

Structure of stratum corneum lipids characterized by FT-Raman spectroscopy and DSC. I. ceramides

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Abstract

Fourier transform Raman spectroscopy and differential scanning calorimetry (DSC) were used to study ceramides type IV as a model substance for the ceramides fraction of stratum corneum lipids. The major part of the Raman spectrum consists of bands owing to the alkyl chain, but there are also bands which belong to the head group of the ceramides. The appearance of the conformationally dependent bands indicates the ordered structure of the hydrocarbon chains. The temperature dependence of the conformationally sensitive bands in the CH₂ stretching region (2800–2975 cm⁻¹), in the chain C–C stretching region (1050–1150 cm⁻¹), and in the low frequency region below 300 cm⁻¹ is used to estimate the degree of order in terms of the relative population of trans and gauche conformers. The C–C stretching modes at 1063 cm⁻¹ and 1130 cm⁻¹ (trans conformation) and the asymmetric CH₂ stretching mode at 2880 cm⁻¹ decrease rapidly near the phase transition temperature and disappear in the liquid state. The temperature and width of the phase transition, derived from Raman data, are similar to these of the DSC study.

Keywords: Raman spectroscopy; DSC; Ceramides; Stratum corneum; Phase transition

1. Introduction

The outermost layer of the skin, the stratum corneum, is the major barrier both to dermal and to transdermal delivered drugs. The stratum corneum is a heterogeneous membrane comprising keratin-rich corneocytes embedded in lipid bilayers. The structure and function of stratum corneum were studied using a variety of techniques such as small angle X-ray diffraction

(Friberg and Osborne, 1985), differential scanning calorimetry (DSC) (Bouwstra et al., 1989), electron microscopy (Swartzenhuber et al., 1989), and electron spin resonance (ESR) (Rehfeld et al., 1990). Increasing interest is currently focused on the molecular structure of the stratum corneum. In the literature, Fourier transformed infrared spectroscopy (FTIR) was applied to study the molecular nature of stratum corneum (Golden et al., 1985; Ongpipattanakul et al., 1994). In recent years, the Fourier transform (FT) Raman spectroscopy was also used to collect vibrational spectra of the human stratum corneum (Barry et al.,

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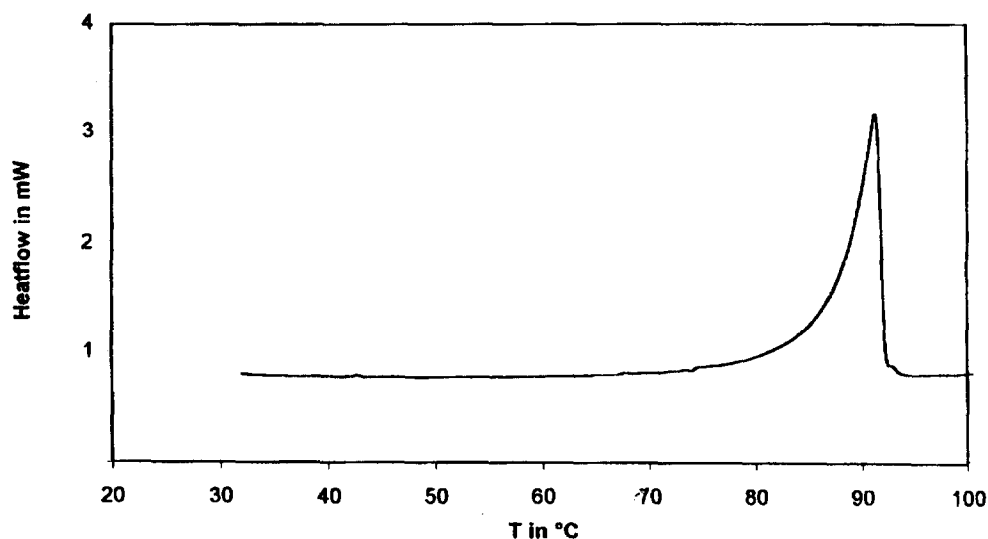


Fig. 1. DSC trace of ceramides type IV.

