Visualizing the penetration of semisolid topical formulations in excised human skin by non-invasive confocal Raman microscopy

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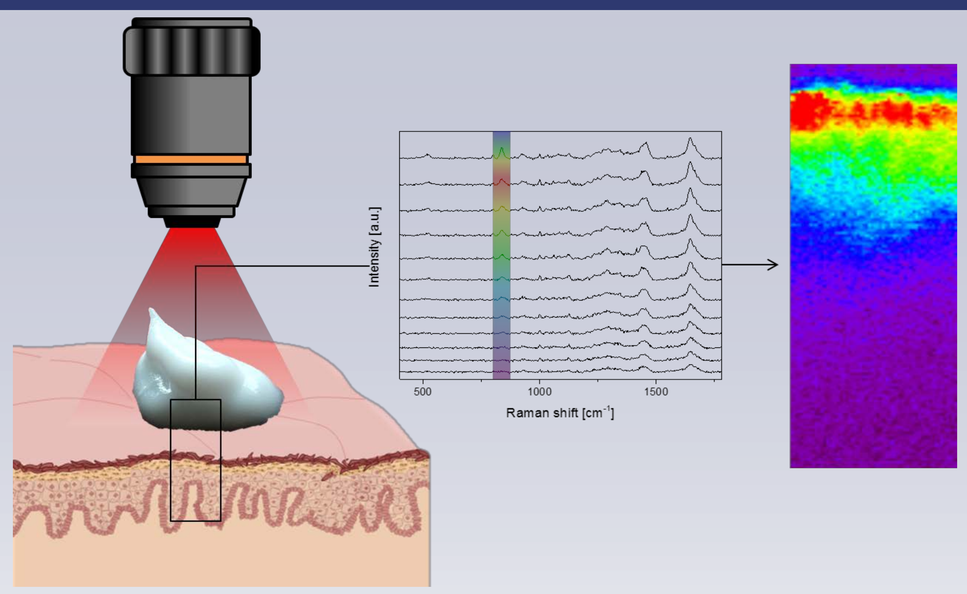
Introduction

The design, manufacture, quality, performance and control of complex semisolid topical drug products collectively requires an understanding of the compositional, physical and structural characteristics of the formulation. It also requires an understanding of how the topical dosage form interacts with human skin. Existing techniques provide limited information about the compositional changes that may occur within a topical product as a result of its interaction with the skin. Also, established analytical techniques often lack the combination of chemical and spatially resolved compositional analysis, which impeding our ability to elucidate potential microstructural changes within the product and the effects of the product on the skin, itself.

In this study, non-destructive, chemically selective confocal Raman microscopy was implemented for the characterization of creams and their interaction with human skin tissue without the need of dyes, labels or destruction of the sample[1,2].



Methodology & Results

Methodology

Six commercial topical acyclovir cream, 5% products were characterized in terms of their compositional arrangement of matter. Also, the influence of mechanical stress ("in use conditions") on the creams was assessed by applying them onto excised full-thickness human skin and monitoring the skin penetration kinetics of a formulation component, propylene glycol, for up to 24 hours within the upper layers of the epidermis. An Alpha300R$^+$ confocal Raman microscope (WITec GmbH, Ulm, Germany) equipped with a 785 nm laser (50 mW) was used to analyze the chemical properties of the creams (integration time 0.1 sec, step size 0.5 µm) and their penetration into excised human skin tissue (integration time 30 sec, step size 2.0 µm). Hierarchical cluster and subsequent basis analysis were performed to create false-color images.

Raman analysis of semisolid products

In the investigated products, acyclovir crystals are suspended in a cream base as visualized by false color Raman images for one U.S. marketed reference cream and one Austrian generic cream, as shown below. The drug crystals (yellow) as well as the cream base (black) show no significant chemical differences between the two cream products, as shown in the respective Raman spectra (A). Size and shape of the drug crystals vary between the products. Comparison of Raman spectra of the drug crystals of all six investigated products with Raman spectra of the different polymorphic forms of acyclovir identified the presence of the polymorphic form V in all products (highlighted in B).

