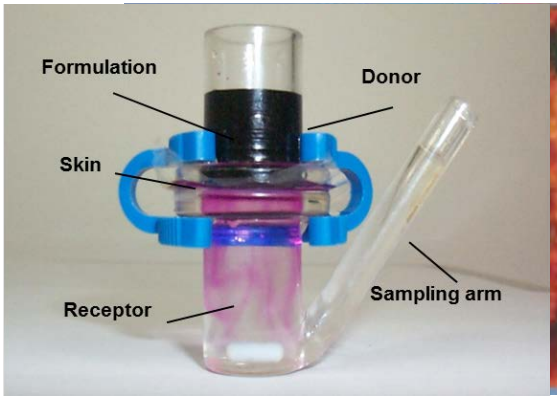

Drug Delivery Australia
Wollongong
23-24 October 2017

Correlation of physicochemical characteristics and *in vitro* permeation test (IVPT) results for acyclovir topical products

Heather Benson

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THE UNIVERSITY OF QUEENSLAND AUSTRALIA

Overview of where we started this study

How can we characterise semisolid products?

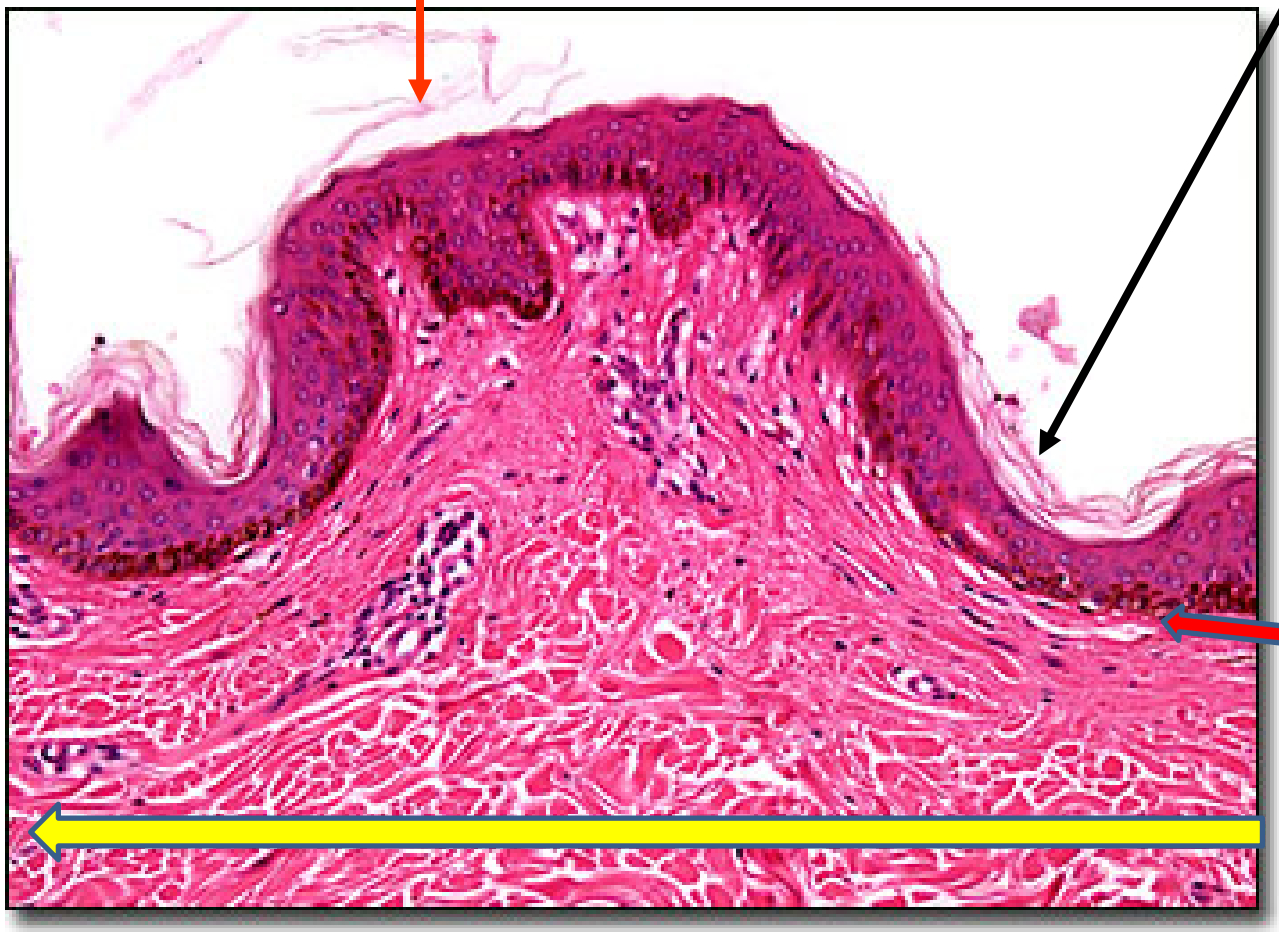
- Q1, Same components as the reference-listed drug product;
- Q2, Same components in same concentration as the reference listed drug product;
- Q3, Same arrangement of matter (microstructure) *(often assumed, but not always, with same components in same concentration)*

How do we define their quality?

- Quality should be by design & testing
- 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

Testing in terms of the skin morphology & sites of action

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

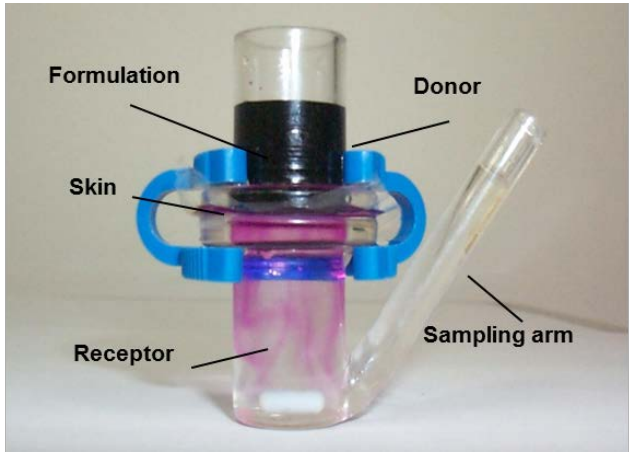
Epidermal membrane sampling site

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

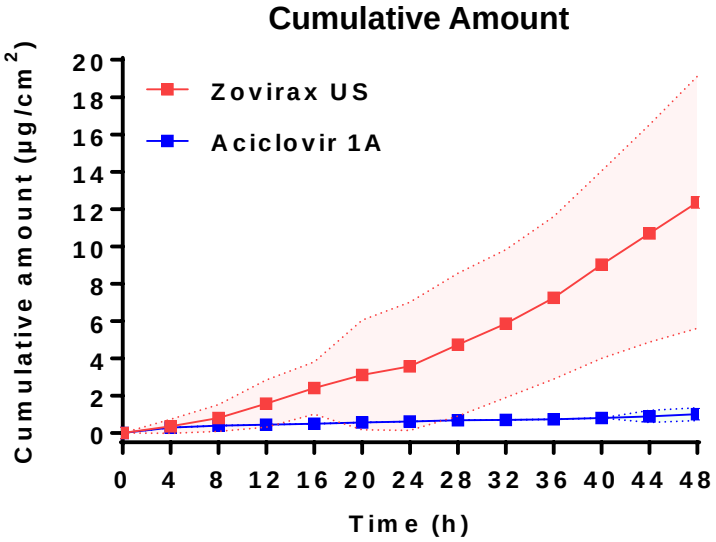
One focus is *In Vitro* Permeation Test (IVPT)

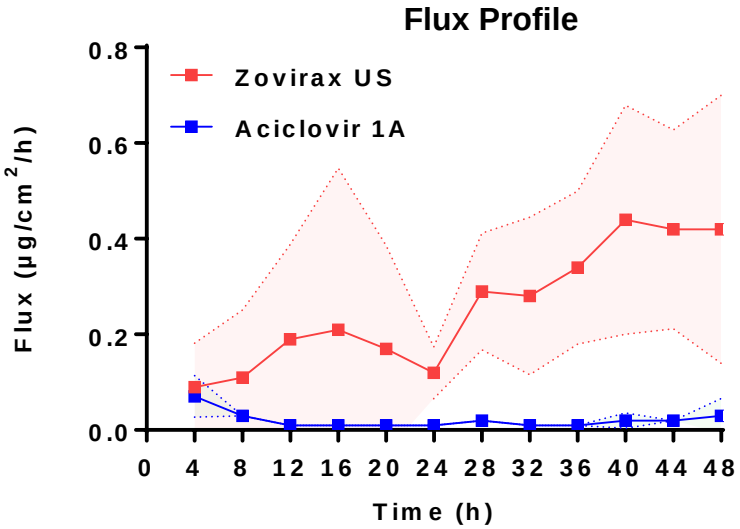
Stratum corneum, epidermis, dermatomed skin & full thickness skin in a static or flow through Franz diffusion cell

