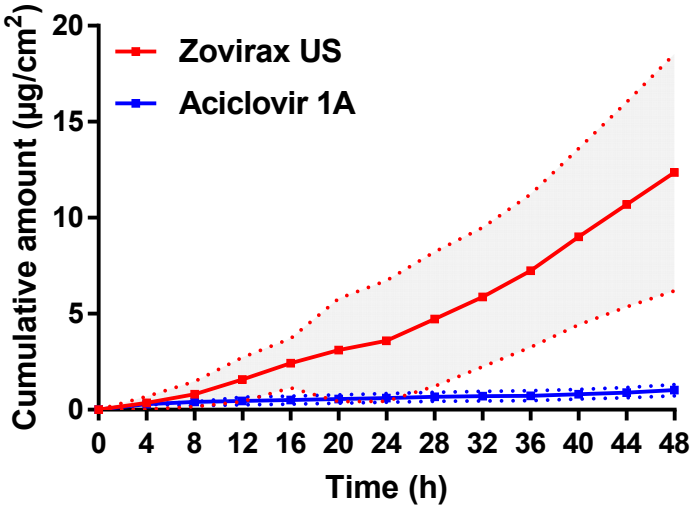
- Propylene glycol (PG) and water, known penetration enhancers, are two excipients present in all products
- Our work has also shown that PG and water can carry solutes into the SC & promote their permeation
- Both are likely to promote direct acyclovir uptake into the stratum corneum
- Potentially, product microstructure (Q3) can impact on acyclovir & enhancer bioavailability to the stratum corneum



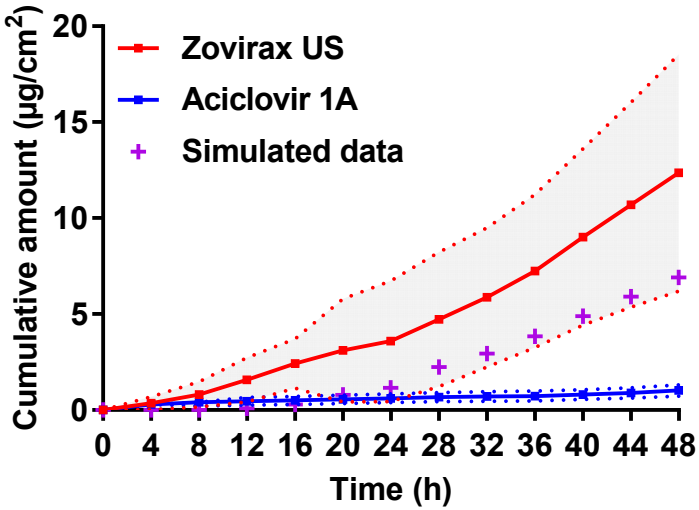
Understanding differences in *IVPT* profiles for acyclovir for 2 products

1. We first consider diffusivity of ACV in SC with no product excipients (PG, water etc.) – SC interactions

Experimental *IVPT* profiles



Can we predict acyclovir permeation theoretically?

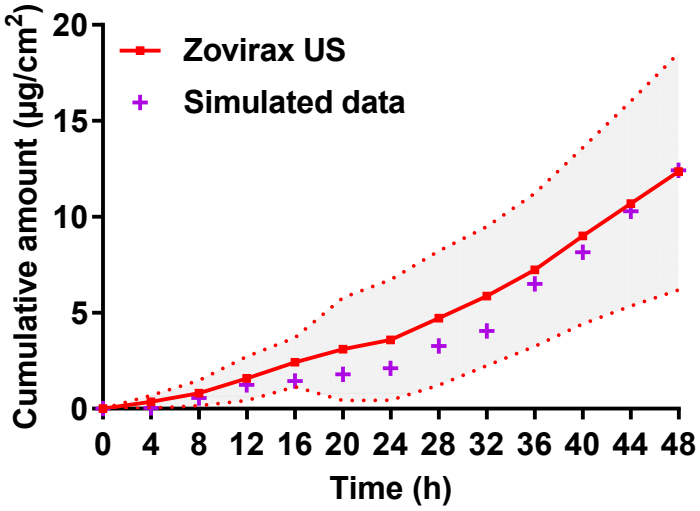


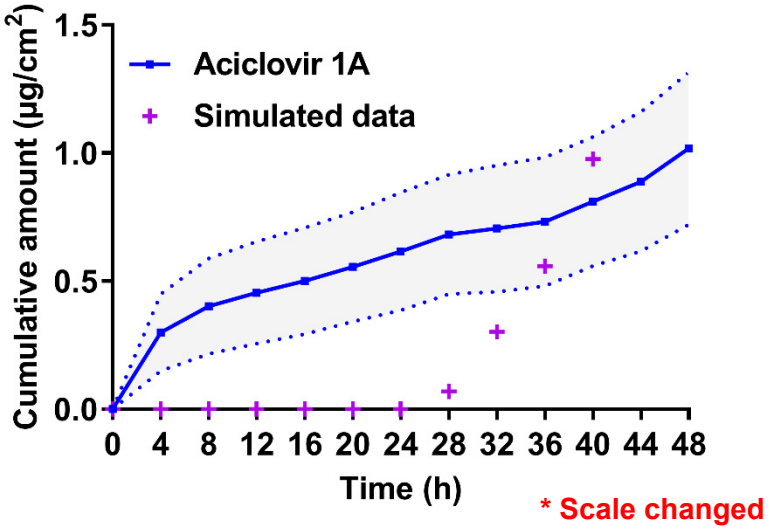
$K_{ACV,SC}$ = 0.24; h_{SC}= 13 µm;
$D_{ACV,SC}$= 2.54 x 10^{-7} µm²/s

The predicted profile by simulation is intermediate between the two observed profiles

Understanding differences in *IVPT* profiles for acyclovir for 2 products

2. Now include impact of PG in SC on Acyclovir permeation predictions





* Scale changed

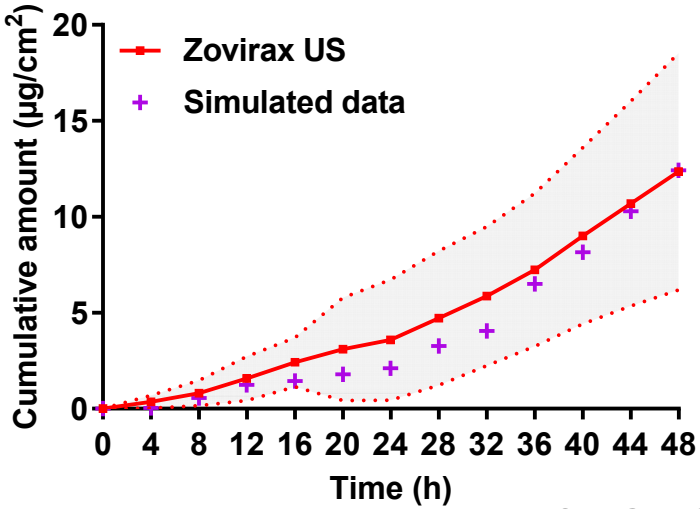
- When the effect of PG, a known ingredient in the formulations and a known solubility and penetration enhancer, is taken into account the simulated profile for Zovirax matches with the *IVPT* data.
- However, Aciclovir 1A still does not fit. Is there something more going on?

