

Investigation of the Temperature Behavior of the Bands Due to the Methylene Stretching Vibrations of Phospholipid Acyl Chains by Two-Dimensional Infrared Correlation Spectroscopy*

ANNE NABET, MICHÈLE AUGER, and MICHEL PÉZOLET †

Centre de Recherche en Sciences et Ingénierie des Macromolécules, Département de Chimie, Université Laval, Cité Universitaire, Québec, Canada, G1K 7P4

The temperature-induced gel-to-liquid crystalline phase transition of dipalmitoylphosphatidylcholine (DPPC) is characterized by a shift towards high frequencies and an increase of the width of the bands due to the methylene stretching vibrations. These spectral modifications are frequently used to measure the conformational order of lipid acyl chains. However, it is not clear whether these bands contain two spectral components due to *trans* and *gauche* conformers or whether they gradually shift with temperature. The temperature-induced gel-to-liquid crystalline phase transition of DPPC has been investigated in the present study by two-dimensional infrared (2D-IR) correlation spectroscopy. Our results show that each methylene stretching band in both the synchronous and the asynchronous maps is characterized by two peaks. The same pattern is also observed when the temperature range is restricted to the gel phase. These results were compared to those obtained by spectral simulations using either a single band that shifts in frequency and gets broader with the increase of temperature (shifting-band model), simulating a continuously evolving one-phase system, or a band made of two components (two-band model), simulating the *trans* and *gauche* spectral contributions of a two-phase system. The results obtained for the asynchronous maps of the simulated spectra indicate clearly that the experimental results cannot be modeled by a pure two-phase system and are best simulated by the shifting-band model.

Index Headings: Two-dimensional infrared; 2D-IR; Lipid conformation order.

INTRODUCTION

The infrared bands due to the CH₂ and CH₃ stretching and bending vibrations have been used extensively to obtain information about the conformation, packing, and orientation of lipid acyl chains. It has been shown that the intensity of the CH₂ rocking and wagging bands and of the CH₃ bending band can be used to quantify the number of *trans* and *gauche* rotamers along the acyl chains of phospholipid bilayer membranes.¹⁻³ From these studies, it appears that an average of four *gauche* bonds per hydrocarbon chain of dipalmitoylphosphatidylcholine (DPPC) are present in the liquid crystalline phase. These studies have also shown that there are more *gauche* bonds at the end of the acyl chains and that the probability of finding *gauche* rotamers increases with increasing the phospholipid chain length.

Structural information is, however, more difficult to obtain from the CH₂ stretching bands since the 2800–3000 cm⁻¹ spectral region is very complex and contains spectral contributions from the methylene and methyl stretching vibrations and from Fermi resonance bands.^{4,5} Nevertheless, the temperature-induced gel-to-liquid crystalline phase transition of phospholipid membranes is characterized by a shift towards high frequencies and an increase of the width of the bands due to the methylene stretching vibrations. These spectral modifications have been used frequently to follow qualitatively the conformational order of the lipid acyl chains and the *trans-gauche* isomerization of the lipid methylene groups.⁶

A comparative study between the conformational order obtained by ²H solid-state nuclear magnetic resonance (NMR) and infrared spectroscopy has shown that a linear correlation exists between the acyl chain conformational order and the frequency of the methylene stretching vibration.⁷ Also, a linear correlation has been observed between the frequency of the CH₂ bands and the order parameter determined by polarized infrared attenuated total reflection (ATR) spectroscopy for these bands.⁸⁻¹⁰ A decrease of the ATR order parameter indicates a decrease of conformational order, which can be interpreted as a reorientation of the phospholipid acyl chains, an increase of the width of the orientation distribution, an increase of the number of *gauche* conformers, or a change in rotational order.⁸⁻¹¹ The linear correlation between the infrared order parameter and the frequency of the CH₂ stretching band was observed for DPPC both in the lamellar crystalline orthorhombic subphase (L_c') and in the liquid crystalline phase (L_a).⁸ Finally, it has also been demonstrated that the frequency of the CH₂ stretching bands is sensitive to the intermolecular coupling and to the librational motions.¹² Large frequency shifts, up to 2.5 cm⁻¹, were observed in the case of high isotopic dilutions of proteated acyl chains in a matrix of perdeuterated acyl chains.^{12,13}

In order to describe the temperature-induced cooperative conformational transition of DPPC, Dluhy et al. have used a two-phase model, using the Zimm–Bragg theory.¹⁴ In this model, a fractional population parameter corresponds to a degree of transition between the gel and the liquid crystalline states. The infrared parameter used was the frequency of the band due to the methylene symmetric stretching vibration of phosphatidylcholine acyl chains. More recently, Kóta et al. concluded that, with the use of the singular value decomposition analysis, both

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† Author to whom correspondence should be sent.

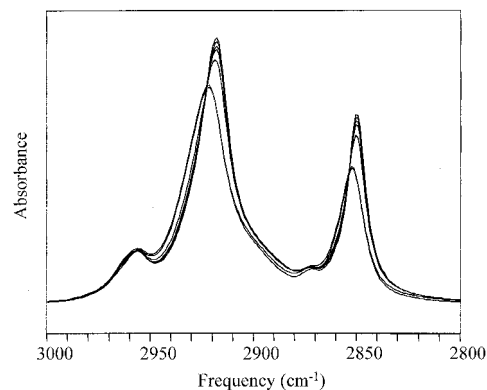


FIG. 1. Temperature dependence of non-normalized infrared spectra of DPPC in the C-H stretching mode region.

the symmetric and antisymmetric CH₂ stretching bands are composed of two bands due to the *trans* and *gauche* conformers spectral contributions.¹⁵

Despite these studies, it is still unclear whether the frequency shift, observed during the gel-to-liquid crystalline phase transition, is caused by an increase in the frequency of the stretching vibrations related to the introduction of *gauche* bonds along the chains or to a decrease of a component associated with *trans* segments in favor of a component due to CH₂ groups in a *gauche* conformation. The temperature behavior of the methylene bands can also be seen as a continuous or coupling process of isomerization while the phospholipid bilayers undergo the temperature-induced gel-to-liquid crystalline phase transition.

