

# On the relationship between the order parameter $\langle P_2(\cos \theta) \rangle$ and the shape of orientation distributions

Claude-Paul Lafrance, Anne Nabet, Robert E. Prud'homme, and Michel Pézolet

**Abstract:** The molecular orientation is generally expressed by an "order parameter,"  $\langle P_2 \rangle$ , which depends on both the angular position and the shape of the orientation distribution. This parameter is an average made over all orientations of the structural units studied in a sample and, consequently, a given  $\langle P_2 \rangle$  value can correspond to different orientation distributions. In this article, model distributions are used to show the relationship between the shape, width, and angular position of the center of the orientation distribution on the  $\langle P_2 \rangle$  coefficient, for the case where the distribution of the molecular chains exhibits cylindrical symmetry with respect to the reference direction. A significant difference is observed between the order parameters calculated for distributions of Gaussian and Lorentzian shapes with similar width at half-height. The variation of the  $\langle P_2 \rangle$  coefficient as a function of the width at half-height,  $W_{1/2}$ , and of the position of the center of the distribution,  $\theta_C$ , is analyzed. Figures showing the range of  $W_{1/2}-\theta_C$  coordinates that can correspond to a given  $\langle P_2 \rangle$  value are presented. As an example, the influence on the order parameter of the disorder between the different domains of phospholipid samples (mosaic spread) and of the conformational disorder in the acyl chains of these molecules is also studied. This example permits the evaluation of the magnitude of the errors that can be introduced in calculations of the tilt angle of the molecular chains in the case of distributions of finite widths or of bimodal character.

**Key words:** orientation, orientation function, phospholipid bilayers, conformational disorder, mosaic spread.

**Résumé :** L'orientation moléculaire est généralement exprimée par un paramètre d'ordre,  $\langle P_2 \rangle$ , dont la valeur dépend à la fois de la position angulaire et de la forme de la distribution d'orientation. Ce paramètre d'ordre constitue une moyenne faite sur toutes les orientations des unités structurales étudiées dans un échantillon et, en conséquence, une valeur de  $\langle P_2 \rangle$  donnée peut correspondre à différentes distributions d'orientation. Des distributions modèles sont utilisées afin de montrer l'influence de la forme, de la largeur et de la position angulaire du centre de la distribution d'orientation sur le coefficient  $\langle P_2 \rangle$ , pour le cas où les chaînes moléculaires possèdent une symétrie cylindrique par rapport à la direction de référence. Une différence significative est observée entre les paramètres d'ordre calculés pour des distributions de formes Gaussienne et Lorentzienne de même largeur à mi-hauteur. La variation du coefficient  $\langle P_2 \rangle$  en fonction de la largeur à mi-hauteur,  $W_{1/2}$ , et de la position du centre de la distribution,  $\theta_C$ , est analysée. Des figures montrant la plage de coordonnées  $W_{1/2}-\theta_C$  pouvant correspondre à une valeur de paramètre d'ordre donnée sont présentées. À titre d'exemple, la variation du coefficient  $\langle P_2 \rangle$  en fonction du désordre entre les différents domaines d'échantillons de phospholipides et en fonction du désordre conformationnel dans les chaînes acyles de ces molécules est également étudiée. Cet exemple permet d'évaluer l'importance des erreurs pouvant être introduites dans le calcul de l'angle d'inclinaison des chaînes moléculaires dans le cas de distributions de largeur finie ou de caractère bimodal.

**Mots clés :** orientation, fonction d'orientation, bicouches de phospholipides, désordre conformationnel, désordre entre les différents domaines.

## Introduction

The occurrence of orientation at the molecular level has a considerable importance in macromolecular science, since it gives

directional properties to materials. In the last two decades, numerous publications have dealt with the characterization of orientation in oriented polymer films and fibers (1–5), in biological membranes (6, 7), and in liquid crystals (3). Measurements of molecular orientation are now commonly conducted in various fields of research, yet the literature gives many examples where conclusions drawn from such measurements seem to overlook limitations related either to the characterization method itself or to the mathematical significance of the order parameter calculated from the experiment.

Recently, Myers and Cooper (8) analyzed some of the errors that can arise in the determination of orientation of polymers by infrared spectroscopy. More specifically, the

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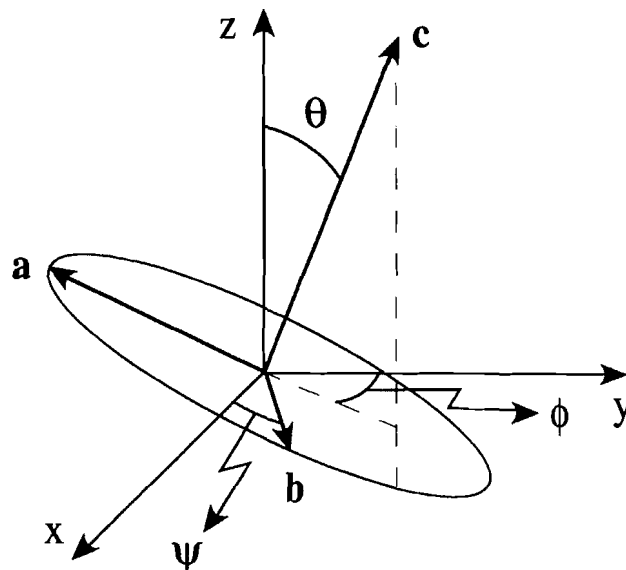
problems caused by the imperfect polarizer behavior, by the improper determination of the chain conformation, and by the assumption of an inaccurate value of the angle between the chain axis and the transition dipole moment were discussed. Furthermore, Ahn and Franses (9) mentioned the possible influence of the width of the orientation distribution on the tilt angle calculated from infrared spectroscopy measurements of Langmuir-Blodgett films. Following this line, the present article focusses on the interpretation of the order parameter, taking into account the fact that orientation distributions at the molecular level are neither necessarily very narrow nor can always be considered as of unimodal character.

Generally, the molecular orientation is expressed by an "order parameter" or "orientation function,"  $\langle P_2(\cos \theta) \rangle$ , or  $\langle P_2 \rangle$ , which is the second-order coefficient of a series expansion of the orientation distribution (1-5). Although all the techniques generally used to study the molecular orientation allow the calculation of the  $\langle P_2 \rangle$  coefficient, wide-angle X-ray diffraction (WAXD) and infrared spectroscopy (IR) are often preferred over other characterization methods. The main advantage of X-ray diffraction is that the orientation distribution of different crystal planes can be measured directly, giving the width, the shape, and the position of the maximum of the distribution. The orientation of the crystal phase is then completely defined, and all the  $\langle P_n \rangle$  coefficients of the series expansion can be determined (1, 10-12). On the other hand, infrared spectroscopy is a simple, fast, and reliable method that can be used to calculate the  $\langle P_2 \rangle$  coefficient for amorphous or crystalline samples, in either solid, gel, or liquid crystalline phases (4, 6).