1992; Williams et al., 1992b; Williams et al., 1993). The FT Raman spectroscopy offers some advantages towards FTIR because of the strong absorption of infrared radiation by water, which presents some problems in FTIR spectroscopy of hydrated lipid bilayers. On the other hand, water shows a weak Raman scattering. This effect allows FT Raman spectroscopy to be used for hydrated probes (Williams et al., 1992a).

Therefore, in this study, FT Raman spectroscopy and DSC were applied to characterize function and structure of stratum corneum lipids. Stratum corneum lipids consist mainly of three fractions: ceramides, fatty acids and cholesterol and its derivatives. We started our investigations with the interesting fraction of the ceramides. The ceramides play an important role in the water-holding properties of the human stratum corneum (Imokawa et al., 1986) and they are required for epidermal barrier function (Holleran et al., 1991). The investigations were carried out using ceramides type IV (Sigma) as a model substance for the fraction of ceramides.

The vibrational properties of hydrocarbon chains and lipids have been the subject of extensive investigation both from experimental and the-

oretical point of view (Harrand, 1983; Huang et al., 1984; Ricard et al., 1985; Brown et al., 1987; Zerbi et al., 1988; Kobayashi et al., 1990). Raman spectroscopy has been widely used for the study of intra- and intermolecular structures of long chain compounds. It is well established to utilize conformationally dependent modes for elucidating the population of trans and gauche conformers, the order/disorder behaviour, phase transitions, the chain packing, and the mobility of chains. So there is a general consensus that the intensity ratio of the asymmetric to the symmetric CH_2 stretching modes can be considered as a measure of the degree of order of the alkyl chains.

2. Materials and methods

2.1. Sample

Ceramides type IV was obtained from Sigma (Sigma Chemical Company St. Louis, Mo. U.S.A.) and was used without further purification. Ceramides type IV contain α -hydroxy fatty acids. For Raman measurements, the sample was

placed in a glass tube and for DSC measurements in a aluminium pan.

2.2. Differential scanning calorimetry

Calorimetric scans were usually performed at 5 K/min on a DSC-2 Perkin-Elmer differential scanning calorimeter. The offset temperature was determined by extrapolation to the baseline of the most rapid rise of the peak as a function of temperature by Perkin-Elmer software. The enthalpy was obtained by integrating the area under the peak of the phase transition.

2.3. Fourier transform Raman spectroscopy

FT Raman spectra were recorded with a Bruker Fourier transform infrared spectrometer IFS 66 equipped with the Raman module FRA 106. A diode pumped Nd:YAG laser which emits radiation at 1064 nm was used as the excitation source. The scattered radiation was collected at 180° to the source. Typical spectra were recorded at a laser power of 300 mW at sample location and a resolution of 4 cm⁻¹. In order to obtain a good signal to noise ratio, typically, 400 scans were coadded for the spectra, corresponding to a measurement time of 10 min. Using the temperature accessory R 495, the temperature dependence of the Raman spectra were studied in the range from 293 to 373 K (stability ± 1 K). The sample was allowed to equilibrate for 15 min to stabilize the temperature before recording each spectrum. The manipulation and evaluation of the spectra were carried out using the Bruker OPUS software package. Generally, Raman intensities were determined as integrated band intensities.

3. Results

In Fig. 1, the differential scanning calorimetry trace of ceramides type IV is given. The probe undergoes an endothermic transition at 89°C. The transition is reversible and reproducible in repeated scans. The enthalpy of ceramides type IV was found to be 112 ± 0.8 J/g.

The Raman spectrum of ceramides type IV at room temperature is shown in Fig. 2. The band assignments according to literature data (Dollish et al., 1973; Fanconi, 1975; Hendra et al., 1977; Snyder and Scherer, 1979; Vergoten and Fleury, 1980; Lin-Vien et al., 1991; Barry et al., 1992) are summarized in Table 1. The major part of the Raman spectrum consists of bands related to the alkyl chain but there are also bands of lower intensity which belong to the head group of ceramides.

The appearance of the conformationally dependent bands $\nu_a(\text{CH}_2)$, $\nu_s(\text{C-C trans})$, $\nu_a(\text{C-C trans})$, and the longitudinal acoustic mode (LAM) clearly indicates the ordered structure of the hydrocarbon chains at room temperature. In particular, the LAM undoubtedly demonstrates the existence of the all-trans (planar zig-zag) conformation of chains.