In the present study, the temperature-induced gel-to-liquid crystalline phase transition of DPPC has been investigated by two-dimensional (2D) infrared (IR) correlation spectroscopy. According to the generalized formalism proposed by Noda, the use of 2D-IR correlation spectroscopy could help to unravel the different components in an infrared spectrum.¹⁶ In theory, the asynchronous correlation map is helpful in the case of noncooperative phenomena, while the synchronous correlation map should help to assign cooperative spectral components due to the temperature-induced gel-to-liquid crystalline phase transition. However, recent studies have pointed out that the interpretations of such maps has to be done carefully.^{17,18} The 2D correlation maps obtained from the spectra of DPPC as a function of temperature are compared in this paper with those obtained by spectral simulations using either a single band that shifts in frequency and gets broader with the increase of temperature (shifting-band model), simulating a continuously evolving one-phase system, or a band made of two components (two-band model), simulating the *trans* and *gauche* spectral contributions of a two-phase system.

MATERIAL AND METHODS

DPPC was purchased from Avanti Polar Lipids (Alabaster, AL). A dispersion containing 10% (w/v) of the phospholipid in water was prepared by heating and cooling this dispersion three times at a temperature 15 °C above and below the main gel-to-liquid crystalline phase transition temperature (42 °C). A drop of the dispersion

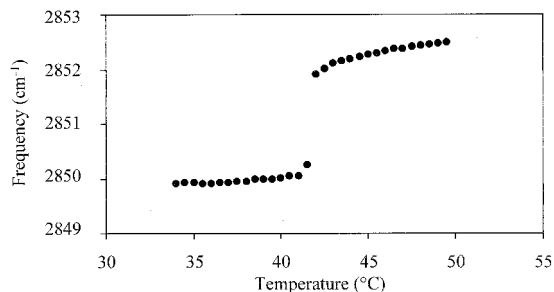


FIG. 2. Temperature dependence of the DPPC CH₂ symmetric stretching mode frequency.

was deposited between two BaF₂ windows separated by a 6 μm mylar spacer. Infrared spectra were recorded with a Nicolet Magna 550 spectrophotometer (Madison, WI) equipped with a narrow-band MCT detector. A total of 250 scans was averaged for the reference spectrum and 100 scans for the lipid spectra at a resolution of 2 cm⁻¹.

The 2800–3000 cm⁻¹ region was truncated and the baseline was fitted with a fourth-order polynomial. We have observed a slight linear decrease of about 10% with increasing temperature of the total area under the 2800–3000 cm⁻¹ spectral region. This observation is probably due to the change of the sample thickness during the temperature increase. The polynomial baseline correction could also affect the integrated intensity of the CH₂ bands, since the polynomial coefficients are not exactly the same for each subtraction due to changes in the shape of the water band with temperature. In order to eliminate intensity fluctuations, all spectra were normalized to the same total area. The baseline subtraction and the spectral normalization were done with the Spectral Calc. software (Galactic Industries Corp., Salem, NH). Finally, the spectral simulations were performed with the MathCad 7 software (Mathsoft, Cambridge, MA).

According to the generalized mathematical formalism of Noda, the experimental approach used in 2D correlation spectroscopy is based on the detection of dynamic spectral variations induced by an external perturbation.¹⁶ In the present study, this perturbation is the effect of temperature on the conformational order of the DPPC hydrocarbon chains. Spectra can thus be expressed as a function of frequency ($\bar{\nu}$) and running index in temperature (t) as $y(\bar{\nu}, t)$. The series of dynamic spectra $\tilde{y}(\bar{\nu}, t)$ was then calculated by subtracting a reference spectrum from each spectrum $y(\bar{\nu}, t)$. The first spectrum, recorded in the gel phase, was used as the reference spectrum $y(\bar{\nu}, 0)$. The dynamic spectra can then be expressed as

$$\tilde{y}(\bar{\nu}, t) = y(\bar{\nu}, t) - y(\bar{\nu}, 0) \quad (1)$$

The calculation has already been done by using different mathematical approaches, such as the continuous Fourier transform,¹⁹ the Hilbert transform,²⁰ or the discrete Fourier transform.^{17,21} All these techniques have been shown to give the same results.

In this paper, the discrete Fourier transform has been used over the N dynamic spectra, giving

$$\tilde{Y}(\bar{\nu}, \tau) = \frac{1}{N} \cdot \sum_{t=0}^{N-1} \left(\tilde{y}(\bar{\nu}, t) \cdot \exp\left(-\frac{2i\pi t\tau}{N}\right) \right) \quad (2)$$

where τ is the running index in the Fourier domain.

