Raman Update

HORIBA JOBIN YVON

L'ORÉAL RECHERCHE

Welcome to the Raman Update, produced by HORIBA Jobin Yvon's Raman Team, to provide our customers, colleagues & friends with up-to-date information in the field of Raman Instrumentation and Application.

As a result of the ongoing collaboration between HORIBA Jobin Yvon and L'ORÉAL, the Winter Edition 2005 of the Raman Update has been dedicated to give you examples of applications in the field of cosmetics and dermatology.

SPECIAL ISSUE

Contents

Confocal Raman Microscopy for Cosmetic Applications

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Introduction

One of the major fields of investigation of L'ORÉAL Research is to improve the understanding of the chemical composition and structure of skin and hair. To enable a better design of cosmetic products, a thorough understanding of the mechanisms and the nature of the interactions of cosmetic ingredients with these substrates is necessary.

Raman is a key analytical method allowing the needed understanding of such mechanisms. In this context, HORIBA Jobin Yvon & L'ORÉAL Research have established a close collaboration in applying the Raman technique under *in vitro* conditions or directly *in vivo*, for hair, skin, nail, eyelashes and model substrates.

Raman spectroscopy is a non destructive technique with a wide range of possible applications in the field of cosmetic research. As this technique can be used non invasively, it is of particular interest for skin and hair research. In many cases, this method can be applied both *in vitro* and *in vivo* to give direct information about the state of skin or hair before and after treatment with cosmetic products.

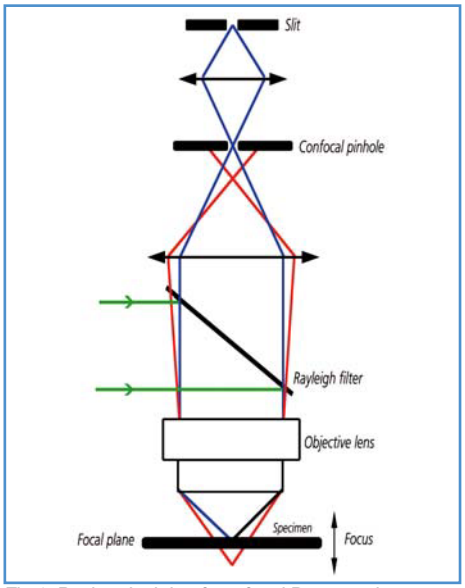

Fig.1: Basic principle of confocal Raman microscopy

Confocal Raman Microscopy

The principle of confocality (Fig. 1) is based on the selection of a restricted collection volume, obtained often by using a small aperture. This dramatically improves lateral and axial resolution by filtering out the signal coming

from out-of-focus or adjacent regions. Another advantage lies in a significant reduction in the fluorescent background generated by the areas encircling the laser focus point. These two advantages play a prominent role in recording high quality Raman data of skin and hair, both at the surface and in depth.

The 3D information recorded by confocal Raman microscopy is crucial to improve our understanding of the skin and hair by in situ analysis of the chemical composition (water, lipids, proteins, sulphur, and amino-acids). It is also very useful for the spatial location of cosmetic ingredients in the substrates as a function of time.

Applications to skin and hair substrates

The outermost layer of skin, the stratum corneum (SC), is of particular interest to cosmetic scientists as this layer is the most influenced by cosmetic products. Hair is composed of a cylindrical cortex about 70 µm in diameter surrounded by an outer protective sheath of overlapping cuticle cells arranged like shingles on a roof (Fig 2). Both substrates are essentially made of a specific class of proteins called keratin, and also contain lipids and water. All these components show distinctive features in Raman spectra.

