Confocal Raman Spectroscopy: In vivo measurement of physiological skin parameters

PTB vienna
Department of Pharmaceutical Technology and Biopharmaceutics

Lisa Binder[a], Lukas Gruber[a], Claudia Valenta[a,b]
University of Vienna, Austria
[a]Department of Pharmaceutical Technology and Biopharmaceutics
[b]Research Platform "Characterisation of Drug Delivery Systems on Skin and Investigations of Involved Mechanisms"
lisa.binder@univie.ac.at

universität wien

Introduction

Confocal Raman spectroscopy (CRS) is a non-invasive and non-destructive analytical technique used in natural sciences and medicine with steadily increasing fields of application. In the area of skin research it has evolved as a convenient approach for evaluation of skin hydration and depth profiling of substance penetration in vitro as well as in vivo. Measurements at different depths below the skin surface allow determining the distribution of substances inside the skin.

The human skin provides a protective barrier against environmental impacts, such as pathogens, microorganisms and water loss. The skin's outermost layer, the stratum corneum (SC), is of utmost importance in fulfilling this function effectively. Physical properties depend on the concentration and distribution of water and other skin components. CRS provides an insight into the complex structure and molecular composition of the SC and deeper skin areas [1].

To this end, in vivo Raman experiments were carried out on voluntary participants. Semi-quantitative concentration profiles for several skin parameters were determined, for example natural moisturizing factor (NMF) and cholesterol. Cholesterol levels were also measured in capillary blood with an optical reflectance system, a device which is routinely used by physicians, researchers and patients in screening and diagnosis of hyperlipidemia.

Experimental Methods

Raman spectroscopy

The analysis was performed using a RiverDiagnostics gen2-SCA Skin Composition Analyzer (RiverDiagnostics, Rotterdam, Netherlands) designed for in vivo investigation of skin. All experiments were carried out on the volar forearm.

Profiles in the fingerprint region were measured in 4 µm steps up to a depth of 50 µm, the signal collection time was 5 s. Water profiles were recorded in 2 µm steps up to a depth of 40 µm using an exposure time of 2 s. Data were analysed using the SkinTools® software version 2.0. At least three individual profiles were obtained from every single participant.

Capillary blood cholesterol measurements

Capillary total cholesterol was determined with the optical reflectance system Accutrend® Plus (Roche Diagnostics GmbH, Mannheim, Germany). A small amount of blood was obtained from the fingertip using a lancing device and applied to the test strip.

The test is based on an enzymatic reaction and results in the formation of a colored product. A simple algorithm converts the reflectance of the test strip to cholesterol concentration.

Determination of SC thickness

To compare skin and blood cholesterol levels a certain skin depth for the skin reference value was selected. It was defined as the first measured cholesterol concentration in the viable epidermis.

As reported in further studies, the water content gradually rises from the upper part of SC to the viable skin zones until it reaches an almost constant value thereafter [2]. Water profile curves were divided into two sections and straight lines were applied along the two different reaches. Then the SC thickness was calculated from the point of intersection of both lines.

References

[1] Caspers, P. J., Lucassen, G. W., Carter, E. A., Bruining, H. A., Puppels, G. J., 2001. In vivo confocal Raman microspectroscopy of the skin: noninvasive determination of molecular concentration profiles. J. Invest. Dermat. 116, 434-442

[2] Egawa, M., Hirao, T., Takahashi, M., 2007. In vivo estimation of stratum corneum thickness from water concentration profiles obtained with Raman spectroscopy. Acta Derm. Venereol. 87, 4-8

Results

The in vivo concentration profiles of various skin parameters were very similar to results reported in the literature and weekly measurements led to comparable findings. Furthermore, it was possible to determine SC thickness through water concentration profiles as described in the Experimental Methods section (Fig. 1). The SC values varied from 12 to 19 µm depending on the participant.

No significant differences in cholesterol concentration profiles were found for repeated measurements over a period of four weeks.

Fig. 2 shows the comparison of fasting total cholesterol concentration with the corresponding results from Raman spectroscopy for two participants. The cholesterol levels in blood and skin seemed to be independent from each other. It is possible that the difference may be caused by the not optimal reproducibility of the Accutrend® Plus device.