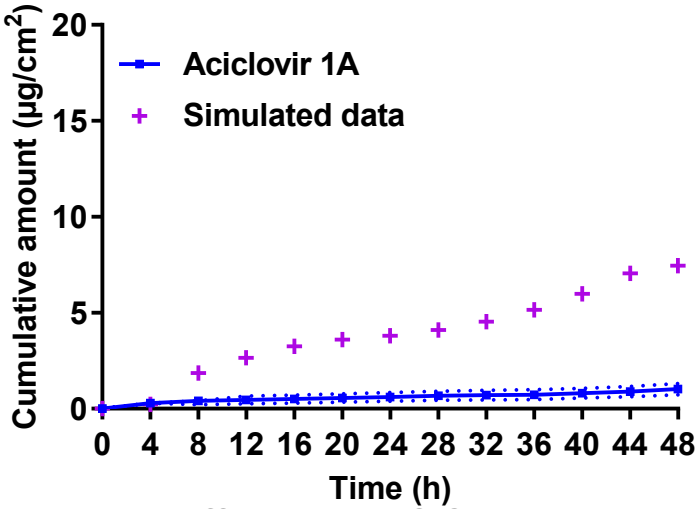
$K_{PG,SC}$ = 0.29; h_{SC}= 13 µm;
$D_{PG,SC}$= 1.03 x 10^{-4} µm²/s

$$D^*_{ACV,SC} = D_{ACV,SC} + 0.00003 \times C_{PG,SC}$$

Understanding differences in *IVPT* profiles for acyclovir for 2 products

3. Now including impact of PG and water in SC and water evaporation from the product





- As well as interactions of PG affecting acyclovir diffusion in SC,
- Evaporation of water from product modifies acyclovir availability, and

$$D_{\mathrm{don,H_2O}} \nabla u_{\mathrm{H_2O}}(x)\vec{n} = \omega\, u_{\mathrm{H_2O}}(x)$$

$D_{donor,water}$= 6.88 µm²/s; ω= 0.02

- Water can modify acyclovir chemical activity and diffusion in SC

$K_{PG,SC}$ = 0.29; h_{SC}= 13 µm;
$D_{PG,SC}$= 1.03 x 10^{-4} µm²/s

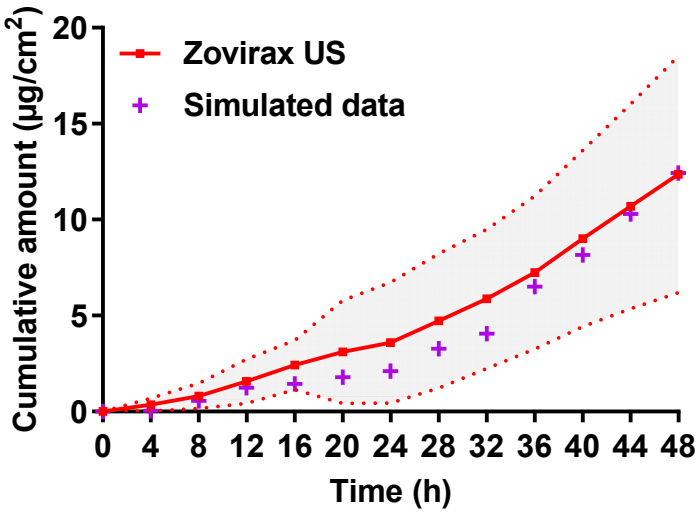
$K_{water,SC}$ = 0.18; h_{SC}= 13 µm;
$D_{water,SC}$= 1.07 x 10^{-3} µm²/s

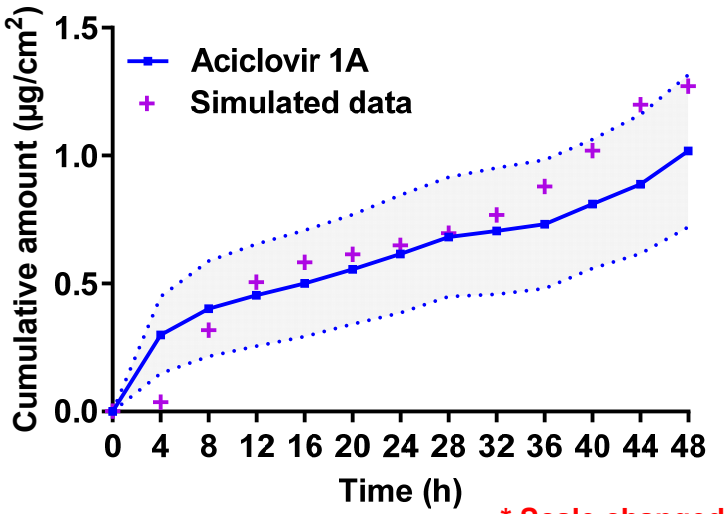
$D^*_{ACV,SC} = D_{ACV,SC} + 0.00003 \times C_{PG,SC} + 0.000043 \times C_{water,SC}$

- Zovirax fits but Aciclovir 1A cannot be fitted.

Understanding differences in *IVPT* profiles for acyclovir for 2 products

4. Now add the availability of acyclovir in the donor for “in-use” conditions



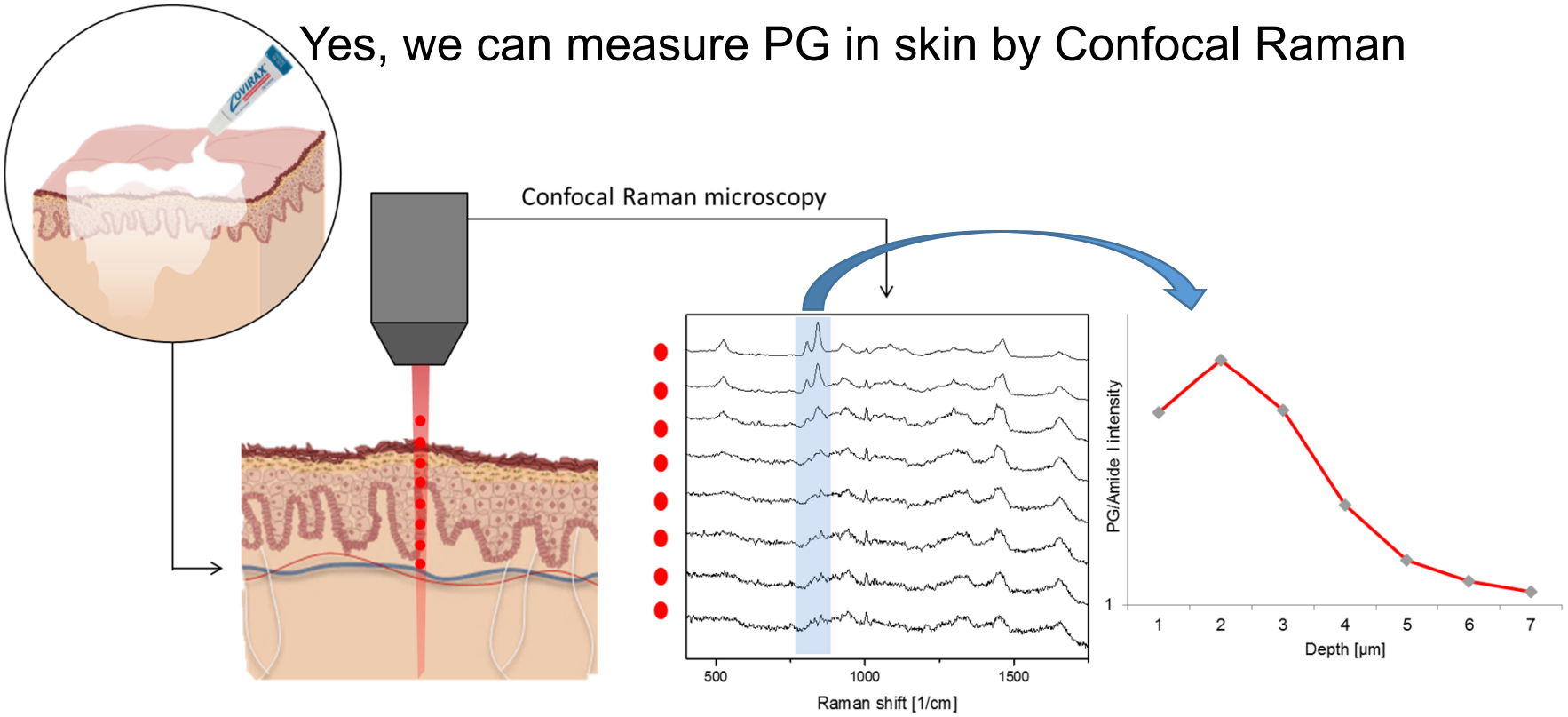


* Scale changed

- Estimated 10% free acyclovir in Zovirax after evaporation (~13.5% before)
- Estimated 1.7% free acyclovir in Aciclovir 1A after evaporation (~14.3% before)
- Now both products fit

Can we verify the theoretical predictions experimentally?