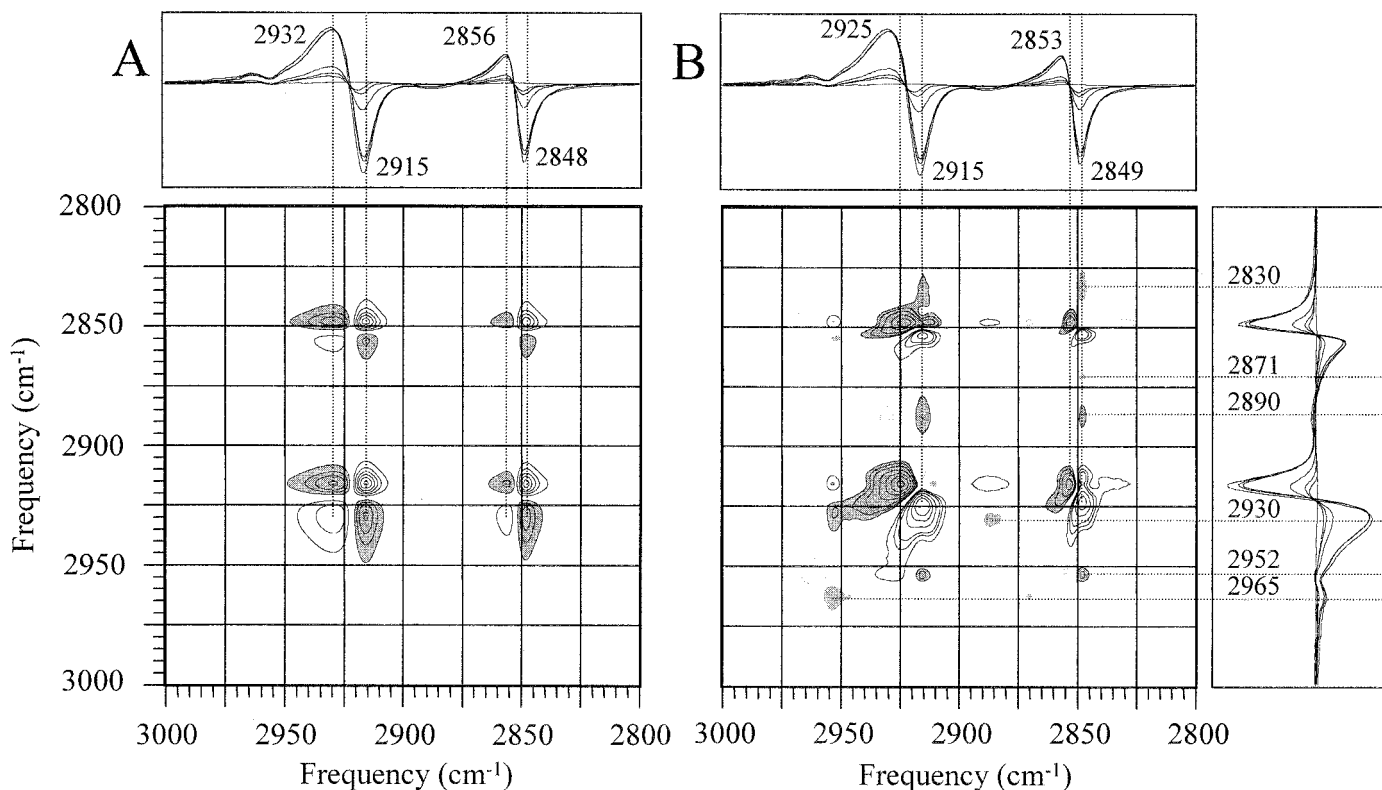


FIG. 3. Synchronous (A) and asynchronous (B) 2D infrared correlation spectra of DPPC calculated over the 34–50 °C temperature range including the gel-to-liquid crystalline phase transition. The shadowed peaks are negative. The asynchronous map intensities are on the order of 1% of those of the synchronous map.

The synchronous $\phi(\bar{\nu}_1, \bar{\nu}_2)$ and asynchronous $\psi(\bar{\nu}_1, \bar{\nu}_2)$ correlations were obtained by using the following formula:

$$\phi(\bar{\nu}_1, \bar{\nu}_2) = \frac{2}{\pi N} \cdot \sum_{\tau=0}^{N/2-1} [\text{Re}\tilde{Y}(\bar{\nu}_1, \tau) \cdot \text{Re}\tilde{Y}(\bar{\nu}_2, \tau) + \text{Im}\tilde{Y}(\bar{\nu}_1, \tau) \cdot \text{Im}\tilde{Y}(\bar{\nu}_2, \tau)] \quad (3)$$

$$\psi(\bar{\nu}_1, \bar{\nu}_2) = \frac{2}{\pi N} \cdot \sum_{\tau=0}^{N/2-1} [\text{Im}\tilde{Y}(\bar{\nu}_1, \tau) \cdot \text{Re}\tilde{Y}(\bar{\nu}_2, \tau) - \text{Re}\tilde{Y}(\bar{\nu}_1, \tau) \cdot \text{Im}\tilde{Y}(\bar{\nu}_2, \tau)] \quad (4)$$

The synchronous 2D spectrum gives autopeaks and cross peaks for vibrations that are time correlated (in phase) with respect to the perturbation, while the asynchronous spectrum contains only cross peaks characteristic of out-of-phase vibrations.¹⁶

RESULTS AND DISCUSSION

Figure 1 shows the infrared spectra of the methylene and terminal methyl group vibrations of the DPPC acyl chains as a function of temperature. When the phospholipid bilayers undergo the gel-to-liquid crystalline phase main transition, the bands due to the symmetric and antisymmetric stretching vibrations of the methylene groups, at approximately 2850 and 2920 cm^{-1} , respectively, shift towards higher frequencies and get broader, resulting in a decrease in the band heights. Figure 2 shows the temperature dependence of the frequency of the band due to the methylene symmetric stretching vibration while the DPPC bilayers undergo the gel-to-liquid

crystalline phase transition. This figure shows that the phase transition is very cooperative and occurs within less than two degrees at 42 °C, in agreement with previous studies.²² It is thus difficult to investigate by 2D correlation spectroscopy the transition alone, since there are not enough points available for the correlation analysis.