The  $\langle P_2 \rangle$  coefficient depends not only on the angular position of the orientation distribution but also on its shape and width, since it is an average made over all orientations in the sample. Thus, the  $\langle P_2 \rangle$  value can be used, for example, to discuss the evolution of orientation in a series of polymer samples deformed to different draw ratios. However, the knowledge of this sole coefficient does not allow the unambiguous determination of the inclination of structural units in a sample. The objective of this paper is, first, to emphasize the fact that the  $\langle P_2 \rangle$  coefficient is an average and to give guidelines on the interpretation of this order parameter. The relationship between the  $\langle P_2 \rangle$  coefficient and the shape, width, and angular position of the orientation distribution will be discussed. A comparison with the calculation of  $\langle P_n \rangle$  coefficients from X-ray diffraction will be made, using model distributions, since WAXD allows the direct measurement of the orientation distribution, and thus offers a practical method to visualize the effect of the shape and position of the distribution on the  $\langle P_2 \rangle$  value. These simulations will demonstrate that the discrimination between different types of orientation distribution, all having the same  $\langle P_2 \rangle$  value, can be made when  $\langle P_n \rangle$  coefficients of higher orders are known. Yet it will be shown that, even in this case, the possibility of differentiating between certain orientation distributions depends on the precision of the  $\langle P_n \rangle$  values obtained from the experiment, and it can still be difficult if only a few coefficients are known ( $n \leq 8$ ).

The final section of this paper presents an evaluation of the errors that can be introduced in angular calculations by assuming a single and narrow orientation distribution model when the actual distribution can exhibit a finite width or possess a bimodal character. For this purpose, the influence on the  $\langle P_2 \rangle$

Fig. 1. The Euler angles  $\theta$ ,  $\phi$ , and  $\psi$  defining the orientation of a set of molecular coordinates  $a$ ,  $b$ ,  $c$  with respect to a macroscopic reference system  $x$ ,  $y$ ,  $z$ .  $\theta$  and  $\phi$  describe the orientation in space of the molecular chain axis  $c$ , while  $\psi$  refers to the rotation of the  $a$  and  $b$  molecular axes around  $c$ .



coefficient of the disorder that can be present in phospholipid samples will be analyzed. Phospholipids are the main constituents of biological membranes and, since the properties of membranes depend on the molecular orientation of their constituents, several studies have appeared in the literature dealing with the orientation of the acyl chains of these molecular assemblies. This system has been chosen to illustrate the influence of the shape of orientation distribution on the order parameter because different types of disorder occur in phospholipid samples. The "mosaic spread," i.e., the disorientation between different domains of phospholipid samples deposited on a support, gives an example of the broadening of an orientation distribution by the superimposition of a second distribution. Besides, the occurrence of conformational disorder, which can be introduced either thermally or by the interaction of phospholipids with peptides and proteins, can be used as a model to illustrate the discrepancy between interpretations of orientation measurements arising when the distribution is of bimodal character rather than unimodal. Figures will be shown to evaluate the influence of both types of disorder in molecular orientation studies.

## Theory

### Characterization of the molecular orientation by a series of Legendre polynomials

The orientation in space of a set of orthogonal axes ( $abc$ ), for example, the crystal axes of an orthorhombic unit cell, can be completely described with respect to a macroscopic reference frame ( $xyz$ ) by using the Euler angles  $\theta$ ,  $\phi$ , and  $\psi$  (1), as shown in Fig. 1. Defining  $c$  as the molecular chain axis, the angle  $\theta$  describes the inclination of the chain with respect to  $z$ ,  $\phi$  describes the position of its projection in the  $xy$  plane, while the angle  $\psi$  describes the rotation of the unit cell around its  $c$  axis. The orientation distribution of structural units in polymer

or biological samples can be characterized by using a variety of mathematical functions, the aim of the procedure being to associate one or several index values to the sample investigated. By reference to the boundaries of the function, these index values are then indicative of the degree and of the type of orientation distribution that is present. With respect to the coordinate system defined in Fig. 1, the orientation distribution of the structural units,  $N(\theta, \phi, \psi)$ , can be mathematically expressed as a series expansion in spherical harmonics (13–16). However, this mathematical treatment can often be simplified by the presence of transverse isotropy of the distribution of the molecular chain axis in the samples, i.e., isotropy in the  $xy$  plane. The problem then reduces to the description of the orientation of a unique axis,  $\mathbf{c}$ , with respect to a reference direction,  $z$ . For distributions of such cylindrical symmetry, the distribution  $N(\theta)$  can be characterized by using a series of Legendre polynomials,  $P_n(\cos \theta)$  (1, 2, 10, 17):

$$[1] \quad N(\theta) = \sum_{n=0}^{\infty} (n + 1/2) \langle P_n \rangle P_n(\cos \theta)$$

where the normalization term  $(n + 1/2)$  ensures the convergence of the series, while the coefficients  $\langle P_n \rangle$  are the values that are determined experimentally. All the odd-order terms in eq. [1] are equal to zero because of the symmetry with respect to the mirror plane perpendicular to the  $z$  axis. The first even-order Legendre polynomials are given by:

$$P_0(\cos \theta) = 1$$

$$[2] \quad P_2(\cos \theta) = \frac{1}{2} (3 \cos^2 \theta - 1)$$

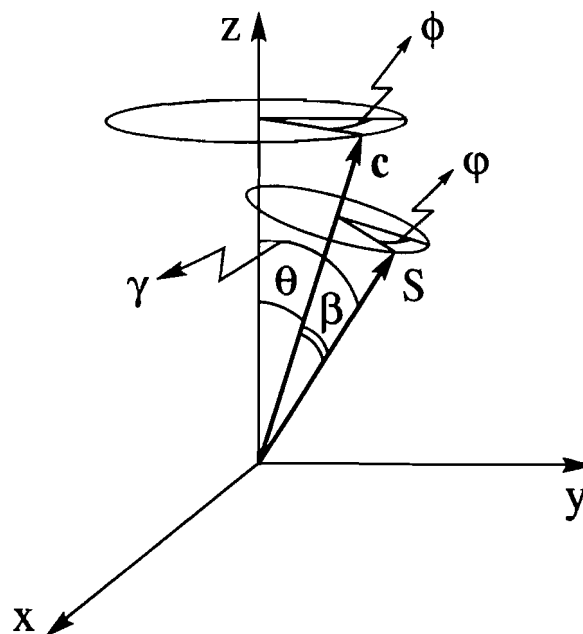
$$P_4(\cos \theta) = \frac{1}{8} (35 \cos^4 \theta - 30 \cos^2 \theta + 3)$$

