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STUDIES ON DETERMINING CONFORMATIONAL ORDER IN *n*-ALKANES AND PHOSPHOLIPIDS FROM THE 1130 cm^{-1} RAMAN BAND *

ROBERT G. SNYDER [a],**, DAVID G. CAMERON [b], HECTOR L. CASAL [b],
DAVID A.C. COMPTON [b] and HENRY H. MANTSCH [b]

[a] *Department of Chemistry, University of California, Berkeley, CA 94720, U.S.A.* and [b] *Division of Chemistry, National Research Council of Canada, Ottawa, Ontario K1A 0R6 (Canada)*

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Conformational disorder in lipid bilayer systems is commonly measured with reference to the intensity of the 1130 cm^{-1} Raman band. However, estimates of the concentration of *gauche* bonds may vary by a factor of six according to the model used to relate intensity and concentration. In an effort to narrow the wide range in these estimates, we have measured the intensity of the 1130 cm^{-1} band of crystalline *n*-$C_{21}H_{44}$ in its orthorhombic and hexagonal phases. On transition to the hexagonal phase, the intensity of the 1130 cm^{-1} band is much reduced. It is assumed that the observed intensity reduction results from the introduction of *gauche* bonds whose number can be independently estimated from other features in the Raman and infrared spectra. From these measurements we conclude that the intensity of the 1130 cm^{-1} band is not linearly related to the concentration of *gauche* bonds and that a disproportionately large decrease in the 1130 cm^{-1} band intensity results from the introduction of a low concentration of *gauche* bonds. Thus previous estimates of *gauche* bond concentrations based on the assumption of a linear relation have tended to greatly overestimate the *gauche* bond concentration. These results derived from experiment are in accord with those of Pink et al. (Pink, D.A., Green, T.J. and Chapman, D. (1980) Biochemistry 19, 349–356) derived from theory.

Introduction

Certain bands in the Raman spectra of lipid bilayer systems are remarkably sensitive to the conformation of the hydrocarbon chains and have been used frequently to monitor conformational disorder in these systems. For the purpose of such measurements, C-C stretching bands, which dominate the region 1150–1000 cm^{-1}, have been most often employed [1–7]. The band near 1130 cm^{-1} is of special interest since it is associated with a relatively well defined type of motion, namely the in phase C-C stretching mode of an all-*trans* segment of the hydrocarbon chain [8,9]. This band is known to undergo a reduction in intensity as *gauche* bonds are introduced into the chain [1]. Quantitative interpretation relating its intensity to the number of *gauche* bonds has proved difficult, however.

Two treatments of this problem are to be found in the literature, one by Gaber and Peticolas (henceforth referred to as GP model [2] and another by Pink, Green, and Chapman (henceforth referred to as PGC model [4]. The two treatments lead to significantly different estimates of the number of *gauche* bonds. The difference is largest for systems that are highly ordered, and in such cases, the gauche bond concentration esti-

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* N.R.C.C. Publication No. 18996.

** To whom correspondence should be addressed.

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mated from the GP model may be greater by a factor of six or more than that estimated from the PGC model.

The difficulty in interpreting Raman spectra in this region of the spectrum has led to a wide range of values reported for the number of *gauche* bonds in the gel state of the dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) bilayer system. For example, just below the pretransition of DPPC, Gaber and Peticolas [2] report that there are between two and three *gauche* bonds per chain while Yellin and Levin [5] report an average of one *gauche* bond per chain for this system at the same temperature.

Infrared studies on the gel state of DPPC by Cameron et al. [10,11] demonstrate that the lateral interchain interactions change continuously with temperature. However, their data seem inconsistent with a substantial increase in conformational disorder as the temperature is increased from −50°C to just below the main transition T_m. In contrast, Gaber and Peticolas [2] conclude that at a temperature just below T_m there is a total of about five *gauche* bonds per chain. According to these authors, three *gauche* bonds are formed below the pretransition temperature while two appear in going through the pretransition. Thus the infrared results appear more in keeping with the much lower estimate of *gauche* bond concentration that results when the PGC model is used.

