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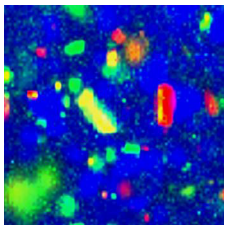
Raman Spectroscopy

Valuable analysis tool for cosmetics and skin characterization



Application Note

Cosmetics RA70

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Abstract

Confocal Raman Spectroscopy is an important analytical tool in various fields and it has shown high efficiency for specific applications in the cosmetic and/or pharmaceutical fields. Its non-invasive behavior, and its high molecular sensitivity make it one of the preferred label-free analytical techniques to characterize materials such as skin and cosmetic products.

In this application note, we show how the Raman technique enables both *in-vivo* and *ex-vivo* chemical analysis of skin or cosmetic products, and the monitoring of the interaction between skin and these products.

Keywords

in-vivo Raman Spectroscopy, *ex-vivo* Raman Spectroscopy, depth profile, skin hydration, hydration moisture cream

Introduction

The skin, the largest organ, plays an extremely important role in protecting our body against various external factors and their effects. Numerous scientific studies have been carried out in order to better understand its structure, its physiology and its interaction with the chemical or physical environment. Health and skin care have often been the focus of these studies.

The most important current studies are focused on the aging of the skin and certain affections such as atopic dermatitis or psoriasis. An important place in these studies is also dedicated to the manufacturing process, control and quality of numerous cosmetic or dermatological products. In this case, the effects of the interaction between the skin and these products will be analyzed.

Using Raman vibrational spectroscopy to manage these studies is a "modern" choice because it is a direct, non-invasive and label-free method, providing rich information about biochemical composition from cell to tissue level. The analysis can be performed at the surface of the skin but also at different depth positions. Thus, the superficial layers of the skin can be characterized in native state or damaged state [1], [2].

Several analysis modes are available [3], [4]. They are based on:

I) Multivariate statistics: Discrimination and classification between 2 types of skin by analysis of the entire Raman spectrum, using statistical and mathematical approaches

II) Discrimination between 2 states of the skin using a bar code. This bar code contains the assembly of particular biophysical links between Raman vibrational characteristics and the specific compositional and chemical changes associated to analyzed states. The Kruskal-Wallis statistical test is usually applied to efficiently discriminate between the Raman frequencies.

III) Molecular descriptors and Raman probes.

The main molecular descriptors are presented in the table 1:

Molecular descriptor		Raman vibrations (cm^{-1})
Water content/ hydration Total water content I(νOH total) / I(νCH) Relative content in bound water: I(νOH bound)/I(νCH)	Total water (nOH) Bound water (nOH) Side chain/proteins (nCH)	3100-3620 3245-3420 2800-3000
Protein secondary structure Amide I band features - main protein to analyze is the keratin	α-helix β-sheet	1656 1672
Lipid conformation and organization Conformation : νCC trans / νCC gauche Organization : νasym CH2 / νsym CH2	νCC trans νCC gauche νasym CH2 νsym CH2	1130/1060 1080 2880 2850
Lipid content	νCH2 νCH	2850 2885

Table1: Main molecular descriptors for Raman analysis of the skin

The skin is an inhomogeneous tissue, composed mainly of several layers with different thicknesses: Epidermis, dermis and hypodermis (as seen in Figure1). The most superficial layer of the epidermis is called Stratum Corneum (SC). This layer is often referred to in the studies of the skin in the cosmetic field. This is not surprising since the SC plays an essential role for healthy skin [5], [6].

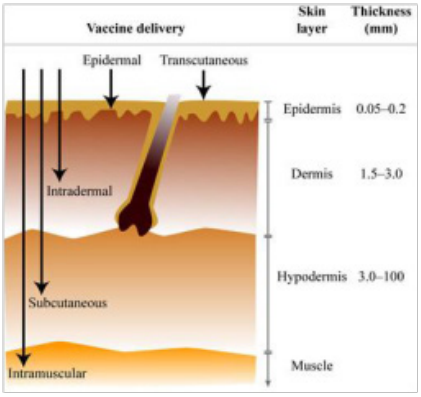

Figure 1: Human skin structure

Indeed, the SC is a protective barrier for external chemical or mechanical factors. It plays a very important role in the barrier function of the skin which has as its main roles:

1) To prevent excessive water loss through the epidermis;
2) To avoid compounds from the environment permeating into the viable epidermal and dermal layers and thereby provoking an immune response.