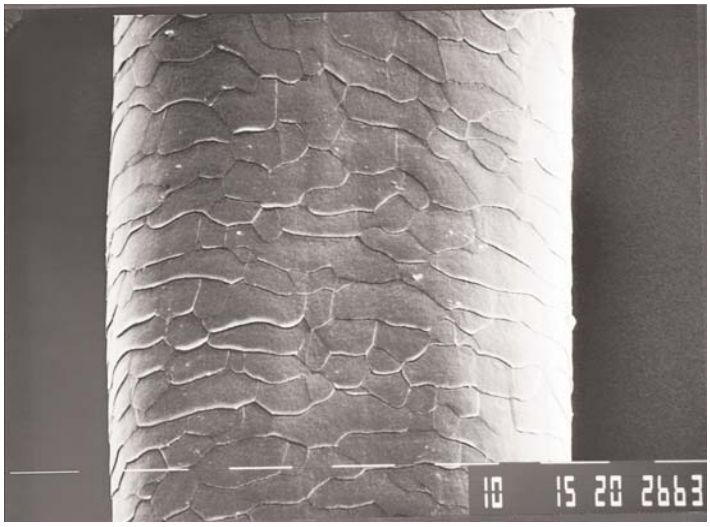

Figure 2: Scanning Electron Micrograph of hair

Investigations of hair through cosmetic treatments

Raman measurements are reported on unpigmented and bleached hair. The chemistry of hair is dominated by the disulfide bonds formed between two keratin molecules via cysteine linkages. The Raman spectra can thus be used to assess the chemical modifications associated with hair bleaching and permanent waving. For example, during the permanent waving of hair (Fig. 3), a decrease in intensity of the 510 cm^{-1} band and a concomitant appearance of a peak at 2568 cm^{-1} (mercaptan) was observed.

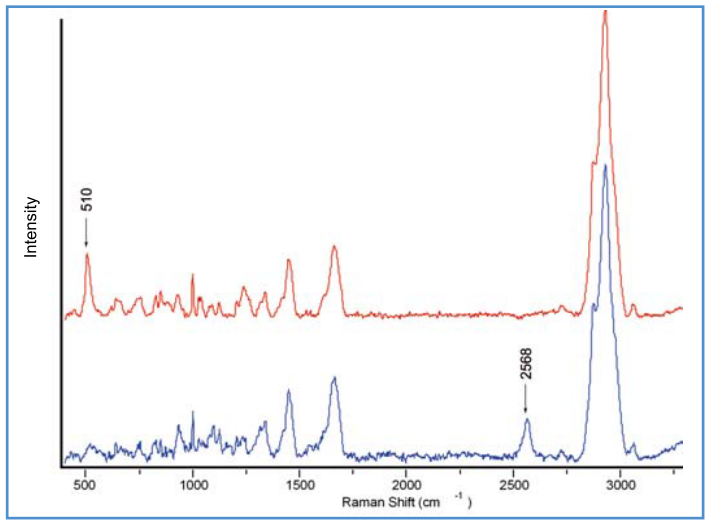

Figure 3: Raman spectra of a virgin hair (red) and of a permed hair (blue)

In depth confocal measurements

In the confocal mode, Raman spectroscopy has the advantage of providing information from the surface of the fiber down to a depth of several microns into the hair. For example, the use of the intensity of the S-S allows one to quantify the oxidation of hair from the cuticle to the depth of the fiber. This non-invasive analysis, requiring minimal sampling preparation (intact fibers), is routinely used to obtain an insight into the structure and the mechanisms which govern the behaviour of hair when bleached or permed.

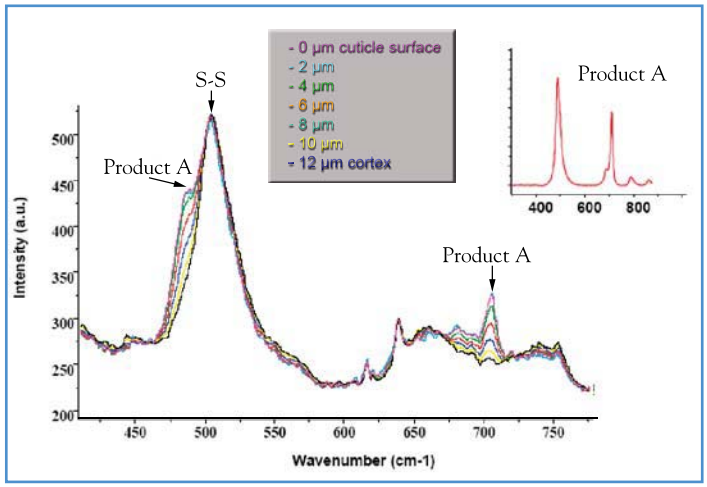

Figure 4: Monitoring the penetration of cosmetic ingredient in the hair

On the other hand, diffusion of molecules can influence the chemical and physical properties of the skin and hair. For instance, the Raman confocal microscope provides a