To help clarify the situation, we have made measurements on the 1130 cm^{-1} Raman band of a less complex system, n-$C_{21}H_{44}$. At low temperatures, the chains of this *n*-alkane are fully extended and are packed in an orthorhombic subcell. The packing is probably similar to that found in DPPC bilayers at low temperatures [12]. At 32.5°C, n-$C_{21}H_{44}$ undergoes a solid-state transition to the 'hexagonal' or 'rotator' phase in which the chain packing tends to be hexagonal [13]. There is a large increase in the concentration of *gauche* bonds in going to the high temperature solid phase [14]. The increase in concentration can be estimated from intensity changes in bands other than the Raman 1130 cm^{-1} band and then compared with the increase derived from the GP and the PGC models on the basis of changes in the intensity of the 1130 cm^{-1} band.

Experimental

The temperature dependence of the 1130 cm^{-1} band was measured in two ways. In the first, the n-$C_{21}H_{44}$ was mechanically mixed with K_2SO_4, and the 986 cm^{-1} band of the K_2SO_4 was used as an intensity reference. In the second, the integrated intensity of the entire C–H stretching region (3000–2800 cm^{-1}) of n-$C_{21}H_{44}$ was used as an internal reference. The results of both sets of measurements were in excellent accord. The quantitative data presented here are from the second set.

The use of the integrated Raman intensity of all C–H stretching modes as an internal standard has been reported earlier in a study of the low-frequency region of the liquid *n*-alkanes [15]. In that study, it was found that the integrated intensity of the C–H bands remained constant to within a few percent in going from the solid to the liquid state. A constant value for the integrated C–H stretching intensity is also indicated in studies on DPPC [3].

Raman spectra of pure n-$C_{21}H_{44}$ were recorded with a computer controlled Spex 14018 double monochromator with a cooled photomultiplier (RCA 31034). The excitation was from the 5145 Å line of a Coherent Radiation CR 12 argon-ion laser giving 220 mW at the sample. The spectral slit width was 2 cm^{-1}. Spectra were recorded at several temperatures ranging from −102 to 37°C. The local sample heating due to the laser beam is estimated to be approximately 3 K. These measurements consisted of four independent experiments with two fresh samples. There was excellent agreement between separate measurements. All temperature effects were found to be reversible.

The spectra in Fig. 1 were measured using a computer controlled double monochromator (Spex 1401) which has been described previously [16]. The excitation was also from the 5145 Å line of an argon-ion laser giving about 200 mW at the sample. The spectra slit width was 1 cm^{-1}.

Results

Raman spectra of n-$C_{21}H_{44}$ in the orthorhombic form at 25°C and in the hexagonal form at 35°C are shown in Fig. 1. In both spectra the

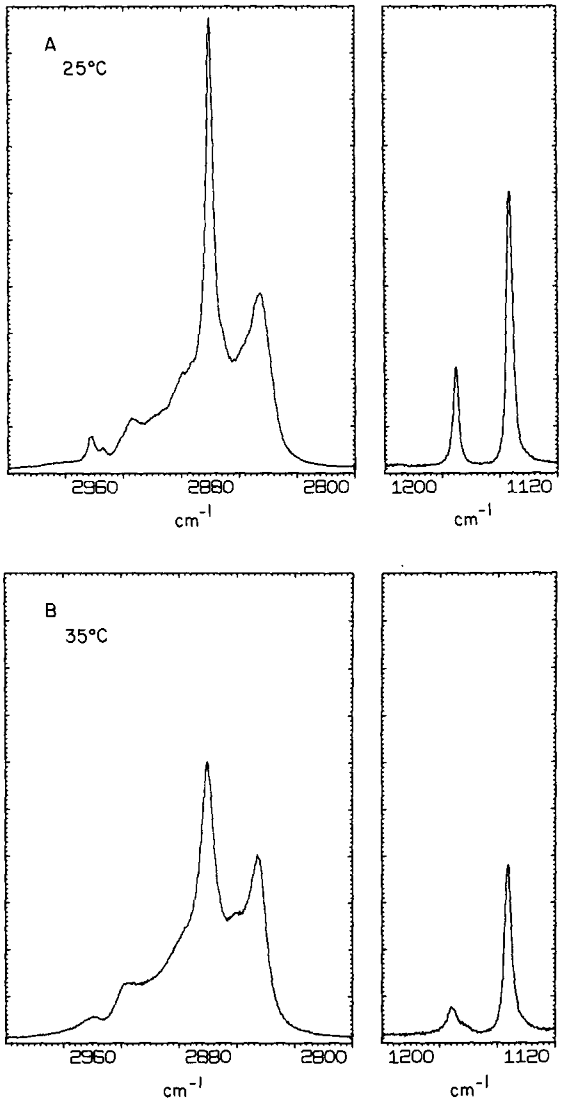

Fig. 1. Raman spectra of crystalline n-$C_{21}H_{44}$. (A) Orthorhombic phase at 25°C. (B) Hexagonal phase at 35°C.

