Fourier Transform Infrared Spectroscopy as a Probe for the Study of the Hydration of Lipid Self-Assemblies. I. Methodology and General Phenomena

WALTER POHLE,[1] CARSTEN SELLE,[1] HARTMUT FRITZSCHE,[1] HANS BINDER[2]

[1] Friedrich-Schiller University Jena, Institute of Molecular Biology, Department of Biophysical Chemistry, Winzerlaer Str. 10, D-07745 Jena, Germany

[2] University of Leipzig, Institute of Experimental Physics I, Linne Str. 5, D-04103 Leipzig, Germany

Received 20 November 1997; revised 1 December 1997; accepted 29 January 1998

ABSTRACT: An algorithm for the study of the gradual hydration of phospholipid assemblies by means of Fourier transform infrared (FTIR) spectroscopy is presented. A complete series of diacyl phosphatidylcholines (PCs) including all possible analogues with palmitoyl and oleoyl residues, namely DPPC, DOPC, POPC, and OPPC, was investigated at room temperature. The lipid samples were prepared as cast films probably consisting of aligned multilamellar bilayers. The range of water activities studied in these films was regulated by adsorption via the gas phase corresponding to relative humidities of between 0 and 100%. Analyses of the IR-spectroscopic data have concentrated mainly on determining the amounts of water incorporated by each lipid as well as the hydration-induced response observed for some absorption bands of the different lipids. The water uptake at high relative humidity (RH) increases with the portion of unsaturated acyl chains in the molecular structure of the PCs. Isothermal phase transitions triggered lyotropically have been detected in demonstrating the occurrence of the main transition in POPC and OPPC films at room temperature. Moreover, it appears that both lamellar phases, the gel as well as the liquid-crystalline phase, are not uniform. They seem to comprise an amazingly large span of order/disorder states of the lipid chains generally depending on the degree of hydration. As exemplified by the significant variation in the onset of wavenumber shifts for the PO_2^- and C=O stretching-vibration modes, obtained as a function of hydration, a sequence of attachment to polar lipid binding sites by water molecules was established for DPPC. © 1998 John Wiley & Sons, Inc. Biospectroscopy 4, 267–280, 1998

Keywords: dipalmitoylphosphatidylcholine; dioleoylphosphatidylcholine; mixed-chain phospholipids; unsaturation; hydration; FTIR spectroscopy; lyotropic phase transition; chain-melting transition

INTRODUCTION

Life has evolved and continues to proceed in aqueous media. Water is amalgamated, down to a molecular level, with all kinds of fundamental biological molecules and is indispensable for maintaining both structural stability and functionality. Recently, there has even been discussion proposing that the hydration state in cells regulates, in a complex way, a number of important activities such as those connected with signal transduction.[1]

Many biomolecules are polymers containing both hydrophilic and hydrophobic sections. In living organisms, in an environment essentially

This paper is dedicated to Professor Helmut W. Meyer on the occasion of his 65th birthday.

Correspondence to: W. Pohle (wapo@molebio.uni-jena.de).

Contract grant sponsor: Thuringian Ministry of Sciences.

Contract grant sponsor: Deutsche Forschungsgemeinschaft; contract grant numbers: SFB 197 and SFB 294.

Biospectroscopy, Vol. 4, 267–280 (1998)

 CCC 1075-4261/98/040267-14

defined by the omnipresence of water, these molecules are driven to adopt secondary and higher-order structures that are organized so as to minimize the extent of unfavorable contact between water and the hydrophobic part of the biopolymers. Thus, hydrophobic regions are generally arranged in the "central core" of such biomolecules so as not to be exposed to water. In this way, water plays a crucial role in the structural organization of proteins (folding)[2–4] as well as nucleic acids (especially for the polymorphism of DNA)[5–7] and carbohydrates.[8,9] However, in the case of lipids, the fourth important class of biomolecules, because amphiphilicity (or amphipathy) is the most pronounced, a more peculiar relationship between water and lipids exists. This relationship largely contributes to the fundamental molecular prerequisites for the unique propensity of lipids to self-organize in supermolecular assemblies, a fact that predestinates this class of chemical compound to provide the frame-giving constituent of biological membranes. In particular, water is essential for the remarkable mesomorphism of lipid systems.[10] Hydration effects are crucial to the fragile interplay of the weak forces and interactions that eventually regulate which phase of a lipid or lipid mixture exists under given conditions. Accordingly, the biological relevance of lipid hydration cannot be overestimated. In single-lipid systems, water content is used as one of the two variables besides temperature in phase diagrams commonly established for characterizing the phase behavior of model membranes.

Interest in lipid hydration has motivated a huge amount of experimental investigation over the last two or three decades resulting, for phospholipids alone, in a large body of relevant data. Although the sheer quantity of articles makes a complete overview extremely difficult, many of the results have been summarized in a number of review articles or textbook chapters, see, for example, Refs. 11–15. In numerous studies using lipid dispersions with excess water, valuable information concerning the hydration state has been deduced.[14] *Ad hoc* investigations of lipid hydration have been performed using lipid samples obtained in two principal ways distinguished by the water-dosage procedure: (1) the solvent is admixtured to lipid in aliquots to get a definite weight-to-weight percentage; (2) water is administered as vapor via gas phase. Samples obtained by the more frequently used procedure (2) were investigated using classical physicochemical methods of lipid research, including differential scanning calorimetry,[16–19] X-ray diffraction,[18,20–22] and NMR spectroscopy.[23–25] In addition methods such as neutron diffraction, hydrodynamics, dilatometry, fluorescence spectroscopy, partition techniques, and, increasingly, theoretical methods have all been utilized for probing lipid hydration. The isopiestic water-dosage protocol as characterized by procedure (2) has been used, on the other hand, mainly in gravimetric experiments.[26–29]

Another valuable tool in lipid research is vibrational spectroscopy with its complementary techniques IR and Raman spectroscopy; this is evidenced by the appearance of several reviews and monographs.[30–34] In particular, FTIR spectroscopy is well suited for following thermotropic lipid phase transitions. Moreover, it provides data on a "submolecular" level, since all the three relevant domains of a PL, that is, headgroups, tails and glycerol backbones, contain IR-active groups whose affiliated absorption bands may be sensitive to both structural changes and reorganization as well as the environmental influences inducing them. This potential of FTIR spectroscopy has, up until now, by no means been completely exhausted.[34]

In spite of frequent application of FTIR spectroscopy in lipid research, investigations involving gradual hydration are less common. In most cases, either anhydrous (sometimes crystalline) or fully hydrated samples have been analyzed.[35,36] Studies of stepwise PL hydration in terms of characterizing lyotropic phase behavior are especially promising in the low-hydration range. One can recognize an overall tendency of the preferred direction of phase boundaries to turn from horizontal to vertical with decreasing water content of the lipids when looking more specifically onto the bulk of respective phase diagrams.[10,13] Vibrational spectroscopy investigations of PL films or multilayers refer mainly to standard compounds such as DPPC,[30,37–39] DMPC[40–42] and DPPE.[30,37,43] Some studies used the FTIR–ATR technique.[30,40,43] Hydration was explicitly addressed in a number of articles, but only in a few cases by adding water via the gas phase.[30,38,41,42,44] FTIR spectroscopy was also used to study the interaction of water with DPPC and DPPE in organic solvents.[37,45]

In this article, a relatively simple approach for probing phospholipid hydration by FTIR spectroscopy is described. The methodology is based on the isopiestic technique (described above) with varying relative humidity and has been utilized previously in studies of oriented films of DNA and DNA complexes (cf. Ref. 46 and references cited in this work). In the DNA experiments, as well as in the lipid experiments described herein, specific

Table I. Comparison of T_m Values for the PCs Under Study as Obtained in Dispersions with Excess Water (From Literature) and Mesomorphic Phases Expected to be Adopted in Films at Room Temperature

Lipid	T_m (°C)	Refs.	Phase Expected in Films at 26°C
DPPC	41	16	L_β
	41.6	54	
DOPC	−16	55	
	−16.5	19	L_α (and/or L_β)
	−18	47	
Dry	48		
POPC	−0.8	56	
	−4.5	57	L_α and/or L_β
10% H_2O	20.3	35	
Dry	68		
OPPC	−7.9	56	L_α and/or L_β
	−11	57	

efforts have been made to get samples as free of water as possible. This makes it possible to study water-depleted PL specimens, which have often been neglected in past studies. The low-hydration region is not only of basic scientific interest since it covers the small number of first, very strong bound water molecules, but it is also biologically meaningful in that it may provide insight into how extremely dry (e.g., sporulated) or frozen organisms are able to maintain their viability.[47,48]