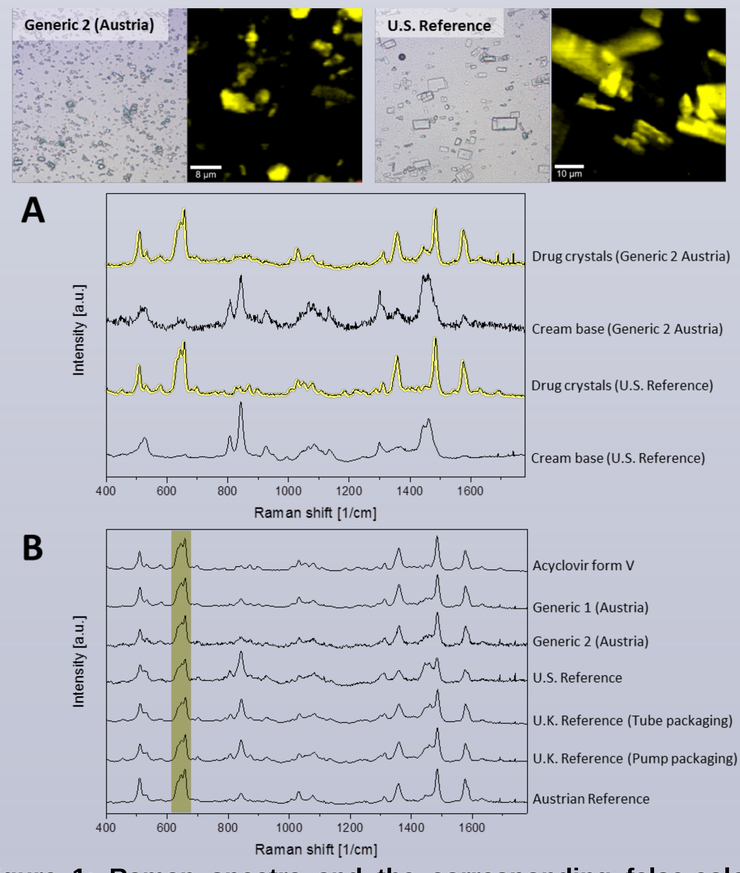


Figure 1: Raman spectra and the corresponding false-color images of two acyclovir creams (A) and comparison of Raman spectra of acyclovir in all six cream products (B).

Effect of mechanical stress on microstructure

Light microscopy and Raman data show that following the application of mechanical stress (pumping), a phase separation is observed in the cream base. The globules observed were comprised of dimethicone. Three-dimensional reconstructions from Raman scans of a U.K. reference cream dispensed from a tube or a pump showed differences in microstructural properties.

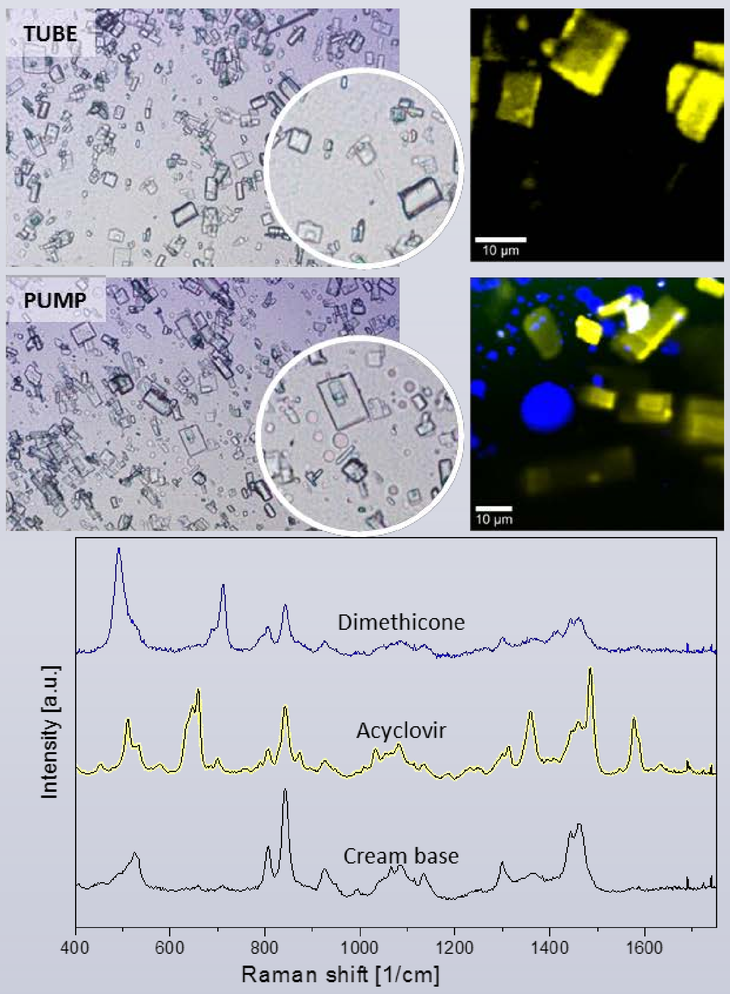


Figure 2: Raman images of U.K. reference acyclovir creams dispensed from in different packaging containers and the respective Raman spectra.