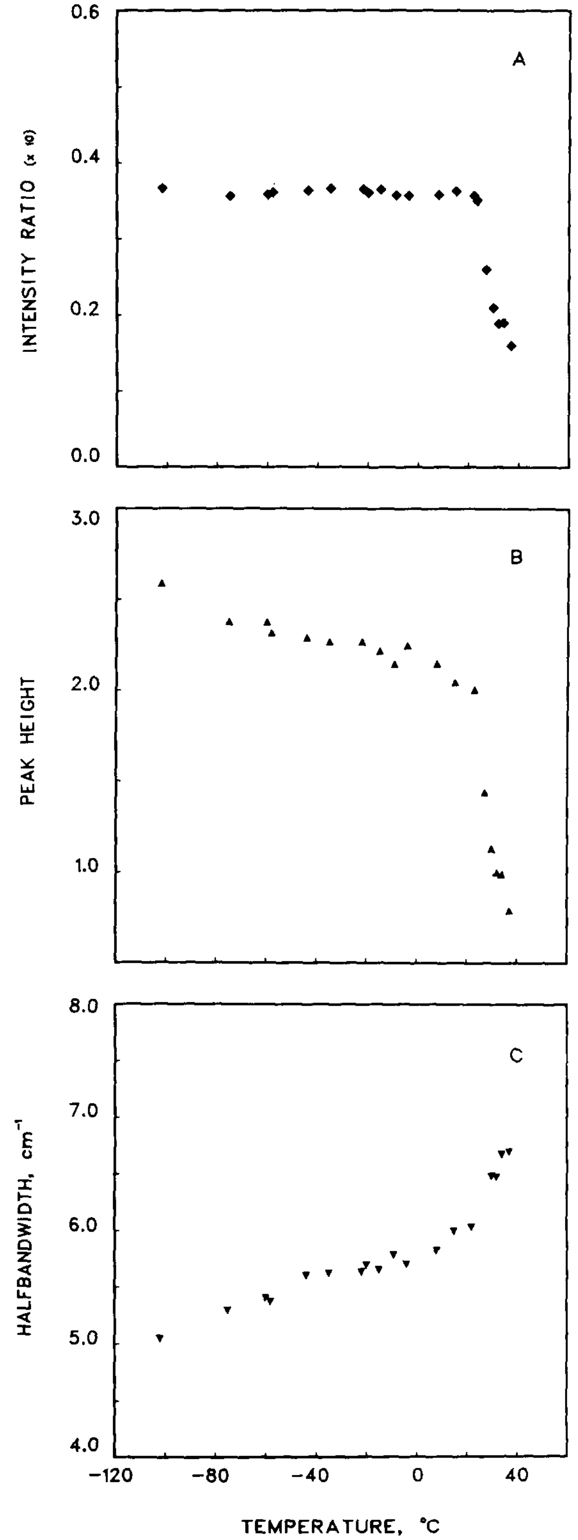

Fig. 2. Temperature dependence of the 1130 cm^{-1} band. The temperatures indicated have not been corrected for heating (estimated to be approx. 3 K) due to the exciting radiation. (A) Intensity ratio versus temperature. The intensity ratio is the integrated intensity of the 1130 cm^{-1} band divided by the integrated intensity in the region 2800–3000 cm^{-1}. (B) Peak height versus temperature. Peak height values were normalized by dividing by the integrated intensity of the 2800–3000 cm^{-1} region. The value 1.0 was assigned to the peak height at 32°C. (C) Band halfwidth versus temperature. (Baseline 1155–1115 cm^{-1})

intensity scale indicated for the 1130 cm^{-1} region is expanded 16:3 relative to that for the C–H stretching region. The spectra have also been scaled so that the integrated areas of the C–H stretching bands are the same for both crystalline forms. This permits direct comparison of the 1130 cm^{-1} bands at the two temperatures.

The temperature dependence of the intensity, peak height, and half-width of the 1130 cm^{-1} band is indicated in Fig. 2. In the orthorhombic phase (−102 to 30°C) the ratio of the integrated intensity of the C–H stretching bands to that of the C–C stretching band is nearly constant. At the transition temperature, however, there is an abrupt decrease (approx. 40%) in the intensity of the 1130 cm^{-1} band.