Figure 3 shows the synchronous and asynchronous maps for the DPPC thermotropic transition and some dynamic difference spectra that have been used for the 2D correlation calculations in the 34–50 °C range of temperature. In the region of the symmetric stretching vibration at 2850 cm^{-1} , the synchronous map is characterized by peaks, observed at 2848 and 2856 cm^{-1} . The peak positions correspond to the maxima and minima of the dynamic difference spectra shown along the sides of the map. These peaks correspond in the asynchronous map to the cross peaks observed at 2849 and 2853 cm^{-1} , which have an elongated shape. In the region of the antisymmetric stretching vibration at 2920 cm^{-1} , the synchronous map shows peaks at 2915 and 2932 cm^{-1} , while in the asynchronous map, the corresponding cross peaks are observed at 2915 and 2925 cm^{-1} . The asynchronous map intensities are on the order of 1% of those of the synchronous map, as observed previously, although not reported, in our laboratory for systems that are not completely synchronous such as polymers and proteins.^{21,23,24,25} These cross peaks have also an elongated shape, as observed in the symmetric stretching vibration spectral region. It can also be observed that the ratio of the 2850 and 2920 cm^{-1} regions is similar for the dynam-

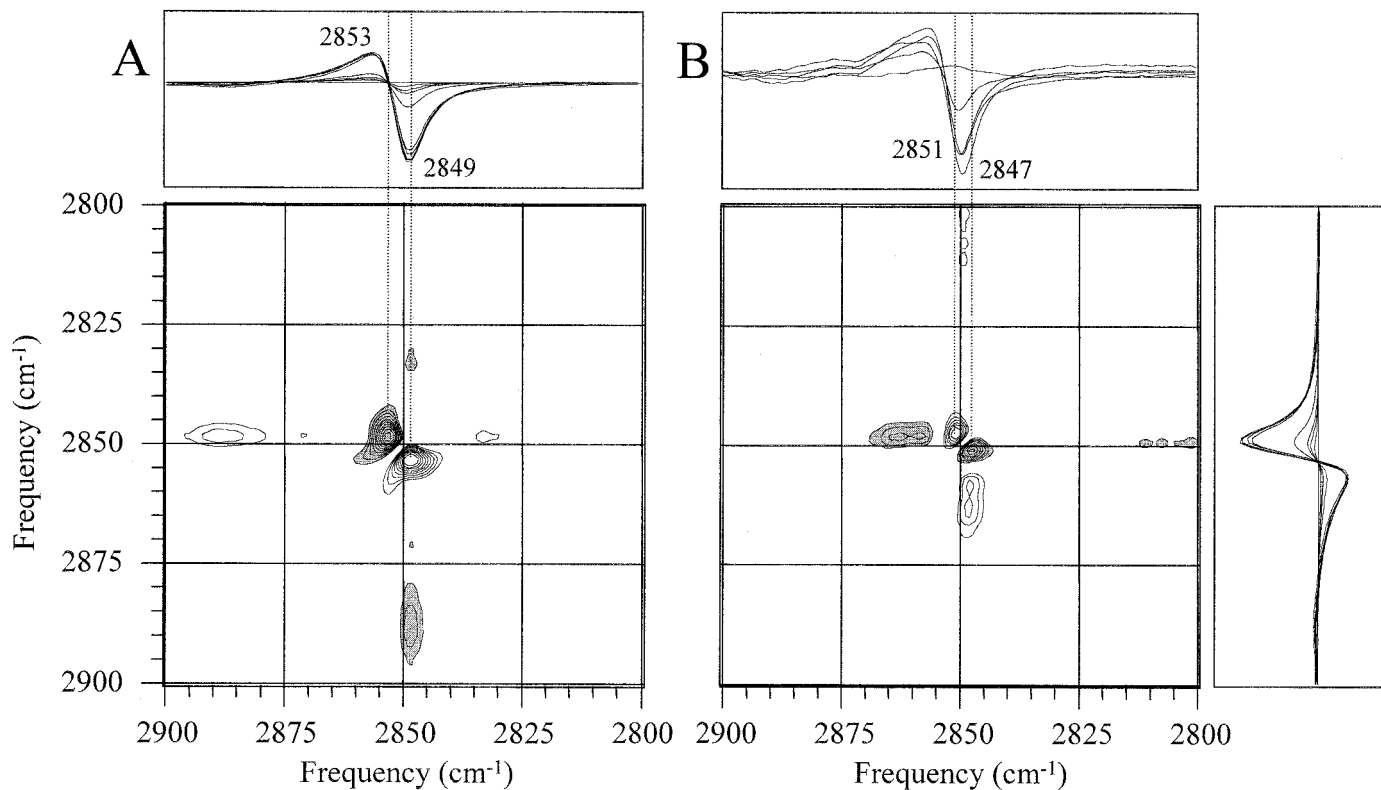


FIG. 4. Asynchronous 2D infrared correlation maps of DPPC calculated for the 34–50 °C temperature range including the gel-to-liquid crystalline phase transition (A), and for the 34–39.5 °C temperature range, in the gel phase (B) in the 2800–2900 cm^{-1} spectral region. The shadowed peaks are negative.

ic 1D spectra and the 2D synchronous map. It has been mentioned in several papers that the frequencies of the synchronous peaks are often not exactly equal to the true frequency values, since, in the synchronous map, intensity variations in the dynamic spectra are most often observed rather than kinetic effects.^{18,19,26} However, if the synchronous peaks correspond to two different spectral contributions, the peak at 2848 cm^{-1} could be assigned to the population of phospholipids that have their acyl chains in the *trans* conformation, while the peak at 2856 cm^{-1} could be assigned to *gauche* conformers.