The  $\langle P_n \rangle$  coefficients of eq. [1] for a given structural unit are obtained by averaging the orientation of all units in the sample. For an isotropic sample, all coefficients are equal to zero, except  $\langle P_0 \rangle$ , which is always equal to unity. For an oriented sample exhibiting an infinitely narrow distribution centered at an angle  $\theta_c$ , the  $\langle P_n \rangle$  coefficients take the value of the corresponding polynomial at this angle. Since all polynomials have their maximum value of unity at  $\theta = 0^\circ$ ,  $\langle P_2 \rangle = 1$  for structural units oriented perfectly parallel to the reference direction, while  $\langle P_2 \rangle = 0$  and  $-0.5$  for a perfect orientation at  $\theta = 54.7^\circ$  and  $90^\circ$ , respectively.

All characterization methods used for molecular orientation studies allow the calculation of the  $\langle P_2 \rangle$  coefficient (1, 2). Raman spectroscopy and polarized fluorescence measurements also give the  $\langle P_4 \rangle$  value, and coefficients up to  $\langle P_8 \rangle$  can be obtained by NMR spectroscopy. Only wide-angle X-ray diffraction allows the calculation of all  $\langle P_n \rangle$  coefficients, because the shape of the orientation distribution of crystal planes is measured directly by this technique.

For a system composed of several nested orientation distributions, each of these showing cylindrical symmetry, for example,  $i$  about the symmetry axis  $z$ ,  $j$  about  $i$ , and  $k$  about  $j$ , the Legendre addition theorem can be used to calculate the relation between the global  $\langle P_n \rangle$  coefficients obtained from the measurement and the coefficients characterizing each distribution. If, in the above example,  $\gamma$  is defined as the angle between  $k$  and the reference direction  $z$ , this theorem states that

**Fig. 2.** Geometric representation for the study of the orientation of a molecular axis  $\mathbf{c}$  inclined at  $\theta$  from the reference direction  $z$ , using measurements made for a structural unit  $\mathbf{S}$  located at  $\beta$  from  $\mathbf{c}$  and at  $\gamma$  from  $z$ . The distributions of  $\mathbf{c}$  about  $z$  and of  $\mathbf{S}$  about  $\mathbf{c}$  both exhibit cylindrical symmetry.



the global  $\langle P_n \rangle_\gamma$  measured for this system is equal to the product of the  $\langle P_n \rangle$  values characterizing each of the distributions:

$$[3] \quad \langle P_n \rangle_\gamma \equiv \langle P_n \rangle_{kz} = \langle P_n \rangle_{kj} \langle P_n \rangle_{ji} \langle P_n \rangle_{iz}$$

This property of the Legendre polynomials can be used to isolate the contributions from nested orientation distributions to the global  $\langle P_2 \rangle_\gamma$  coefficient calculated from the experiment. Let us consider the determination of the  $\langle P_2 \rangle_{cz}$  coefficient, which characterizes the orientation of the molecular chain  $\mathbf{c}$  with respect to the reference direction  $z$ , by taking measurements relative to a structural unit  $\mathbf{S}$ , which is inclined at an angle  $\beta$  from  $\mathbf{c}$ , as is depicted in Fig. 2. The structural units studied could be, for example, the normal to a set of crystal planes studied by WAXD, or the dipole transition moment of a vibration studied by infrared spectroscopy. Since both the distributions of  $\mathbf{S}$  with respect to  $\mathbf{c}$  and of  $\mathbf{c}$  with respect to  $z$  exhibit cylindrical symmetry, then that of  $\mathbf{S}$  with respect to  $z$  also possesses this symmetry. Using eq. [3], the measured  $\langle P_2 \rangle_\gamma$  or  $\langle P_2 \rangle_{S_z}$  coefficient characterizing the orientation of the units  $\mathbf{S}$  inclined at the angle  $\gamma$  with respect to  $z$  is related to the  $\langle P_2 \rangle_{cz}$  coefficient by:

$$[4] \quad \langle P_2 \rangle_\gamma \equiv \langle P_2 \rangle_{S_z} = \langle P_2 \rangle_{S_c} \langle P_2 \rangle_{cz}$$

Since the angle  $\beta$  is generally considered as fixed, i.e., the orientation distribution of  $\mathbf{S}$  about  $\mathbf{c}$  is infinitely narrow, then  $\langle P_2 \rangle_{S_c} = P_2(\cos \beta)$ , and the  $\langle P_2 \rangle_{cz}$  is calculated by the equation:

$$[5] \quad \langle P_2 \rangle_{cz} = \frac{\langle P_2 \rangle_{S_z}}{P_2(\cos \beta)}$$

## Discussion

### Relationship between the $\langle P_2 \rangle$ coefficient and the orientation distribution

The relationship between the  $\langle P_2 \rangle$  coefficient and the shape and angular position of the orientation distribution can be illustrated through the calculation of the  $\langle P_n \rangle$  coefficients from the intensity distribution measured by wide-angle X-ray diffraction,  $I(\theta)$ . Considering Fig. 2, it can be seen that the fraction of chains  $c$  oriented at any angle  $\theta$  is proportional to the circumference of the base of the cone described by the rotation of  $c$  about  $z$ , i.e., it is proportional to  $N(\theta) \sin \theta$ . For a given set of crystal planes, the diffracted intensity measured at an angle  $\theta$ ,  $I(\theta)$ , is proportional to the number of planes inclined at this angle, and the  $\langle P_n \rangle$  coefficients are thus given by (1, 10, 17):

$$[6] \quad \langle P_n \rangle = \frac{\int_0^{\pi/2} I(\theta) \sin \theta P_n(\cos \theta) d\theta}{\int_0^{\pi/2} I(\theta) \sin \theta d\theta}$$

The model orientation distributions  $I(\theta)$  used here are obtained by a 50% Gaussian – 50% Lorentzian sum, the functions being defined by:

$$[7] \quad \begin{aligned} \text{Gaussian: } I_G(\theta) &= I_C \exp[-\ln 2((\theta - \theta_C)/\omega_{1/2})^2] \\ \text{Lorentzian: } I_L(\theta) &= I_C [1 + (\sqrt{2} - 1)((\theta - \theta_C)/\omega_{1/2})^2]^{-2} \end{aligned}$$

where  $I_C$ ,  $\theta_C$ , and  $\omega_{1/2}$  represent, respectively, the maximum intensity, the center, and the half-width at half-height of the distribution. For the Lorentz-type function, the expression in the square brackets is raised to a  $-2$  power instead of  $-1$  for a classical Lorentzian, which causes a faster decrease of the tail of the distribution. The function then becomes equivalent to a Pearson VII function with an adjustable parameter  $m = 2$  (11, 12). This shape of Lorentzian function is used here because, for highly oriented polymers, the orientation distribution of the crystal planes measured by wide-angle X-ray diffraction exhibits a mixed Gaussian–Lorentzian character, which is well approximated by a 50%G–50%L sum of the functions given in eq. [7] (10–12). Moreover, as will be discussed further, the analysis of data from the literature indicates that the 50%G–50%L sum also gives a good fit of the shape of the mosaic spread in supported phospholipid samples.