By increasing the temperature, we have observed changes in three different regions of the Raman spectrum. Fig. 3 illustrates the temperature dependence of the CH₂ stretching vibrations and of the C-C skeletal stretching vibrations, respectively. These changes are thermal reversible.

3.1. Methylene stretching vibrations

As expected in the Raman spectra of ceramides type IV, the stretching vibrations of methylene groups are located in a very complex band spread over the 2800–3000 cm⁻¹ region. The striking feature on increasing temperature is that the intensity of the asymmetric CH₂ stretching line at 2880 cm⁻¹ decreases and disappears in the underlying background above the melting point. As we have already mentioned, it is standard in vibrational spectroscopic studies on lipids to take the intensity ratio $I(\nu_a(\text{CH}_2))/I(\nu_s(\text{CH}_2))$ as a measure of the relative population of the trans and gauche conformers and with it as a degree of the order of the alkyl chain.

In order to determine the integrated intensity of the $\nu_s(\text{CH}_2)$ and $\nu_a(\text{CH}_2)$ lines, we have decomposed the overlapping bands by using the OPUS fit procedure. Assuming six bands with Gaussian line shape, we obtain good fits. Fig. 4 shows

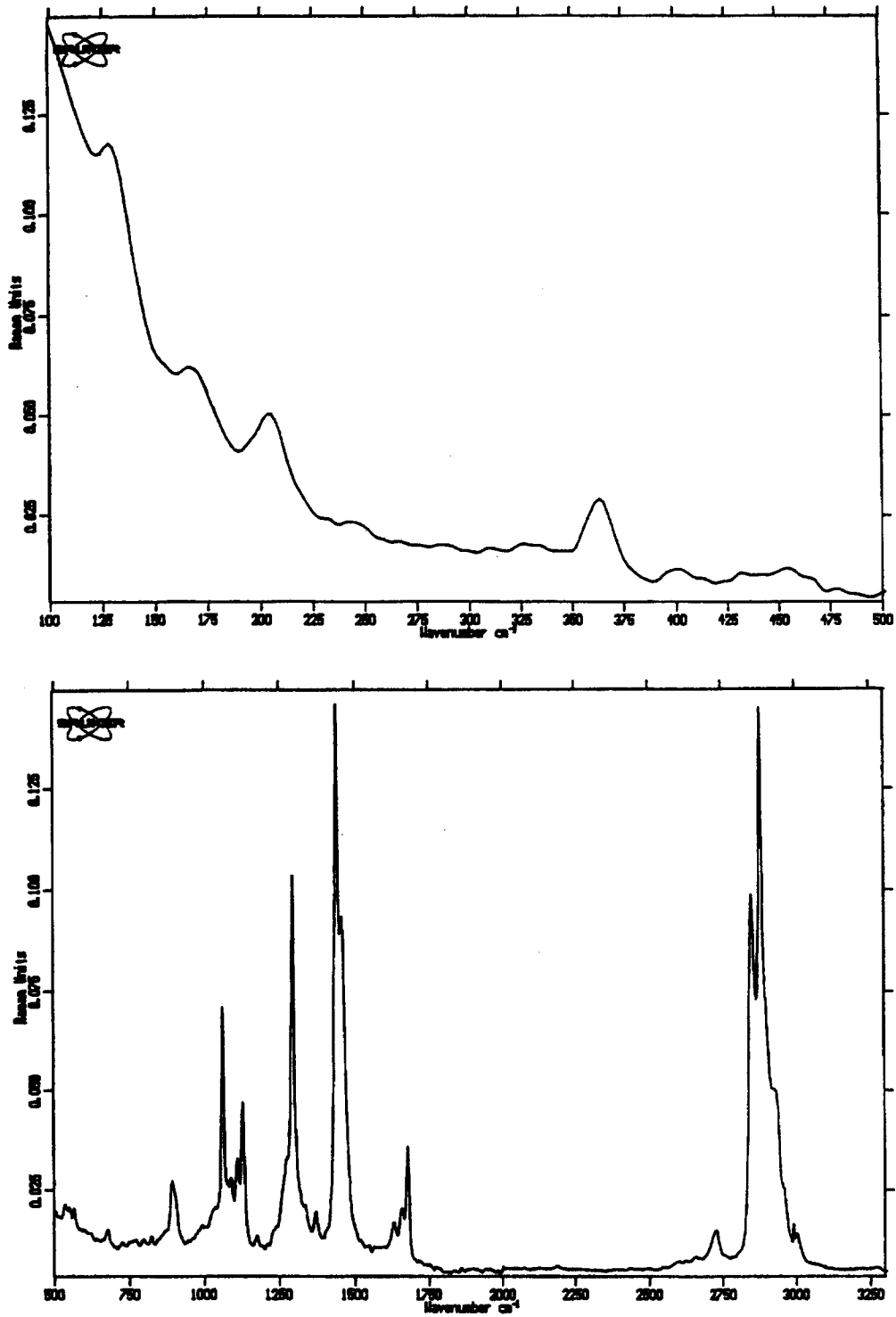


Fig. 2. Raman spectrum of ceramides type IV at room temperature. Top: range 100–500 cm^{-1} , spectrum multiplied by factor 2. Bottom: range 500–3300 cm^{-1} , spectrum in the range 2000–3300 cm^{-1} divided by factor 3.