In-vivo approach

Exploring *in-vivo* the surface of the skin with Raman technique

A typical Raman spectrum of the skin can be found Figure 2:

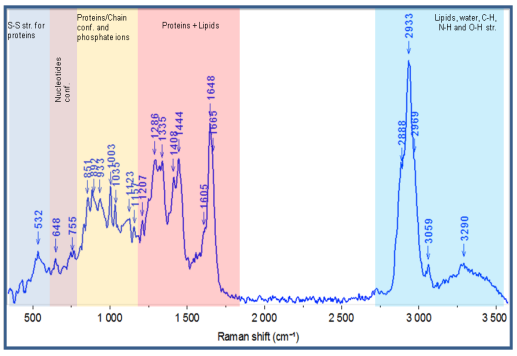

Figure 2: Typical Raman spectrum of the skin and general Raman bands assignment

Using the Raman micro-probe, a single Raman spectrum on the surface of the skin in different regions of the body can be measured (as illustrated in Figure 3). The complete spectral range (400-3600 cm^{-1}) is acquired in one shot.

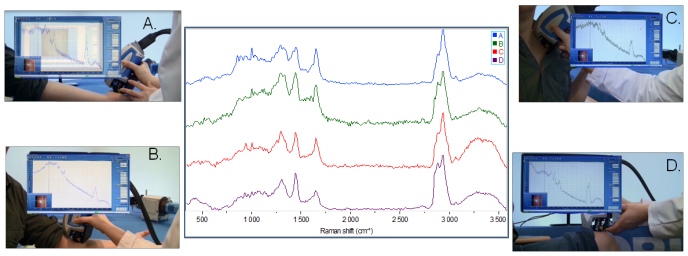

Figure 3: Examples of Raman spectra of the skin measured with the flexible micro-probe on the different regions of the body. The spectra displayed in the middle of the image are baseline, corrected post-measurement. (A) Palm, (B) Forearm, (C) Neck, (D) Leg

The micro-probe offers true confocal performance for optimized depth resolution, allowing superficial human skin layers to be distinguished, as well as their behavior to cosmetic and/or pharmaceutical products. The compact design, the patented integrated visualization camera enabling simultaneous video observation and spectrum acquisition, and the portability make this Raman device easy to use to obtain efficient results [4].

In-vivo depth profiling of the skin with Raman technique

Cutaneous water content is known to play an important role in different skin functions and its deficiency is associated with several dermatological dysfunctions (skin aging, psoriasis, atopic dermatitis…). Loss of water from the skin must be carefully regulated, as it is a function dependent on the complex nature of the SC.

Studying SC using the Raman spectroscopy method paves the way to:
I) The direct characterization of skin hydration (Figure 4);
II) The estimation of the SC thickness and lipids content (Figure 5);
III) The follow-up of the cosmetic products penetration into the skin (Figure 6)

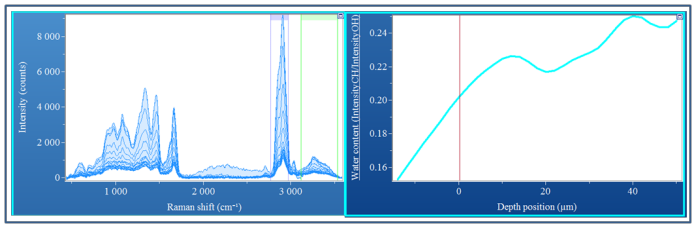

Figure 4. Example of in vivo Raman depth profile of the skin. The blue curve (right side image) shows the water content in the superficial layers of the skin. It represents the intensity ration between CH and OH intensities.