Yes, we can measure PG in skin by Confocal Raman

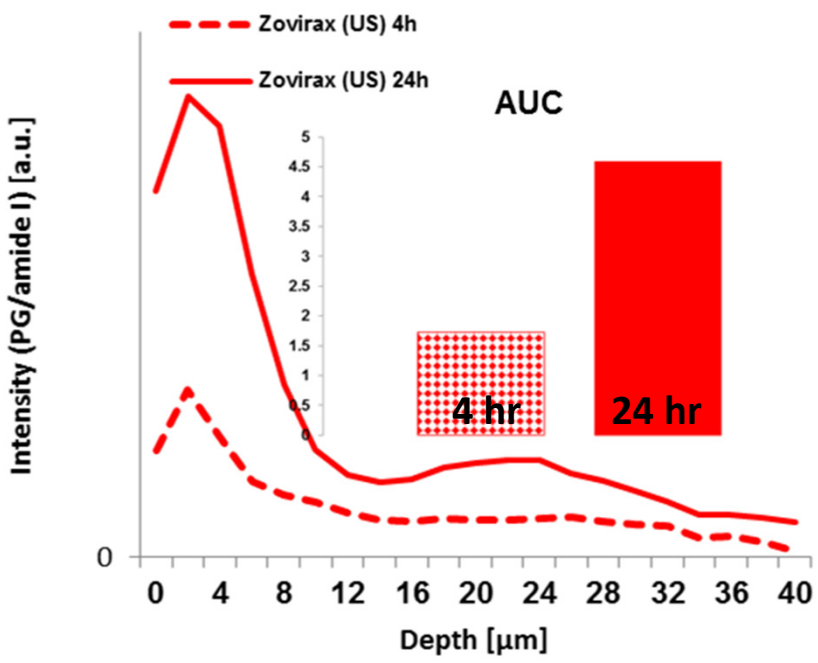


- After incubation of the sample on the skin, excess cream is removed
- With the Confocal Raman microscope, vertical line scans are acquired from the skin surface downwards in z-direction

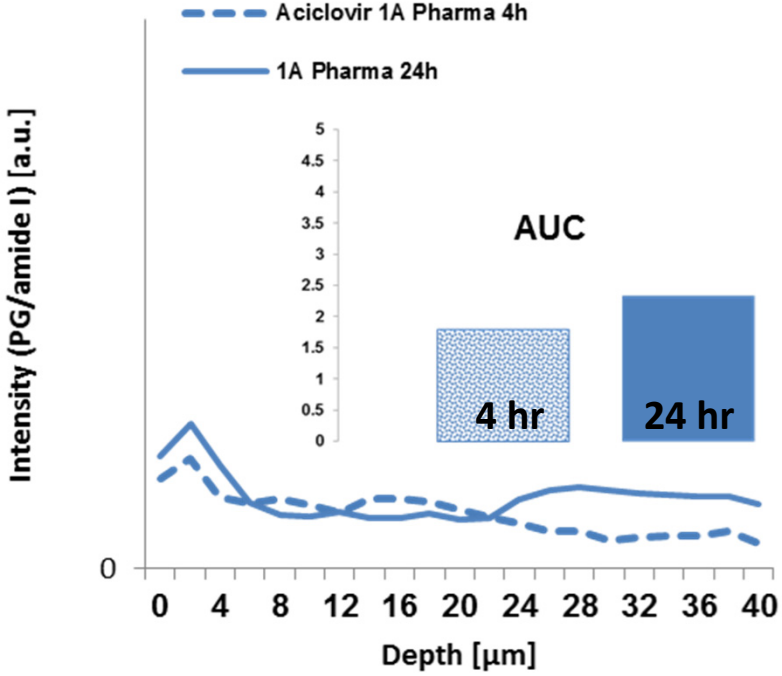
- In the resulting Raman spectra, a formulation-associated peak (here highlighted is a characteristic peak of PG) is normalized by a skin-derived peak (amide I around 1641 cm^{-1})
- The normalized Raman intensity of PG is then plotted against the penetration depth to create a depth profile

We find…

Zovirax (US)



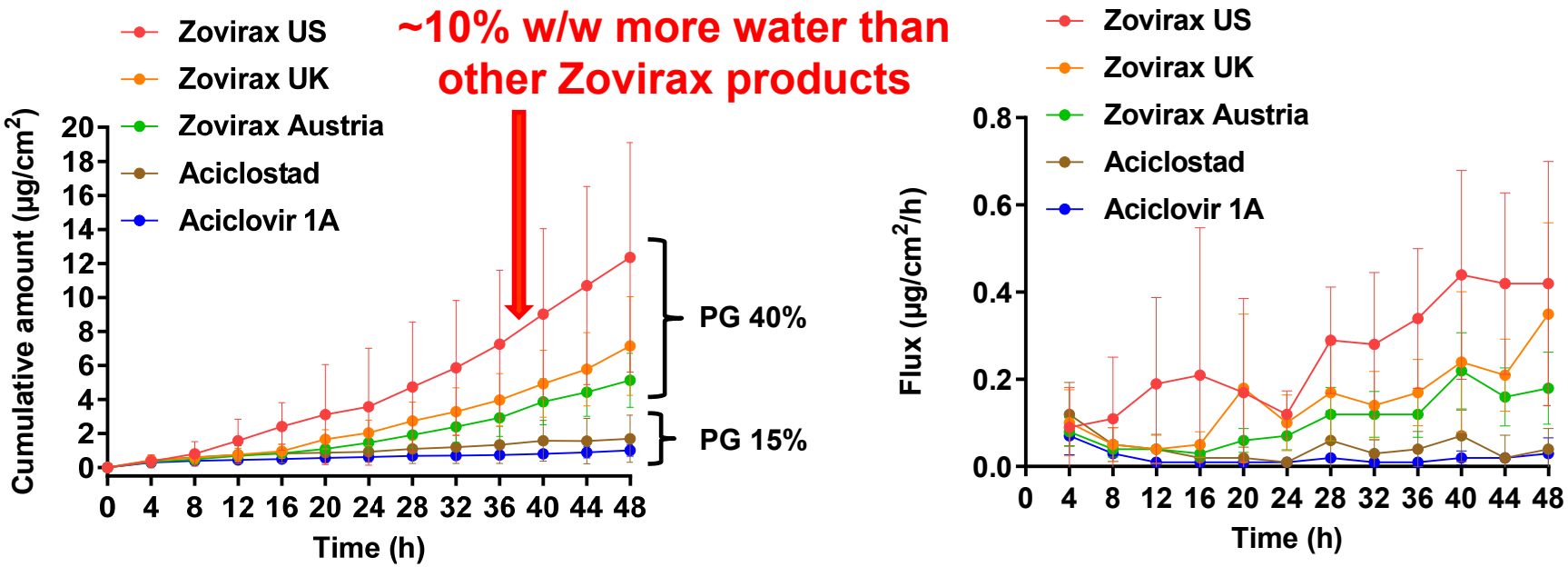
Aciclovir 1A



- Zovirax (US) has 2.5 times the PG content of Aciclovir 1A*
- PG uptake in the SC increases 2.5 fold over time after Zovirax (US) application but not after Aciclovir 1A.