Dluhy et al. have already proposed a two-state model to describe the temperature-induced cooperative conformational transitions of phospholipids observed by infrared spectroscopy.¹⁴ Using singular value decomposition, Kóta et al. have also calculated that the CH_2 groups in the *trans* conformation give components at 2851 and 2919 cm^{-1} , while the CH_2 groups in the *gauche* conformation give components at 2859 and 2932 cm^{-1} .¹⁵ These frequencies are close to those observed in the synchronous and asynchronous maps in Fig. 3. In the case where the asynchronous map would be sensitive only to kinetic effects, the presence of these peaks would, curiously, indicate that some populations of *trans* and *gauche* isomers do not vary synchronously during the phase transition. The peaks observed in Fig. 3B at 2849 and 2915 cm^{-1} would then, also, be associated with the *trans* conformer spectral contributions, while those at 2853 and 2925 cm^{-1} would be associated with the *gauche* conformers.

Weak cross peaks can also be observed at 2890 and 2952 cm^{-1} in asynchronous correlation with 2849 and 2915 cm^{-1} in the asynchronous map (Fig. 3B), but these

peaks do not correspond to specific intensity changes in the dynamic spectra. They may be associated with the Fermi resonance band, expected at approximately 2890 cm^{-1} for the extended polymethylene chain and the antisymmetric vibration of the terminal methyl group, which is observed at 2953 and 2962 cm^{-1} for *n*-paraffins in the crystalline phase, when the vibration is not degenerated.⁵ Weaker cross peaks can also be observed at 2830 and 2871 cm^{-1} in correlation with 2849 and 2915 cm^{-1} , at 2930 cm^{-1} in correlation with 2890 cm^{-1} , and at 2965 cm^{-1} in correlation with 2871 and 2952 cm^{-1} (shown in dashed lines in Fig. 3B). Although the 2830 cm^{-1} position could be associated with baseline fluctuations, the other positions have already been observed by Snyder et al.⁵ The 2871 and 2965 cm^{-1} positions can be associated with the symmetric and antisymmetric stretching vibrations of the terminal methyl groups, respectively, while the 2930 cm^{-1} position could be associated with the Fermi resonance between the methyl symmetric stretching and umbrella vibrations.⁵ These results show that 2D correlation infrared spectroscopy is a powerful technique to unravel weak bands in complex infrared spectra.

In order to determine whether the peaks, observed in Fig. 3 for the correlation analysis performed over the entire range of temperature, are also observed over a smaller temperature range, calculations were performed on the first 12 spectra before the gel-to-liquid crystalline phase transition. Figure 4 shows the comparison of the asynchronous maps of DPPC for the 34–50 °C range (A) and the 34–39.5 °C range (B). In order to simplify the analysis, only the 2800–2900 cm^{-1} spectral region, containing the symmetric stretching band, is shown on this fig-

ure. While Fig. 4A shows two intense peaks centered at 2849 and 2853 cm^{-1} for the whole transition temperature range, Fig. 4B shows the two corresponding peaks at the 2847 and 2851 cm^{-1} positions, for the gel phase range. It is interesting to note that the lower contour line of the peaks centered at 2849–2853 cm^{-1} has an elongated “butterfly” shape similar to the shape observed by Gericke et al. and Czarnecki, in the case of simulated spectra generated for a band shifting during a perturbation.^{17,18} For the smaller temperature range, the shape of the 2847–2851 cm^{-1} asynchronous peak is more symmetric. Czarnecki has also observed that asynchronous peaks become more symmetric in the case of very small frequency shifting.¹⁸ This result is most likely due to the fact that the band, shifting during the perturbation, may induce intensity variations that do not occur at the same speed for all frequencies. Finally, the analysis of the results presented in Fig. 4 indicates that the characteristic shape of a band shifting is present in the gel phase range, as well as over the whole gel-to-liquid crystalline phase main transition temperature range.

Figure 4 also shows broad peaks along and beside the main peak positions for the asynchronous maps obtained for both the small and whole temperature ranges. These positions seem to correspond to the baseline fluctuations. The relative intensity of the broad peaks is higher in the case of the small temperature range, as the relative intensity of the main bands is in the difference spectra in comparison to the baseline fluctuations. Figure 4 also shows that the two main peaks are present in the case where the frequency of the symmetric stretching band varies linearly, as in the gel phase temperature range, or undergoes the cooperative melting transition, as observed in Fig. 2. However, the presence of broad peaks in the asynchronous maps indicates that the 2D correlation intensity is sensitive to both the kinetics and the spectral intensities. It is, therefore, of primary interest to compare the experimental results obtained for DPPC with simulated systems in which several spectral parameters are controlled.

