LOW-WAVENUMBER NIR-FT-RAMAN STUDIES OF WATER STRUCTURE IN HUMAN AND ANIMAL SKIN: BIOMEDICAL ASPECTS

M. Gniadecka¹, H.C. Wulf¹, T. Larsen²⁴, N. Rastrup Andersen², K. Birklund Andersen², L. Simonsen², K. Lilto[p³, P. Westh³ and O. Faur[ov Nielsen⁴

¹ Department of Dermatology, Bispebjerg Hospital, Bispebjerg Bakke 23, 2400 Copenhagen, Denmark; E-mail: hcw01@bbh.hosp.dk
² Department of Spectroscopy and Department of Pharmaceutical Formulation, LEO Pharma, Industriparken 55, 1750 Ballerup, Denmark; E-Mail: niels.rastup@leo-pharma.com
³ Department of Biology and Chemistry, Roskilde University, 4000 Roskilde, Denmark; E-mail: pwesth@ruc.dk
⁴ Department of Chemistry, University of Copenhagen, Universitetsparken 5, 2100 Copenhagen, Denmark; E-mail: ofn@kiku.dk

Keywords: water structure, skin diseases, human and animal skin, protein/water interactions, low-wavenumber Raman scattering

Abstract: This contribution reports on experimental studies of water in human and animal skin by low-wavenumber Raman scattering. The water structure is related to human skin diseases. A comparison is performed between the water structure in human normal skin and animal skin used as a model for human skin in biomedical applications.

Water is a major constituent of all living systems. Human skin contains around 70% of water. In healthy skin most of the water molecules are bound to biological macromolecules and a water structure like the one in pure liquid water is not present. It is not easy to distinguish between a liquid like water structure and water bound to biomolecules, because individual water molecules take part in a dynamical exchange of hydrogen atoms from one molecule to another and water molecules are exchanged between different sites. This problem has been excellently stated in the mini review by Lewitt and Park [1] “Water: now you see it, now you don’t”. They ask four general questions about water molecules interacting with proteins: (1) where are they? (2) How long do they stay there? (3) How strongly do they interact with the protein? (4) How do they affect protein structure and stability? Lewitt and Park [1] mention that three key techniques of modern structural research are: crystallography, NMR-spectroscopy and computer modeling. Advantages by these methods are that structural information is directly obtained. However, none of these methods can easily be applied to structural studies of water in skin samples.

Raman spectroscopy with cw-laser excitation reflects the dynamics on a pico-second time scale and faster. Thus Raman spectroscopy gives a snapshot of the interacting water/biomolecule system. The water stretching and bending vibrations are only slightly perturbed by differences in water hydrogen bonding. The low-wavenumber region is much more sensitive, because this part of the spectrum depends directly on the intermolecular hydrogen bonds. However, Rayleigh scattering yields a very intense band extending to Raman shifts of several hundred wavenumbers (cm⁻¹). This band tends to hide weaker bands from intermolecular water vibrations. By use of the so-called \( R(\bar{\nu}) \)-representation the band due to Rayleigh scattering is converted to a plateau, and weak vibrational features of water below 400 cm⁻¹ are more easily seen [2-5]. \(^2\)H-, \(^17\)O- and \(^18\)O- isotopic substitution in water showed that a band with a maximum around 180 cm⁻¹ in the \( R(\bar{\nu}) \)-representation was caused solely by oxygen displacements [2,4]. The hydrogen atoms in water are not involved in the vibrational mode for this band. Fig.1 illustrates potential energy curves for two different oxygen displacements. A water molecule with four neighbours (shown to the left in Fig.1) exhibits a vibrational motion. The corresponding band is observed at 180 cm⁻¹ in the \( R(\bar{\nu}) \)-representation. With only three neighbours the potential changes as shown in the right part of Fig.
1. This corresponds to a translation of a water molecule and no vibrational feature is observed in the spectrum. A water molecule surrounded by 4 other molecules is characteristic of liquid water. Thus, the 180 cm\(^{-1}\) band can be taken as a characteristic band for a bulk-like liquid water structure.

![Potential functions for four and three coordinated water molecules.](image)

**Fig. 1.** Potential functions for four and three coordinated water molecules.

Skin biopsies were collected from malignant melanoma (5 patients), basal cell carcinoma (5 patients), pigmented nevi (8 patients), seborrheic keratosis (5 patients) and normal skin (5 young and 3 aged individuals). NIR-FT-Raman spectra were recorded with excitation at 1064 nm. The total water content was in each case estimated from the intensities of the OH-stretching band at 3250 cm\(^{-1}\). No significant differences between benign and malignant tumours (benign: seborrheic keratosis and pigmented nevi; malignant: malignant melanoma and basal cell carcinoma) were observed with an exception of seborrheic keratosis, in which the water content was decreased. However, the low-wavenumber spectra in the \(R(\bar{v})\)-representation revealed an increase of water with a bulk-like structure in malignant skin tumours. A similar change in water structure was observed for photo aged skin [6].

Animal skin is used as model for human skin in laboratory studies of skin properties. Water content and structure in skin from pig ears, guinea pig and mouse were compared to human skin. Differences in loss of bulk water were observed for human skin samples after freezing and thawing.

Water/collagen samples with various contents of water were used as model systems for water/collagen interactions in skin.

**Acknowledgements:**
MG, PW and OFN want to thank the Danish Research Academy for financial support, (grant no. 51-00-0312).

**References:**