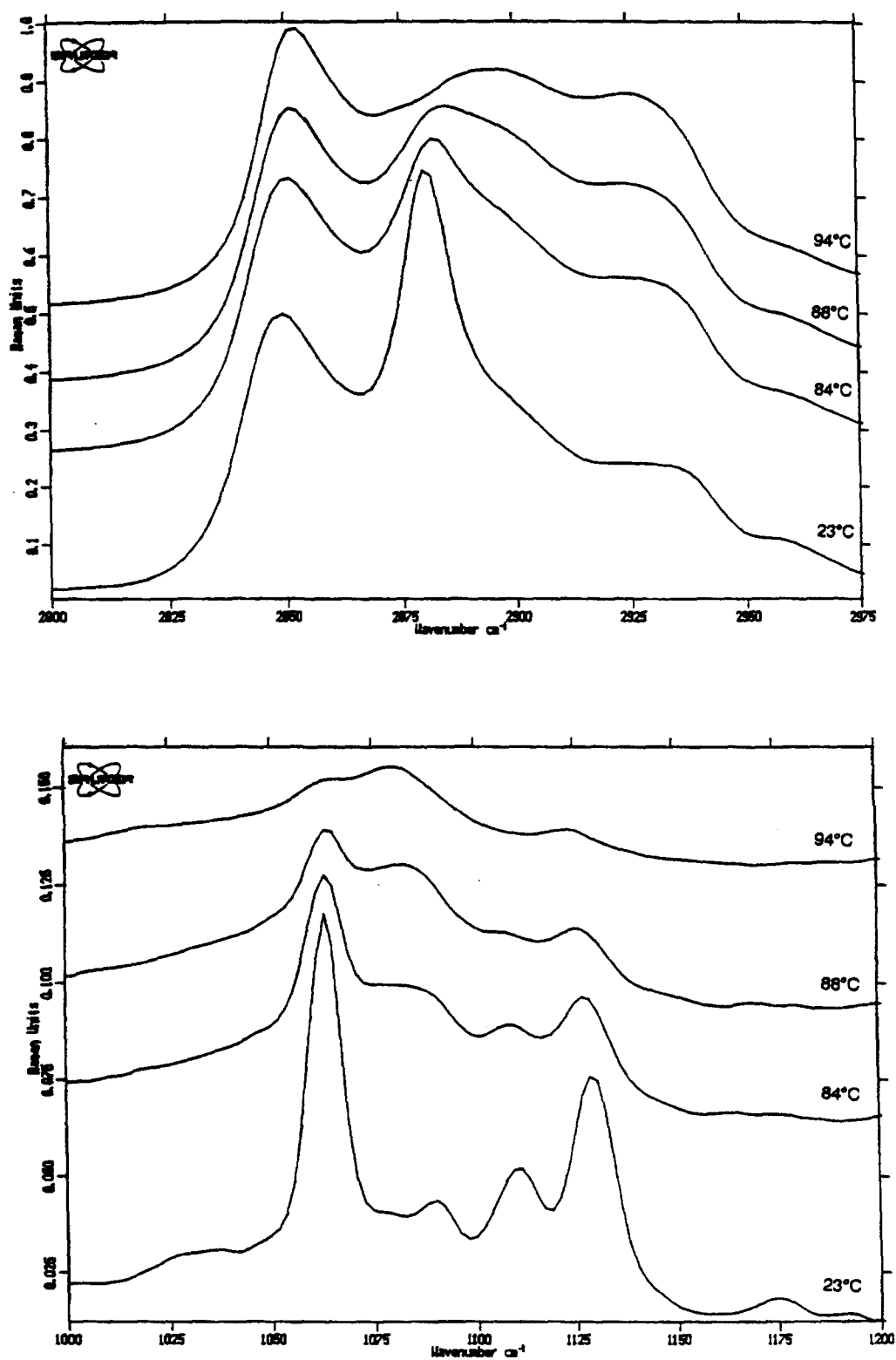


Fig. 3. Temperature dependence of the Raman spectra of ceramides type IV. Top: range 2800–2975 cm^{-1} . Bottom: range 1000–1200 cm^{-1} .

Table 1
Observed FT-Raman bands for ceramide type IV at 293 K and assignments according to literature data^a

Band position (cm ⁻¹)	Assignment
131 vw	Rotational mode about the long axis of the alkyl residue
169 vw	Longitudinal acoustic mode (LAM)
205 w	Longitudinal acoustic mode (LAM)
363 w	δ (CCC) skeletal backbone
677 w	δ (O = C-N) amide IV
891 mw	CH ₃ rocking, tt chain-end conformation
1063 m	ν_a (C-C), alkyl chain in all-trans conformation
1090 w	ν (C-C), alkyl chain disordered
1110 w	ν (C-C), alkyl chain disordered
1130 m	ν_s (C-C), alkyl chain in all-trans conformation
1175 w	Rocking CH ₂ , alkyl chain
1272 sh	Amide III
1295 s	In-phase CH ₂ twisting, alkyl chain
1369 w	Wagging CH ₂ , alkyl chain
1438 s	} CH ₂ scissoring, splitting due to Fermi resonance with CH ₂ rocking
1459 s	
1628 w	Amide I
1654 w	Amide I
1678 m	ν (C = C)
2727 m	ν (CH) aliphatic
2847 vs	ν_s (CH ₂)
2880 vs	ν_a (CH ₂)
2895 s,br,sh	} Fermi resonance ν_s (CH ₂) and overtones of CH ₂ scissoring
2927 s	
2960 m, sh	ν_a (CH ₃)
3002 m	ν (CH) olefinic

^av, very; s, strong; m, medium; w, weak; br, broad; sh, shoulder; δ , deformation; ν_a , asymmetric stretching; ν_s , symmetric stretching.