This report on phospholipid hydration is subdivided for convenience. The first part is more general and describes the methodology of obtaining and interpreting the FTIR-spectroscopic data. In the second part, the details of some specific cases will be discussed aimed at elucidating the interplay of hydration and conformational (or phase) effects. To this end, a series of PCs distinguished by a systematic variation of the nature of the acyl chains was designed and studied: two symmetric diacyl PCs, disaturated DPPC, diunsaturated DOPC, and their mixed-chain analogues POPC and OPPC. Except for a few relevant reports[49–53] unsaturated PLs have not yet been extensively investigated using FTIR. One interesting, desirable aspect of this selection of PLs is that, under the conditions chosen (RT), gel as well as liquid-crystalline lipid phases can be adopted. This is suggested by the calorimetric data in Table I exhibiting the range that covers the bulk of main-transition temperatures measured; the average T_m values in excess water of the four lipids formed on the basis of the data found in Marsh's handbook[13] and in LIPIDAT[58] are given by 41.4, −17, −3, and −8°C for DPPC, DOPC, POPC, and OPPC, respectively. At high water content, DPPC consequently adopts the gel phase at room temperature while unsaturated PCs are in the L_α phase. In the films used here, the same phase affiliation can strictly be expected only for DPPC whereas the data reported for water-depleted unsaturated PCs permits speculation as to which phase(s) might be formed in terms of RH for each of them (see Table I).

METHODS

Lipid Sample Preparation

DPPC was from Bachem Biochemica (Heidelberg, Germany), DOPC, POPC, and OPPC were from Sigma Co. (Munich, Germany). All compounds were used without further purification, their uniformity was confirmed by thin layer chromatography. UV-grade chloroform from Baker (Deventer, Netherlands) was used as a solvent.

Films of these lipids were prepared by casting about 60 μL of their chloroformic solutions (concentration 10 mg/mL) directly onto IR-transparent ZnSe (Irtran 44) windows and evaporating the solvent while simultaneously stroking the solution unidirectionally and gently with a spatula.

Hydration Experiments

After completely removing the solvent, the films were placed into specially designed IR cells. The cells are similar to gas cells and were developed from the standard nujol cells obtained from Graseby Specac (supplied by LOT Oriel, Langenberg, Germany). A second identical cell was used for measuring the background. Hydration experiments themselves were performed *in situ* according to two different protocols. In the standard procedure, dehydration–rehydration cycles were scanned according to stationary conditions. The water content of the lipid films was widely varied by changing the RH surrounding the samples between 0 and 100%. This was achieved by loading the appended flasks with water or saturated solutions of the appropriate salts. The equilibration times necessary to reach saturation of a given PL at each RH were carefully measured and will be considered below in "Water Adsorption." Unsaturated PCs have been found to be stable during

these long-term measurements. In a second series of experiments, lipid dehydration was followed in a nonstationary mode by starting from the sample equilibrated at high RH (usually 98%). After exchanging the salt solution, the RH is reduced continuously in the cell, resulting in lipid dehydration. FTIR spectra were recorded at fixed time intervals until equilibration at the relevant lower-hydration (usually 0% RH) level was reached. Although the actual RH values at the times of measurement are not known in each case, the water content of the film could be controlled via the absorbance of the prominent water absorption band near 3400 cm^{-1} (for details, see below). The data points obtained in this dynamic way agree well with the results of measurements using the isopiestic method. Certain deviations may arise in systems where phase transitions occur (see part II of this series). The reverse procedure, starting from dry film and following lipid hydration continuously after moistening the surrounding atmosphere, was also performed.

FTIR Measurements

Infrared spectra were recorded at 26 ± 1°C using a IFS-66 FTIR spectrometer (Bruker, Karlsruhe, Germany) in the DTGS-detection mode. Although the instrument was purged with an air-drying apparatus (Zander, Essen, Germany), in addition a shuttle device accounting for a significant "extra"—elimination of atmospheric contamination was used. Incomplete water vapor compensation was corrected where necessary by subtracting gas-phase spectra of water from the lipid spectra and subsequent smoothing. A resolution of 2 cm^{-1} and a zero-filling factor of 2 were used. For the equilibrium and dynamic measurements, respectively, 32 and 2 scans were applied. An apodization function according to Hepp and Genzel has been applied. Data processing was done using the OPUS (Bruker) and GRAMS (Galactic Industries, Salem, New Hampshire, USA) software packages. Wavenumbers of the peak maxima were determined using the OPUS peak-picking routine in the standard mode (accuracy 0.1 cm^{-1}).

RESULTS AND DISCUSSION

Films prepared by the hand-stroking orientation method give rise to much more distinct IR spectra than those obtained by spontaneous spreading of the multibilayers (Fig. 1). This finding can be understood in terms of a significantly more homogeneous type of specimen arising in the former case especially where sample thickness is concerned. It has been known for many years that differences in thickness within a sample can cause tremendous deterioration with respect to the absorbance of infrared bands.[59] In general, sample homogeneity should be achieved more easily for lipid lamellae in the liquid-crystalline phase than in the more rigid gel phase. This expectation is experimentally confirmed by the finding that differences in the respective DOPC spectra are not as pronounced (data not shown) as for DPPC. In conclusion, it can be established that the quality of the infrared spectra obtained for lipid films depends very critically on the sample-preparation method and can be improved by hand-stroking the material.

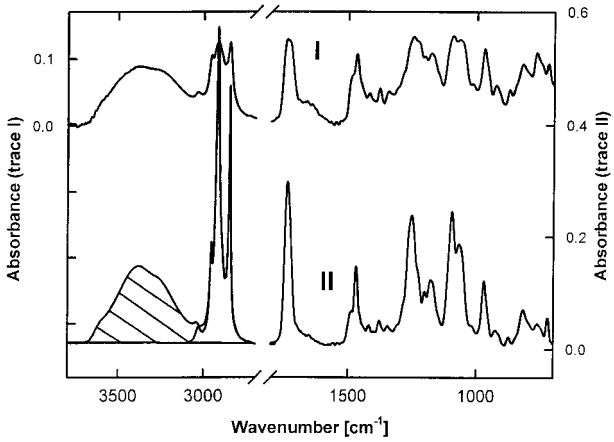

Figure 1. Comparison of typical FTIR spectra for DPPC (at 23% RH); the multibilayers presumably existing in the films have been formed either by spontaneous orienting of the lipid molecules in the sample (I, above) or by the same (amount of) material when additionally oriented by gentle hand-stroking (II, below).

In principle, the results reflect the effects of hydrating the PCs in two different ways: (1) directly by following the characteristics of the absorption bands due to water itself, for example, adsorption isotherms can be measured spectroscopically, and (2), more indirectly probing the lipid response to the variation of the water content in the samples by monitoring the spectral changes undergone by appropriate PL vibrational bands. In the latter case, the involvement of certain lipid sites in the water binding process can be revealed at least in a qualitative sense (see section "Spectral region primarily due to the polar lipid groups").

Table II. Relaxation Times τ Due to Water Uptake by a POPC Film at Different Temperatures

RH_{start} (%)	RH_{end} (%)	τ (30°C) (min)	τ (10°C) (min)
0–	12	(44)	85
12–	34	26	54
34–	58	40	77
58–	75	30	120
75–	84	70	193
84–	92	164	
92–	97	400	314
97–	100	720	

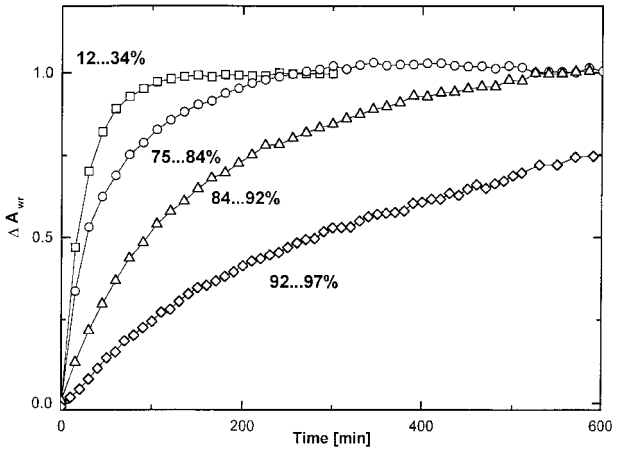

Figure 2. Kinetics of water adsorption of a POPC film measured for different RH intervals as specified.

