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## NIR-FT RAMAN SPECTROSCOPY OF THE MUMMIFIED SKIN OF CHIRIBAYA MUMMIES FROM PERU

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Near infrared Fourier transform (NIR-FT) Raman spectroscopy is an analytical, non-destructive technique based on analysis of laser light reflected from the sample. It provides information about the molecular structure of the sample. NIR-FT Raman spectroscopy was employed here to investigate molecular structure of skin samples from mummified bodies from the Chiribaya culture from the Southern Peruvian desert (1500 years before present). In the spectra of dark pigmented mummy in comparison to temporary dried skin a marked loss of protein amide I ( $1640\text{-}1680\text{ cm}^{-1}$ ) and amide III ( $1220\text{-}1290\text{ cm}^{-1}$ ) band intensities was found, indicating major loss of protein or changes in the secondary protein structure. These molecular changes were less pronounced in the spectra of light pigmented mummies. Moreover a strong peak at  $1300\text{ cm}^{-1}$  and an increased intensity of the peak at  $2850\text{ cm}^{-1}$  was observed. The band at  $1300\text{ cm}^{-1}$  is characteristic for twisting and wagging  $\text{CH}_2$  vibrations in lipids and the  $2850\text{ cm}^{-1}$  band represents lipid CH stretching vibrations. These spectral changes suggested an increased lipid content in Peruvian mummy skin (light coloured mummies) compared with contemporary skin. We ascribe this increased lipid intensity in the skin of the light coloured Peruvian mummies to embalming, by which means a better preservation is achieved.

**Key Words:** Mummies, proteins, conservation, embalming.

*Espectroscopia Near-Infrared Fourier Transform Raman (NIR-FT) es una técnica analítica no destructiva basada en la luz de laser reflejada por la muestra. Esta técnica sirve para generar información sobre la estructura molecular de la muestra. La espectroscopía de Raman NIR-FT fue utilizada para investigar la estructura molecular de muestras de piel de cuerpos momificados de la Cultura Chiribaya del Sur del Perú (1500 A.P). El espectro de las momias de color oscuro o mas pigmentadas en comparación con la piel seca es una pérdida de la intensidad de banda de las proteínas amido I ( $1640\text{-}1680\text{ cm}^{-1}$ ) y amido II ( $1220\text{-}1290\text{ cm}^{-1}$ ) indicando un cambio en la estructura de la proteína secundaria. Estos cambios moleculares fueron menos obvios en el espectro de momias con menor pigmentación. Sin embargo, se observó una señal muy fuerte a  $1300\text{ cm}^{-1}$  y una intensidad mayor a  $2850\text{ cm}^{-1}$ . La banda de  $1300\text{ cm}^{-1}$  se caracteriza por las vibraciones en los lípidos y la banda de  $2850\text{ cm}^{-1}$  representa las vibraciones de los lípidos CH. Estos cambios espectrales sugieren un incremento en los contenidos de los lípidos de las momias con piel clara comparada con la piel moderna. Pensamos que el incremento de lípidos en las momias con piel clara podría deberse al embalsamamiento.*

**Palabras Claves:** Momias, proteínas, conservación, embalsamar.

Raman spectroscopy has been used for studying the molecular structure of complex biological samples ([Edward et al. 1995](#); [Gniadecka et al. 1997](#); [Lawson et al. 1997](#); [Williams et al. 1996](#)). Interest in this technique is growing because this method is non-destructive and does not require any sample preparation. Raman spectroscopy is thus well suited for archeological studies where the best possible preservation of the sample material is very important.

The processes by which human or animal bodies are preserved are of interest since they reflect the cultural development and the habits of our ancestors. Mummification can either represent a natural process of drying (often seen for the mummies found in deserts) or an artificial process where the body had been treated with different substances to promote preservation (the best known examples are Egyptian mummies recovered from pyramid or rock tombs). It is not always clear whether the body was naturally or artificially mummified, an issue of great importance in anthropological and archeological research. It is often assumed that the mummies found in dry areas (deserts) are naturally mummified. Surprisingly, however, some differences are often found in the appearance of the skin from bodies found in the same area which may suggest differences in the process of mummification. Therefore, new techniques that examine the constitution of samples are needed to assess the mummification processes.