method of studying the penetration of molecules with a spatial resolution of a few micrometers from the periphery of a fiber to the center (Fig. 4).

Characterisation of skin: *in vitro* and *in vivo* Raman studies

The application of Raman spectroscopy for monitoring topically applied substances is also investigated on the isolated stratum corneum and/or skin biopsies. Concentration profiles are measured in order to monitor the distribution of substances as a function of depth. Since Raman spectroscopic measurements can be performed repeatedly on the same skin area, the effects of the molecules can be studied as a function of time (Fig. 5).

The molecular selectivity of the method provides the means to separate the Raman signal of the product from the skin signal (inset of Fig. 5). This is a great advantage when the effects of a product are to be studied without interference from the product itself.

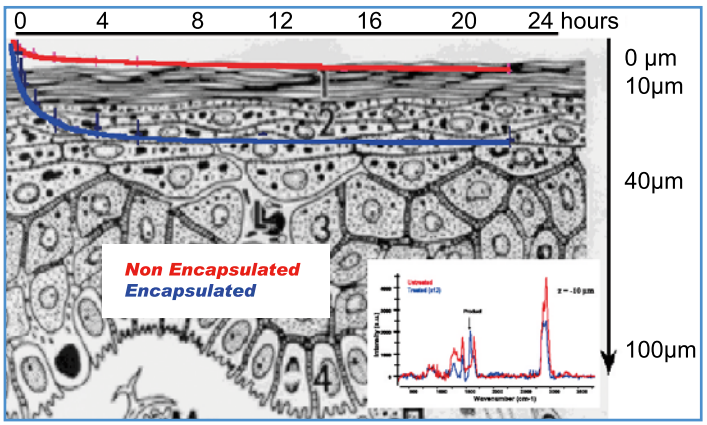

Figure 5: Results highlighting the encapsulation effect on the penetration of a cosmetic ingredient in the stratum corneum

The assessment of the water concentration profile in the stratum corneum (SC) provides crucial information regarding the water-holding capacity and the barrier properties of the skin. The evolution of the water content in the SC can indeed be determined by calculating the area below a part of the complex broad band in the range 3100-3600 cm^{-1} of the normalised spectra with respect to the CH band (2810-3030 cm^{-1}) (Fig. 6). This enables rapid, automated determination of water concentration profiles for the SC. In comparison to the profile recorded before the hydration, this measurement demonstrates the effect of moisturising cream.

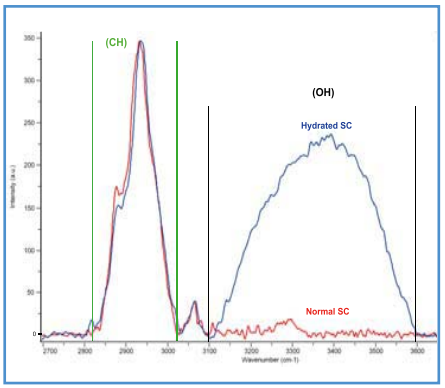

Figure 6: Raman spectra of untreated (red) versus hydrated (blue) stratum corneum

The fantastic advantage of this technique is the possibility to carry out experiments under *in vitro* as well as *in vivo* conditions.

In vivo Raman analysis of skin

A new *in vivo* confocal Raman probe (Fig. 7) has been designed, by HORIBA Jobin Yvon, to reach a spatial resolution equivalent to that obtained under a confocal microscope (Fig. 8), whilst being less restrictive in terms of sampling. Initial experiments have been carried out at L'ORÉAL Research and have demonstrated the relevance of this instrument for the *in vivo* characterisation of skin.