Water Adsorption

The amount of adsorbed water was monitored using the integral absorbance A_w of the broad $\nu_{1,3}$OH band centered near 3400 cm^{-1} due to the stretching vibrations of water. As an internal standard, we used the total integrated absorbance of the overall C—H stretching-vibration bands between ca. 2800 and ca. 3050 cm^{-1}. This term $A^t_{CH(lipid)}$ $(=A_{lt})$, is expected to be at most only a little influenced by hydration in the absence of phase transitions. The estimation results in a relative, calibrated water-adsorptivity parameter determined, for given RH values, and denoted for the ith spectrum as $A_{wr,i} = A_{w,i}/A_{lt,i}$. In Figure 1 the spectral decomposition is illustrated for DPPC. Since both palmitoyl and oleoyl chains contain 14 CH_2 groups and one (terminal) CH_3 group, A_{lt} values for either lipid used in this study could be directly compared in a first approximation.

First, the kinetics of water adsorption by lipids were carefully probed in a set of preexperiments. For either PC, the time necessary to essentially complete hydration was relatively short, in accord with a previous report.[41] In a detailed study using POPC, it turned out that the equilibration times measured depended on both the RH interval and the temperature. The data are collected in Table II and partly illustrated in Figure 2. The kinetics was well fitted by exponential growth functions yielding the relaxation times τ listed in Table II. The results demonstrate that the relaxation times increase in terms of enhancing RH and decrease with rising temperatures within the interval considered. At RT and small RHs, the waiting times for achieving equilibrium can be chosen fairly short (1–2 h), but at higher RHs a wait of between several hours up to overnight is necessary.

From the IR spectra, adsorption isotherms can be derived by plotting A_{wr} versus RH. Figure 3 shows a typical example obtained for OPPC. As for the other lipids, the curve approximately follows the course of a type II or type IV isotherm according to the BET classification. Although stringent specification between these two types is not possible due to lack of high-RH data, the data indicate a strong binding of water to the PCs, especially for the primarily bound molecules. The more detailed gravimetric adsorption data of Jendrasiak et al.[27,28] reveal a type IV isotherm for both DOPC and egg lecithin.

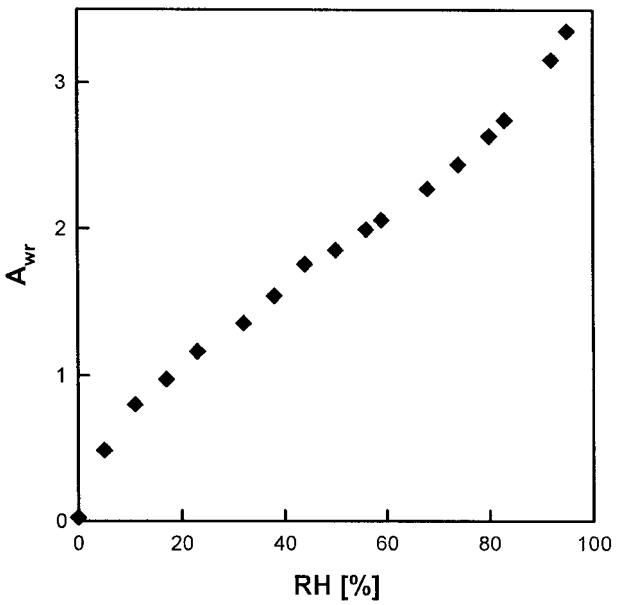

Figure 3. Depiction of the water-adsorptivity parameter, A_{wr}, versus RH for OPPC revealing a typical adsorption isotherm as measured by FTIR spectroscopic investigations of PC films.

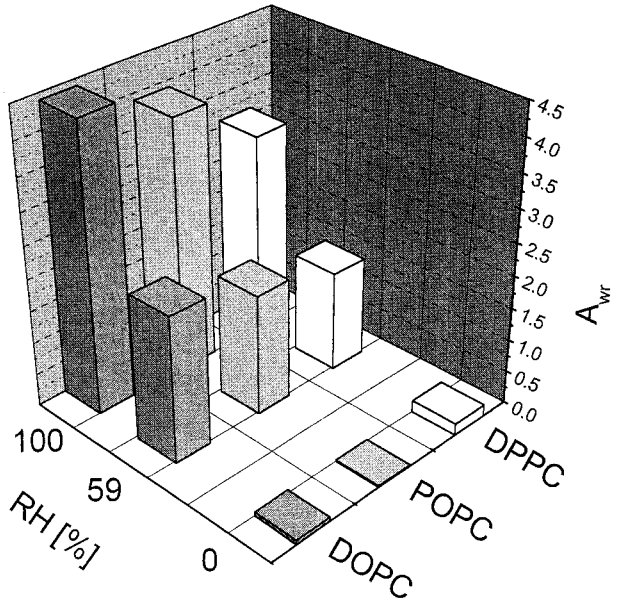

Figure 4. Schematic comparison of the water uptake by the DPPC, POPC, and DOPC films estimated at different stages of hydration according to RH values of 0, 59, and 98%.

Since A_{wr} is a normalized parameter, the adsorption isotherms for all the PLs studied can be quantitatively compared with respect to their capacity to take up water. As demonstrated by the schematic drawing in Figure 4, the PCs with unsaturated acyl chains, in particular DOPC, are able to absorb significantly higher amounts of water than DPPC especially at higher RHs. This sequence which is, for the PCs with equal acyl chains, in agreement with previous data[14,26,27] reveals a significant influence of the chemical nature of the hydrocarbon chain on the extent of lipid hydration. The hydrophobic acyl chains can be confidently assumed as not being directly involved in the process of water binding. The significant difference between DOPC and DPPC may be understood as follows. The cis configuration of the C=C double bond of the acyl chains in DOPC introduces a substantial structural disturbance in the apolar lipid region thus causing a profound destabilization of the gel phase leading to the relatively low T_m value for DOPC (see Table I). This special structural feature in the chain region may *a priori* result in an increased cross-sectional area of DOPC, and, more importantly, induce this type of lipid to adopt a fluid phase, most probably L_α, in films at room temperature, whereas DPPC exists in the gel phase under these conditions. The cross-sectional area is well known as being significantly larger if the lipid is in the liquid-crystalline rather than the gel phase.[12,60] This provides some "extra" space in the headgroup region of DOPC, which can accommodate more water molecules than in the case of saturated-chain lipids. It seems plausible that the water-adsorption capacity data for the mixed-chain PLs lie between those for lipids with uniform acyl chains (see POPC values in Fig. 4, unreported results for OPPC are very similar). As shown by the first set of columns in Figure 4, all the PCs studied contain a certain amount of residual water at zero RH conditions, the largest is for DPPC. This finding is in accord with previous data characterizing PCs in general as hygroscopic substances,[16] always containing some residual water,[61] and demonstrating a portion of particularly strong (phosphate) bound water molecules in lipid crystals.[62,63] In this respect, there is a qualitative difference between PCs and certain PEs: DOPE, for instance, can be dried to a state of complete water removal when applying exactly the same experimental conditions as for the lecithins.[64] It is also straightforward that the amount of residual water associated with the PCs under zero RH conditions is apparently higher in DPPC than in the unsaturated PCs. The reason for this is presumably the same as for the higher overall water-uptake capacity of the latter, namely the smaller cross-sectional area in DPPC due to its more closely packed intralayer structure which retains primarily bound water molecules more tightly.

In order to get an idea of how many water molecules might be adsorbed at each stage of hydration, one can correlate a series of A_{wr} values obtained spectroscopically for several lipids with the corresponding water-per-lipid ratios determined gravimetrically at the same RH. The extinction coefficient of the OH stretching-vibration band of water is assumed to be a constant over the hydration range. This estimation based on the data from different gravimetric studies[26,27,29] yields the water-per-lipid stoichiometry or hydration number, n_w, on a basis that A_{wr} unity corresponds to about 3.0 ($\pm$0.3) water molecules per lipid. Maximum A_{wr} values of ca. 2.5 and 4 for DPPC and DOPC and tentative n_w (at RH = 100%) values of about 7 and 12, respectively, are found as a result of this procedure. The values shown in Figure 4 are averages from a series of measurements (DPPC: 6, DOPC: 8, POPC: 5). It should be mentioned that the water-per-lipid stoichiometry at zero RH is actually rather small with $n_w < 1$ (even the highest corresponding figure found, in this series, for DPPC is only about 0.5).