- Long history
- Robust
- Simple
- Precise
- Reproducible



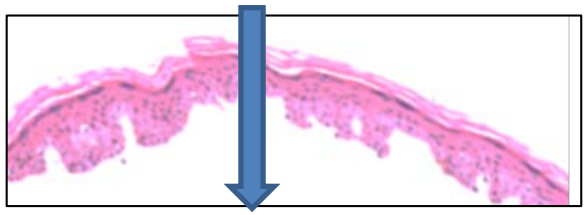
Here, epidermal membranes for 2 Acyclovir products





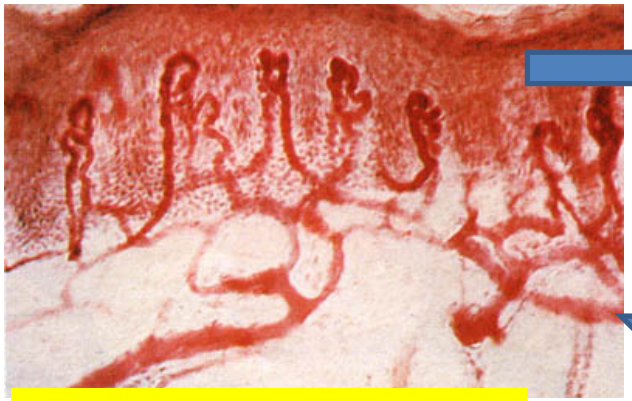
Data shown as mean ± 95% Confidence Interval (CI)
Each point is the mean of 9* (3 donors & 3 replicates per skin)

In principle, one can also estimate *in vivo* profiles from *in vitro* permeation test (IVPT) data



In vitro permeation test (IVPT) results for epidermal membrane

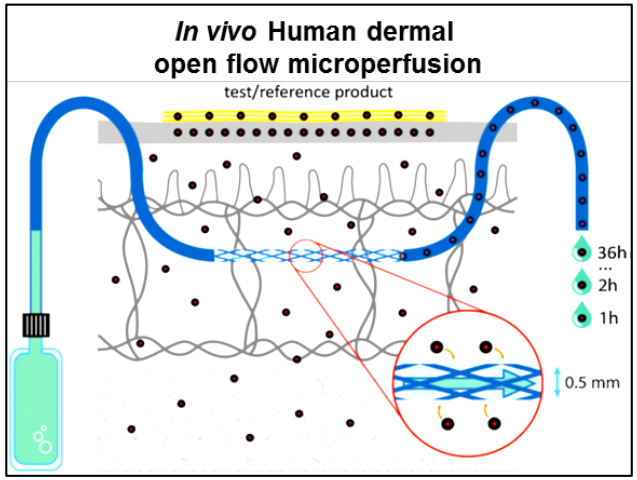
* Convolution with *In vivo* disposition in dermis



In vivo dermis sampling site

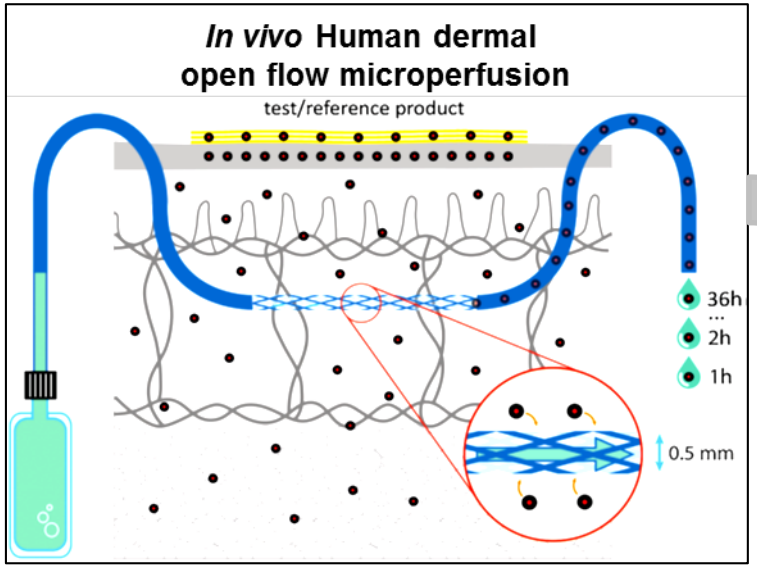
Removal by blood supply superficial to dermis sampling probe

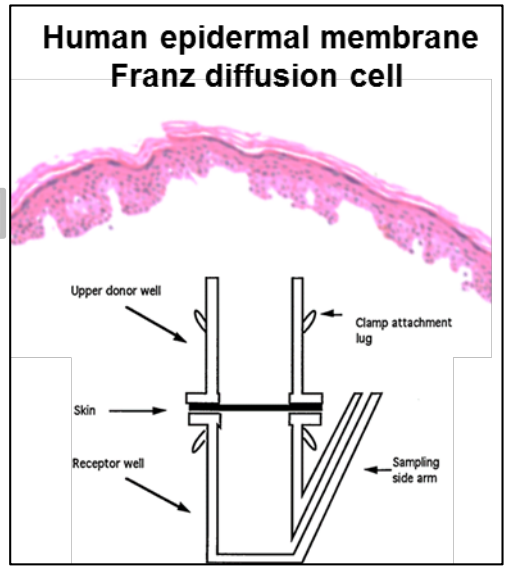
Transport to deeper layers by diffusion and convective dispersion



In vivo dermis sampling site output

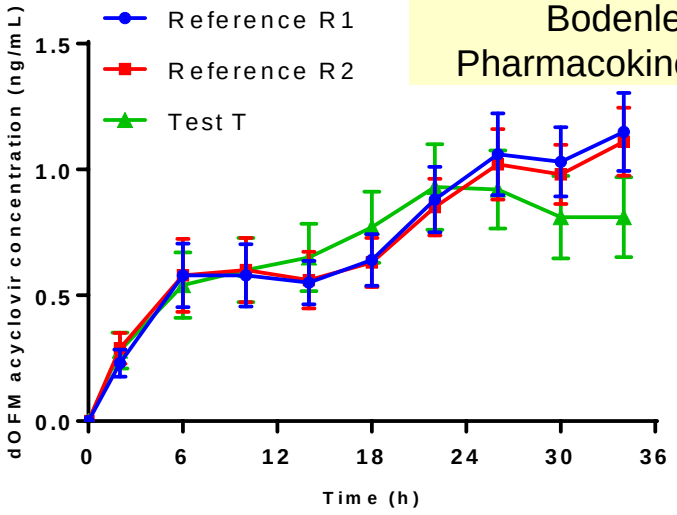
And two examples in practice - *In vivo* vs. *In vitro*





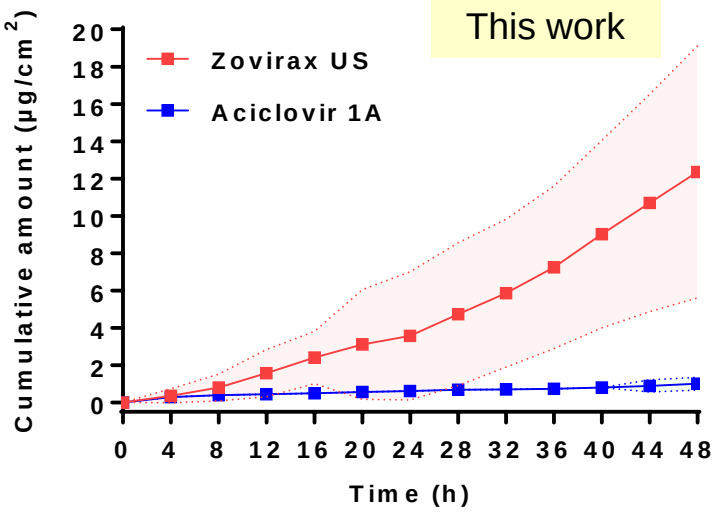
***In vivo* concentration in dermis vs. time**