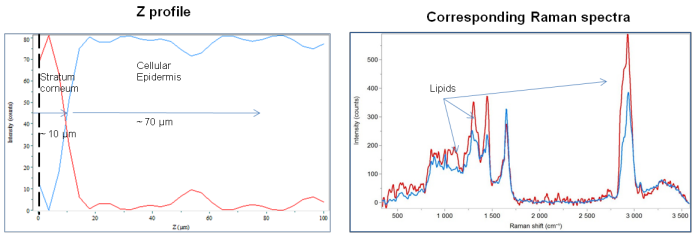

Figure 5. Since the Stratum corneum is very rich in lipids, it is possible to use the Raman signature of the lipids to estimate the thickness of this layer. Dashed line indicates the surface of the skin (origin for the measurement)

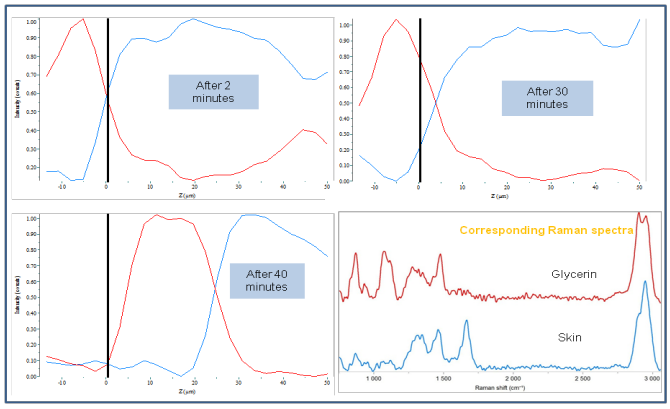

Figure 6. Penetration profiles of the glycerin through the skin over time. Dark line indicates the surface of the skin (SC+Epidermis)

Ex-vivo approach

Cosmetic and/or dermatological products are obviously analyzed and characterized more often by *ex-vivo* methods. Since these products will be in contact with the human body, it is necessary to control their manufacture from the early stages, and their quality. The post-manufacturing step is essential and involves testing their interactions with the skin, or the tissue analyzed.

Fast chemical tracking of compounds and identification within a formulation

Many scientific and technical studies have successfully shown that the Raman technique is a powerful imaging technique with non-negligible advantages for the cosmetic and even pharmaceutical industries. The Raman image is extremely rich in chemical/molecular information. The technique reaches exceptional spatial and spectral resolutions and thanks to the optimized confocal coupling, small details of the sample are localized and identified.

Formulation of different types of moisture creams can be routinely checked in order to ensure the quality control and the content uniformity of the product.

Below is presented a Raman image of a highly hydrating cream sample (Figure 7). The sample is deposited directly into a microscope slide glass and no other preparation is needed. Long working objectives are used, as the surface of the sample is rough. One can note the distribution and the chemical identification of important compounds within the formulation. For this hydrating cream, the distribution of the Niacynamide is crucial as they have an important concentration. Other compounds as Titanium Oxide or Sodium Lauryl Sulphate are observed.

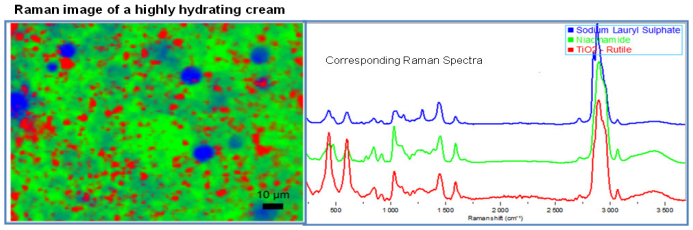

Figure 7. Raman image showing spatial distribution of products in a a highly hydrating cream. Each color correspond to a different chemical compound.

The distribution of an active pharmaceutical ingredient within a dermatologic cream (Figure 8) is also possible by performing Raman imaging. In this case also, no sample preparation is required.