As the temperature is increased through the hexagonal phase, the intensity of the 1130 cm^{-1} band continues to decrease. Continuous change in the structure of the hexagonal phase with temperature is indicated also by the behavior of intermolecular band splitting observed in the infrared spectra (Casal, H.L., Cameron, D.G., Mantsch, H.H. and Snyder, R.G., unpublished data) and by increases in the intensity of bands assigned to nonplanar chain conformations [14].

The peak intensity of the band (Fig. 2b) shows greater dependence on temperature than the integrated intensity. A decrease of about 24% occurs over the temperature range from −100°C to just below the transition. The peak intensity just below the transition temperature is reduced by about 60% after the temperature is increased to within a few degrees of the melting point. Although in the orthorhombic phase the peak intensity decreases steadily with increasing temperature, the halfwidth of the band increases so that the product of half-width and the normalized peak intensity yields intensities whose temperature dependence is similar to that of the integrated intensity.

Discussion

The assignment of the 1130 cm^{-1} band

The theoretical justification for using the 1130 cm^{-1} band as a measure of conformational order rests in large part on the results of normal coordinate calculations on the *n*-alkanes. The Raman spectra of the crystalline *n*-alkanes and the infrared spectra of those members having an odd-number of carbon atoms show a band near 1130 cm^{-1} that has been assigned to the totally symmetric C–C stretching mode of the fully extended chain [8,9]. Relative to other bands, the 1130 cm^{-1} band is weak in the infrared and strong in the Raman. Less complete analyses are available for conformationally disordered *n*-alkanes due to the complexity of these systems. However, those calculations do suggest that, within the longer trans or nearly trans segments of the disordered chain, there occurs a symmetric C–C stretching vibration that is similar in form and near in frequency (±5 cm^{-1}) to the symmetric C–C stretching mode of the fully extended chain [17].

The computational results are supported by experiment. The Raman spectra of the liquid *n*-alkanes show an intense band near 1130 cm^{-1}. However, this band is less intense than it would be were the sample a solid (in which case all the chains would be fully extended) so that it is clear that the effect of *gauche* bonds is to decrease the intensity of the 1130 cm^{-1} band. On the other hand, the intensity of this band in the spectrum of the liquid is much too large to be accounted for by fully extended rotamers since their concentration in the liquid state is very low. Thus its intensity must be attributed to contributions from *trans* segments in the liquid state. It has been proposed by analogy that, in the case of lipids, the intensity of the 1130 cm^{-1} band is related to the number and length of the *trans* segments.

Two models

Derivations of a quantitative relation between the observed intensity of the 1130 cm^{-1} band and the ratio of the numbers of *trans* to *gauche* bonds require a vibrational model for calculating the intensity contribution of each rotamer. In the GP model [2], each *trans* segment is assumed to contribute independently to the 1130 cm^{-1} band and the contribution is assumed to be proportional to the number of C–C bonds in the segment. An 'order parameter' is defined in terms of the ratio of the intensity of the 1130 cm^{-1} band of the lipid bilayer to that of the same sample in the crystalline state, in which case all bonds are presumed to be in the trans state. This ratio, normalized with an internal intensity standard, is taken as a linear measure of the number of *trans* bonds present, a value of one indicating all bonds are *trans*, and a value of zero indicating no bonds are *trans*.

In the PGC model [4] the coupling between the symmetric C–C stretching vibrations of two *trans* segments separated by a *gauche* bond is taken into account. This coupling causes the two segments to vibrate mutually out of phase [17], and its effect is to amplify the intensity decrease per *gauche* bond at low *gauche* bond concentration. As a result, the application of the PGC model leads to a lower concentration than does the GP model.

Since, in the PGC model, intensities are dependent on the location of *gauche* bonds, a statistical mechanical model of the bilayer is included in the formulation. The PGC model accounts for the observed intensity changes in the 1130 cm^{-1} band of DPPC bilayers from −150°C through the melting temperature, the discontinuous behavior at the pretransition excepted.

Interpretation of the n-$C_{21}H_{44}$ data

The observed decrease in the intensity of the 1130 cm^{-1} band will be analyzed in terms of the PGC and GP models after we briefly summarize some relevant aspects of the structure of the hexagonal phase.