Bodenlenz et. al. Clin Pharmacokinet (2017) 56:91–98



Cumulative amount permeated vs. time

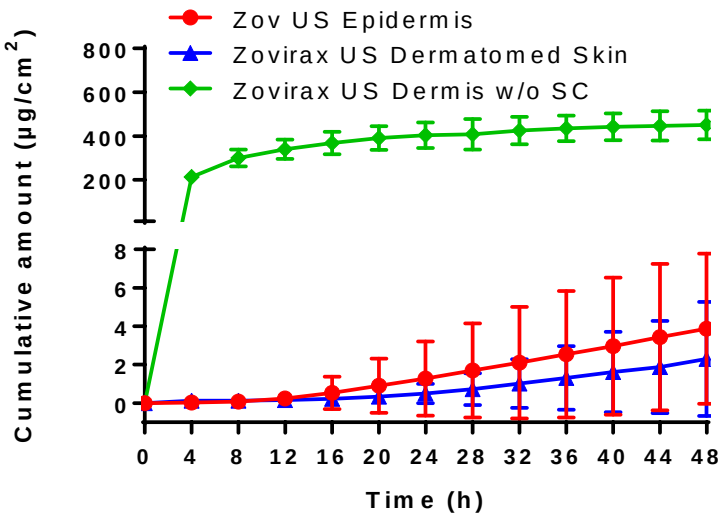
This work

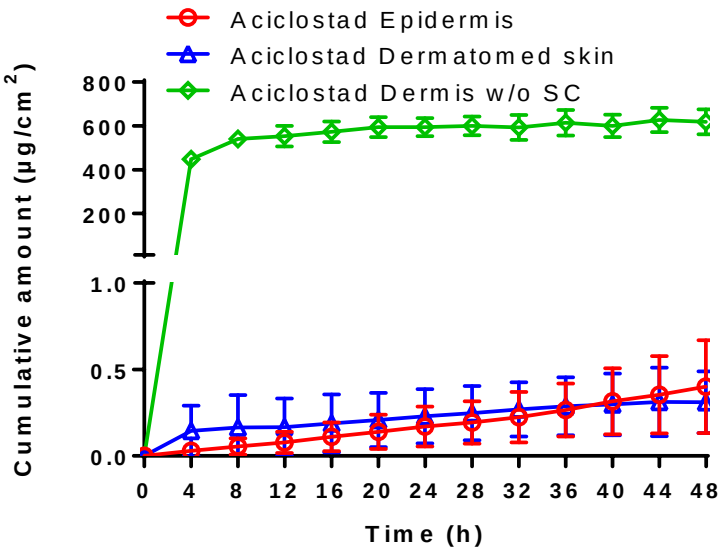


In Vitro Permeation Test (IVPT) Studies: epidermal membranes v dermatomed skin

We found similar permeation profiles for 2 acyclovir products using human epidermal membranes & dermatomed skin

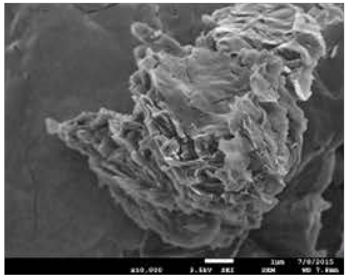
- Dermal membranes: confirm SC is main underlying barrier
- Either epidermal membranes or dermatomed skin could be used in IVPT studies
- Skin barrier integrity is an important control component to get right.

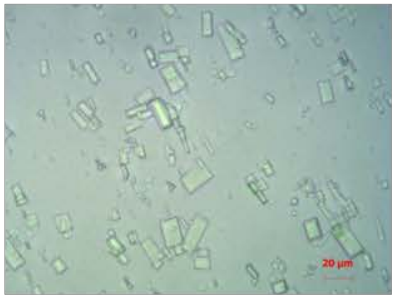


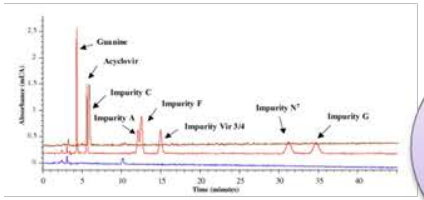


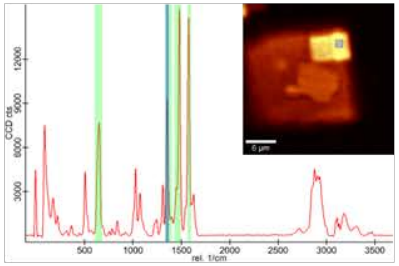
Data shown as mean ± 95% Confidence Interval (CI)
Each point is the mean of 9* (3 donors & 3 replicates per skin)

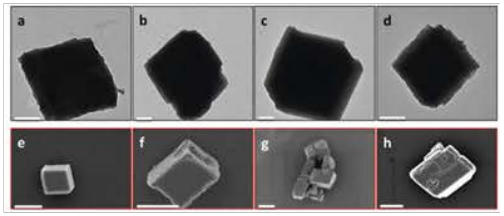
***In vitro* testing for product quality** by an articulated battery of physicochemical tests - potential critical quality attributes, i.e. Q3

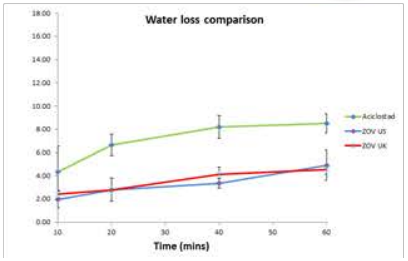


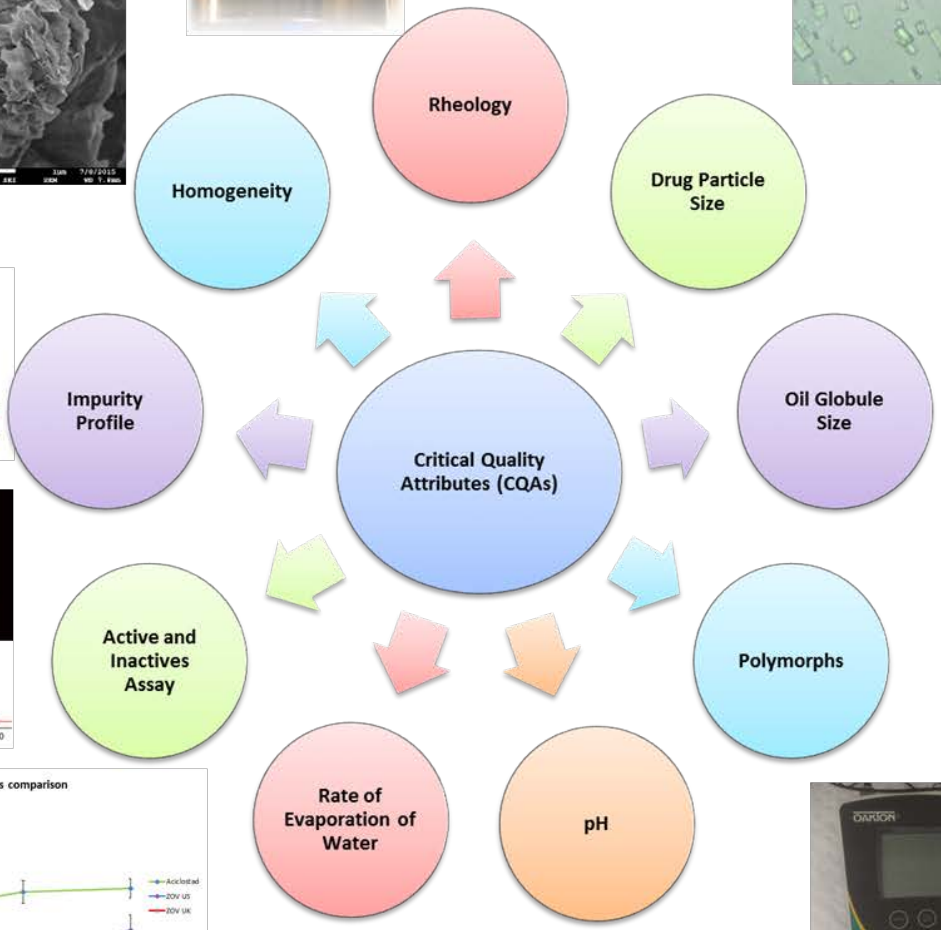












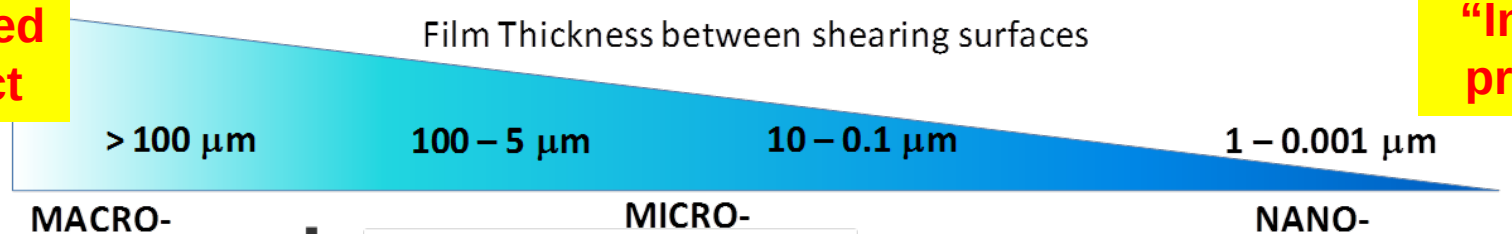
Rheology and tribology as particular critical quality attributes