* Trottet, L., H. Owen, P. Holme, J. Heylings, I. P. Collin, A. P. Breen, M. N. Siyad, R. S. Nandra and A. F. Davis (2005). "Are all aciclovir cream formulations bioequivalent?" Int J Pharm 304(1-2): 63-71.

What happens with other acyclovir products? IVPT



Data shown as mean ± 95% CI; Each point is the mean of 9* (3 donors & 3 replicates per skin)

- Trottet has suggested that PG is major determinant of acyclovir permeation
- The difference between Zovirax reference products and the Austrian "generic products" is largely due to difference in PG content
- Zovirax (US) has ~10% more water than Zovirax (UK) and Zovirax (Austria)
- Possible impact of other excipients and Q3?

Trottet, L., H. Owen, P. Holme, J. Heylings, I. P. Collin, A. P. Breen, M. N. Siyad, R. S. Nandra and A. F. Davis (2005). "Are all aciclovir cream formulations bioequivalent?" Int J Pharm 304(1-2): 63-71.

Composition of Acyclovir products

Other excipients also vary & may matter!

Zovirax (USA)	Zovirax (UK)	Zovirax (Austria)	Aciclostad (Austria)	Aciclovir-1A (Austria)
Water	Water	Purified water	Water	Water
Propylene glycol	Propylene glycol	Propylene glycol	Propylene glycol	Propylene glycol
Mineral oil	Liquid Paraffin	Liquid Paraffin	Liquid Paraffin	Viscous Paraffin
White petrolatum	White soft paraffin	White Vaseline	White Vaseline	White Vaseline
Cetostearyl alcohol	Cetostearyl alcohol	Cetostearyl alcohol	Cetyl alcohol	Cetyl alcohol
SLS	SLS	SLS		
Poloxamer 407	Poloxamer 407	Poloxamer 407		
	Dimethicone 20	Dimethicone 20	Dimethicone	Dimethicone
	Arlacel 165	Glyceryl Mono Stearate	Glyceryl Mono Stearate	Glyceryl Mono Stearate
	Arlacel 165	Polyoxyethylene stearate	Macrogol stearate	Polyoxyethylene stearate

Summary of Acyclovir product quality attributes

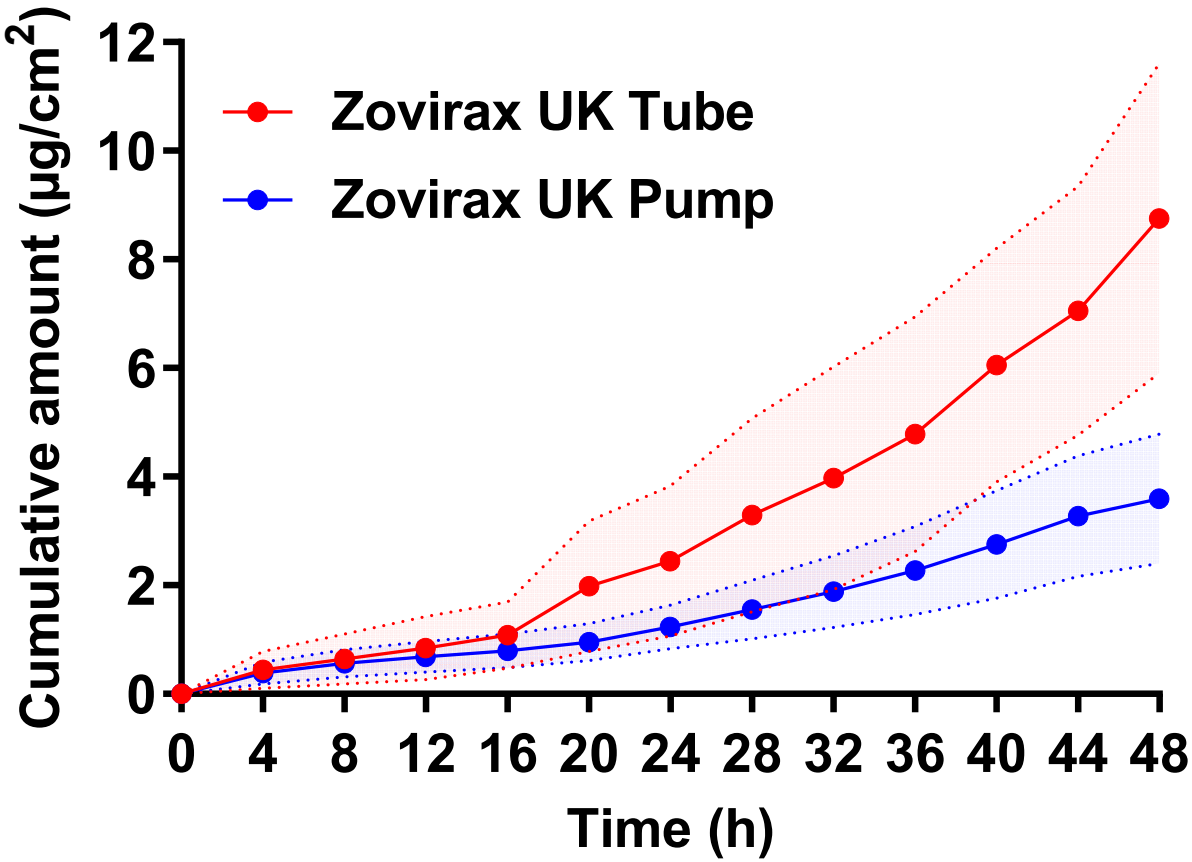
Quality Attribute	Zov US	Zov UK	Zov Austria	Aciclostad	1A Pharma
pH	6.4	7.2	6.8	4.6	5.9
Polymorphs	No difference in polymorphic forms				
Crystal Shape/Crystal habit	Rectangular			Irregular	
Predominant particle size range (µm)	5 -10	5 -10	5 -10	0 - 5	0 - 5
Excipients	NA	Different from RLD		Different from RLD	
Zero Shear Rheology	NA	Different from RLD		Similar to RLD	
Water Content (% w/w)	? (~33)	≈ 25	≈ 25	≈ 60	≈ 60
Loss of Water (% w/w)	17.8 ± 1.6	23.4 ± 3.2	21.0 ± 1.9	55.9 ± 4.9	53.2 ± 4.3
Globule Size	No globules visible	Globules in pump product	No globules visible	Globules Apparent	
Microstructure (without inclusions)	Wavy surfactant like features			Globules Apparent	
IVPT					
Cumulative amount 48 hrs (µg/cm^2)	11.0 ± 2.7	7.2 ± 1.5	5.1 ± 0.7	2.2 ± 0.6	1.0 ± 0.2
AUC – Flux curve	11.3 ± 2.6	6.3 ± 1.3	4.4 ± 0.6	1.8 ± 0.5	0.8 ± 0.2
Jmax (µg/cm^2/h)	0.44 ± 0.11	0.35 ± 0.09	0.22 ± 0.04	0.12 ± 0.03	0.07 ± 0.02
Tmax (h)	40	48	40	4	4

NA: Not Applicable

Q1, Q2 is important. What about Q3?