[Williams et al. \(1995\)](#) carried out Raman spectroscopic studies of the skin of a late Neolithic man (Iceman) whose body was preserved by the freeze-drying process in a glacial field on the border of Italy and Austria. This study proved that it is possible to obtain good quality spectra of mummified skin and it was possible to ascribe chemical changes in the naturally mummified skin to changes in structure of proteins ([Edward et al. 1995](#); [Lawson et al. 1997](#); [Williams et al. 1996](#)). Recently, we obtained Raman spectra of the skin from 15th century mummies ([Gniadecka et al. 1997](#)) found in two graves located in a rock cleft near the abandoned settlement of Qilakitsoq, Northwest Greenland ([Hansen et al. 1991](#)). These skin spectra also showed structural changes of proteins similar to those alterations observed in the Ice-man skin. This confirmed the hypothesis that favourable local conditions: low temperature, low air humidity and protective shelter against rain and snow promoted natural mummification and stopped the decaying process, which needs water and temperatures above 4° C.

Recently, around 500 mummies were found in the Peruvian desert (Chiribaya Alta region) which are stored now at the Mallqui Center in Ilo. Cultural and environmental conditions have permitted the extraordinary preservation of organic material in the desert coast of the extreme south. Most of the bodies were buried wrapped in textiles in cysts opened in the ground at a depth of one meter under the surface. Despite that all bodies were considered to be naturally mummified, their skin differed in colour. Most of the bodies were light brown in colour, while some were very darkly pigmented. The question arises also whether the darkly pigmented bodies were processed differently, e.g. by artificial mummification. In this paper, we report the near-infrared Fourier transform (NIR-FT) Raman spectra of the skin from these mummies. The advantage of the skin as a material for investigations is that it is easily available, it is usually preserved in mummified bodies, and the sampling does not require the destruction of the mummy. Also, the eventual presence of embalming substances are most likely to be seen as residue in the skin.

## Experimental

Two skin punch specimens (3 mm in diameter) from each mummy were taken. Skin samples were collected from five Peruvian mummies with various skin pigmentation (four light brown and one dark brown). A biopsy from normal, dried contemporary skin were also included for comparison.

Raman spectra were obtained using a FRA 100 system or FRA 106 Raman module on a Bruker IFS 66 optics system (Bruker, Karlsruhe, Germany); the 1064 nm line from a Nd:YAG laser was used as exciting wavelength. Biopsies were placed in stainless steel cups and the laser beam was focused on the sample spot of approximately 100  $\mu\text{m}$  in diameter. For each sample of mummified skin, 1000 scans were obtained over 30 min with 20 mW laser power. This power was chosen in order to minimise the strong, broad fluorescent background which could interfere with the spectra and to avoid sample degradation. For each biopsy of contemporary skin, 250 scans at a laser power of 300 mW were taken over 10 min. In no case were smoothing procedures, normalisation of the spectra or white light corrections performed.

## Results and Discussion

Raman spectra of mummified skin and dried skin are presented in [Figure 1](#).