exemplary the results of the decomposition of the CH stretching modes at room temperature and 6 K below the melting transition. The broad line at 2883 cm⁻¹ seems to be a good approximation for the broad background owing to the Fermi resonance interaction. Temperature induced shifts of the peak positions occur for the asymmetric and symmetric CH₂ stretching mode and for the 'Fermi resonance' band (Fig. 5). Variations of the bandwidth are noticeable only for the broad 'Fermi resonance' band (see Fig. 6). As shown in Fig. 7, with increasing temperature the intensity ratio $I(\nu_a(\text{CH}_2))/I(\nu_s(\text{CH}_2))$ continuously decreases and tends to zero at the phase transition temperature T_c .

3.2. Skeletal stretching vibrations

As illustrated in Fig. 3, the intensity of the symmetric and asymmetric C-C stretching vibrations of the alkyl chain in all-trans conformation decreases with increasing temperature. We have examined the integrated intensity of the ν_a (C-C) band with respect to that of the triplet around 1650 cm⁻¹. The triplet around 1650 cm⁻¹ belongs to modes of the rigid head group. The temperature dependence of the intensity ratio $I(\nu_a(\text{C-C}))/I(\nu \sim 1650 \text{ cm}^{-1})$, as displayed in Fig. 7, is very similar to that of the methylene stretching mode.