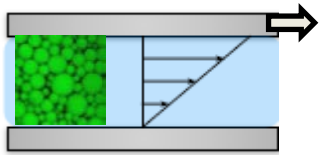
In-use physics: Multiple scales of deformation

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

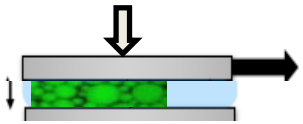
Packaged product

"In-use" product

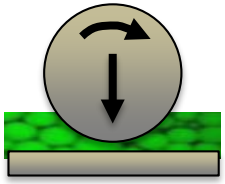




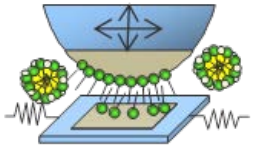
Rheology[1] (fixed gap)



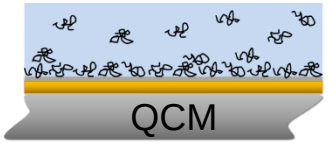
Narrow gap / GDR[2] (thin film)



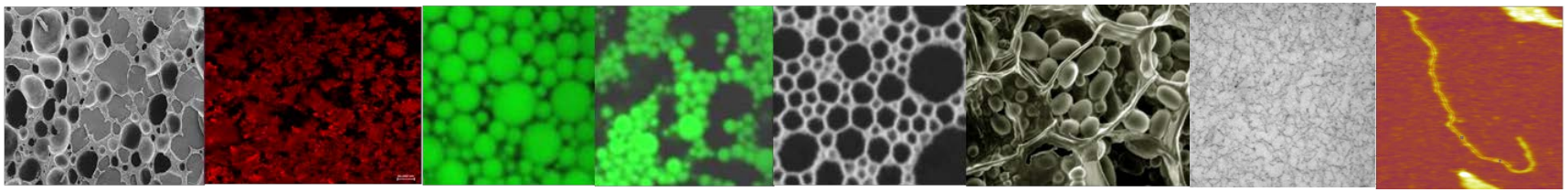
Tribology[3] (fixed load)



Micro & Nano Mechanics[4]



Adsorbed polymer films[5]

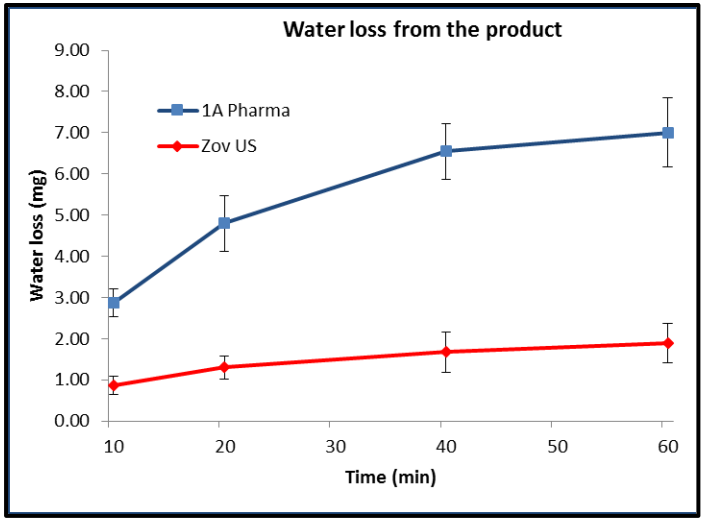


Adaptation of slide courtesy of Prof. Jason Stokes, UQ

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 properties
  - ❖ Evaporation also differs
- 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

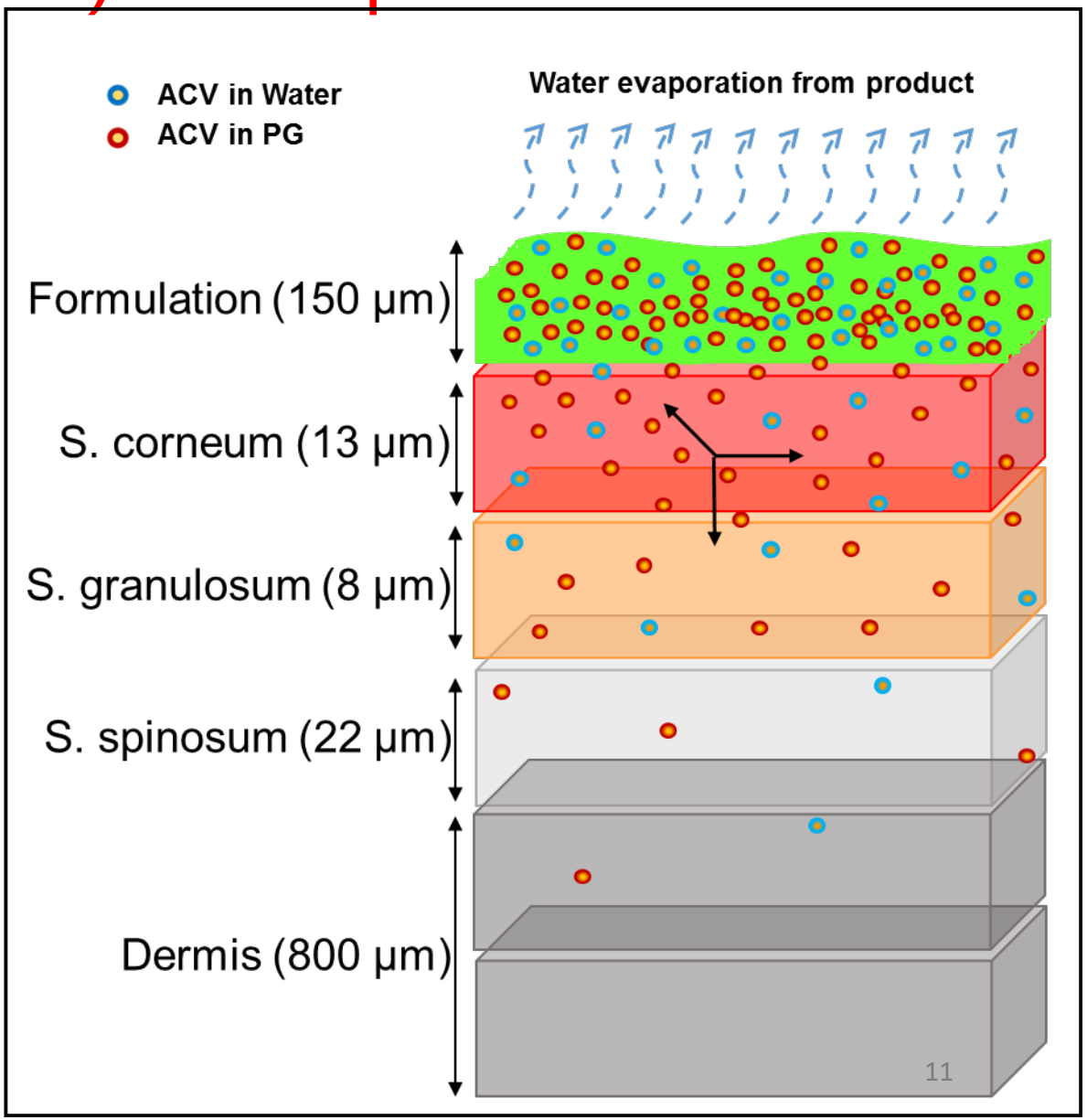
Ingredient Name	Zovirax (U.S.)	Aciclovir 1A Pharma (Austria)
Acylovir concentration	5% w/w	5% w/w
Propylene glycol (PG)	**40% w/w**	**15% w/w** *1
Water Content	**≈ 1/3 w/w**	**≈ 2/3 w/w**
Other Ingredients:	Cetostearyl alcohol Mineral oil Poloxamer 407 Sodium lauryl sulfate Water White petrolatum	White 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