As temperature is increased from just below the orthorhombic-to-hexagonal transition, the lattice parameters of the lower temperature form change abruptly towards values associated with hexagonal crystal symmetry. Müller [18] suggested that at higher temperatures the chains were undergoing increased amplitudes of rotation-twisting type motions about their long axes. With an increasing loss of orientational correlation between chains, the effective (statistical) symmetry tends to become hexagonal. This view is still generally accepted.

Earlier vibrational studies have made the point that the chains in the hexagonal phase are predominately *trans* as they are in the lowest temperature phase. Thus the most prominent features in the infrared spectrum of hexagonal *n*-$C_{29}H_{60}$ [19] and the Raman spectrum of hexagonal *n*-$C_{19}H_{40}$ [20] were found to be essentially the same as those in the spectra of the *n*-alkanes in their orthorhombic form. Band splitting due to intermolecular coupling in the orthorhombic phase was not present in the hexagonal phase and this can be accounted for in a plausible manner in terms of differences in the two crystal structures.

There are, however, substantial differences between the spectra of the orthorhombic and hexagonal phases that cannot be accounted for in terms of intermolecular effects. These differences, which consist, in part, of bands that appear in the hexagonal phase but not in the orthorhombic phase, may be readily explained in terms of a low concentration of *gauche* bonds in the hexagonal phase. Most of these bands can be attributed to localized methyl group vibrations that are characteristic of rotamers having *gauche* bonds near the ends of the molecule while some are due to conformational defects within the chain [14]. All of the bands indicative of a *gauche* population increase as the temperature is raised in the hexagonal phase, paralleling the continuous decrease in the intensity of the 1130 cm^{-1} band.

We have estimated the concentration of *gauche* bonds in the hexagonal phase of *n*-$C_{21}H_{44}$ from the intensity of the longitudinal acoustic mode (LAM-1) band that appears in the Raman spectrum near 110 cm^{-1}. The LAM-1 band is unique to the all-*trans* conformation of the *n*-alkanes [21]. In going to nonplanar conformations, the form of this vibration is drastically modified and its frequency is displaced away from its value for the all-*trans* conformer [22]. An exception occurs when the otherwise all-*trans* form has a *gauche* bond at the end of the molecule. In that case, the frequency of LAM-1 is displaced upward a few wavenumbers [15]. Thus the intensity of the LAM-1 band is a linear measure of the combined concentration of all-*trans* and end-*gauche* conformers.

Our Raman measurements on this band in the case of *n*-$C_{21}H_{44}$ indicate that less than one *gauche* bond per molecule is introduced into the otherwise all-*trans* chains during the transition to the hexagonal phase. This estimate is based on an observed decrease of 10 ± 10% in the LAM band intensity in going from a temperature a few degrees below to a few degrees above the transition temperature. If the temperature is further increased to within a few degrees of the melting point, the reduction from the orthorhombic value is 50 ± 20%. A shoulder appears on the high frequency side of LAM-1 for the hexagonal phase which is probably due to the presence end-*gauche* forms. From the intensity of the shoulder relative to the main band, we estimate perhaps 20 ± 10% of the molecules are end-*gauche* conformers, a number that is in line with the results of earlier infrared measurements [14]. In any case, these results indicate that the overall *gauche* bond concentration is well below one per chain. The value is in keeping with that of 0.7 'defects' per chain estimated by Strobl [23] for n-$C_{33}H_{68}$ from X-ray measurements.

The observed decrease in the intensity of the 1130 cm^{-1} band at the transition corresponds to at least six *gauche* bonds per chain according to the GP model and to about one *gauche* bond per chain according to the PGC model. It is clear that the latter model leads to a more accurate number.

Finally, we note that the present interpretation of the observed decrease in intensity differs from, and corrects our earlier preliminary communication reporting that the observed change was due primarily to intermolecular effects [24].

Conclusions

We conclude from our Raman measurements on n-$C_{21}H_{44}$ that, while the 1130 cm^{-1} band does provide a useful monitor of change in conformational order in bilayer systems, its integrated intensity is a nonlinear measure of *gauche* bond concentration, i.e., the introduction of a low concentration of *gauche* bonds leads to a disproportionally large reduction in the intensity of the 1130 cm^{-1} band. Consequently, many earlier estimates of *gauche* bond concentrations are high because a linear relation between intensity and concentration was assumed. The markedly lower estimate derived from the PGC model [4] is consistent with the results of recent infrared measurements on lipid bilayer systems that indicate a low concentration of gauche bonds in the gel phase of DPPC [10,11].

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