Table 1 gives  $\langle P_2 \rangle$  values calculated using a Gaussian, a Lorentzian, and a sum of 50% Gaussian – 50% Lorentzian functions, for distributions centered at  $\theta_C = 0^\circ$  with full-widths at half-height ( $W_{1/2} = 2\omega_{1/2}$ ) between  $5^\circ$  and  $20^\circ$ . It is observed that the shape of the orientation distribution has a considerable influence on the order parameter. For example, the  $\langle P_2 \rangle$  coefficient calculated for the Lorentzian and the Gaussian–Lorentzian sum shows a significant deviation from unity when  $W_{1/2} = 10^\circ$ , whereas that obtained for the Gaussian function is still close to the value for perfect parallel orientation. It has been assumed in different studies that the shape of orientation distributions can be well approximated by a Gaussian function (9, 18, 19). However, the exponential form of this type of function produces a rapid decrease of the tail of the curves, and mathematical simulations made using a Gaussian or another function

**Table 1.**  $\langle P_2 \rangle$  coefficient calculated for orientation distributions generated using the Gaussian and Lorentzian functions defined in eq. [7] and a 50%–50% sum of these functions for different values of the full-width at half-height of the distribution,  $W_{1/2}$  (in degrees).

$W_{1/2}$	Gauss	50% G + 50% L	Lorentz
5	1.00	0.98	0.97
10	0.98	0.94	0.91
15	0.96	0.89	0.85
20	0.94	0.85	0.79

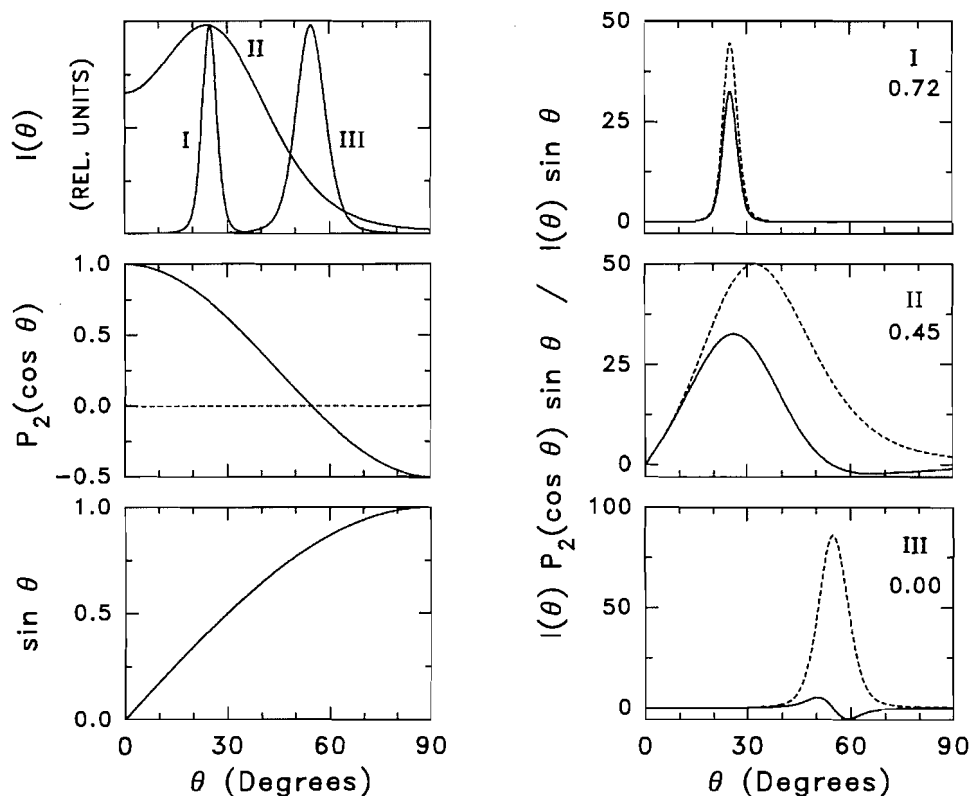
of exponential form generally fall below the experimental distributions in the low-density region of the curves (10, 18, 20, 21). Comparisons made between orientation distributions measured by X-ray diffraction and mathematical simulations have shown that, for oriented polymers, a function of mixed Gaussian and Lorentzian character (Pearson VII function) gives a much better fit than the Gaussian shape (10–12, 21).

Figure 3 illustrates the calculation of the  $\langle P_2 \rangle$  coefficient for three different orientation distributions defined by a 50% Gaussian – 50% Lorentzian sum. The distribution curves,  $I(\theta)$ , are shown on the left-hand side of the figure, together with the curves showing the variation as a function of  $\theta$  of the second-order Legendre polynomial  $P_2(\cos \theta)$  and of the sine function. Distributions I and II are centered at  $\theta_C = 25^\circ$ , with a full-width at half-height of  $5^\circ$  and  $40^\circ$ , respectively, while distribution III has its maximum at  $54.7^\circ$ , with  $W_{1/2} = 10^\circ$ . The expressions evaluated in the numerator and denominator integrals of eq. [6] are plotted in the three graphs on the right-hand side of Fig. 3 for the different orientation distributions. The  $\langle P_2 \rangle$  value is calculated from the ratio of the area under the full curve divided by that under the dotted curve. For the narrow distribution I, the shape of these two curves is identical, and the value of the  $\langle P_2 \rangle$  coefficient, 0.72, is close to the value of the  $P_2(\cos \theta)$  function at  $\theta = 25^\circ$ , which is equal to 0.732. Distribution II multiplies the Legendre polynomial curve over a broader range, showing significant intensity up to angles where  $P_2(\cos \theta) < 0$ . The full curve corresponding to the numerator of eq. [6] thus takes negative values at  $\theta > 54.7^\circ$ . Moreover, the dotted curve relative to the normalization term  $I(\theta) \sin \theta$  defines a considerable area in comparison with the full curve, and is shifted towards the high angles because the sine function increases with  $\theta$ . These two factors give a much lower  $\langle P_2 \rangle$  value of 0.45 for distribution II as compared to that obtained for the intensity distribution I, even if both exhibit their maximum at  $\theta_C = 25^\circ$ .