It has been established that the hydration

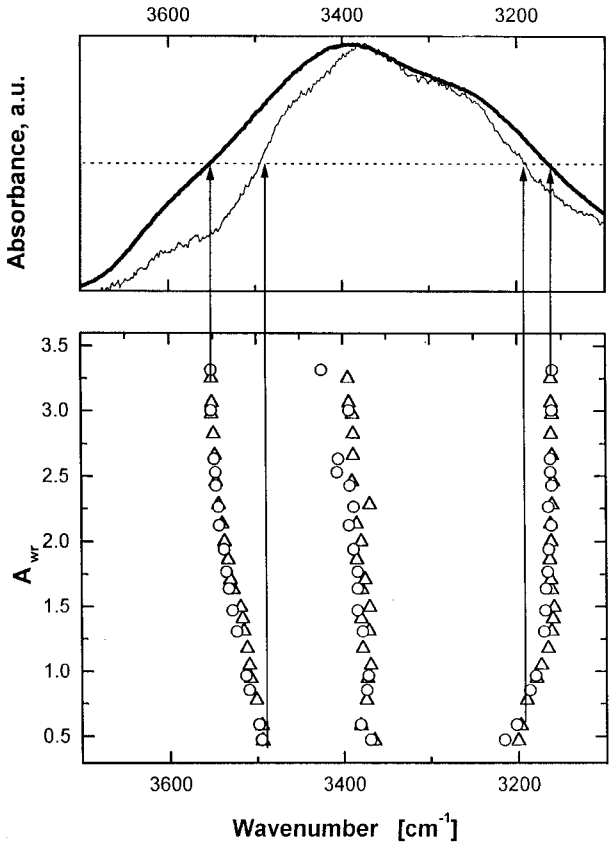

Figure 5. Wavenumbers of the overall maxima as well as of the left-side and right-side flanks (estimated at half-height positions) of the $\nu_{1,3}$OH band due to water obtained for definitely hydrated (○) DOPC and (△) POPC in terms of the parameter A_{wr}.

numbers obtained in isopiestic experiments of water adsorption at an RH towards 100% are significantly smaller than those found by other techniques using aqueous dispersions as samples.[12,14] In the latter case n_w values of up to 40 for DOPC have been reported. This phenomenon was ascribed to the so-called vapor pressure paradox.[12]

Analyses of the $\nu_{1,3}$OH band arising from the water contained in the lipid films give rise to the scheme in Figure 5, which demonstrate nearly identical patterns for DOPC and POPC. Going to lower hydration levels is accompanied by a weak, continuous decrease of the overall wavenumber of this $\nu_{1,3}$OH band interpretable as indicating an enhancement of the fraction of the more strongly bound water molecules present in the lipid sample. Hydration-induced increase of the wavenumber of $\nu_{1,3}$OH-absorption-band maximum of water was previously also observed for DPPC.[37,39,45] At the same time in the low water-activity region, the $\nu_{1,3}$OH band becomes narrower as revealed by the slightly converging half-height wavenumbers. This finding can be understood in terms of a more uniform hydrogen-bond strength distribution due to the preferably and more strongly bound and therefore more immobilized water molecules. An even stronger $\nu_{1,3}$OH band-narrowing effect at low RH was found for DODPC.[44]

Water-Induced Changes in the Lipid Spectra

Spectral Region Primarily Due to the Apolar Lipid Tail

In this first part of the two-article series, the νCH_2 bands will be discussed primarily. In Figure 6, $\nu_s CH_2$, the peak-maxima wavenumbers of the $\nu_s CH_2$ band, taken from nonstationary rehydration scans, are plotted versus the water content (A_{wr}) for films of each of the PCs studied. The curves of the symmetrical diacyl lipids are largely linear and lie almost parallel to the hydration axis. The ordinate values at ca. 2850 and ca. 2854 cm^{-1} confirm that as expected (see Table I) DPPC and DOPC have adopted a lamellar gel phase and a fluid, most probably the L_α, phase, respectively. In contrast, the $\tilde{\nu}_s CH_2$ values of POPC and OPPC increase dramatically upwards at a particular A_{wr} value slightly above 1. The dependency of $\tilde{\nu}_a CH_2$ on A_{wr} (data not shown) has a shape very similar to that obtained for $\tilde{\nu}_s CH_2$. In principle, dehydra-

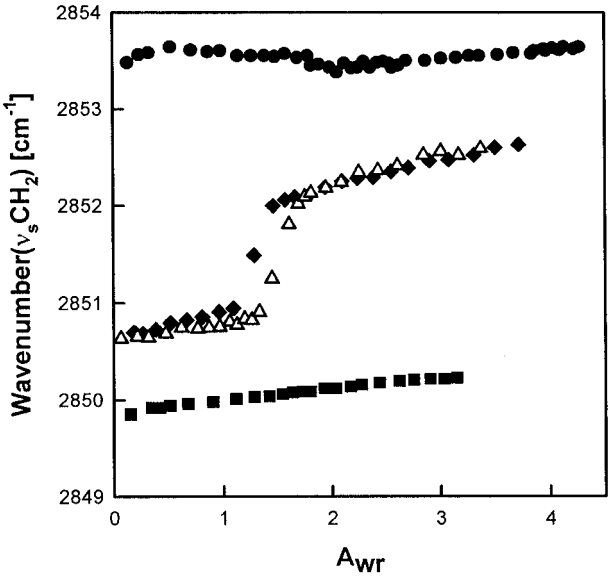

Figure 6. Dependence of the wavenumbers of the bands due to the symmetric stretching vibration of the chain CH_2 groups of (■) DPPC, (●) DOPC, (♦) OPPC, and (△) POPC on the water content of the pertinent films obtained for rehydration scans at 26°C.

tion scans lead to the same finding. These features are phenomenologically the same as previously observed when demonstrating the existence of the thermotropic main transition of lipids by FTIR spectroscopy.[31,34,65] This coincidence is encouraging for assigning the steep decrease/increase of the $\tilde{\nu}_s CH_2$ and $\tilde{\nu}_a CH_2$ wavenumbers observed for POPC and OPPC dehydration/rehydration as unraveling acyl-chain freezing/melting and, thus, indicating the occurrence of the main transition which in this case is, however, lyotropically induced. Since excluding direct water binding to methylene groups appears justified, the effect of bound water molecules here is clearly an indirect one. Strong successive water attachment to the PC headgroups (a process which is quantitative in character) leads, presumably via an increase in the related cross-sectional area, and, consequently, by a successive deterioration of the van der Waals binding between the acyl chains, to a progressive destabilization and eventual collapse of the gel phase for the benefit of the liquid-crystalline phase (a process which is qualitative in character). The wavenumber jumps, that is, the main-transition hydration levels, are observed at approximately the same A_{wr} values for these two lipids, with a weak tendency towards smaller A_{wr} for OPPC, as demonstrated by the plots in Figure 6. The latter observation is in accord with the lower T_m value for OPPC compared to POPC (each in fully hydrated samples, see Table I). As far as we are aware, this, together with a similar finding for DODPC,[44] is the first clear-cut IR-spectroscopic evidence for a lyotropically induced isothermal main transition as illustrated by a continuous set of data points covering the complete RH scale in lipids.

The main-transition temperatures of lipids generally increase steadily from their standard values determined in dispersions with excess water when the degree of hydration of the samples is diminished. This is due to a concomitant gel-phase stabilization.[16,17,41] For instance, Scherer et al. have evaluated a T_m increase of 0.44°C per RH-unit decrease for DMPC from the data of their Raman measurements.[41] Applying the same evaluation procedure to POPC and OPPC would accordingly result in a T_m change of approximately −0.5°C/% RH. In view of the relevant data for DOPC (T_m of dry DOPC at 48°C,[47] see Table I) gel phase occurrence could be suggested also in this case. However, in the case of DOPC, the present data does not give any indication of gel-phase formation. This is true even at the lowest water activities achieved in the films (RH = 0), that is, $\Delta T_m/\Delta RH$ relation for gel-phase stabilization appears quite different for DOPC, smaller than for either POPC or OPPC.