Need to consider specific case when Q1 and Q2 are the same

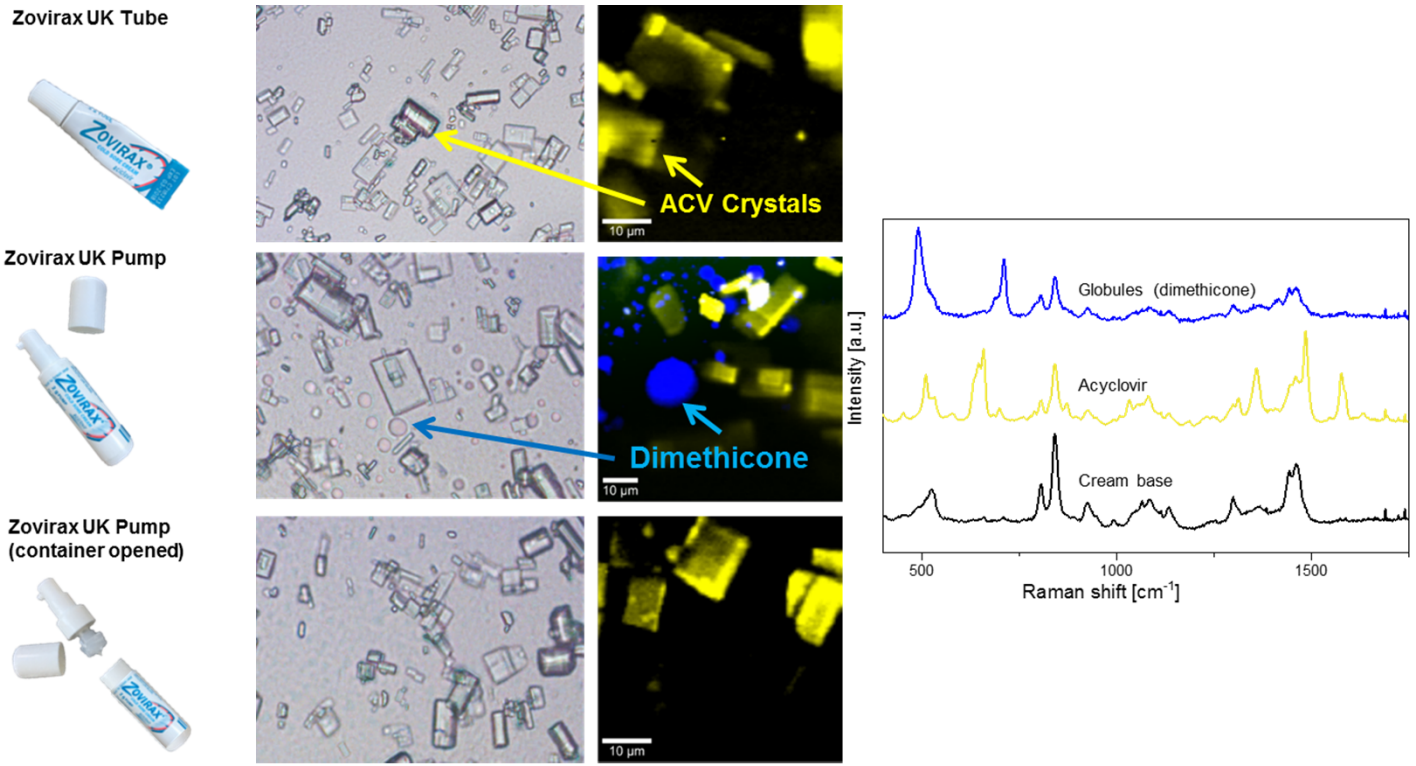
- The Q1 and Q2 of acyclovir packaged in a tube and a pump dispenser are the same;
- But their IVPT profiles differ – Why?



Using confocal Raman & rheology to assess impact of dispensing on Q3 metamorphosis & IVPT

- Confocal Raman suggests that pumping affects the crystal habit for acyclovir and leads to the formation of dimethicone globules
- Rheology suggests that the packaged tube and pump have a similar yield stress but that the product after pumping is higher – due to dimethicone agglomeration?

Yield stress from strain sweep (Pa)



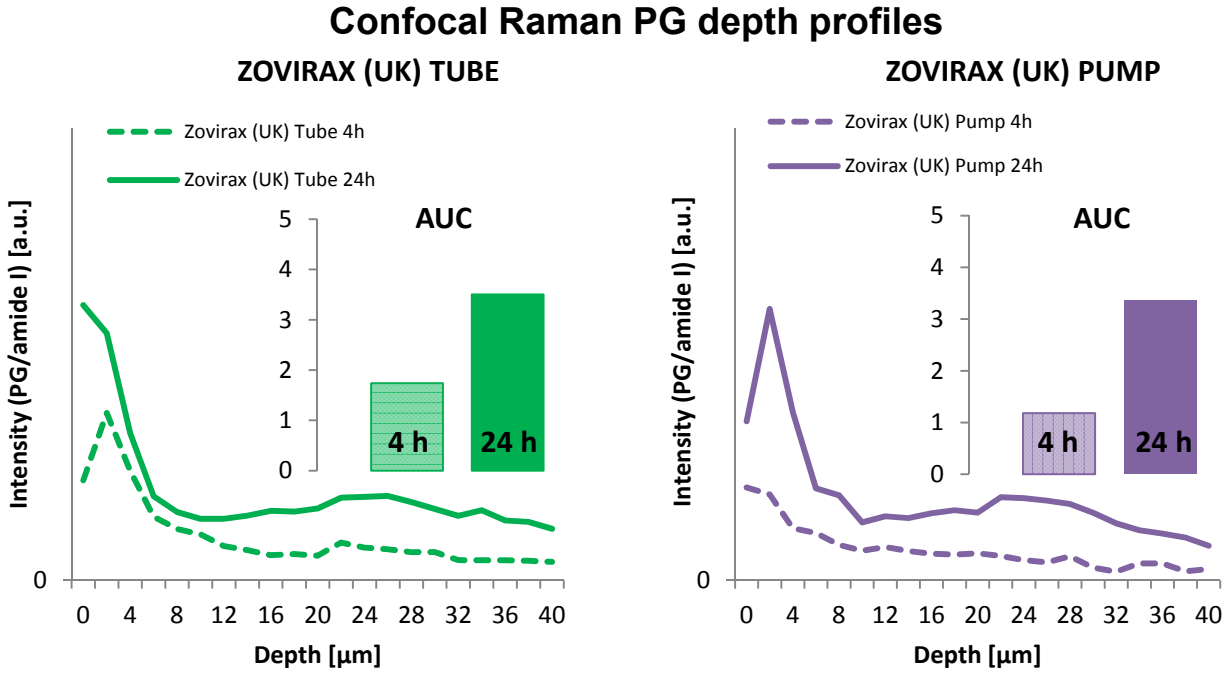
78 ± 1.3

182 ± 0.6

70 ± 10

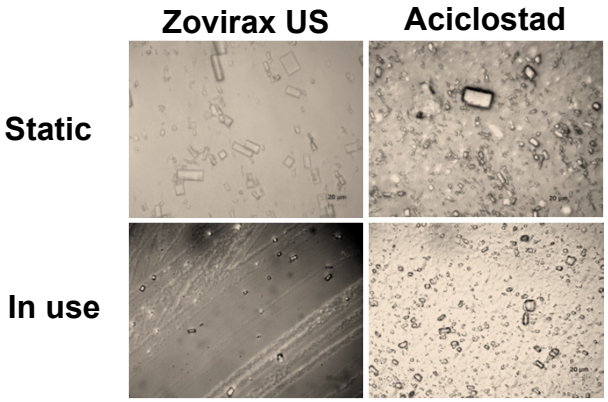
Correlation of Q3 microstructure with performance (Example I)

- Reflections on the differences in IVPT permeation flux with the Q3 differences? Impact of pumping on Q3
- Pumping leads to agglomeration of dimethicone (in which ACV is poorly soluble), i.e. a change in product microstructure (Q3)
  - Does the dimethicone agglomeration on the skin surface act as a potential additional barrier to acyclovir permeation?
  - Does this also include affecting the the bioavailability of the enhancer (PG)?