Distribution III is centered at the angle where the  $P_2(\cos \theta)$  polynomial is equal to zero. Therefore, the numerator of eq. [6] gives both positive and negative areas in the corresponding graph of the right-hand side of Fig. 3 and the  $\langle P_2 \rangle$  coefficient is negligible, even if the  $I(\theta)$  curve exhibits a considerable orientation. Thus, an orientation measurement giving  $\langle P_2 \rangle = 0$  can be interpreted either as an indication of an ordered system centered around  $\theta = 54.7^\circ$ , or as a completely isotropic system.

A given  $\langle P_2 \rangle$  value can correspond either to a narrow distribution centered at the angle  $\theta_{\text{narrow}}$  where the Legendre polynomial is equal to the calculated coefficient, or to a broad distribution centered at an angle between  $\theta_{\text{narrow}}$  and  $0^\circ$  (positive  $\langle P_2 \rangle$  values) or between  $\theta_{\text{narrow}}$  and  $90^\circ$  (negative  $\langle P_2 \rangle$  values).

**Fig. 3.** Calculation of the  $\langle P_2 \rangle$  coefficient from eq. [6] for three different model intensity distributions  $I(\theta)$ . The  $\langle P_2 \rangle$  values for distributions I, II, and III are given in the corresponding graphs of the right-hand side of the figure, showing the curves for  $[I(\theta) P_2(\cos \theta) \sin \theta]$  (solid line) and  $[I(\theta) \sin \theta]$  (dotted line).



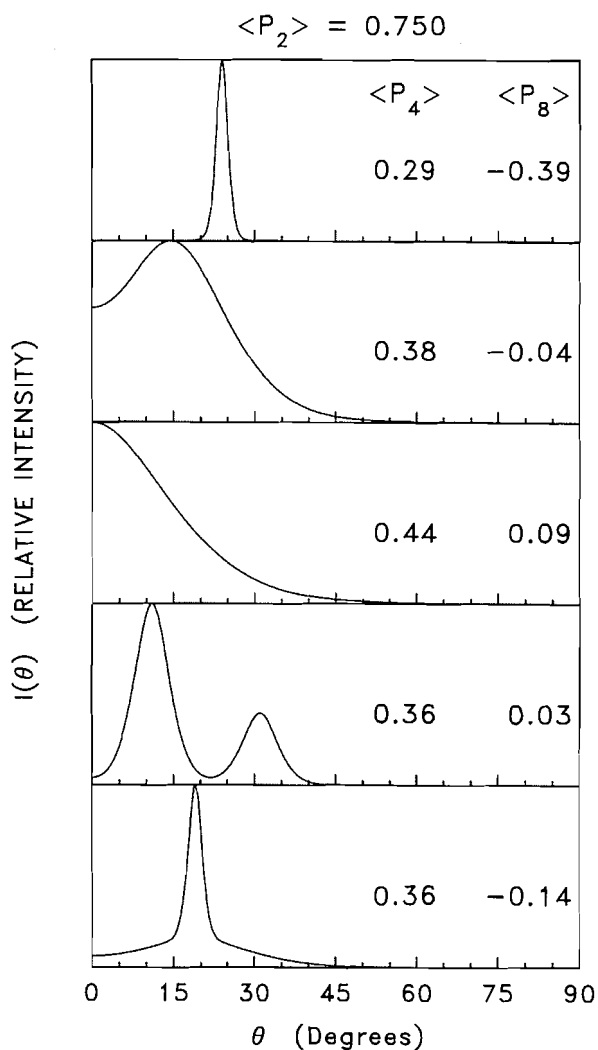
values). This is illustrated in Fig. 4, which presents different orientation distributions that were constructed using a 50% Gaussian – 50% Lorentzian sum, or the addition of two such functions. Despite the obvious differences in the position and in the width of the peaks, all distributions give the same  $\langle P_2 \rangle$  coefficient. The first distribution is centered at  $\theta_C = 24^\circ$  and its  $\langle P_2 \rangle$  coefficient takes the value of the polynomial at this angle, 0.750, since it is sufficiently narrow ( $W_{1/2} = 2.5^\circ$ ). The second and third distributions are broader than the first one and are centered at angles  $\theta_{\text{narrow}} < 24^\circ$ . The two distributions at the bottom of the figure show that it is not possible to determine whether this order parameter corresponds to a unimodal or a bimodal distribution. To obtain this information, it is necessary to calculate higher order  $\langle P_n \rangle$  coefficients, using X-ray diffraction, NMR, Raman, or polarized fluorescence spectroscopy. The knowledge of the  $\langle P_2 \rangle$  and  $\langle P_4 \rangle$  coefficients, with sufficient accuracy, can rule out some types of orientation distribution, as it is seen by comparing the  $\langle P_4 \rangle$  values in Fig. 4, but the unambiguous discrimination between some distributions requires still higher order coefficients. Since the precision on  $\langle P_n \rangle$  coefficients obtained in orientation studies is at best 0.01 (for example, by comparing results from different crystal planes in WAXD (10–12, 22) or from different IR bands), the distinction between the second, fourth, and fifth distributions displayed in Fig. 4 is not possible from their  $\langle P_4 \rangle$  values, but could be made on the basis of the  $\langle P_8 \rangle$  coefficients.

The interpretation of the type and degree of orientation can-

not be made on the sole basis of the  $\langle P_2 \rangle$  value unless the coefficient is close to the boundaries of the  $P_2(\cos \theta)$  polynomial.  $\langle P_2 \rangle$  coefficients close to 1.0 or to  $-0.5$  clearly indicate that the sample studied is highly oriented, with its chains oriented nearly parallel or perpendicular to the reference direction, respectively. However, a  $\langle P_2 \rangle$  value close to zero can be obtained for an isotropic sample, or for a highly oriented sample where the molecular chains are oriented around  $\theta = 54.7^\circ$ , or for a sample possessing a broad orientation distribution at an intermediate angle.