We have thus simulated two cases, a continuously evolving one-phase system in which a single band shifts towards higher frequencies and becomes broader with increasing temperature, and another one in which the band due to the CH_2 symmetric vibration is assumed to be composed of two components that do not change in frequency but in relative intensity with temperature, as in a two-phase system. A mixture of 50% Lorentzian and 50% Gaussian bands was created, and the series was generated in 10 steps. In the case of spectra modeling the one-phase system, the center position and the full width at the half-maximum (FWHM) linearly shift from 2850 to 2854 cm^{-1} and 10 to 15 cm^{-1} , respectively, by steps of 10%. Spectra modeling the two-phase system were made of two bands, simulating the *trans* and *gauche* conformer spectral contributions, centered at 2850 and 2854 cm^{-1} , with FWHM of 10 and 15 cm^{-1} , respectively. In order to compensate for the width of the band, the height of the *gauche* component was corrected so that the areas of the *trans* and *gauche* components were equal. One component decreases in favor of the other by steps of 10% in intensity. The spectra for both systems were then normalized, no baseline fluctuation or noise was added, and they were done at a 2 cm^{-1} resolution. It should be

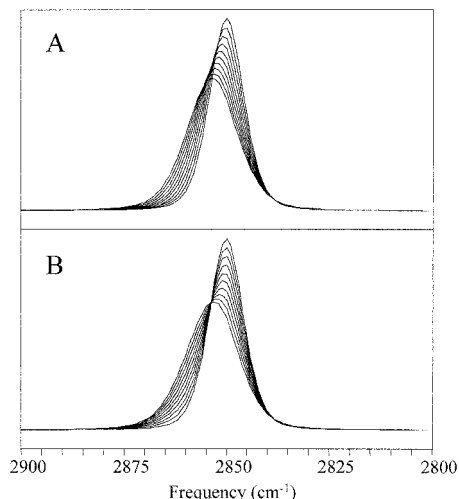


FIG. 5. Synthetic spectra obtained for the shifting-band model (A) and for the two-phase model (B).

noted that the Zimm–Bragg thermodynamic theory of cooperative conformational transitions, which takes into account the large frequency gap occurring during the transition, could not help for this study since it intrinsically involves a two-phase system.¹⁴ The simulated spectra are shown in Fig. 5A for the shifting-band system and 5B for the two-phase system. In the last case, an isosbestic point is clearly observed at 2854 cm^{-1} , as expected for a two-phase system.

Figure 6 shows the synchronous (A) and the asynchronous (B) maps obtained for the shifting-band model. The synchronous map exhibits two peaks at 2848 and 2856 cm^{-1} , while the asynchronous map exhibits two elongated peaks close to the diagonal at 2849 and 2853 cm^{-1} , and two other peaks at 2847 and 2862 cm^{-1} , suggesting again the “butterfly” pattern already observed by Gericke et al.¹⁷ The elongated shape is characteristic of a shifting band, while the other two peaks are artifacts due to intensity modulations in the dynamic spectra. The relative intensities in both maps are on the order of 1%, as observed experimentally.

Figure 7 shows the synchronous (A) and the asynchronous (B) maps obtained for the two-phase system. The synchronous map exhibits four peaks at almost the same frequencies as observed for the simulations of the shifting band (Fig. 6A), indicating that the synchronous map is not helpful for distinguishing the shifting-band from the two-band models in the cooperative process of the *trans*–*gauche* isomerization of the acyl chains. On the other hand, the asynchronous map exhibits only a “noisy” pattern, in which the global intensity is about 10^{-20} , compared to a value of 10^{-5} for an intense synchronous peak observed in Fig. 7A. This result is in agreement with the fact that two-dimensional correlation spectroscopy is useful to discriminate different kinetics. In fact, for a perfectly synchronous or cooperative process such as that modeled by the two-phase system simulation, the *trans* and *gauche* spectral contributions behave perfectly synchronously and give only noise in the asynchronous map. Although the synchronous map fails to discriminate between the shifting-band and the two-band models, the pattern and the relative intensities observed in Fig. 4 for