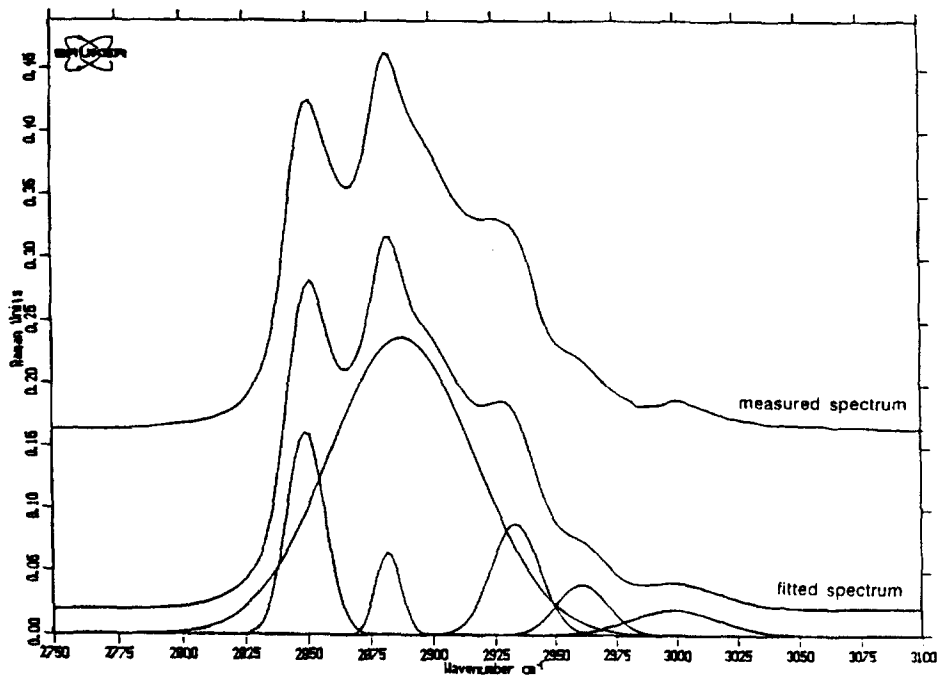
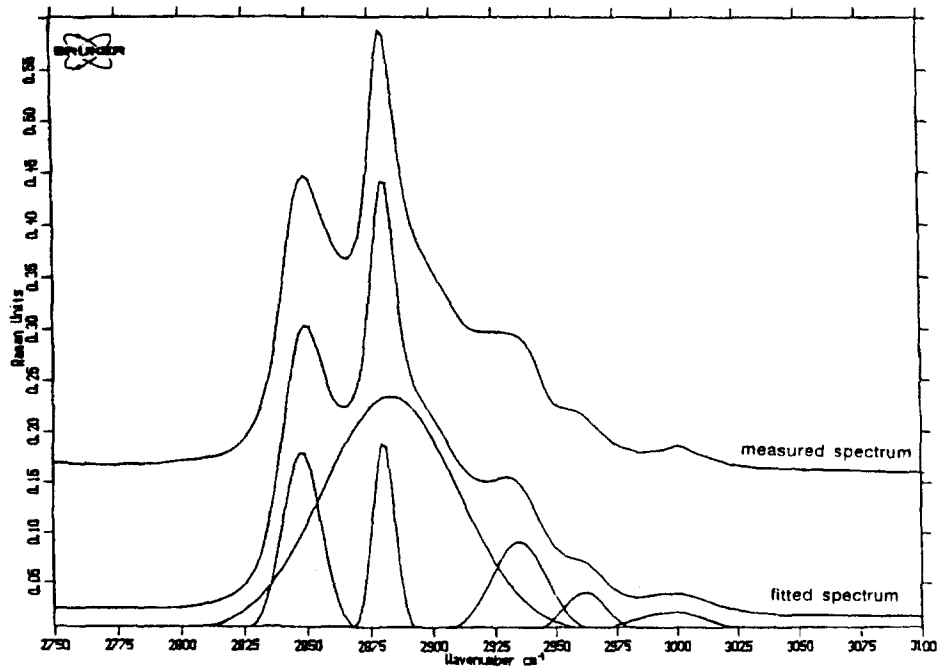


Fig. 4. Decomposition of the methylene stretching band. Top: $T = 23^\circ\text{C}$. Bottom: $T = 84^\circ\text{C}$.