Figure 5a shows a three-dimensional representation of the variation of the  $\langle P_2 \rangle$  coefficient as a function of the width at half-height  $W_{1/2}$  and of the position  $\theta_C$  of a mixed Gaussian – Lorentzian orientation distribution. As stated above, for a very narrow distribution, the  $\langle P_2 \rangle$  coefficient is equal to the value of the second-order Legendre polynomial (eq. [2]) at the angle corresponding to the center of the distribution. Thus, for  $W_{1/2} = 0^\circ$ , the  $P_2(\cos \theta)$  curve is obtained, with a maximum of unity at  $\theta = 0^\circ$  and a minimum of  $-0.50$  at  $\theta = 90^\circ$ . The broadening of the orientation distribution causes a decrease of the absolute value of the  $\langle P_2 \rangle$  coefficient, except for distributions centered around  $\theta = 54.7^\circ$ , which are always characterized by a  $\langle P_2 \rangle$  value near zero. Figure 5b gives iso- $\langle P_2 \rangle$  curves obtained by joining the  $\theta_C$  and  $W_{1/2}$  coordinates corresponding to identical  $\langle P_2 \rangle$  values, i.e., these curves represent the intersection between horizontal planes and the three-dimensional surface of Fig. 5a. It can be observed that the  $\langle P_2 \rangle$  values near

**Fig. 4.** Values of the  $\langle P_4 \rangle$  and  $\langle P_8 \rangle$  coefficients, for different orientation distributions, all having the same order parameter  $\langle P_2 \rangle = 0.750$ .

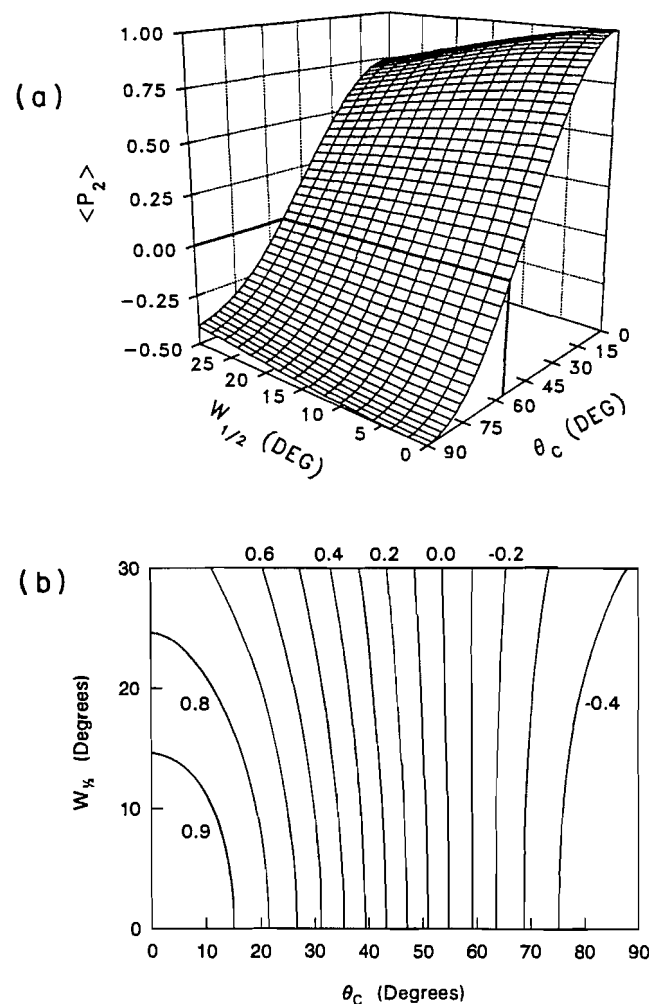


the extremum of the function vary significantly with the width and position of the center of the distribution. For example, a  $\langle P_2 \rangle$  coefficient equal to 0.90 can be obtained for a narrow distribution located at  $\theta_c = 15^\circ$  as well as for a distribution with  $W_{1/2} = 14.5^\circ$  and centered at  $\theta_c = 0^\circ$ . In comparison, for the  $\langle P_2 \rangle = 0.50$  curve, the  $\theta_c$  value varies only by  $8^\circ$ , from approximately  $35^\circ$  to  $27^\circ$ , when the width of the distribution increases from nearly zero to  $W_{1/2} = 30^\circ$ . For unimodal distributions, Fig. 5b can be used to determine the range of  $\theta_c - W_{1/2}$  coordinates corresponding to a given value of the  $\langle P_2 \rangle$  coefficient.

#### Calculation of angles from the $\langle P_2 \rangle$ coefficient

In many instances, the  $\langle P_2 \rangle$  coefficient is used as an orientation index, which indicates the evolution of the molecular orientation in a series of samples. For example, the  $\langle P_2 \rangle_c$  value is frequently plotted as a function of the draw ratio,  $\lambda$ , in order to study the variation of the molecular chain orientation occurring during the deformation of polymers. However, the  $\langle P_2 \rangle$  coefficient is also sometimes used to calculate the angle between a given structural unit in the sample and the reference

**Fig. 5.** (a) Three-dimensional representation of the variation of the  $\langle P_2 \rangle$  coefficient as a function of the full-width at half-height ( $W_{1/2}$ ) and of the position of the maximum ( $\theta_c$ ) of the orientation distribution. (b) Iso- $\langle P_2 \rangle$  curves for different values of the coefficient as a function of  $W_{1/2}$  and  $\theta_c$ .

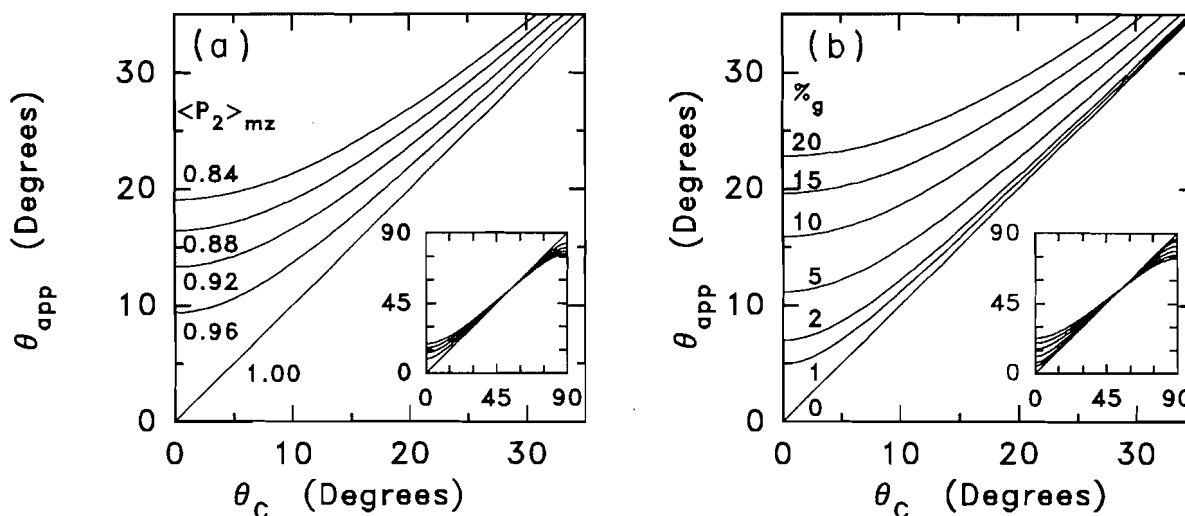


direction. For example, infrared measurements of the  $\langle P_2 \rangle_\gamma$  coefficient for two different bands can be used to determine the angle  $\beta$  between the dipole transition moment and the molecular chain for the second vibration, provided that this angle is known for the first one and that the orientation distribution of the chains and of both vibrations exhibit cylindrical symmetry. In other cases, the  $\langle P_2 \rangle_\gamma$  coefficient is also used to calculate the apparent tilt angle,  $\theta_{app}$ , of the molecular chains with respect to the reference direction,  $z$ :