Very recently, some reservations have emerged concerning the unambiguity of correlating the CH_2 stretch wavenumbers stringently with the number of gauche conformers in lipid acyl chains.[66] The minor uncertainty arising from whether discontinuous $\tilde{\nu}CH_2$ changes do indicate lipid main transitions can be eliminated by considering a further infallible IR-spectroscopic criterion for that phase transition. The appearance of the wagging-deformation band progression between 1350 and about 1180 cm^{-1} [31,34,53,67] observed for POPC and OPPC simultaneously with the wavenumber decrease of the νCH_2 bands is, though occurring with weaker relative intensity than in DPPC (data not shown), further evidence that the chain-freezing transition in the mixed-chain lecithins actually takes place.

For the critical hydration level triggering the chain-melting transition of the mixed-chain PCs, a hydration number can be evaluated for both POPC and OPPC to n_w values of approximately 4. For POPC, this estimate is in excellent agreement with recent NMR data.[68]

On further inspection of Figure 6, it may be noticed first that the $\tilde{\nu}_s CH_2$ of DOPC scatters around 2853.5 cm^{-1} over the whole range of water activities, whereas the corresponding value for DPPC shows a systematic hydration-induced increase of about 0.3 cm^{-1}. The figure for DOPC suggests that the lipid maintains a highly disordered phase, presumably L_α, throughout all introduced hydration levels, even after long drying times (several days). Smaller inflections of the curve at very low (<0.5) and medium (ca. 2) A_{wr} values will be discussed in part II of this work. The corresponding data for DPPC may demonstrate a slight increase in the fraction of gauche conformers (and/or in the librotorsional mobility of all-*trans* segments[66]) in its acyl chains with progressive hydration. Obviously, even small changes in water activity in DPPC at temperatures remote from T_m noticeably influence the conformation or state of order of the hydrocarbon chains. This conclusion is corroborated by data for mixed-chain PCs. As shown in Figure 6 for both POPC and OPPC, the wavenumbers of their respective $\nu_s CH_2$ bands are found, at an A_{wr} slightly below their main-transition points, at values of about 2850.5 cm^{-1}, that is, at figures still somewhat above those obtained for DPPC. This is an-

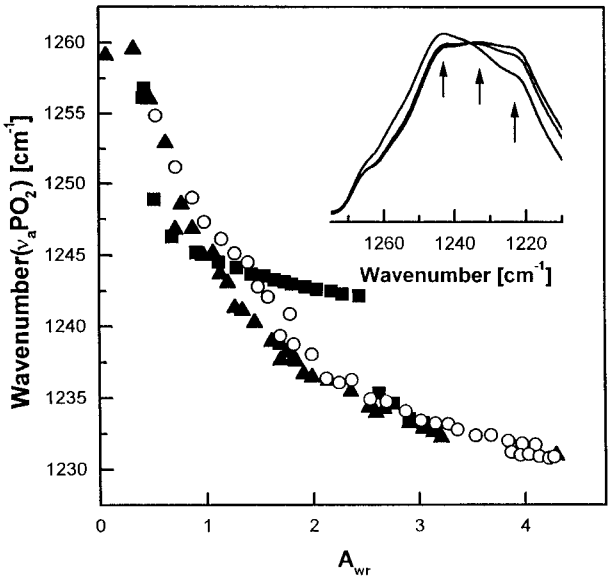

Figure 7. Wavenumber shifts for the antisymmetric PO_2^- stretching-vibration bands of (■) DPPC, (○) DOPC, and (▲) POPC obtained in terms of A_{wr}. Arrows in the insert indicate CH_2 wagging-band progressions interfering the $\tilde{\nu}_a PO_2^-$ mother band.

other indication that the gel phase of PCs may involve a fairly wide span of chain-order states. The same appears to be true for such chains in the case of the liquid-crystalline phase. The $\tilde{\nu}_s CH_2$ values for POPC as well as OPPC are slightly above the main transition at 2852.5 cm^{-1}. For DOPC, which is at room temperature considerably above the main transition, they are at ca. 2853.5 cm^{-1} (Fig. 6). Accepting the correlation between $\tilde{\nu}_s CH_2$ and chain order as valid, this "interlipid" comparison unravels for both phases, L_α and L_β, a range of "intraphase" chain fluidities each corresponding to not less than about 1 cm^{-1}. Incidentally, the same phenomenon can be also seen when looking at much of the data obtained from temperature-dependent IR-spectroscopic measurements in fully hydrated lipid dispersions: in the temperature regions adjacent to T_m on both sides, slight increases in $\tilde{\nu}_s CH_2$ values are observed.[31,33,34] As to the L_α phase, a strict correlation between $\tilde{\nu}_s CH_2$ and the chain order as determined by NMR spectroscopy was found[52] thus supporting the above interpretation. A search for a similar relationship in the gel phase failed because of technical restrictions of NMR spectroscopy.[52]

The variability of order states for acyl lipid chains in either the lamellar gel or fluid phase, must be regarded fairly substantial since the difference in $\nu_s CH_2$ wavenumbers determined for the main transition, is itself often not more than 2 cm^{-1}. This is true for experiments reported both here and in the literature (temperature runs).[31,34]

Such considerations are indicative of the high degree of sensitivity of the FTIR spectroscopic methodology used.

Spectral Region Primarily Due to the Polar Lipid Groups

Absorption bands arising from moieties belonging to the polar, hydrophilic region of PLs are predominately situated in the 700–1800 cm^{-1} spectral range. The most significant to be addressed in hydration studies are the stretching modes due to the potentially water-binding PO_2^- and C=O groups.[35]

In Figure 7, the dependencies of $\tilde{\nu}_a PO_2^-$ on A_{wr} for DOPC, POPC, and DPPC are shown. The $\tilde{\nu}_a PO_2^-$ decrease found upon hydration is, with the exception of DPPC, continuous and with about 30 cm^{-1} exorbitantly strong. The results of semiempirical quantum-chemical calculations are in accord with the idea that this wavenumber shift is caused by water molecules hydrogen-bonded to phosphate.[69] It has been demonstrated frequently by experimental data on DNA[69,70] as well as on PLs[37,71,72] that $\tilde{\nu}_a PO_2^-$ is considerably more sensitive against environmental influences, as hydration, than $\tilde{\nu}_a PO_2^-$ where the hydration-induced shift amounts to 5–6 cm^{-1} (see Fig. 8). This diverse experimental behavior has been qualitatively explained by the results of previous model calculations simulating PO_2^- hydration effects.[69]

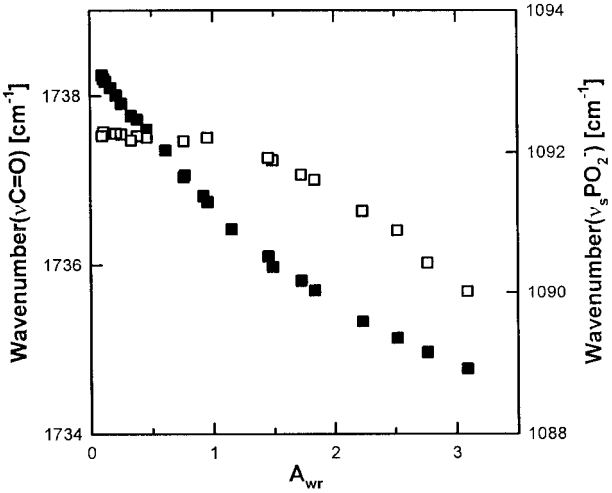

Figure 8. Wavenumber displacements drawn for the C=O (open symbols) and symmetric PO_2^- stretching-vibration bands (full symbols) of DPPC as found in dependence on A_{wr}.

The curves for all these lipids as well as for OPPC (data not shown) are nearly identical, and only the plot of DPPC displays a pronounced deviation essentially given by a striking wavenumber jump at a particular critical A_{wr} value near 2.5. The similarity of the $\tilde{\nu}_a PO_2^-$-versus-A_{wr} plots at least for DOPC, POPC, and OPPC, together with the high sensitivity of this wavenumber as illustrated by a total shift of about 30 cm^{-1}, supports the proposition that $\tilde{\nu}_a PO_2^-$ is a suitable measure alternative to A_{wr} for reliably indicating the degree of hydration at least in fluid lipids. It may be tempting to explain the discontinuity in the curve for DPPC in terms of either a conformational change or a phase transition. However, as the spectra shown in the inset in Figure 7 suggest, it is rather due to a modulation introduced by the overlapping progression bands arising from the methylene wagging vibration. This assumption is eventually supported by the fairly smooth graph due to the $\nu_s PO_2^-$ band of DPPC depicted in Figure 8. Consequently, the discontinuity of the $\tilde{\nu}_a PO_2^-$ plot for DPPC in Figure 7 appears to originate in the fact that, on gradual varying of the center of gravity of the overall PO_2^- band on hydration (i.e., water binding to the headgroup region), the peak maximum switches abruptly since the two relevant CH_2-wagging progression-bands (at ca. 1222 and 1244 cm^{-1}, see arrows in Fig. 7) strongly interfere. This behavior illustrates once more the fact that analyses of the $\nu_a PO_2^-$ band of PLs resting in the gel phase must be performed with caution.