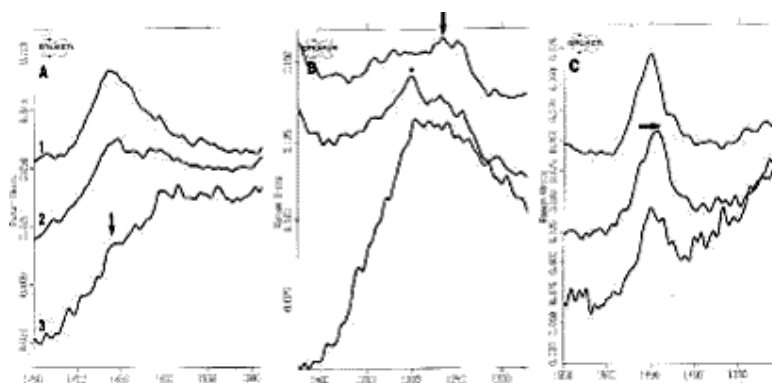


Figure 1. NIR-FT Raman spectra of dried contemporary skin (1) and mummified skin (2), light pigmented and dark pigmented (3); (A) decrease of the amide I vibrations in proteins in the dark pigmented skin (marked with an arrow); (B) the amide III vibrations in proteins (marked with an arrow) and increase of the  $\text{CH}_2$  twisting and wagging vibrations of lipids in the light pigmented mummified skin (marked with asterisks); (C) shift to lower wavenumbers of the  $\text{d}(\text{CH}_2)$ ,  $\text{d}(\text{CH}_3)$  band of proteins and lipids (marked with an arrow).

Bands from tissue proteins are found in the wavenumber region from 1640 to 1680  $\text{cm}^{-1}$  (amide-I) , 1220 to 1300  $\text{cm}^{-1}$  (amide-III), and from 1440-1460  $\text{cm}^{-1}$  d(CH<sub>2</sub>), d (CH<sub>3</sub>) ([Liu et al. 1991](#); [Manor et al. 1991](#); [Tu 1986](#)). The latter band, however, overlaps with? (CH<sub>2</sub>) scissoring vibrations from lipids ([Lin-Vien et al. 1991](#); [Thomas et al. 1976](#)). In the samples from the dark pigmented Peruvian mummy the relative amide I band intensities were greatly reduced ([Figure 1A](#)) and a decrease in the overall band intensity in the amide III region was observed ([Figure 1B](#)) in comparison to normal contemporary skin. The origin of these spectral changes cannot be completely elucidated, but they reflect changes in protein content and and/or changes in the secondary protein structure. Loss of and damage to the collagen fibres (which provide the major protein component of the skin) have previously been suggested to be responsible for the changes in the amide I and III regions in Raman spectra of the Iceman skin ([Edward et al. 1995](#); [Lawson et al. 1997](#); Williams et al. 1996). Strikingly, the spectral changes in samples of the 1000 year-old dark pigmented Peruvian mummy were very similar to those found in the 5200 year-old Iceman. It implies that most changes in molecular structure take place in a relatively short time, probably during the period between death and the completion of the natural mummification process.

Inspection of the spectra from the lightly pigmented mummies from Peru revealed interesting and unique spectral changes. A marked intensity increase of lipid characteristic bands was found. Namely, the CH stretching band near 2850  $\text{cm}^{-1}$  (not shown) and CH<sub>2</sub> twisting and wagging vibrations around 1300  $\text{cm}^{-1}$  ([Figure 1B](#)). Moreover, the d (CH<sub>2</sub>), d (CH<sub>3</sub>) peak of proteins and lipids was shifted to lower wavenumber, suggesting a more pronounced lipid component ([Figure 1C](#)). The decrease in amide I band intensity was less extensive in lightly pigmented Peruvian mummies when compared with the darkly pigmented one. We conclude that a better preservation of the skin has occurred in the light-pigmented mummies, presumably because of artificial mummification. This is a challenging hypothesis because all Chiribaya mummies have previously been considered to be naturally mummified. The reason why some bodies were artificially mummified while other left untreated is an important anthropological and archeological issue.

## Conclusions

Our findings give an example of the use of NIR-FT Raman spectroscopy for studies on mummification. Raman spectroscopy is undoubtedly a useful technique to determine the degree of degradation in the protein component of the skin (represented mainly by collagen). Changes in amide-I and III modes (reduction in peak height) probably take place in a relative short time after mummification (<1000 years) and remain stable for prolonged periods of time (minimum 5000 years). NIR-FT Raman spectroscopy can also detect the presence of new chemical substances in the skin, as in the case of lightly pigmented Peruvian mummies where evidence for a lipid-like material was found in the skin. This material is thought to represent (an) embalming material(s). Thus, NIR-FT Raman spectroscopy is a useful tool to determine whether the mummy had been naturally mummified or embalmed.

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