$$[8] \quad \theta_{app} = \arccos \left[ \frac{2\langle P_2 \rangle_\gamma}{3P_2(\cos \beta)} + \frac{1}{3} \right]^{1/2}$$

However, such angular calculations are justified only in the case of very narrow orientation distributions, since the  $\langle P_2 \rangle_\gamma$  coefficient depends on both the shape and the angular position of the center of the distribution, as was discussed in the preceding section. Therefore, it is important to evaluate the magnitude of the errors that can be introduced when calculations are made using eq. [8], assuming a narrow and unimodal ori-

**Fig. 6.** Curves describing the apparent angle  $\theta_{\text{app}}$  (eq. [8]) between the phospholipid acyl chain axis and the normal to the surface of the sample as a function of the molecular chain orientation  $\theta_C$ : (a) for different values of the mosaic spread order parameter  $\langle P_2 \rangle_{\text{mz}}$ ; (b) for different fractions of methylene groups in and between *gauche* conformers  $x_g$  (in %).



entation distribution whereas the real shape of this distribution is unknown. To achieve this, it is convenient to analyze results of studies where calculations of this sort are made. Orientation studies of phospholipid bilayers by infrared spectroscopy have been chosen for this purpose, since this characterization method gives the  $\langle P_2 \rangle$  coefficient only, whereas the orientation of the lipid acyl chains can be affected by different types of disorder.

#### Orientation measurements of phospholipid bilayers

Phospholipid molecules, which are the main constituents of biological membranes, are built of two acyl chains linked to a polar head group (6). These molecules form bilayer structures, where the carbon chains form the inner hydrophobic part of the membrane, while the polar heads are exposed to the aqueous media on both sides of the membrane. The properties of these molecular assemblies are temperature dependent, with a phase transition occurring at the so-called "melting temperature,"  $T_m$ . In the gel phase ( $T < T_m$ ), the conformation of the carbon chains is generally considered as all-*trans*, whereas in the liquid crystalline phase ( $T > T_m$ ), *gauche* bonds are present in the acyl chains. Attenuated total reflectance (ATR) and tilted angle transmission infrared spectroscopy are frequently used to investigate the molecular orientation in phospholipid bilayers (6, 7).

The  $\langle P_2 \rangle_\gamma$  coefficient measured for samples deposited on a solid substrate, as it is done in orientation studies of biological membranes by infrared spectroscopy, can be considered as resulting from three different orientation contributions. First, the angle  $\beta$  between the transition dipole moment and the molecular chain introduces a factor equal to  $P_2(\cos \theta)$  in the calculation, assuming that the orientation of the dipole with respect to the chain can be represented by a delta function, as is done in eq. [5]. It is noteworthy that Zbinden (23) and, more recently, Myers and Cooper (8) pointed out that there can be inaccurate assumptions regarding the  $\beta$  values for vibrations that are frequently used in orientation studies. In cases where

discrepancies are obtained when the molecular chain axis orientation is calculated from different vibrations, this factor should thus be considered. The  $\langle P_2 \rangle_\gamma$  coefficient also takes into account two orientation distributions, one resulting from the inclination of the chains with respect to the normal to the surface of the membrane, and the other from the disorder of the different membrane domains on the surface of the support. This latter type of disorder is generally referred to as the mosaic spread. Using the Legendre addition theorem (eq. [3]), the  $\langle P_2 \rangle_\gamma$  value measured for such samples can be written as:

$$[9] \quad \langle P_2 \rangle_\gamma = \langle P_2 \rangle_{\text{mz}} \langle P_2 \rangle_{\text{cm}} P_2(\cos \beta)$$

where the subscripts mz and cm refer to the orientation distributions of the membrane domains with respect to the support and to that of the molecular chains in the membrane, respectively.

Values of  $W_{1/2}$  ranging from  $5^\circ$  to  $15^\circ$  are common for the mosaic spread of supported samples measured in studies of biological membranes (18, 24–30). However, knowing the width at half-height of the distribution is not sufficient to assess the effect of this disorder on the measurements, since the shape of the distribution is also important, as is demonstrated in Table 1.  $\langle P_2 \rangle_{\text{mz}}$  values of  $0.92 \pm 0.02$  were calculated for freeze-fractured multilayered purple membrane samples studied by electron microscopy measurements (18, 31), which showed an orientation distribution curve with  $W_{1/2} \approx 12.5^\circ$  (18). A comparison with the results presented in Table 1 indicates that the shape of such a distribution is definitely not Gaussian, and would better be described by a 50% Gaussian – 50% Lorentzian distribution. A curve-fitting analysis of the results of Stamatoff et al. (28), who studied multilayered DPPC samples by X-ray diffraction, also indicates that the mosaic spread distribution in biological membranes is not purely Gaussian. The exponential form of the Gaussian function produces a rapid decrease of the tail of this distribution, which falls below the experimental points. Comparing

the Gaussian, Lorentzian, and the 50%G–50%L curves, the latter showed the best correlation with the experimental data.

It can thus be concluded that the mosaic spread in phospholipid bilayer samples exhibits a shape that can be approximated by a Gaussian–Lorentzian sum. Considering the values given in Table 1 for this type of distribution, it appears that the influence of the  $\langle P_2 \rangle_{mz}$  coefficient in orientation studies cannot be neglected. Figure 6a shows curves that demonstrate the deviation between the apparent angle  $\theta_{app}$  calculated by eq. [8] and the actual center  $\theta_C$  of narrow orientation distributions (delta functions) for different  $\langle P_2 \rangle_{mz}$  values. It is observed that the superimposition of the mosaic spread over the molecular chain distribution can cause a significant discrepancy between these two angles, particularly near  $\theta_C = 0$  and  $90^\circ$ .