Assessing skin penetration

Raman microscopy was used to acquire penetration depth profiles of the cream products within human skin, monitoring the propylene glycol component. From the resulting Raman spectra, a formulation-associated peak is selected and normalized by a skin-derived peak to account for depth attenuation of the Raman signal. The intensity is subsequently plotted against the penetration depth.

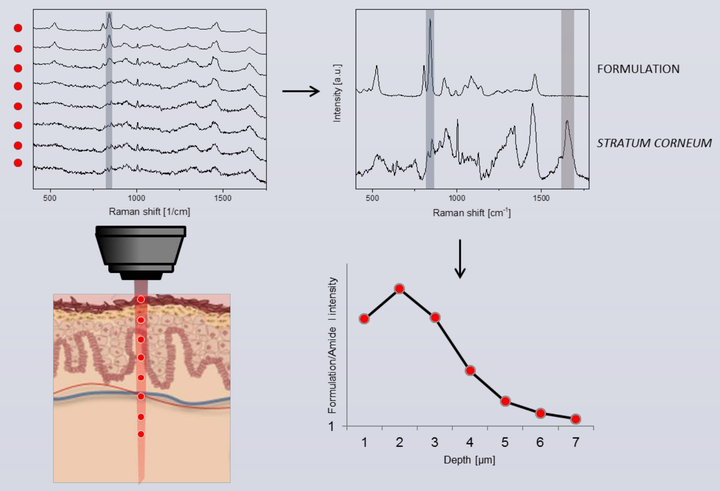


Figure 3: Acquisition of Raman skin penetration profiles.

The epidermal penetration profiles of propylene glycol from six acyclovir creams (4 reference creams, 2 Austrian generic creams) was assessed by Raman microscopy after 4 h and 24 h of incubation on the skin (A). After 4 h, no significant difference was observed between the penetration profiles. After 24 h, the U.S. reference product (red) appeared to show higher Raman intensities of propylene glycol in the uppermost skin layer and deeper penetration compared to other reference and generic creams (yellow, green). These findings for higher propylene glycol permeation correlated with higher acyclovir penetration evaluated by an in vitro permeation test (IVPT) using Franz cells (B).

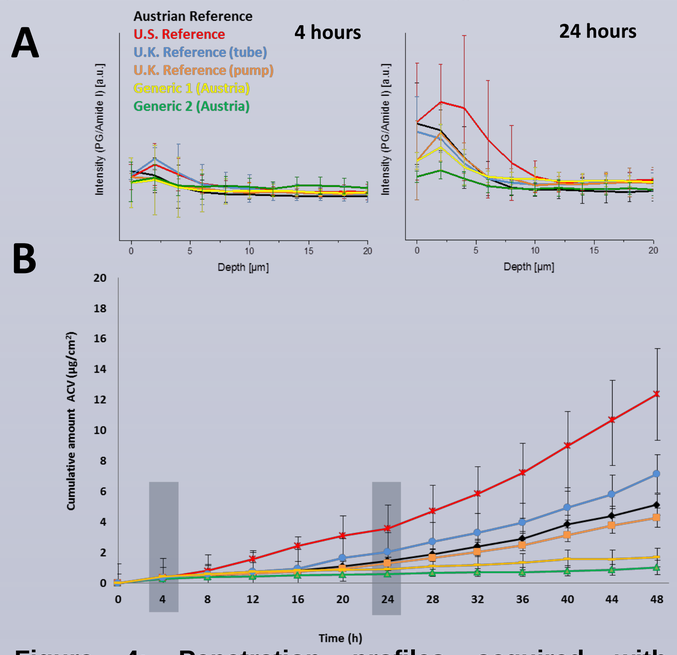


Figure 4: Penetration profiles acquired with Raman microscopy (A) and Franz cell IVPT studies (B).

Conclusions

- Confocal Raman microscopy successfully facilitated the characterization of semisolid topical products by visualizing crucial differences between international reference acyclovir creams and Austrian generic creams.
- Mechanical stress induced by pumping the U.K. reference cream during dispensing, influenced the microstructure of the cream compared to the same product within the pump canister prior to dispensing, or dispensed from a tube.
- Epidermal permeation profiles for the creams studied by confocal Raman microscopy (CRM) monitoring the propylene glycol component of the cream appeared to correlate with acyclovir penetration studied using IVPT studies.
- The novel approaches reported here provide direct insights into the interaction of topical products with skin, and illustrate how complementary, orthogonal approaches can be used to characterize/compare topical product performance.

ACKNOWLEDGEMENT
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References
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