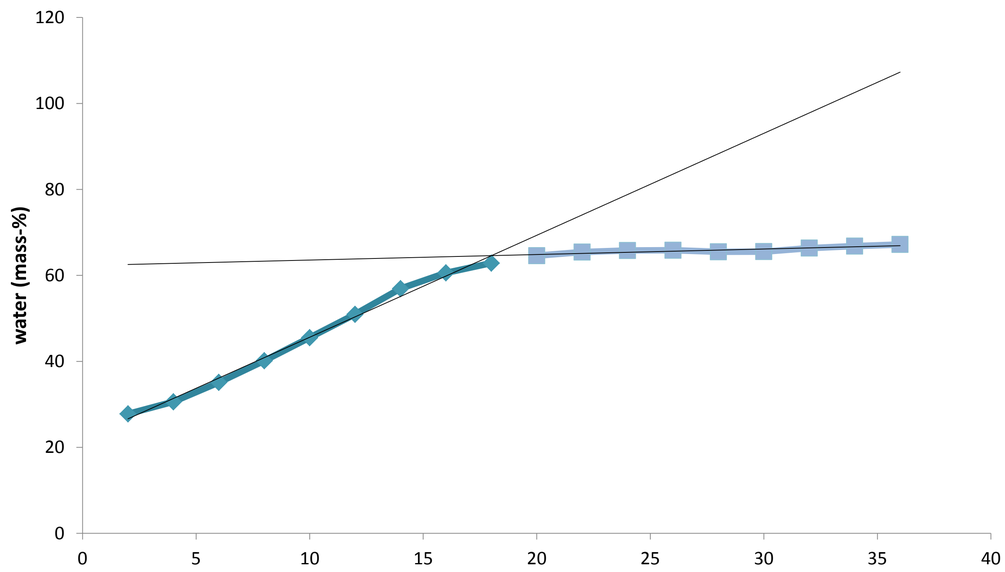

Fig. 1: Exemplary diagram of the determination of SC thickness from water concentration profile. The point of intersection marks the boundary of the SC.

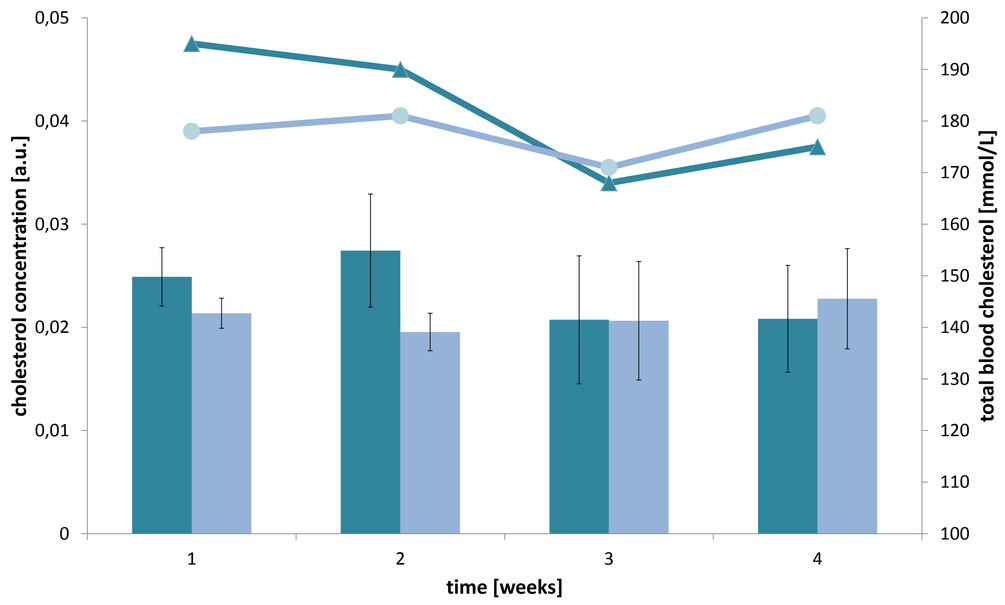

Fig. 2: Cholesterol concentration in skin (bars) and blood (lines) for two voluntary participants. The presented skin values are means and ±SD of at least 4 individual experiments for each participant (n ≥ 4).

Conclusion

Raman spectroscopy is a rapid and simple technique for molecular concentration profile measurements as well as SC thickness determination.

A distinct correlation could not be determined between the two cholesterol concentrations yet but a connection cannot be precluded. Further studies with a bigger number of participants are planned to investigate the relation of cholesterol levels in blood and skin in more detail. Particular attention should be paid on a more precise method to determine the fasting total cholesterol concentration in blood.

Acknowledgements

This work was financed by the research platform "Characterisation of Drug Delivery Systems on Skin and Investigation of Involved Mechanisms", University of Vienna.