International Journal of Pharmaceutics, 81 (1992) R11–R14

IJP 10046

Rapid Communication

Fourier transform Raman spectroscopy A novel application for examining human stratum corneum

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(Received 7 February 1992)
(Accepted 12 February 1992)

Key words: Raman spectroscopy; Human skin; Infrared spectroscopy; Stratum corneum

Summary

Fourier transform Raman spectroscopy has been used for the first time to characterise human stratum corneum. Raman and infrared spectra were compared and differences, for example due to water effects or response linearity, were noted. The technique should prove valuable in fundamental and practical studies with human and animal skin.

The barrier function of human skin depends mainly on its outermost layer, the stratum corneum, which is a heterogeneous membrane comprising keratin-rich corneocytes embedded in multiple lipid bilayers. The molecular basis for the barrier nature of the stratum corneum is currently attracting much interest and has recently been probed by Fourier transform infrared (FTIR) spectroscopy (e.g., Golden et al., 1986; Williams and Barry, 1989; Mak et al., 1990). We now present data on the molecular structure of the stratum corneum determined using Fourier transform Raman (FT-Raman) spectroscopy, and compare the information provided by this technique with that obtained from FTIR.

In order to discern fully the vibrational modes of a molecule, one needs to examine both IR and Raman spectra. There are some similarities between the information provided by both techniques, but a fundamental difference is that the selection rules for IR and Raman spectroscopy are not identical. A molecule absorbs IR radiation when the dipole moment changes during molecular vibration. The Raman process is a scattering effect and results from an induced dipole moment, dependent on a change in molecular polarisability during a vibration. The complementarity of the two techniques is shown by the rule of mutual exclusion; for a molecule with a centre of symmetry (for example, benzene), vibrational transitions in the IR and Raman spectra are

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mutually exclusive. Hence, a molecule may shown an IR absorption band for a particular vibration, but not a Raman band, or vice versa depending on molecular symmetry. Other non-centresymmetric molecules often exhibit important intensity differences between the Raman and IR bands. Also, since the Raman spectrum of water is weaker than that from the IR, the swamping effect of aqueous material frequently noted in the IR is minimised in the Raman spectrum.

For this study, stratum corneum samples were prepared from human epidermal membranes after the method of Kligman and Christophers (1963). Transmission FTIR spectra of stratum corneum samples were obtained using a Nicolet 740i FTIR spectrometer (100 scans; 4 cm^{-1} resolution) and the same samples were also used to obtain FT-Raman spectra from a Nicolet System 800 FT-Raman spectrometer (100 scans; 4 cm^{-1} resolution). Representative spectra are given in Fig. 1, which compares the data from the two spectroscopic techniques.

The most striking difference between the Raman and IR spectra arises from the O-H stretch (about 3250 cm^{-1}) and H-O-H bend (about 1620 cm^{-1}) of water which are strong features in the IR spectrum but have only a very weak Raman scattering. This effect allows FT-Raman spectroscopy to be performed readily on hydrogels and aqueous solutions, and any problems associated with sample drying during IR studies may be avoided.

Both spectra show considerable detail concerning C-H stretching-features in the range 2700–