In Figure 8, the wavenumbers of the $\nu C{=}O$ and $\nu_s PO_2^-$ bands of DPPC are depicted side-by-side in terms of the water content in the film. In both cases, hydration-induced wavenumber downward shifts are observed according to theoretical predictions made on the basis of quantum-chemical calculations.[69,73] Whereas the direction of the wavenumber shift for the PO_2^- stretching modes is in complete agreement with literature data (see above), the situation is not as simple where the $\nu C{=}O$ band is concerned. In several recent articles,[35,40] a hydration-induced wavenumber decrease of this band similar to our case is reported. In other articles the behavior of this band is not as straightforward with largely hydration-independent wavenumber characteristics and/or the occurrence of splitting and shoulders.[36–38,45] In the present study, fairly symmetric $\nu C{=}O$ bands have been observed. These are displaced for all measurements more or less to lower wavenumbers upon hydration. In this article we will limit the discussion to DPPC. The relation between the respective $\tilde{\nu}C{=}O$-versus-A_{wr} functions for the three lipids containing unsaturated chains are more complex and will be considered in detail in the companion article of this work. Interpretation of the spectral behavior of the lipidic $\nu C{=}O$ band has been controversial for some time. This is mainly due to the fact that this band is not uniform and consists of at least two subspecies appearing either directly in the spectra (visible as splitting or shoulders) or after deconvolution procedures were applied. Taking into consideration the relevant known facts, the most conclusive idea might be to assign differently hydrated carbonyl-group populations to the two or more subspecies contributing to this band.[36,74] This interpretation is in accord with our finding that, with increasing hydration, the fraction of "water-bound" $C{=}O$ groups will be enhanced, and, consequently, $\tilde{\nu}C{=}O$ will be lowered.

Ruling out the existence of a lyotropic phase transition for DPPC at RT, as justified by both the smooth curve characteristics for DPPC in Figure 6 and 8 as well as previous data (Table I), suggests a hydration-dependent ranking of water-binding sites: the different (in terms of A_{wr}) onsets of relevant wavenumber shifts suggest that water molecules bind to the phosphate groups first before $C{=}O$ groups are attached. This concept is plausible since the phosphate moiety as a constituent of the lipid headgroup is, in general, positioned in the middle of the hydrophilic region, whereas the carbonyl groups, next to the hydrophilic/hydrophobic boundary, will be reached, in the course of a stepwise increasing hydration, only subsequently by water molecules. Previous work reports the phosphate groups as acting as primary water-binding sites for DPPC.[38,62,63]

The wavenumbers of the stretching-vibration bands due to the esterified $C{-}O$ and $P{-}O$ moieties at about 1170 and 820 cm^{-1}, respectively, are compared in Figure 9. In contrast to the νPO_2^- and $\nu C{=}O$ bands (Figs. 7 and 8), the wavenumbers of these two bands increase with hydration. Where the phosphate groups are concerned, this oppositely directed spectral behavior of adjacent single bonds and bonds with a pronounced double-band character is in agreement with theoretical predictions.[38] Moreover, it appears that the greater part of wavenumber shifting for the $\nu P{-}O$ band is concentrated much more towards the lower-hydration region than for the $\nu C{-}O$ band. In principle this confirms the conclusions drawn above concerning the sequence of water binding

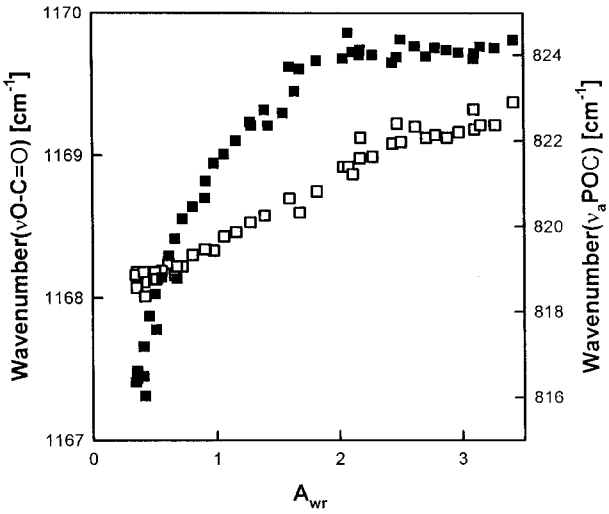

Figure 9. Wavenumber displacements drawn for the νC—O (open symbols) and ν_aP—O bands (full symbols) of DPPC plotted in dependence on A_{wr}.

to the relevant PL sites in the headgroup and at the polar/apolar interface, respectively.

CONCLUSIONS

The process of phospholipid hydration, of considerable biological importance, has been studied. In the present article, Fourier-transform infrared spectroscopy has been used for investigating the gradual hydration of PLs in model-membrane systems. Cast aligned films consisting of multibilayers formed by a number of lecithins were examined at room temperature under conditions covering a wide range of water activities. Four different lecithins with systematic variations in their apolar tail region, namely the dipalmitoyl and dioleoyl PCs together with their mixed-chain analogues POPC and OPPC, were studied. PL hydration can be followed using IR-spectroscopy in two principal ways: either more or less directly by the water absorption bands or indirectly via the PL bands involved in and, thus, responding to water binding. The overall water uptake by PCs is dependent on the nature of the apolar hydrocarbon tails and increases for lipids with oleoyl chains. This finding can be ascribed to structural and/or superstructural consequences due to unsaturation in the oleoyl chains and the larger molecular area available for the hydrated lipid headgroups.

The mixed-chain PCs studied stand out from the other lipids by exhibiting isothermal main transitions at a particular water activity corresponding to a hydration number of about 4. The wavenumbers of the CH_2 methylene stretching-vibration bands are clearly in accord with the idea that, among the lipids studied, both the lamellar gel (in DPPC as well as in dry POPC and OPPC) and the lamellar liquid-crystalline phases (presumably in DOPC as well as in hydrated POPC and OPPC) are formed under the ambient conditions. Moreover, hydration causes a very slight, but steady wavenumber increase for the νCH_2 bands at water activities above and below the one at which chain melting occurs. These data give clear-cut evidence for a substantial extent of nonuniformity existing in both phases, L_β and L_α, constituted most probably by significant variations in the conformational order of their fatty-acid chains.

Furthermore, there are characteristic differences in the hydration-dependent behavior of the infrared bands of the PC headgroups and polar/apolar interfaces. These differences particularly refer to both the onset and the extent of wavenumber shifts. Generally, phosphate groups are already affected at smaller hydration levels than carbonyl groups. In principle, the data permit conclusions concerning the sequence in which lipid binding sites are attached by water molecules or which PL subdomains are involved in the process of water binding to be drawn. The results obtained once more emphasize the potential usefulness of FTIR techniques in lipid research. The data can be regarded as being fairly complementary to the data obtained by other methods. This is mainly due to the versatility of IR spectroscopy permitting direct monitoring of the extent of water uptake, as well as some clarification of structural and/or superstructural events on a submolecular level.

The authors have to thank the Thuringian Ministry of Sciences (TMWFK) as well as the Deutsche Forschungsgemeinschaft (DFG) for providing funds to support these studies, the latter with SFB No. 197 and SFB No. 294 (H.B.). Furthermore, they are indebted to Drs. Anne S. Ulrich and Ruthven N.A.H. Lewis for a critical reading of the manuscript and to Mrs. W. Scheiding for technical assistance.