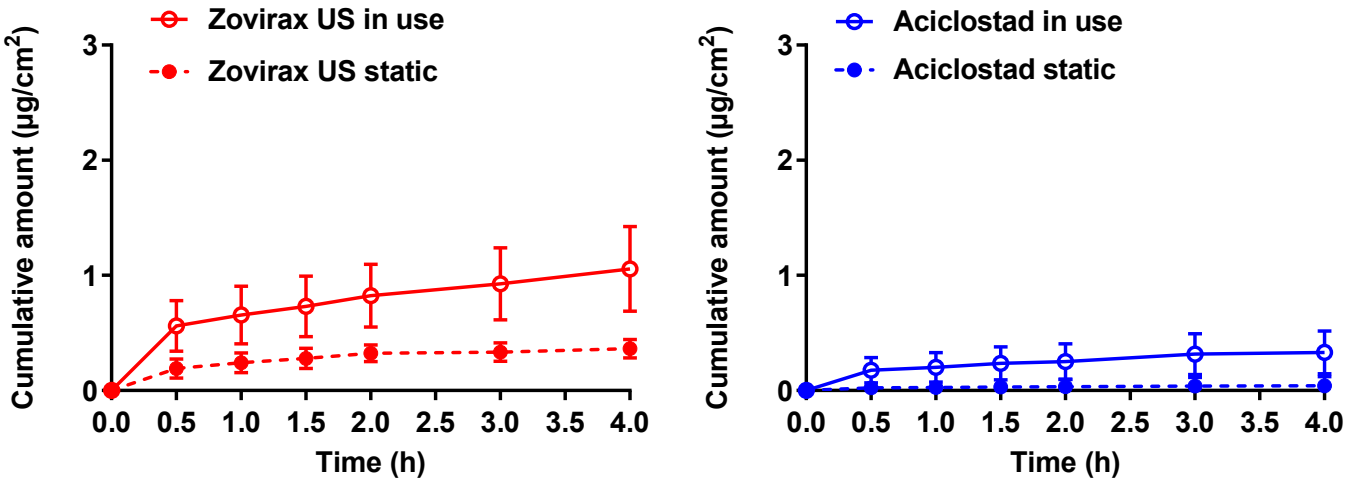


Does how a product is applied to the skin also change the product microstructure (Q3) and resulting IVPT?

- In use (rubbing onto the skin for 30sec) led to a reduction in acyclovir particle size and redistribution of acyclovir in the various phases



The IVPT for both Zovirax and Aciclostad suggests that rubbing enhances permeation and that this effect is more pronounced for the Zovirax product – indeed the ratio for rubbing/static amount permeated for Zovirax is 8-10 times higher than Aciclostad.



Transition – Acyclovir to metronidazole products

- Acyclovir products have enabled us to understand the impact of variations in:
  - The nature of the excipients (Q1)
  - Product composition (Q2) and
  - Product microstructure (Q3)

  on acyclovir *IVPT* profiles and, in particular, that significant differences arise in the *IVPT* profiles between the Zovirax group of products and two Austrian "generic" products
- In principle, *IVPT* can be related to *in vivo* microperfusion data in their discrimination between products but we have not shown a consistent *in vitro-in vivo* relationship across the various products as yet
- We have shown that how products are used can have a major impact on *IVPT* outcomes

- Can we show similar findings for the more lipophilic active metronidazole?

Composition of Metronidazole products as per prescribing information

Excipients vary & may matter!

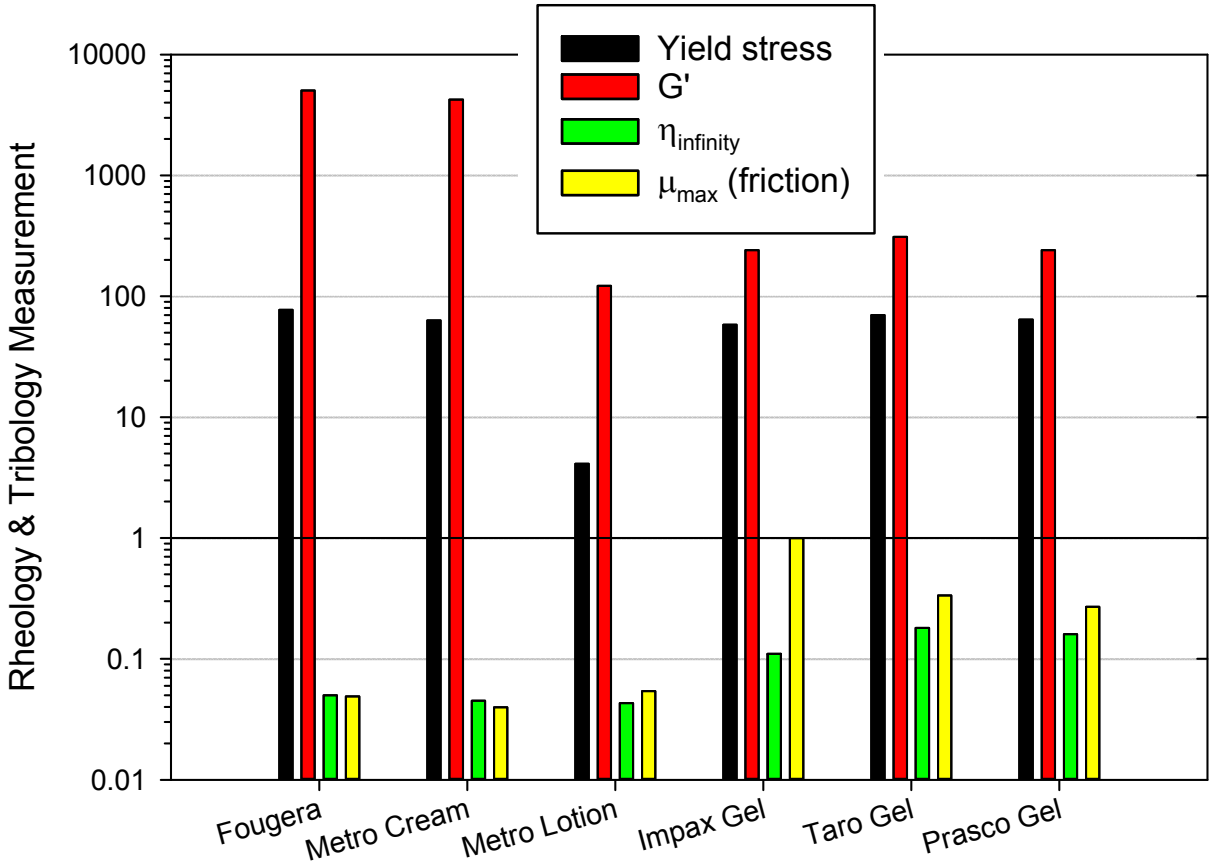
Metro Cream RLD 0.75% (Galderma)	Fougera Cream Generic 0.75%	Metro Lotion RLD 0.75%	Prasco Gel RLD 0.75%	Impax Gel Generic I 0.75%	Taro Gel Generic II 0.75%
Benzyl alcohol	Benzyl alcohol	Benzyl alcohol	Carbomer 940	Carbomer 940	Carbomer 940
Emulsifying wax	Emulsifying wax	Carbomer 941	Edetate disodium	Edetate disodium	Edetate disodium
Glycerin	Glycerin	Cyclomethicone	Methylparaben	Methylparaben	Methylparaben
Isopropyl palmitate	Isopropyl palmitate	Glycerin	Propylene glycol	Propylene glycol	Propylene glycol
Purified water	Purified water	Glyceryl stearate	Propylparaben	Propylparaben	Propylparaben
Sorbitol solution	Sorbitol solution	Light mineral oil	Purified water	Purified water	Purified water
Lactic acid and/or sodium hydroxide to adjust pH	Lactic acid and/or sodium hydroxide to adjust pH	PEG-100 stearate	Sodium hydroxide	Sodium hydroxide	Sodium hydroxide
		Polyethylene glycol 400			
		Potassium sorbate			
		Purified water			
		Steareth-21			
		Stearyl alcohol			
		Sodium hydroxide and/or lactic acid to adjust pH			