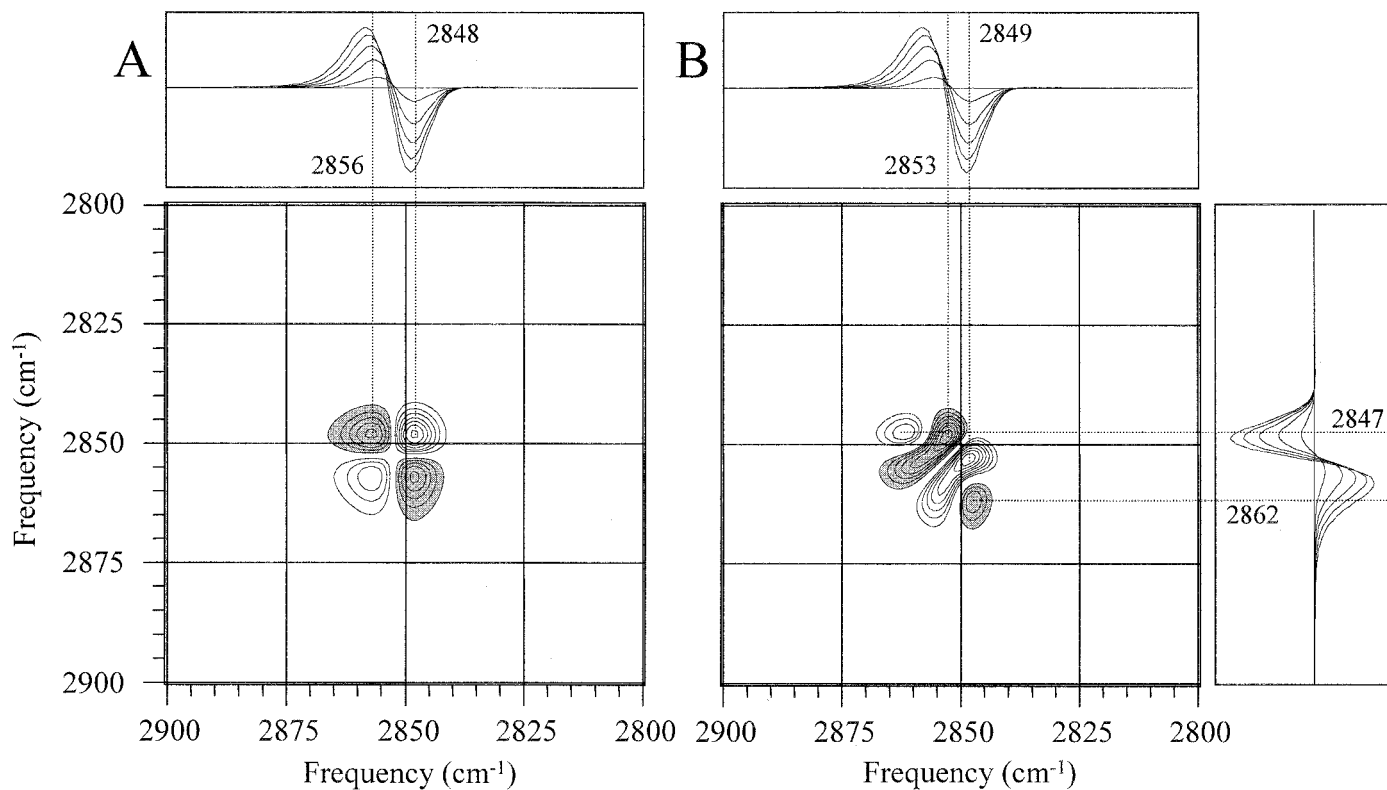


Fig. 6. Synchronous (A) and asynchronous (B) 2D infrared correlation maps of normalized simulated spectra made of one band centered at 2850 cm^{-1} with a width of 10 cm^{-1} , shifting linearly to 2854 cm^{-1} and getting broader to a width of 15 cm^{-1} . The asynchronous map intensities are on the order of 1% of those of the synchronous map.