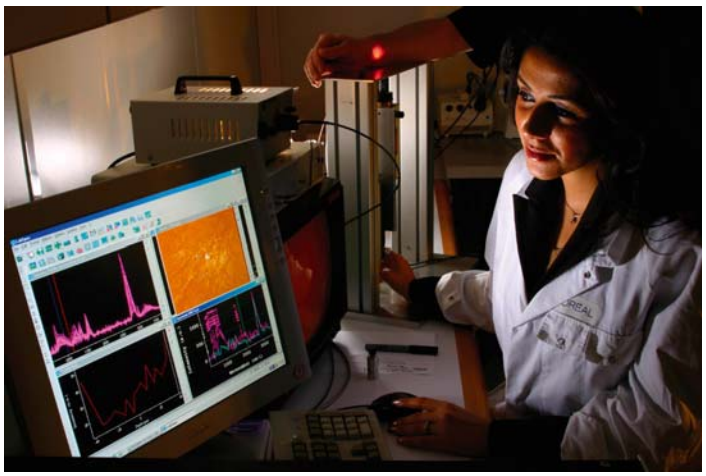

Figure 7: Confocal Raman probe installed at L'ORÉAL

Such a fiber optic probe enables remote measurements to be conducted under confocal conditions, and to achieve a spatial resolution in the order of a few micrometers.

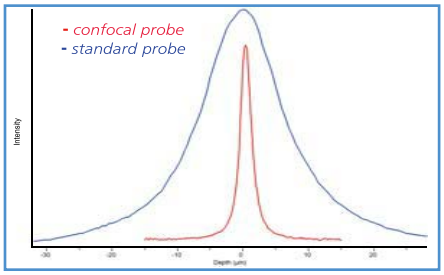

Figure 8: Determination of the depth of field of a confocal (about 2 µm) versus standard (about 16 µm) probe with a 100 x LWD objective. This is done by measuring the silicon mode intensity versus depth.

These measurements are further aided by the device being compact and hence easy to handle. An integrated high definition camera provides visualisation of the sample and laser spot simultaneously, for precise area selection and laser focusing optimisation.

A piezo-electric device allows control of the high precision axial translation of the objective throughout depth profiling from the top surface down to a defined depth within the sample. This scanning device is directly controlled by the data acquisition software (LabSpec, HORIBA Jobin Yvon), which enables automated depth profiling procedures.

This new *in vivo* confocal Raman probe provides the high spatial resolution required to study the different layers of the skin and to detect in-depth variations in the molecular composition of the SC between different anatomical sites and different volunteers (Fig. 9).
The confocal Raman probe also enables the determination of *in vivo* molecular gradients of water and topically applied substances to the stratum corneum.

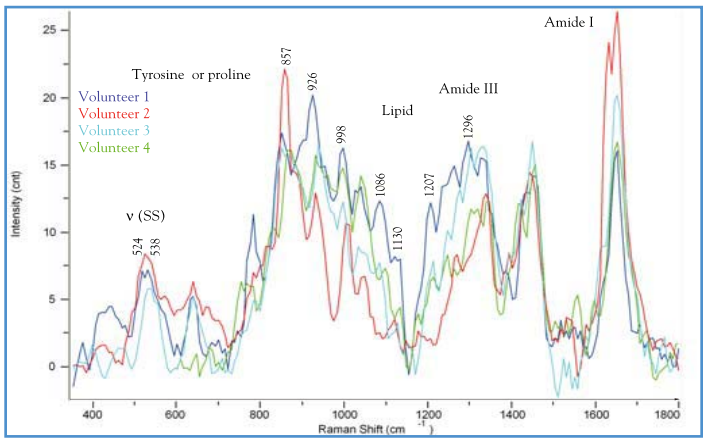

Figure 9: Investigation of stratum corneum composition on different volunteers at the same anatomical site

Although at an early stage, the cosmetic applications of Confocal Raman Microscopy already demonstrate a high potential to better understand the chemical composition of skin and hair, *in vitro* and *in vivo*.

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