Overview of Metronidazole product quality attributes

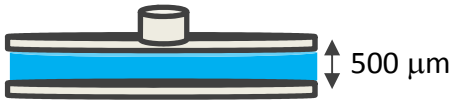
Test	Creams		Lotion	Gels		
	Metro Cream RLD	Fougera Cream Generic	Metro Lotion RLD	Prasco Gel RLD	Impax Gel Generic 1	Taro Gel Generic 2
pH	5.0 ± 0.3	5.3 ± 0.3	5.1 ± 0.1	4.8 ± 0.1	5.4 ± 0.1	5.2 ± 0.1
Polymorphs	No difference in polymorphic forms					
Crystal Shape/Crystal habit upon drying on Skin	No crystals	Rectangular crystals	Irregular crystals	Rectangular and Branched crystals		
Excipients	Similar as per prescribing information (PI)		Different from cream composition	Similar composition in between them as per PI and different from creams		
Loss of Water	Lower than other products		In between creams and gels	Higher than creams and similar among them		
Globules	Globular structure		Globular structure	No globules appeared		
Microstructure (Without inclusions)	Classic emulsion based microstructure		Classic emulsion based microstructure	Visible polymer matrix		
IVPT						
Cumulative amount 48 hrs (µg/cm^2)	45.1 ± 4.4	51.8 ± 4.9	35.3 ± 6.1	12.3 ± 1.6	9.7 ± 0.8	13.8 ± 2.1
AUC – Flux curve	44.2 ± 5.4	53.0 ± 8.0	29.3 ± 6.5	13.4 ± 2.9	10.2 ± 1.7	15.6 ± 3.7
Jmax (µg/cm^2/h)	1.5 ± 0.3	1.9 ± 0.4	1.1 ± 0.4	0.6 ± 0.1	0.6 ± 0.1	0.9 ± 0.3
Tmax (h)	24	16	32	8	8	4

Rheology and Tribology of Metronidazole Creams

- **Aim:** To evaluate 'in use' properties of Metronidazole creams/lotions/gels.
- **Measurement includes:** shear stress sweep (apparent yield stress), linear viscoelasticity (G'), viscosity at high shear rates (η at 10,000 s^{-1}), & lubrication/tribology (friction, μ_{max}).
- **Result Summary:** several samples that have similar low-shear rheology (G', yield stress) are differentiated by their high-shear η and lubrication properties.

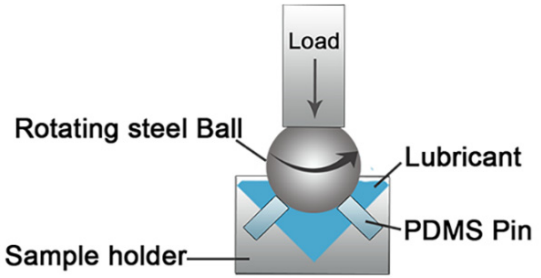


Rheology:



Thin film rheology:

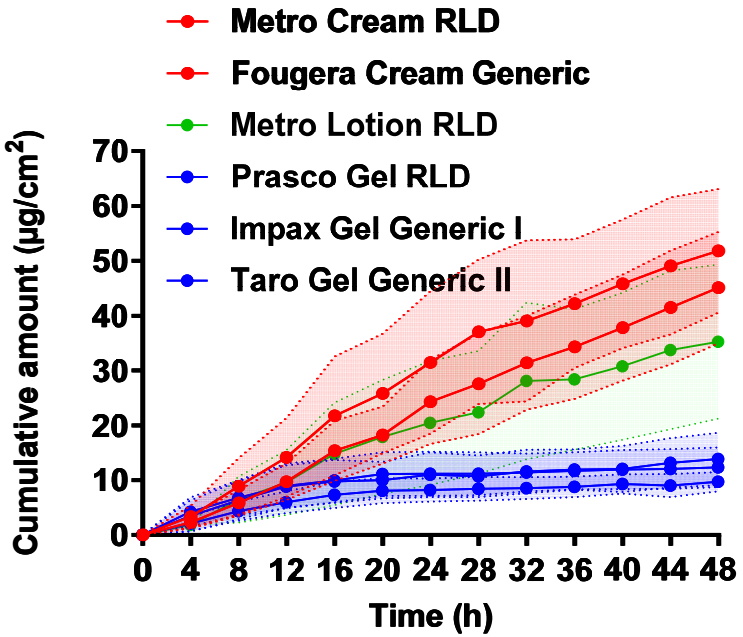
Tribology measure:

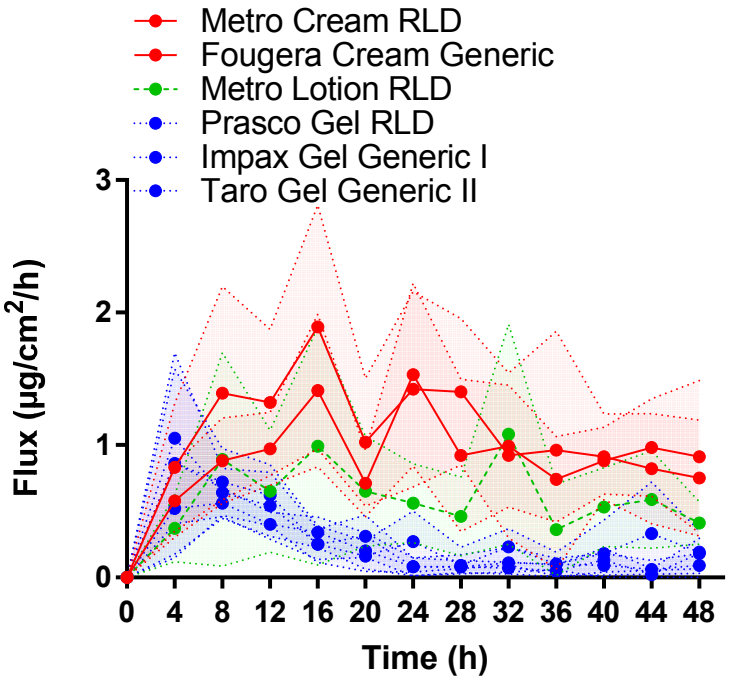


Work by Prof Jason Stokes, Dr Heather Shewan and Dr Yousuf Mohammed from UQ

Q1, Q2 and Q3 variations between product classes - Does this impact on IVPT?

- Q1, Q2 and Q3 could vary between product classes - Is this associated with change in IVPT?





Data shown as mean ± 95% CI; Each point is the mean of 9* (3 donors & 3 replicates per skin)

Meaning in parallels?

- IVPT cream ≥ lotion > gel and
- Tribology (friction) cream ≤ lotion < gel

Why are the gels and creams non-bioequivalent – how do these products differ?