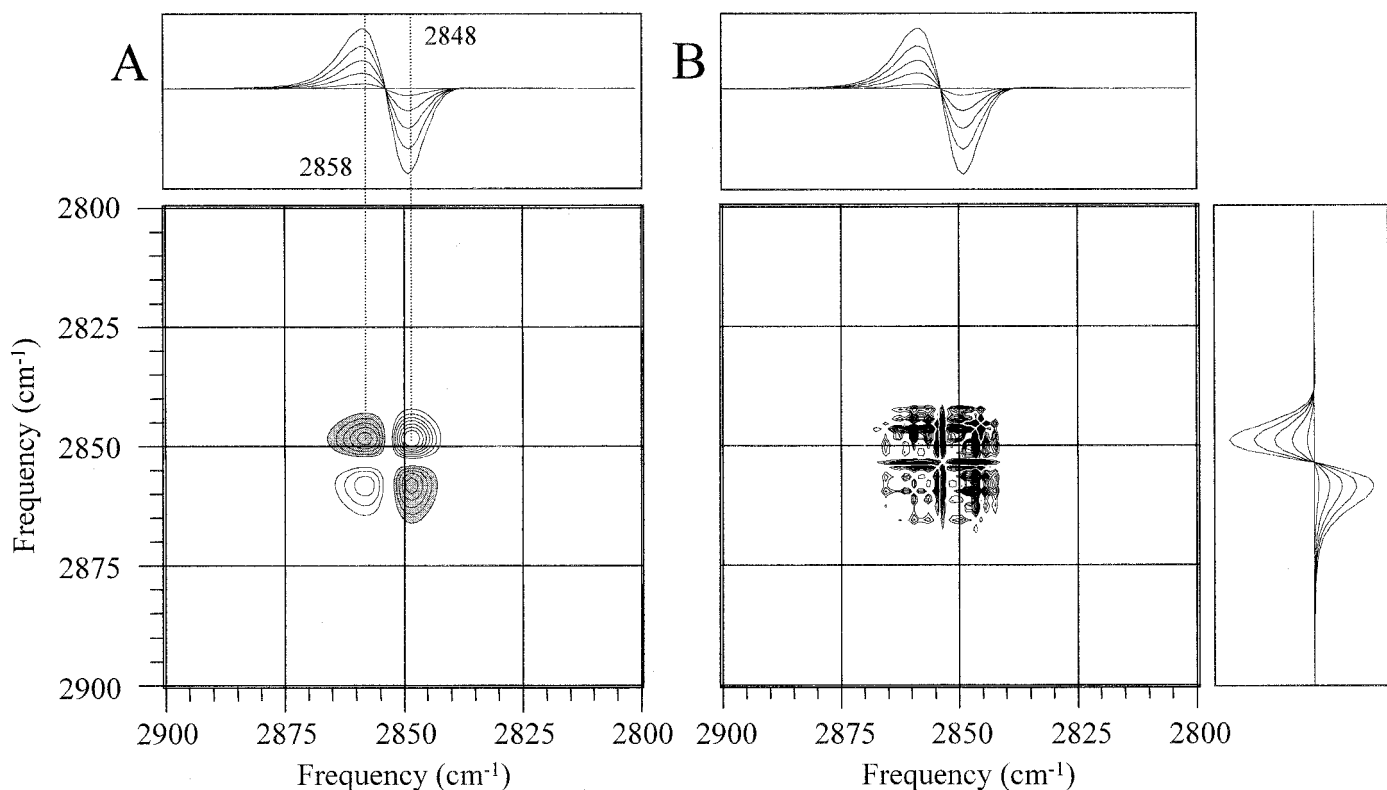


Fig. 7. Synchronous (A) and asynchronous (B) 2D infrared correlation maps of normalized simulated spectra of one band made of two components. The first component, centered at 2850 cm^{-1} with a width of 10 cm^{-1} , decreases linearly in favor of a component centered at 2854 cm^{-1} with a width of 15 cm^{-1} . The asynchronous map intensities are on the order of $10^{-15}\%$ of those of the synchronous map.

the asynchronous map of DPPC is closer to the pattern observed for the shifting band simulation shown in Fig. 6B. Our results clearly show that the methylene stretching bands cannot be described as a pure system of two bands of constant frequency and width assigned to the *trans* and *gauche* conformations. It rather suggests that the bands shift gradually in frequency with temperature, as observed experimentally. The origin of this shift can be associated with several effects.

It has been observed in Raman spectroscopy that the methylene stretching vibration frequency of specifically deuterated dimyristoylphosphatidylcholine varies with the position of the labeled CD₂ group in the phospholipid acyl chain.²⁷ Each conformational state would then give rise to a different frequency, and the observed bands would result from the superposition of several overlapping bands. The shifting-band model is in agreement with this result and indicates that the *trans-gauche* isomerization process does not involve two specific frequencies for each conformational state. It seems also that the frequency distribution observed in the width of the stretching band in the gel or the liquid crystalline phase does not take into account the different frequencies of the CH₂ vibrations along the chain. Kodati et al. have observed that the frequency of the DPPC CH₂ stretching vibrations is sensitive to temperature when DPPC acyl chains are enclosed in clathrate matrices which inhibit the formation of *gauche* conformers, indicating that the frequency is also controlled by the librational motions.¹² Also, Pohle et al. have shown that the hydration of DPPC results in a small shift of the methylene symmetric stretching vibration frequency of about 0.3 cm⁻¹.²⁸ It appears then that the frequency shifting of the CH₂ stretching band observed during the DPPC acyl chain disordering is complex and does not involve only the *trans-gauche* isomerization.

These results suggest that the width of the spectral component due to the *trans* conformation may not remain constant over the entire gel-to-liquid crystalline phase transition and may get broader while it decreases in intensity in favor of the spectral component due to the *gauche* conformers. In fact, we have observed that, for DPPC, the frequency calculated by evaluating the center of gravity of the 2850 cm⁻¹ band, at 50% of its height, increases by 10% of the whole transition in the gel state (below the phase transition), while the bandwidth at 50% of the band intensity increases by 20% of the whole transition for the same temperature range. Since the frequency of the bands due to the methylene stretching vibrations is directly related to the conformational order of phospholipids, and thus to the degree of the phase transition, the fact that the relative broadening of the 2850 cm⁻¹ band in the gel phase is more important than its relative frequency shift clearly indicates that the gel-to-liquid crystalline transition of DPPC is not described properly by a pure two-phase system.^{12,14} Our results instead suggest that there is a continuous broadening of the *trans* component with temperature. Such a pseudo two-phase system could account for the large increase of the bandwidth below the main phase transition and for the peaks observed in the asynchronous maps.

Finally, in order to isolate the *trans-gauche* isomerization effect on the stretching band frequency, the inter-

molecular coupling and the Fermi resonance band due to the CH₃ stretching and bending should be eliminated. DPPC with CD₃ groups was not available for this study. However, we have measured the spectra of DPPC in DPPC-d₆₂ bilayers, isotopically diluted in proportions smaller than 10%. For proteated DPPC diluted in a matrix of deuterated DPPC, a similar “butterfly” pattern has been observed in the asynchronous correlation map (results not shown). According to these results, it is most likely that the *trans-gauche* isomerization effect is a coupled and continuous effect, inducing a continuous shift and broadening of the methylene stretching bands of phospholipid acyl chains.

CONCLUSION

Two-dimensional infrared correlation spectroscopy has been used in the present study to determine whether the CH₂ stretching vibrations of phospholipid acyl chains are made of one or two spectral components. The experimental 2D correlation maps obtained for pure DPPC bilayers as a function of temperature have been compared to simulated spectra obtained for shifting-band and two-band models. For the two-phase system, the simulation in which the intensity of the *trans* spectral component decreases simultaneously with the increase of the *gauche* component gives only noise in the asynchronous 2D map. However, for the shifting-band model, the simulation in which the band, due to the stretching vibration of the phospholipid acyl chain CH₂ groups, shifts towards higher frequencies gives a characteristic pattern of elongated shape for the asynchronous map, similar to that observed in the DPPC experimental spectra. Although the comparison between the DPPC thermal process and the spectral simulations demonstrates that the *trans-gauche* isomerization cannot be described by a simple two-phase model, it appears clearly that the frequency shift is a complex mechanism involving many effects, such as librational motions and intermolecular coupling. Our results, however, support the hypothesis that the width of the spectral component due to the *trans* conformers changes with temperature.

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