ABBREVIATIONS AND SYMBOLS

A_w	room temperature
A_{wr}	normalized absorbance of the $\tilde{\nu}$OH band of water (in a lipid film)

ATR	attenuated total reflectance
BET	Brunauer–Emmett–Teller
DMPC	dimyristoylphosphatidylcholine
DODPC	phosphatidylcholine
DOPC	1,2-dioleoyl-*sn*-glycero-3-phosphocholine or dioleoylphosphatidylcholine
DOPE	dioleoylphosphatidylethanolamine
DPPC	dipalmitoylphosphatidylcholine
DPPE	dipalmitoylphosphatidylethanolamine
FTIR	Fourier transform infrared
IR	infrared
L_α	liquid-crystalline phase
L_β	gel phase
NMR	nuclear magnetic resonance
n_w	hydration number
$\tilde{\nu}$	wavenumber
νC—O	stretching vibration of the O—C(=O) unity
νC=O	carbonyl stretching vibration
νO—C=O	stretching vibration of the O—C=O unity
$\nu_a CH_2$	antisymmetric stretching vibration of methylene CH bonds
νP—O	antisymmetric stretching vibration of the P—O(—C) unity
$\nu_a PO_2^-$	antisymmetric stretching vibration of the PO_2^- moiety
ν_aP—O—C	antisymmetric stretching vibration of the P—O—C unity
$\nu_s CH_2$	symmetric stretching vibration of methylene CH bonds
$\nu_s PO_2^-$	symmetric stretching vibration of the PO_2^- moiety
$\nu_{1,3}$OH	O—H stretching vibration of water
OPPC	1-oleoyl, 2-palmitoylphosphatidylcholine
PC	phosphatidylcholine
PE	phosphatidylethanolamine
PL	phospholipid
POPC	oleoylphosphatidylcholine
RH	relative humidity
RT	room temperature
T_m	main-transition temperature

REFERENCES

1. D. Häussinger, "Regulation der Zellfunktion durch den Hydratisierungszustand," *Naturwissenschaften,* **83,** 264–271 (1996).
2. C. Tanford, "The hydrophobic effect and the organization of living matter," *Science,* **200,** 1012–1018 (1978).
3. M. M. Teeter, "Water–protein interactions: Theory and experiment," *Annu. Rev. Biphys. Biophys. Chem.,* **20,** 577–600 (1991).
4. W. Blokzijl and J. B. F. N. Engberts, "Hydrophobe Effekte—Ansichten und Tatsachen," *Angew. Chem.,* **105,** 1610–1648 (1993).
5. R. E. Franklin and R. G. Gosling, "The structure of sodium thymonucleate fibers. I. The influence of water content," *Acta Cryst.,* **6,** 673–677 (1953).
6. H. M. Berman, "Hydration of DNA: Take 2," *Curr. Opinion Struct. Biol.,* **4,** 345–350 (1994).
7. W. Pohle and M. Bohl, "Infrared spectroscopic data favor a global mode hydration in determining DNA conformation by water activity," *Biospectroscopy,* **1,** 101–104 (1995).
8. S. A. Galema, J. B. F. N. Engberts, H. Hoiland, and G. M. Forland, "Informative thermodynamic properties of the effect of stereochemistry on carbonate hydration," *J. Phys. Chem.,* **97,** 6885–6889 (1993).
9. Y. Marcus, "Water binding by organic molecules," *Cell Biochem. Funct.,* **13,** 157–163 (1995).
10. G. Cevc, "Isothermal lipid phase transitions," *Chem. Phys. Lipids,* **57,** 293–307 (1991).
11. H. Hauser, "Lipids," in *Water—A Comprehensive Treatise,* ed. by F. Franks, Plenum Press, New York, London, 1975, pp. 209–304.
12. R. P. Rand and V. A. Parsegian, "Hydration forces between phospholipid bilayers," *Biochim. Biophys. Acta,* **988,** 351–376 (1989).
13. D. Marsh, Ed., *CRC Handbook of Lipid Bilayers,* CRC Press, Boca Raton, Ann Arbor, Boston, 1990.
14. T. J. McIntosh and A. D. Magid, "Phospholipid hydration," in *Phospholipids Handbook,* ed. by G. Cevc, Marcel Dekker, Inc., New York, Basel, Hong Kong, 1993, pp. 553–578.
15. G. L. Jendrasiak, "The hydration of phospholipids and its biological significance," *J. Nutr. Biochem.,* **7,** 599–609 (1996).
16. B. D. Ladbroke and D. Chapman, "Thermal analysis of lipids, proteins and biological membranes—A review and summary of some recent studies," *Chem. Phys. Lipids,* **3,** 304–367 (1996).
17. M. Kodama, M. Kuwabara, and S. Seki, "Successive phase-transition phenomena and phase diagram of the phosphatidylcholine-water system as revealed by differential scanning calorimetry," *Biochim. Biophys. Acta,* **689,** 567–570 (1982).
18. G. Bryant, J. M. Pope, and J. Wolfe, "Low hydration phase properties of phospholipid mixtures," *Eur. Biophys. J.,* **21,** 223–232 (1992).
19. A. S. Ulrich, M. Sami, and A. Watts, "Hydration of DOPC bilayers by differential scanning calorimetry," *Biochim. Biophys. Acta,* **49,** 39–50 (1994).
20. D. M. Small, "Phase equilibria and structure of dry and hydrated egg lecithin," *J. Lipid Res.,* **8,** 551–557 (1967).
21. A. Tardieu, V. Luzzati, and F. C. Reman, "Structure and polymorphism of the hydrocarbon chains