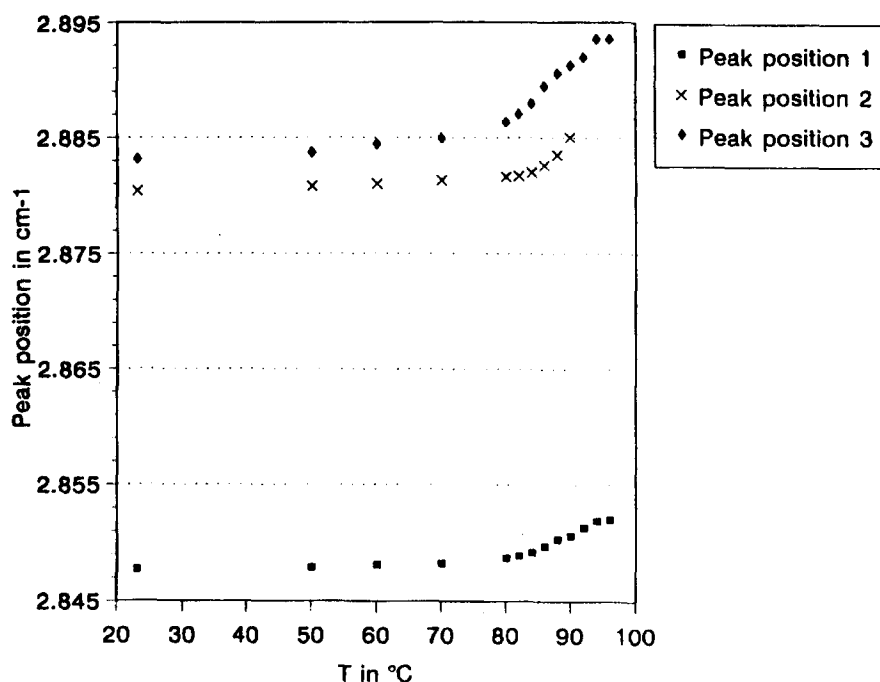


Fig. 5. Temperature dependence of the peak position of the $\nu_s(\text{CH}_2)$ (Peak position 1), $\nu_a(\text{CH}_2)$ (Peak position 2) and 'Fermi resonance' band (Peak position 3), obtained by decomposition of the spectra.

3.3. Low frequency region below 300 cm^{-1}

The bands of weak intensity in the low-frequency region could be monitored up to a temperature of about 6 K below the phase transition temperature T_c . With increasing temperature, the intensity of the longitudinal acoustic modes decreases whereas its band position remains unchanged within the error limits. Unfortunately, a detailed analysis seems to be not reasonable because of the rather large uncertainty due to the very weak intensities of the lines. Nevertheless, the temperature dependence of the LAM indicates that alkyl chains in the planar all-trans conformation exist certainly up to a few degrees below the melting point.

4. Discussion

In the present work, the ceramides type IV were used as a model substance for the ceramides fraction of stratum corneum. Ceramides type IV

are obtained from the cerebroside fraction which contains α -hydroxy fatty acids. The sphingosine composition of bovine brain cerebroside are as follows: 88.5% C18-sphingene, 8.5% C18-sphinganine, and 3% C20-sphingene of which the sphinganine does not possess a double bond in the 4,5 position (Curatolo and Jungalwala, 1985). In view of the amide-linked acyl chain, the hydroxy fatty acid cerebroside consist of primarily C18:0, C22:0, C24:0 and C24:1. The content of different chain length in ceramides type IV is the reason for the broad phase transition in the DSC trace. This broad phase transition is also to be seen in the temperature dependence of the Raman spectra (Fig. 7). At the beginning of the melting process about 75°C, indicated in the DSC curve, the number of alkyl chains in all-trans conformation decreases to nearly 70% of that at room temperature. The temperature of the phase transition determined with DSC is nearly the same as that from the Raman data, although a direct comparison is not possible because the temperature/time regime is not the same. The appearance