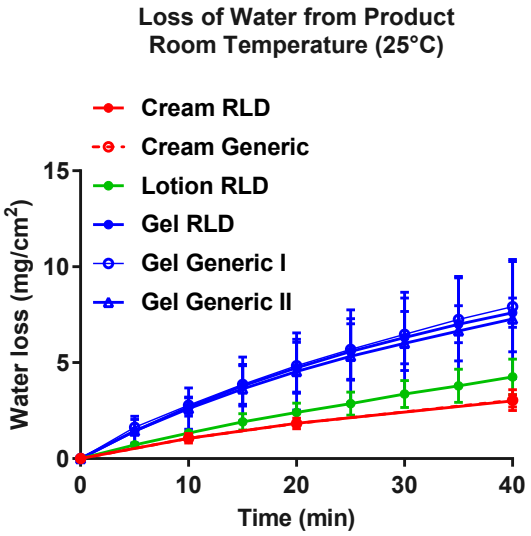
- Q1 (content) and Q2 (amounts)
  - thermodynamic activity &
  - enhancer effects

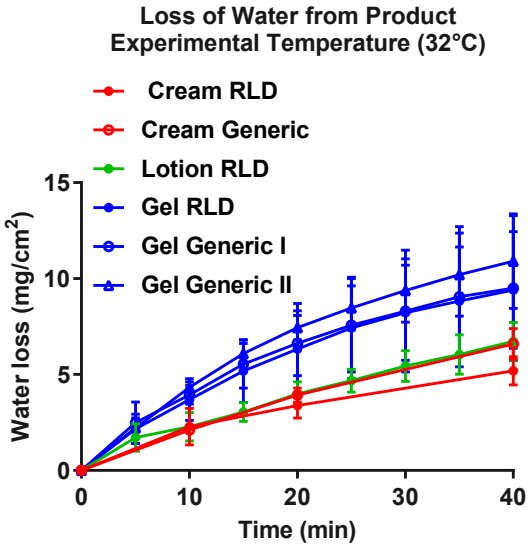
- Microstructure differences
  - Qualitative and quantitative differences may be present; but here we emphasize – all three different product classes (Creams, Lotions and Gels) have unique structural features

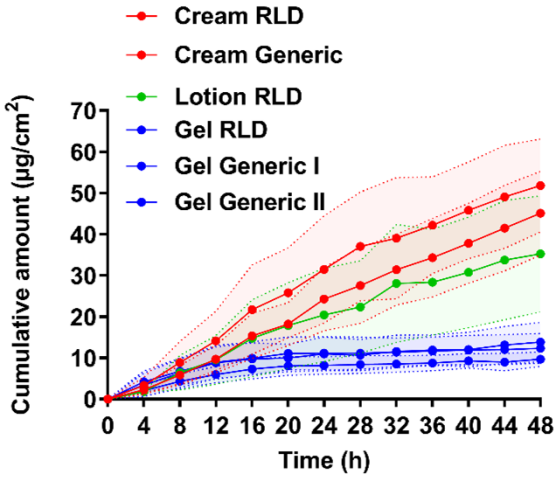
- How did the different microstructures affect Quality and Performance?
  - Emulsion based microstructures could presumably have better solubilisation and hence more available drug – we are in the process of simulating the amount of Metronidazole in each of the products under static as well as in use conditions.
  - Textural properties and spreading would be different
  - Evaporation

Product drying

- The Gels have a very high water content and would therefore evaporate much quicker?
  - ❖ How would this impact the Metronidazole in solution?
- We observed the product drying on the skin surface
- To what extent does this contribute to the observed IVPT differences?

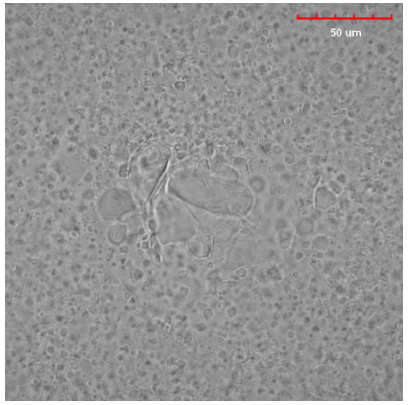






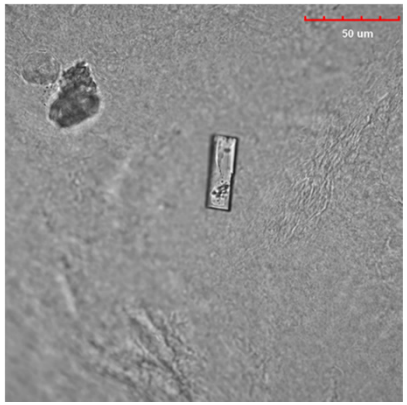
Crystal structure upon drying Metronidazole products

Cream RLD



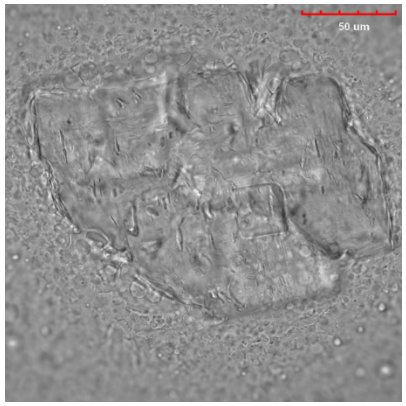
No Crystals

Lotion RLD



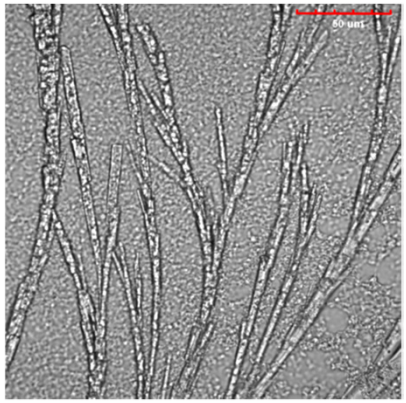
Rectangular Crystals

Cream Generic

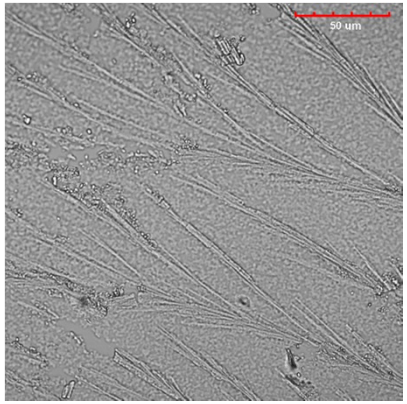


Rectangular Crystals

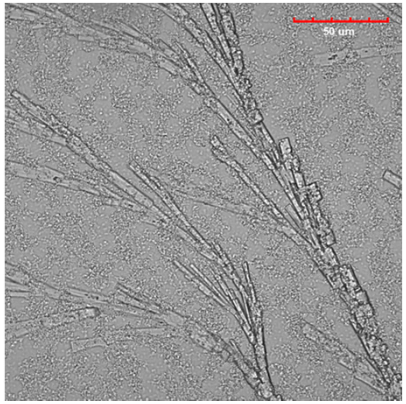
Gel RLD



Gel Generic -1



Gel Generic - 2



Rectangular Crystals forming branched structures

Conclusions

- How far have we come?
  - We have developed an elaborate tool box of methods for evaluation of Quality Attributes.
  - Some of these attributes have been found to be critical to product performance
  - We have also developed different product performance testing tools (IVPT) in varied conditions (Skin prep, donor dose, receptor phase, application methods etc.)
- Where to from here?
  - Our goal is to further develop these techniques and test the whole range of semisolid product microstructures with molecules of different physicochemical properties
  - Ultimately, these tools should be able to facilitate a quality and timely generic product approval process

Acknowledgements

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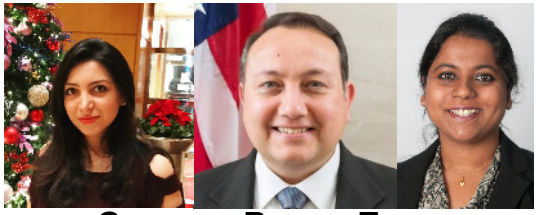
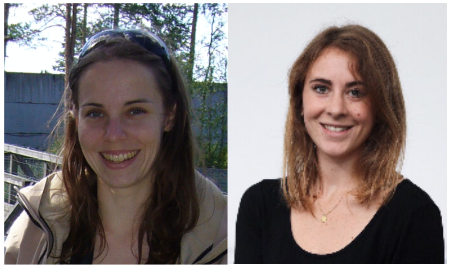
FDA Team



Germany Raman Team



Germany Simulation and Modelling Team

Queensland Team

South Australia Team

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