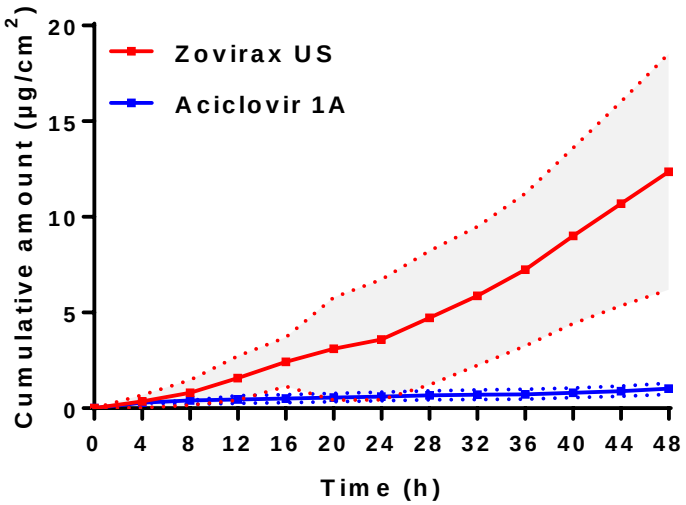
- Propylene glycol (PG) and water, known penetration enhancers, are two excipients present in all products
- Our work has also shown that PG and water can carry solutes into the SC & promote their permeation
- Both are likely to promote direct acyclovir uptake into the stratum corneum
- Potentially,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



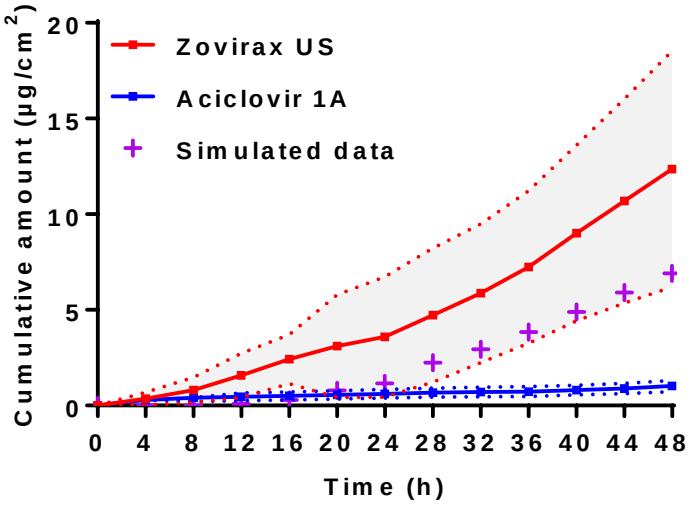
Understanding differences in *IVPT* profiles for acyclovir for 2 products

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?

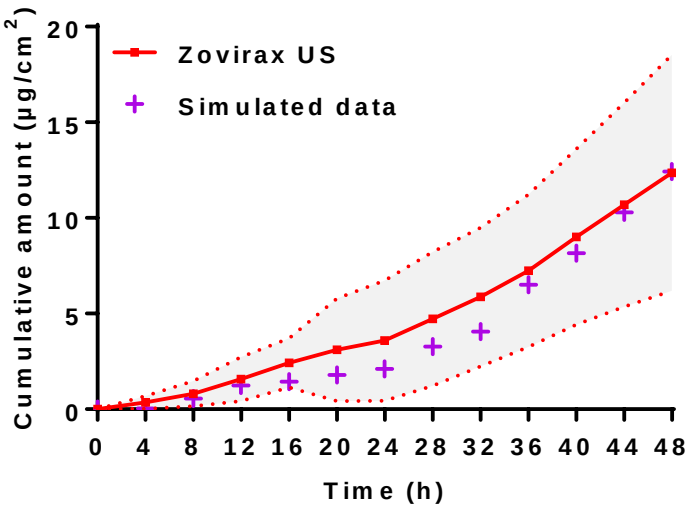


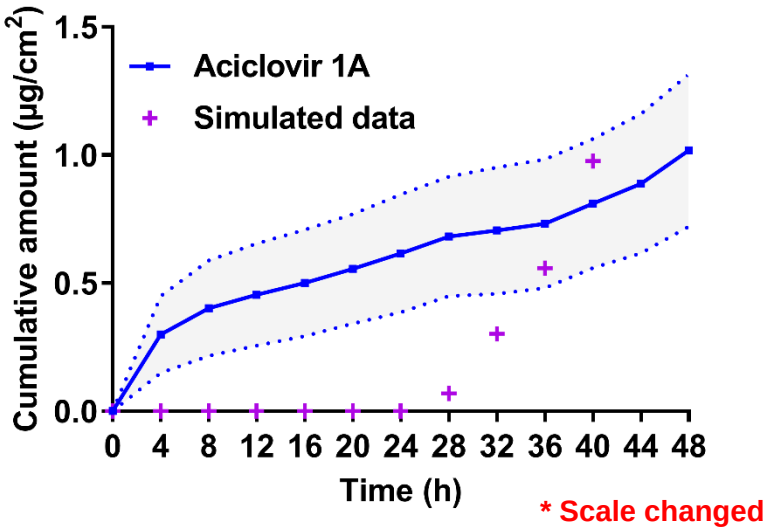
$K_{ACV,SC}$ = 0.24; h_{SC}= 13 µm;
$D_{ACV,SC}$= 2.54 x 10^{-7} µm²/s

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

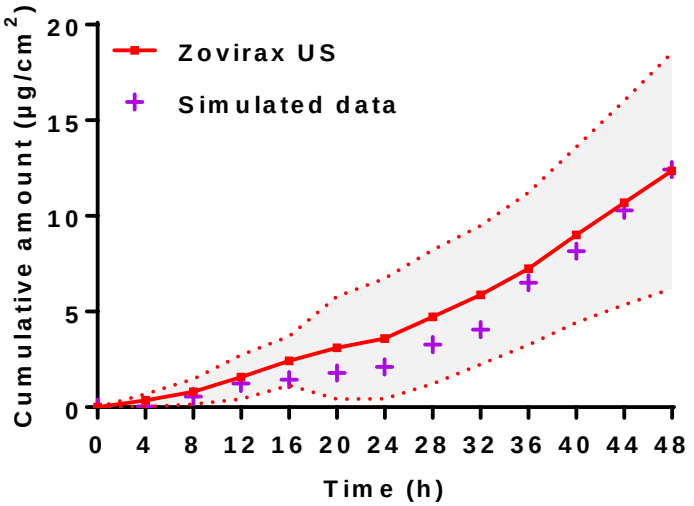
- When the effect of PG, a known ingredient in the formulations and a known solubility and penetration enhancer, is taken into account the simulated profile for Zovirax matches with the *IVPT* data.
- However, Aciclovir 1A still does not fit. Is there something more going on?

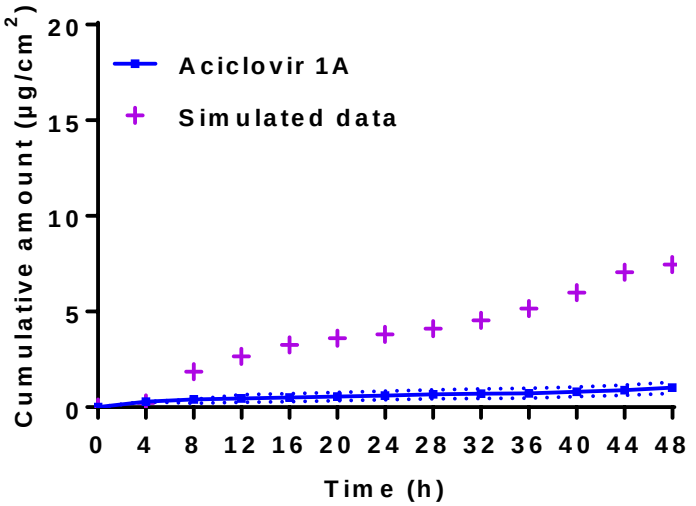
$K_{PG,SC}$ = 0.29; h_{SC}= 13 µm;
$D_{PG,SC}$= 1.03 x 10^{-4} µm²/s