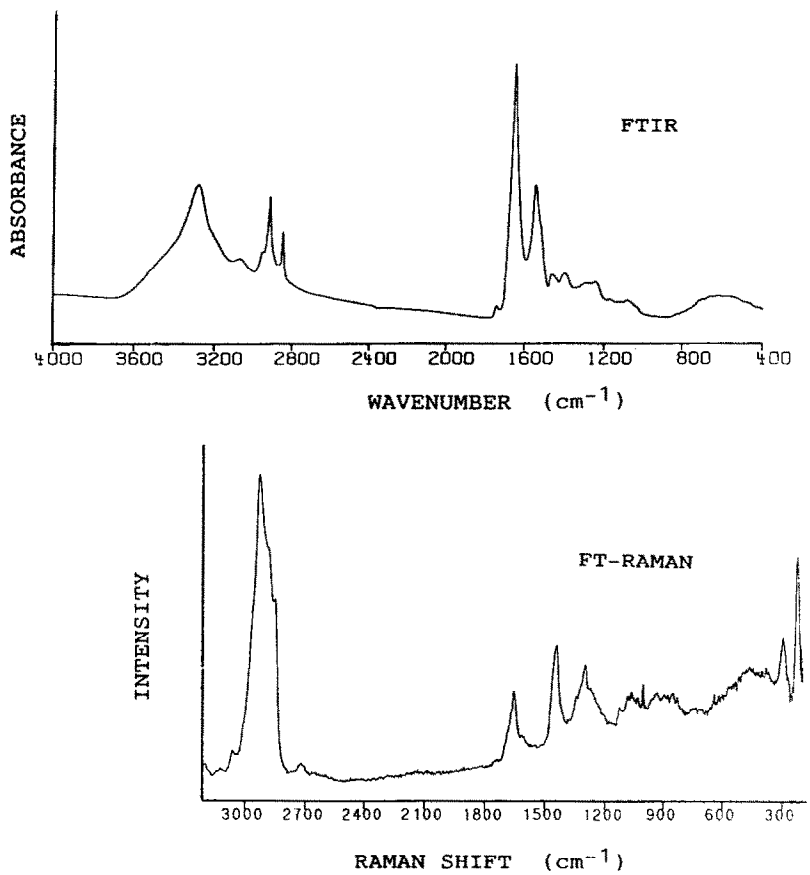

Fig. 1. Vibrational spectra of human stratum corneum; FTIR, Fourier transform infrared spectrum; FT-Raman, Fourier transform Raman spectrum.

3100 cm^{-1}. These vibrations are associated with intercellular lipid bilayers, and detailed studies of molecular arrangements within these bilayers and their modifications by chemical penetration enhancers are currently ongoing in our laboratories.

The spectral features in the range 1200–1700 cm^{-1} provide information as to the functionality of molecules within the stratum corneum. Principal features in this range of the IR spectrum are the amide I (C = O stretch at about 1655 cm^{-1}) and amide II (N-H bend at about 1550 cm^{-1}) absorbance bands. The FT-Raman spectrum is somewhat different; whilst the amide I band is still apparent, it is reduced in intensity to a relatively weak feature when compared with the intense absorbance in the IR spectrum. The amide II band is weak in the Raman spectrum whilst bands assignable to the CH_2 scissoring and to the amide III band (CH_2 twisting at about 1300 cm^{-1}) are present in the Raman spectrum but are weak in the IR spectrum. Also of interest in this spectral range is the poor spectral resolution (baseline separation) in the IR spectrum between the amide I and amide II peaks. This may be due to an underlying H-O-H water bending motion at about 1620 cm^{-1}, which is as previously mentioned a strong IR feature. For quantitative work the presence of this band is undesirable, and even relatively small alterations in water content during a study could greatly affect peak heights and intensity ratios. Also, it is notable that the amide I peaks are located at 1652 cm^{-1} in both the FTIR and FT-Raman spectra, bands which, along with the amide III Raman peak at about 1300 cm^{-1}, are consistent with an α-helix protein assignment in the stratum corneum (Carey, 1982). This contrasts with recent X-ray evidence suggesting that intracellular keratin occurs in the β-form (Garson et al., 1991).

The Raman spectral region 1000–250 cm^{-1} provides fundamental skeletal information about the molecules, such as longitudinal acoustical modes with bands arising from C-C stretching vibrations. This spectral range is complementary to the far-IR region which requires specialised detectors and experimental conditions for analysis, and is an area we are currently investigating.

Clearly, the spectra show that the two techniques are complementary. However, FT-Raman spectroscopy does hold several advantages over FTIR, in addition to those factors mentioned above. For biological macromolecules, Raman bands are usually sharper than IR bands and Raman spectroscopy has been successfully applied to study proteins, DNA and viruses (e.g., Clark and Hester, 1986), although to our knowledge the present article is the first report of FT-Raman spectroscopy of human skin.

A principal advantage of FT-Raman spectroscopy over FTIR is that the intensity of Raman scattering is directly proportional to the concentration of the scattering species, whereas IR absorbance does not relate linearly to absorber concentration. This is clearly an important factor in quantitative analytical studies, although there are also attendant problems in quantitative Raman spectroscopy with source stability and with refractive index and molecular environment changes. The principal disadvantage with Raman spectroscopy at present is that the technique is generally less widely available than IR spectroscopy.

These preliminary studies have indicated the value of FT-Raman spectroscopy for investigations into the barrier nature of human skin. The technique, with its linearity of response, should prove valuable in studies on the nature of healthy and diseased skin, transdermal drug and pollutant permeation and of the mechanisms of action of penetration enhancers. Current studies are aimed at probing some facets of these aspects.

Acknowledgment

The authors thank Nicolet Instruments Ltd for assistance with this study.

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