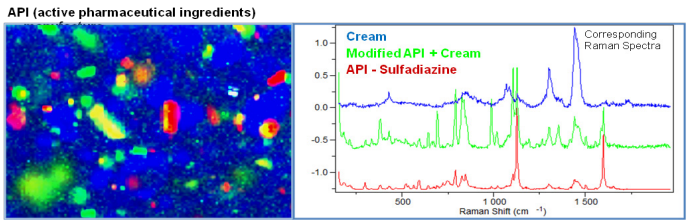

Figure 8. Raman image showing spatial distribution of API within a cream

Following cosmetic products through the skin layers

Delivery of molecules into the human skin is one of the main issues in dermatology and cosmetology. The conventional methods such as Franz diffusion cells are unable to provide the accurate localization of actives in the skin layers [7], [8].

The next example is real evidence shows that, due to its advantages, confocal Raman spectroscopy is useful on this specific field [8].

Raman imaging was performed in order to investigate the penetration of hyaluronic acid (HA) of different molecular weights on human skin sections (Figure 9). Three HA derivatives were used: Cristalhyal (1000–1400 kDa), Bashyal (100–300 kDa), and Renovhyal (20–50 kDa) (SOLIANCE, Bazancourt, France).

The first step was to measure the reference Raman spectra of these HA and to identify the spectral features that can be used to discriminate them from those of the skin. Multivariate analysis is essential in data analysis.

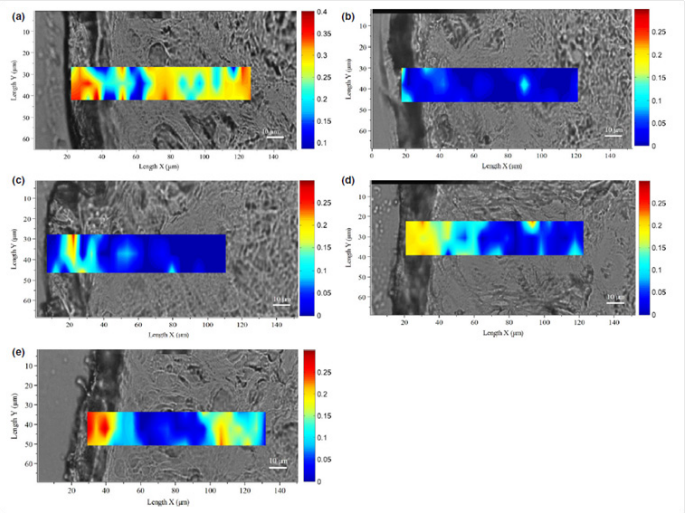

Figure 9. Permeation and localization of various hyaluronic acids (HA) after fitting by their reference spectra and by comparison to the:
(a) Positive control (glycerin treated skin)
(b) Negative control (water treated skin)
(c) Cristalhyal (1000–1400 kDa HA)
(d) Bashyal (100–300 kDa HA)
(e) Renovhyal (20–50 kDa HA)

The Raman images are overlaid on the video image. Higher accumulation of the product is indicated by the red color. By analyzing these images one can note that Renovhyal HA is highly present in the deepest epidermis layers, whereas Bashyal HA is localized in the superficial layer of the epidermis under the stratum corneum. The Cristalhya HA has the highest molecular weight and is observed only in the stratum corneum. For an easy comparison, positive and negative controls are used. We observe that there is no HA signal in negative control, while for positive control the glycerin signal is present at full epidermal depth, thus highlighting the high diffusion of glycerin through the human skin.

These results show that HA permeation increases with decreasing molecular weight of HA. This is in agreement with other studies demonstrating that low molecular weight HA (50 kDa), was associated with significant improvement in skin hydration and wrinkle depth, which is due to better penetration.

Conclusion

Confocal Raman spectroscopy is a powerful tool to be used in routine research in the cosmetic field, *in-vivo* and *ex-vivo* configurations.

Due to its advantages, it enables the detection and the localization of chemical molecules at a micrometric resolution, in a label-free, non-destructive manner and with a limited sample preparation.
Cosmetic products can be tested during the manufacturing process to optimize the process and the quality of products.
By measuring depth profiles of the skin, we have access to important molecular information allowing us to describe the composition and conformation, and to track the penetration of cosmetic molecules.

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