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

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

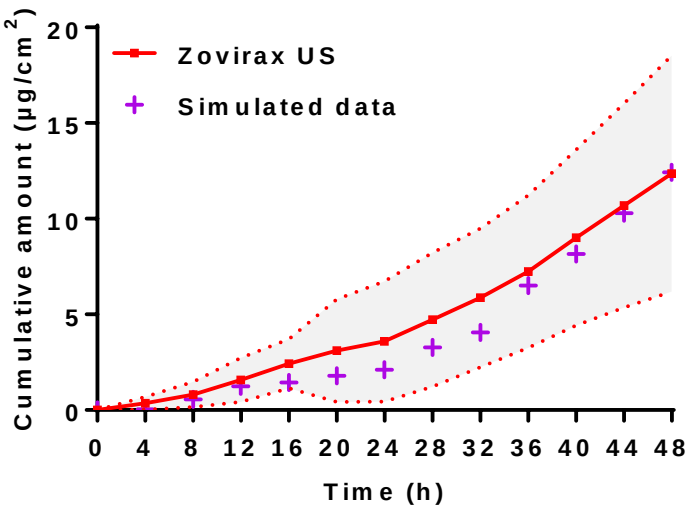
$K_{water,SC}$ = 0.18; h_{SC}= 13 µm;
$D_{water,SC}$= 1.07 x 10^{-3} µm²/s

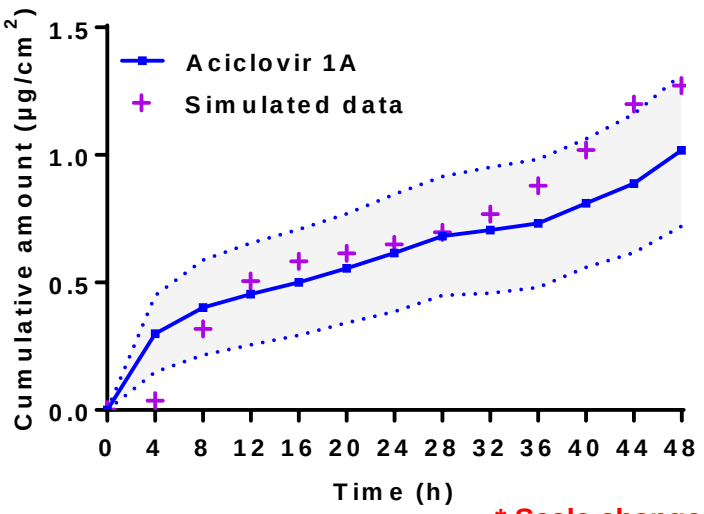
$D^*_{ACV,SC} = D_{ACV,SC} + 0.00003 \times C_{PG,SC} + 0.000043 \times C_{water,SC}$

- 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



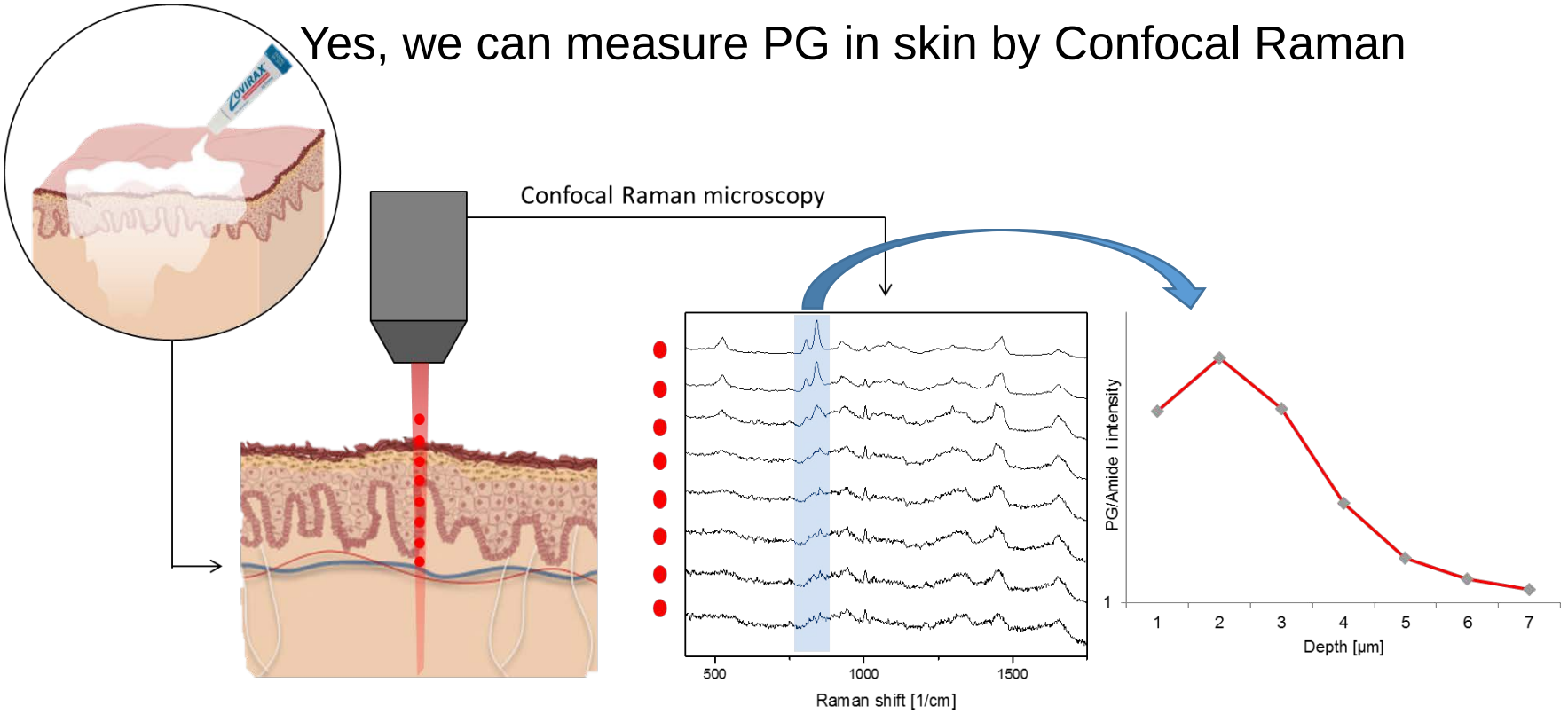


*** Scale changed**

- 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

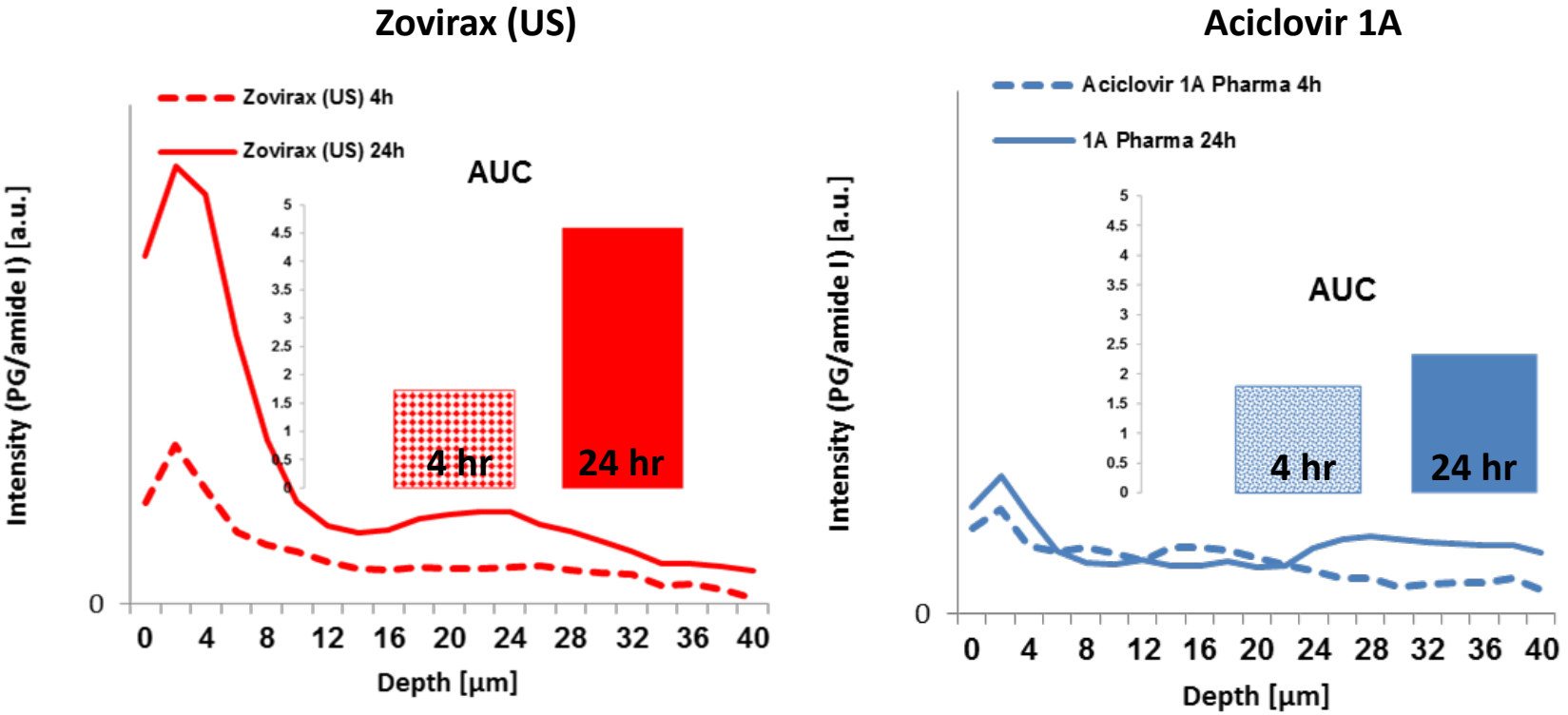
Can we verify the theoretical predictions experimentally?