Another factor that could affect the interpretation of orientation measurements of phospholipids is the presence of *gauche* conformers in the acyl chains of the lipids. In the liquid crystalline phase, the presence of *gauche* conformers precludes the definition of a chain axis, and orientation measurements by IR spectroscopy can thus only be considered as indicative of the average orientation of the studied vibrations. For the gel phase, the tilt angle between the acyl chains and the normal to the bilayer could be calculated using eq. [8] in cases where the preparation conditions provide a narrow ( $W_{1/2} < 5^\circ$ ) mosaic spread, or if a good estimate of the  $\langle P_2 \rangle_{mz}$  coefficient can be obtained for samples prepared by a given procedure. Nevertheless, provided that this condition is fulfilled, it is still necessary to determine the shape of the orientation distribution.

For phospholipids in the gel phase, it is frequently assumed that the acyl chains of the lipid are in the all-*trans* conformation (32). However, several studies by infrared (6, 33–35), NMR (36, 37), and Raman (38, 39) spectroscopy have shown that approximately a 2–7% fraction of *gauche* conformers is probably already present at temperatures below the transition, accounting for an average of 0.5–1.0 *gauche* bond per chain. The *gauche* rotamers are probably located mostly in kinks (*gtg'*) or jogs (*gttg'*) (34, 40, 41), which are not too disruptive of side-to-side order in the bilayer, or form end-*gauche* methyl groups (38). Moreover, the insertion of peptides or proteins in membranes can also introduce conformational disorder in the phospholipid acyl chains. A simple calculation (40) can be made to evaluate the influence of the nonplanar conformers on the orientation measurements of lipid acyl chains. Let us consider a bimodal distribution of the orientation of the methylene groups, with a first population of *trans* conformers oriented along the molecular chain axis, and a second oriented at  $60^\circ$  from this axis, with respective fractions  $x_t$  and  $x_g$ . Neglecting the presence of double *gauche* conformers, the  $x_g$  population is composed of the  $\text{CH}_2$  groups located at the *gauche* bonds and of those between the *g* and *g'* conformers. The global  $\langle P_2 \rangle_{\text{CH}_2}$  coefficient for this bimodal distribution is given by:

$$[10] \quad \langle P_2 \rangle_{\text{CH}_2} = x_t \langle P_2 \rangle_t + x_g \langle P_2 \rangle_g$$

It is assumed again that the orientation distributions of the  $x_t$  and  $x_g$  populations both exhibit cylindrical symmetry with respect to the reference direction and that the orientation of the  $\text{CH}_2$  groups in the  $x_g$  fraction can be represented by a delta function inclined at  $60^\circ$  from the acyl chain axis. Then,  $\langle P_2 \rangle_t = \langle P_2 \rangle_{cz}$  and the Legendre addition theorem leads to:

$$[11] \quad \langle P_2 \rangle_g = \langle P_2 \rangle_{cz} P_2(\cos 60^\circ)$$

where the subscripts *z* and *c* represent the reference direction and the molecular chain axis, respectively. Using  $x_g = (1 - x_t)$  and introducing eq. [11] in eq. [10] gives the equation:

$$[12] \quad \langle P_2 \rangle_{\text{CH}_2} = (1.125x_t - 0.125) \langle P_2 \rangle_{cz}$$

Assuming that  $\langle P_2 \rangle_{cz} = P_2(\cos \theta_C)$ , i.e., the orientation distribution of the acyl chains centered at  $\theta_C$  is very narrow, the  $\langle P_2 \rangle_{\text{CH}_2}$  coefficient calculated by eq. [12] can be used to evaluate, through eq. [8], the apparent angle between the chains and the membrane normal,  $\theta_{app}$ . Figure 6b presents curves describing the variation of  $\theta_{app}$  as a function of the orientation of the molecular chain axis  $\theta_C$  for different percentages of methylene groups in the  $x_g$  population. These curves show that, even for a fraction  $x_g \leq 5\%$ , the presence of the *gauche* conformers in the phospholipid hydrocarbon chains can result in a significant error in the calculation of the chain tilt in the membrane. As it was observed in Figs. 5 and 6a, distributions oriented around  $\theta_C = 0^\circ$  are influenced more strongly than those centered at higher angles. However, even for phospholipids with chains oriented at  $20^\circ$ – $35^\circ$  from the reference direction, a range that encompasses the tilt angle values measured for several phospholipids (29, 42–44), *trans-gauche* isomerization could introduce some errors in the determination of the chain tilt angle by IR spectroscopy measurements at temperatures below the transition temperature. For example, for molecular axis orientations  $\theta_C$  of  $20^\circ$  and  $35^\circ$ ,  $\theta_{app}$  values of  $21.1^\circ$  and  $35.5^\circ$  are calculated, respectively, if  $x_g = 2\%$ , while values of  $22.6^\circ$  and  $36.2^\circ$  are obtained for  $x_g = 5\%$ .

## Conclusions

This article presents an analysis of the relationship between the order parameter  $\langle P_2 \rangle$  and the width, the shape, and the position of the maximum  $\theta_C$  of the orientation distribution. It is shown that distributions of different widths, shapes, and  $\theta_C$  can give the same order parameter. Therefore, to determine unambiguously the characteristics of the actual orientation distribution, the  $\langle P_2 \rangle$  coefficient is generally not sufficient and higher order coefficients are necessary. In cases where only the  $\langle P_2 \rangle$  is known, the tilt angle  $\theta_C$  between the molecular chain axis and the reference direction can be calculated from the order parameter only if the distribution is unimodal and very narrow because, in this particular case, the  $\langle P_2 \rangle$  coefficient is close to the value of the  $P_2(\cos \theta_C)$  polynomial. For broad distributions, or for distributions featuring more than one maximum, the  $\langle P_2 \rangle$  value has to be considered as an average, which cannot be used to calculate the tilt angle.

The above conclusions have also been applied to the determination of the acyl chain orientation in phospholipid samples, frequently studied by infrared spectroscopy, a method that allows only the calculation of the  $\langle P_2 \rangle$  coefficient. The order parameter obtained for supported phospholipid samples contains three different contributions, arising from the mosaic spread, from the orientation of the lipid molecules in the bilayers, and from the orientation of the transition dipole moment of the vibration used for the calculation. Our simulations show that the presence of mosaic spread and that of conformational disorder cannot be neglected in orientation studies. The anal-



ysis of the probable influence of these two parameters indicates that errors of a few degrees can be made in the determination of the tilt angle of the acyl chains of the lipids. The deviations between the apparent tilt angle and the true center of the orientation distribution are larger for distributions centered close to 0 or 90°.

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