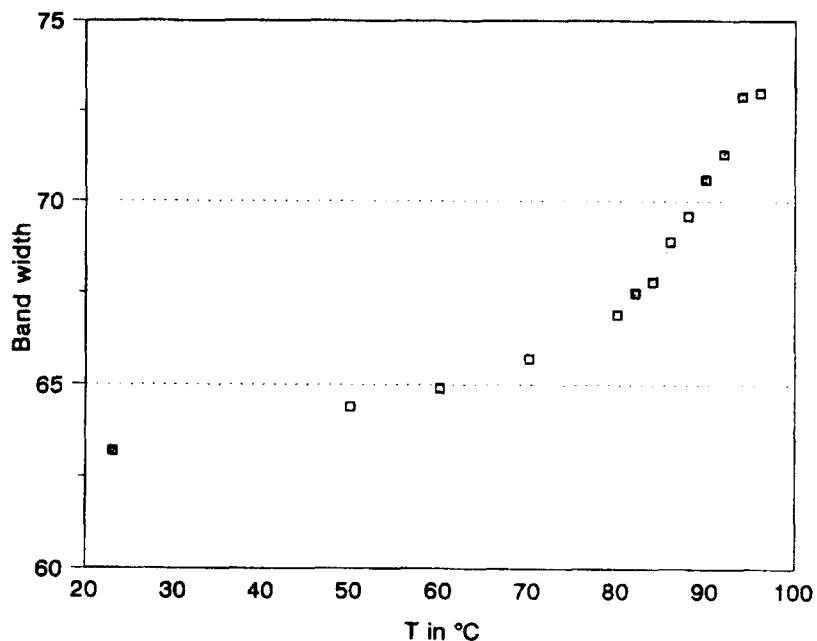


Fig. 6. Temperature dependence of the band width of the 'Fermi resonance' band, obtained by decomposition of the spectra.

of the longitudinal acoustic modes at 169 cm^{-1} and 205 cm^{-1} hints also at $(\text{CH}_2)_n$ chains in

all-trans conformation with different chain lengths.

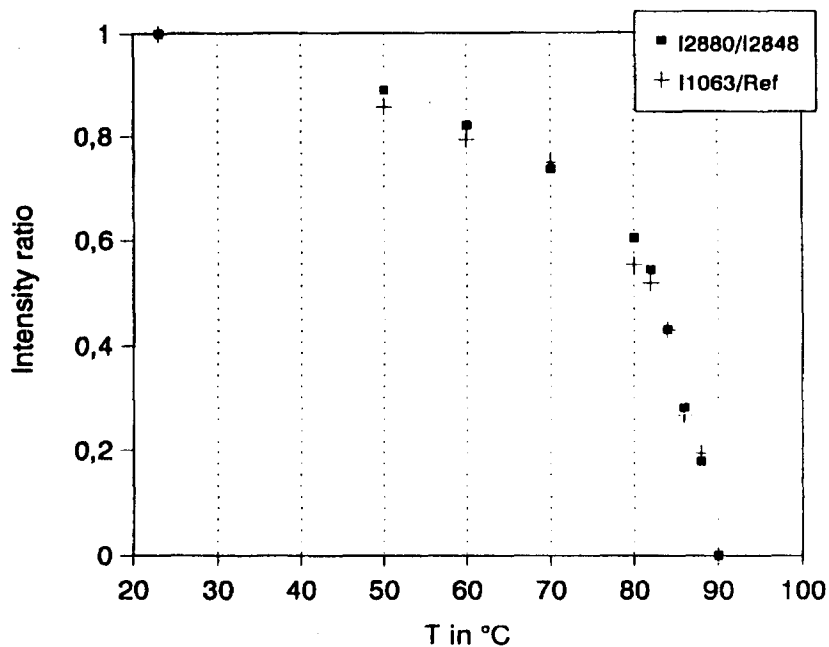


Fig. 7. Temperature dependence of the normalized intensity ratios $I(\nu_a(\text{CH}_2))/I(\nu_s(\text{CH}_2))$ and $I(\nu_a(\text{C-C}))/I(\text{Ref.})$. Ref: triplet around 1650 cm^{-1} .

In summary, we have demonstrated that the Fourier transform Raman spectroscopy is a useful method to characterize the molecular structure of stratum corneum lipids such as ceramides. In addition to DSC studies on the phase behaviour of ceramides type IV, Raman spectroscopic investigations elucidate the molecular aspects of the melting process. It was demonstrated that ceramides type IV exhibit a highly ordered structure of the hydrocarbon chains in the crystalline state. From the temperature dependence of Raman band intensities of conformationally sensitive modes, we have obtained a quantitative description of the conformational changes connected with the melting process. Further studies concerning the interactions between ceramides and cholesterol, and fatty acids, respectively, are in progress.

Acknowledgements

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