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

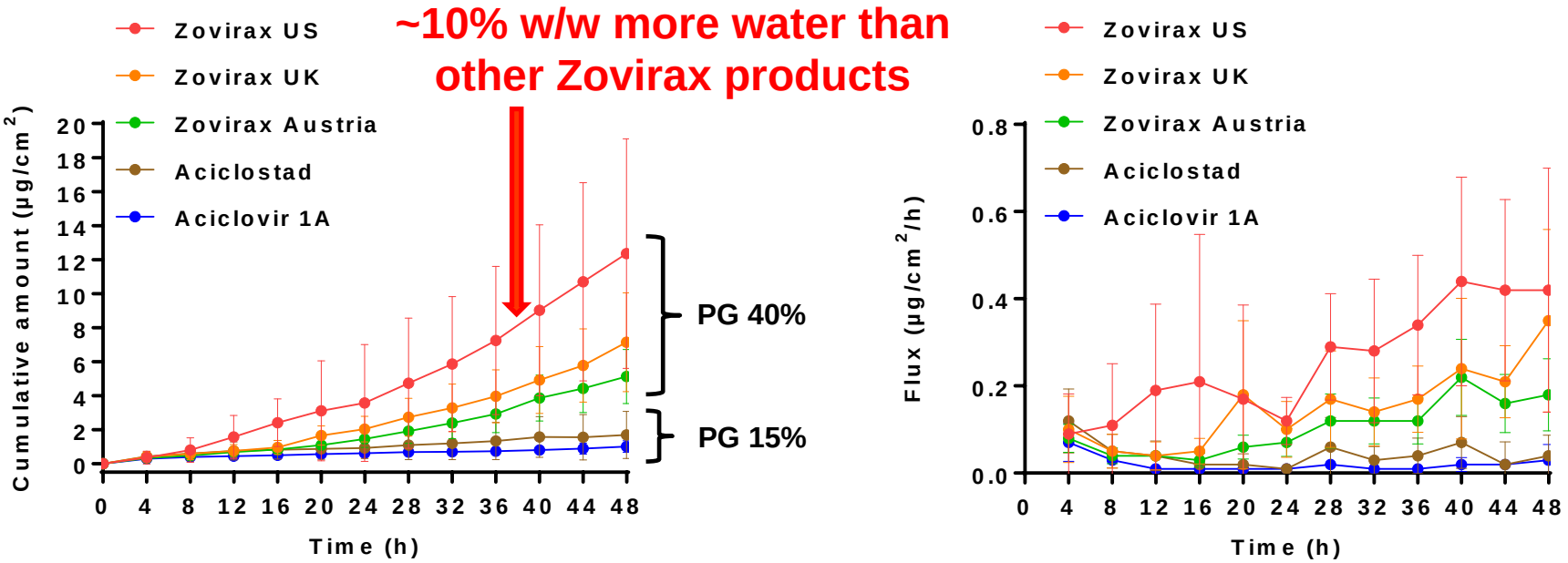
We find…



- Zovirax (US) 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

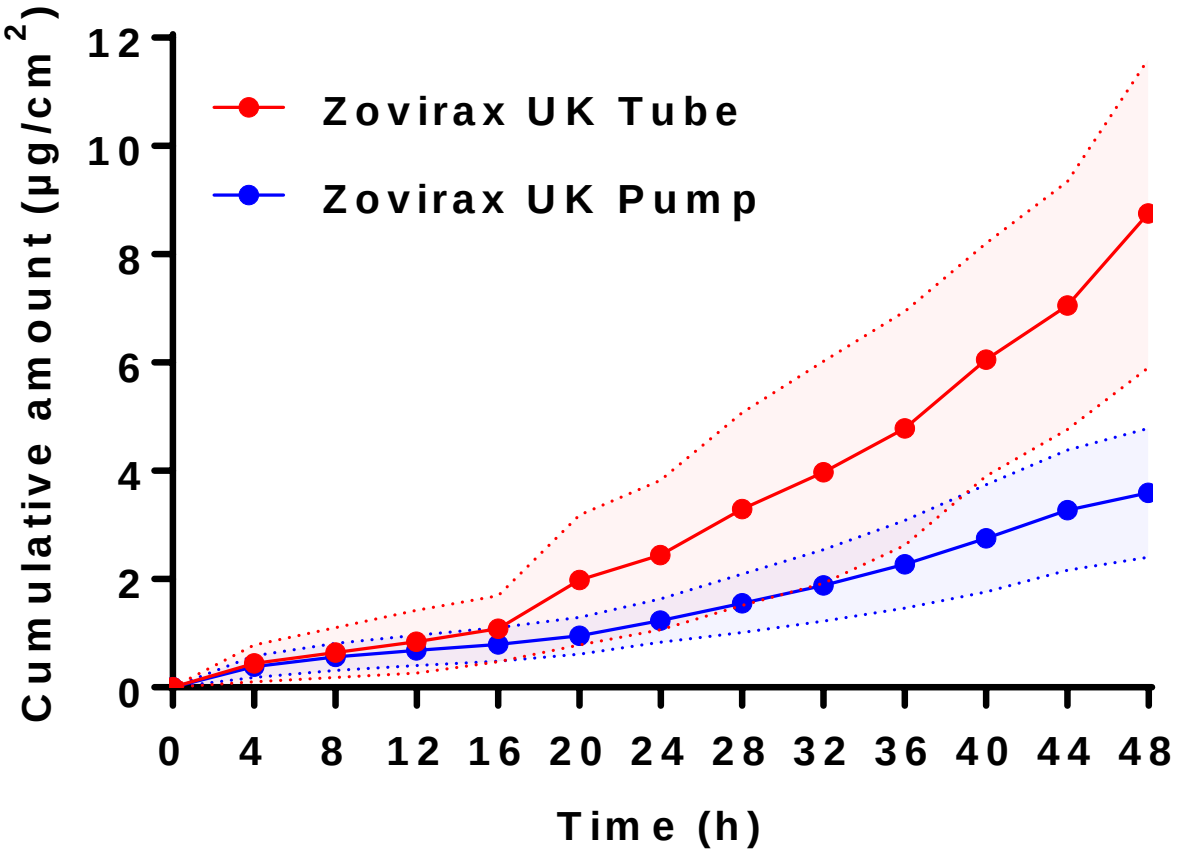
Quality Attribute	Zov US	Zov UK	Zov Austria	Aciclostad	1A Pharma
pH	6.4	7.2	6.8	4.6	5.9
Polymorphs	No difference in polymorphic forms				
Crystal Shape/Crystal habit	Rectangular			Irregular	
Predominant particle size range (µm)	5 -10	5 -10	5 -10	0 - 5	0 - 5
IVPT (Cumulative amount 48 hrs µg/cm^2)	11.0 ± 2.7	7.2 ± 1.5	5.1 ± 0.7	2.2 ± 0.6	1.0 ± 0.2
Excipients	NA	Different from reference product		Different from reference product	
Zero Shear Rheology	NA	Different from reference product		Similar to reference product	
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	

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

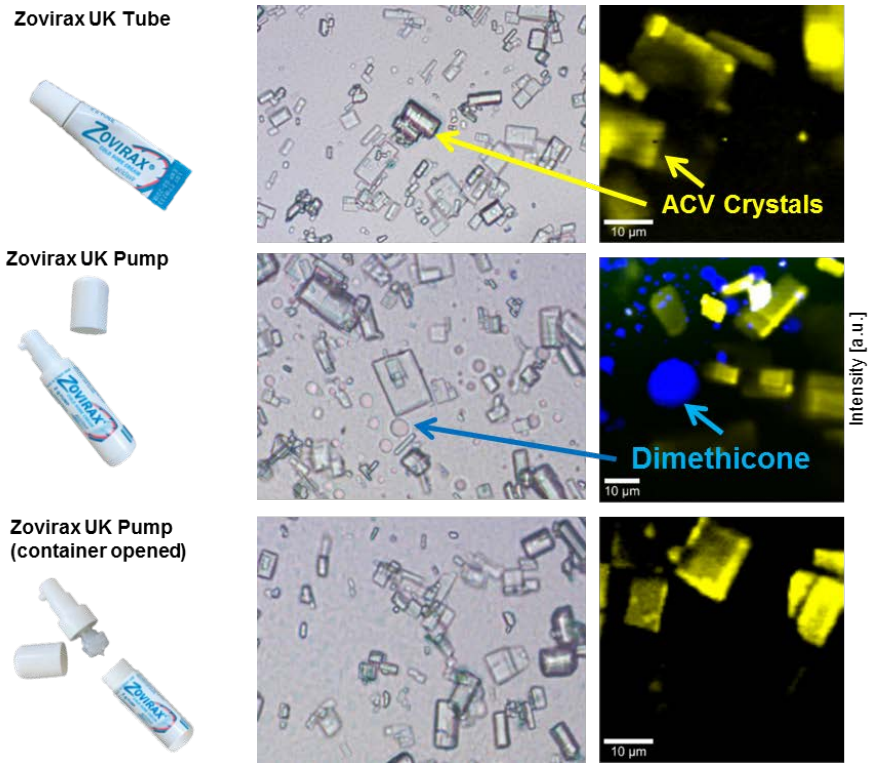
- Confocal Raman suggests that pumping affects the crystal habit for acyclovir and leads to the formation of dimethicone globules
- Rheology suggests that the packaged tube and pump have a similar yield stress but is higher in the product after pumping– due to dimethicone agglomeration?

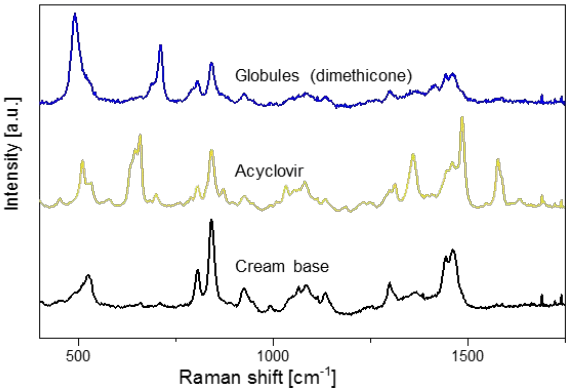
Yield stress from strain sweep (Pa)

78 ± 1.3

182 ± 0.6

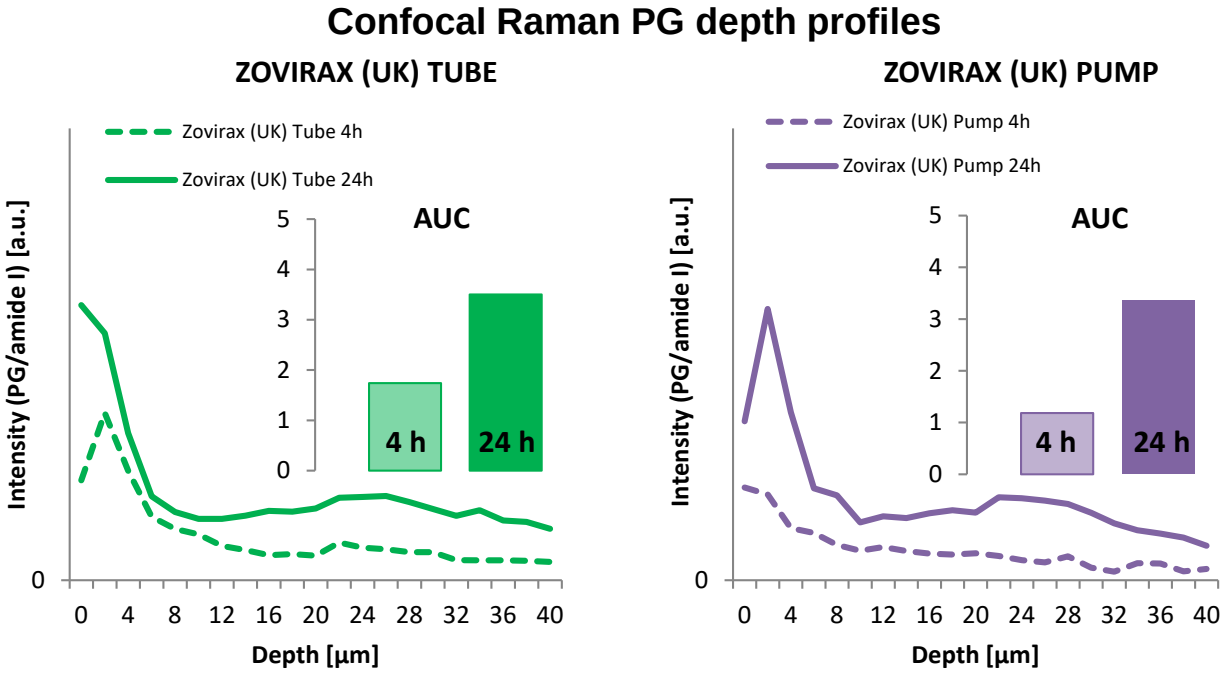
70 ± 10





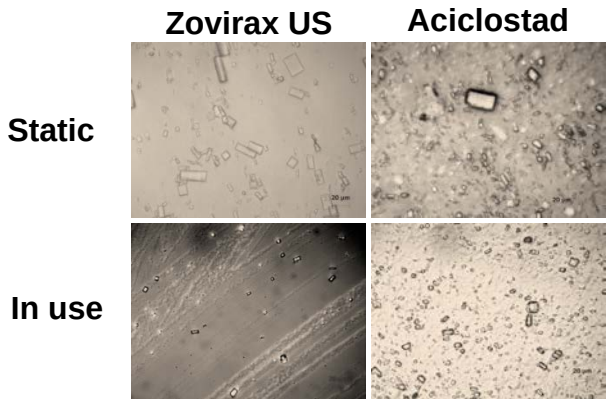
Correlation of Q3 microstructure with performance (Example I)

- 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 affect the bioavailability of the enhancer (PG)?

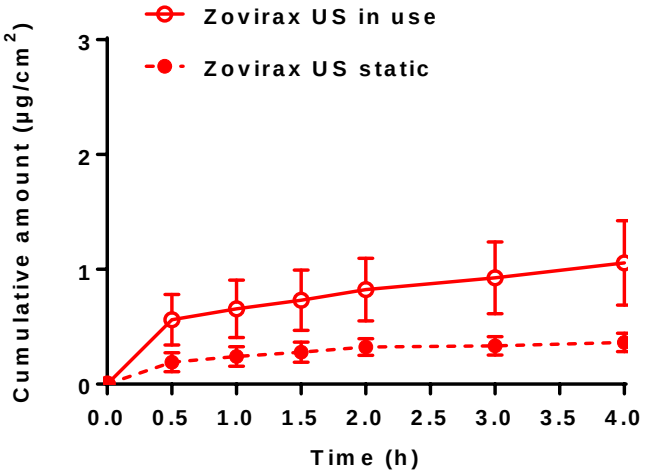


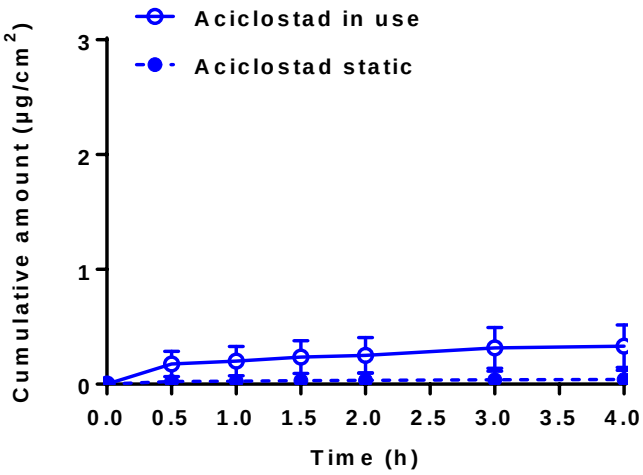
Does how a product is applied to the skin also change the product microstructure (Q3) and resulting IVPT?

- 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.





Summary – Acyclovir 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 further work is required to establish a consistent *in vitro-in vivo* relationship across the various products
- We have shown that the way in which products are used can have a major impact on *IVPT* outcomes

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

Conclusions

- How far have we come?
  - We have developed an 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

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