of lipids: A study of lecithin–water phases," *J. Mol. Biol.,* **75,** 711–733 (1973).
22. G. Klose, B. König, H. W. Meyer, G. Schulze, and G. Degovics, "Small-angle x-ray scattering and electron microscopy of crude dispersions of swelling lipids and the influence of the morphology on the repeat distance," *Chem. Phys. Lipids,* **47,** 225–234 (1988).
23. E. G. Finer and A. Darke, "Phospholipid hydration studied by deuteron magnetic resonance spectroscopy," *Chem. Phys. Lipids,* **12,** 1–16 (1974).
24. B. Bechinger and J. Seelig, "Conformational change of the phosphatidylcholine headgroup due to membrane dehydration; a ^{2}H-NMR study," *Chem. Phys. Lipids,* **58,** 1–5 (1991).
25. A. S. Ulrich and A. Watts, "Molecular response of the lipid headgroup to bilayer hydration monitored by ^{2}H-NMR," *Biphys. J.,* **66,** 1441–1449 (1994).
26. P. H. Elworthy, "The adsorption of water vapor by lecithin and lysolecithin, and the hydration of lysolecithin micelles," *J. Chem. Soc.,* 5385–5389 (1961); "Sorption studies on phosphatides. Part II. Sorption of water vapor by a synthetic lecithin and cephalin," *J. Chem. Soc.,* 4897–4900 (1962).
27. G. L. Jendrasiak and J. H. Hasty, "The hydration of phospholipids," *Biochim. Biophys. Acta,* **337,** 79–91 (1974).
28. G. L. Jendrasiak and J. C. Mendible, "The phospholipid head-group orientation: Effect on hydration and electrical conductivity," *Biochim. Biophys. Acta,* **424,** 149–158 (1976).
29. G. Klose, B. König, and F. Paltauf, "Sorption isotherms and swelling of POPC in H_2O and ^2H_2O," *Chem. Phys. Lipids,* **61,** 265–270 (1992).
30. U. P. Fringeli and H. H. Günthard, "Infrared membrane spectroscopy," in *Membrane Spectroscopy,* ed. by E. Grell, Springer-Verlag, Berlin, Heidelberg, New York, 1981, pp. 270–332.
31. H. L. Casal and H. H. Mantsch, "Polymorphic phase behavior of phospholipid membranes studied by infrared spectroscopy," *Biochim. Biophys. Acta,* **779,** 381–402 (1984).
32. D. C. Lee and D. Chapman, "Infrared spectroscopic studies of biomembranes and model membranes," *Bioscience Rep.,* **6,** 235–256 (1986).
33. H. H. Mantsch and R. N. McElhaney, "Phospholipid phase transitions in model and biological membranes as studied by infrared spectroscopy," *Chem. Phys. Lipids,* **57,** 213–226 (1991).
34. R. N. A. H. Lewis and R. N. McElhaney, "Fourier transform infrared spectroscopy in the study of hydrated lipids and lipid bilayer membranes," in *Infrared Spectroscopy of Biomolecules,* ed. by H. H. Mantsch and D. Chapman, Wiley-Liss, New York, Chichester, Brisbane, Toronto, Singapore, 1996, pp. 159–202.
35. P. T. T. Wong and H. H. Mantsch, "High-pressure infrared spectroscopic evidence of water binding sites in 1,2-diacyl phospholipids," *Chem. Phys. Lipids,* **46,** 213–224 (1988).
36. R. N. A. H. Lewis, R. N. McElhaney, W. Pohle, and H. H. Mantsch, "Components of the carbonyl stretching band in the infrared spectra of hydrated 1,2-diacylclycerolipid bilayers: A reevaluation," *Biophys. J.,* **67,** 2367–2375 (1994).
37. A. Bertoluzza, S. Bonora, G. Fini, and M. A. Morelli, "The role of water in biological systems. An infrared study on phospholipid–water interactions," *Can. J. Spectrosc.,* **29,** 93–98 (1984).
38. J. Grdadolnik, J. Kidric, and D. Hadzi, "Hydration of phosphatidylcholine reverse micelles and multilayers—An infrared spectroscopic study," *Chem. Phys. Lipids,* **59,** 57–68 (1991).
39. J. Grdadolnik, J. Kidric, and D. Hadzi, "An FT–IR study of water hydrating dipolmitoylphosphatidylcholine multibilayers and reversed micelles," *J. Mol. Struct.,* **322,** 93–103 (1994).
40. L. Ter-Minassian-Saraga, E. Okamura, J. Umemura, and T. Takenaka, "Fourier transform infrared attenuated total reflectance spectroscopy of hydration of dimyristoylphospatidylcholine multibilayers," *Biochim. Biophys. Acta,* **946,** 417–423 (1988).
41. J. R. Scherer, S. Kint, B. A. Bolton, and G. F. Bailey, "Raman spectra of hydrated phospholipid bilayers. Part I. Measurement of humidity-dependent phase transitions," *J. Mol. Struct.,* 245–257 (1990).
42. S. Kint, P. H. Wermer, and J. R. Scherer, "Raman spectra of hydrated phospholipid bilayers. 2. Water and head-group interactions," *J. Phys. Chem.,* **96,** 446–452 (1992).
43. H. Akutsu, Y. Kyogoku, H. Nakahara, and K. Fukuda, "Conformational analysis of phosphatidylethanolamine in multilayers by infrared dichroism," *Chem. Phys. Lipids,* **15,** 222–242 (1975).
44. H. Binder, A. Anikin, B. Kohlstrunk, and G. Klose, "Hydration induced gel states of the dienic lipid 1,2-bis(2,4-octadecadienoyl)-sn-glycero-3-phosphorylcholine and their characterization using infrared spectroscopy," *J. Phys. Chem.,* **101,** 6618–6628 (1997).
45. J. B. Davenport and L. R. Fisher, "Interaction of water with egg lecithin in benzene solution," *Chem. Phys. Lipids,* **14,** 275–290 (1975).
46. W. Pohle and H. Fritzsche, "Infrared spectroscopy as a tool for investigations of DNA structure and DNA-ligand interactions," *J. Mol. Struct.,* **219,** 341–346 (1990).
47. D. V. Lynch and P. L. Steponkus, "Lyotropic phase behavior of unsaturated phosphocholine species: Relevance to the mechanism of plasma membrane destabilization and freezing injury," *Biochim. Biophys. Acta,* **984,** 267–272 (1989).
48. S. B. Leslie, E. Israeli, B. Lighthart, J. H. Crowe, and L. M. Crowe, "Trehalose and sucrose protect both membranes and proteins in intact bacteria during drying," *Appl. Environ. Microbiol.,* **61,** 3592–3597 (1995).
49. A. Nilsson, A. Holmgren, and G. Lindblom, "Fou-

rier-transform infrared spectroscopy study of dioleoylphosphatidylcholine and monooleoylglycerol in lamellar and cubic liquid crystals," *Biochemistry,* **30,** 2126–2133 (1991).
50. K. H. Cheng, "Infrared study of the polymorphic phase behavior of dioleoylphosphatidylethanolamine and dioleoyolphosphatidylcholine mixtures," *Chem. Phys. Lipids,* **60,** 119–125 (1991).
51. B. J. Litman, E. N. Lewis, and I. W. Levin, "Packing characteristics of highly unsaturated bilayer lipids: Raman spectroscopy studies of nultilamellar phosphatidylcholine dispersions," *Biochemistry,* **30,** 313–319 (1991).
52. V. R. Kodati and M. Lafleur, "Comparison between orientational and conformational orders in fluid lipid bilayers," *Biophys. J.,* **64,** 163–170 (1993).
53. A. Gericke, D. J. Moore, R. K. Erukulla, R. Bittman, and R. Mendelsohn, "Partially deuterated phospholipids as IR structure probes of conformational order in bulk and monolayer phases," *J. Mol. Struct.,* **379,** 227–239 (1996).
54. M. Wong, F. H. Anthony, T. W. Tillack, and T. E. Thompson, "Fusion of dipalmitoylphosphatidylcholine vesicles at 4°C," *Biochemistry,* **21,** 4126–4132 (1982).
55. J. R. Silvius and R. N. McElhaney, "Effects of phospholipid acyl chain structure on thermotropic phase properties: 2. Phosphatidylcholines with unsaturated or cyclopropane acyl chains," *Chem. Phys. Lipids,* **25,** 125–134 (1979).
56. P. J. Davis, B. D. Fleming, K. P. Codbear, and K. M. W. Keough, "Gel to liquid-crystalline transition temperatures of water dispersions of two pairs of positional isomers of unsaturated mixed-acid phosphatidylcholines," *Biochemistry,* **20,** 3633–3636 (1981).
57. J. F. Santaren, M. Rico, J. Guilleme, and A. Ribera, "Thermal and ^{13}C-NMR study of the dynamic structure of 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine and 1-oleoyl-2-palmitoyl-sn-glycero-3-phosphocholine in aqueous dispersions," *Biochim. Biophys. Acta,* **687,** 231–237 (1982).
58. M. Caffrey, *Lipid Thermotropic Phase Transitions, NIST Standard Reference Database 34. Standard Reference Data,* National Institute of Standards and Technology; Gaithersburg, 1993.
59. R. N. Jones, "The absorption of radiation by inhomogeneously dispersed systems," *J. Amer. Chem. Soc.,* **74,** 2681–2683 (1952).
60. H. Heller, M. Schaefer, and K. Schulten, "Molecular dynamics simulation of a bilayer of 200 lipids in the gel and in the liquid-crystal phases," *J. Phys. Chem.,* **97,** 8343–8360 (1993).
61. E. Baer, "On the crystallization, structure and infrared spectra of saturated L-α-lecithins," *J. Amer. Chem. Soc.,* **75,** 621–623 (1953).
62. R. H. Pearson and I. Pascher, "The molecular structure of lecithin dihydrate," *Nature,* **281,** 499–501 (1979).
63. H. Hauser, I. Pascher, R. H. Pearson, and S. Sundell, "Preferred conformation and molecular packing of phosphatidylethanolamine and phosphatidylcholine," *Biochim. Biophys. Acta,* **650,** 21–51 (1981).
64. W. Pohle and C. Selle, "Fourier-transform infrared spectroscopic evidence for a novel lyotropic phase transition occurring in dioleoylphosphatidylethanolamine," *Chem. Phys. Lipids,* **82,** 191–198 (1996).
65. I. M. Asher and I. W. Levin, "Effects of temperature and molecular interactions on the vibrational infrared spectra of phospholipid vesicles," *Biochim. Biophys. Acta,* **468,** 63–72 (1977).
66. V. R. Kodati, R. Eljastimi, and M. Lafleur, "Contribution of the intermolecular coupling of librotorsional mobility in the methylene stretching modes in the infrared spectra of acyl chains," *J. Phys. Chem.,* **98,** 12191–12197 (1994).
67. N.-C. Chia, C. Vilchèze, R. Bittman, and R. Mendelsohn, "Interactions of cholesterol and synthetic sterols with phosphatidylcholines as deduced from infrared CH_2 waging progression intensities," *J. Amer. Chem. Soc.,* **115,** 12050–12055 (1993).
68. G. Klose, S. Eisenblätter, and B. König, "Ternary phase diagram of mixtures of palmitoyl-oleoyl-phosphatidylcholine, tetraoxyethylene dodecyl ether, and heavy water as seen by ^{31}P and ^{2}H NMR," *J. Colloid Interface Sci.,* **172,** 438–446 (1995).
69. W. Pohle, M. Bohl, and H. Böhlig, "Interpretation of the influence of hydrogen bonding on the stretching vibrations of the PO_2-moiety," *J. Mol. Struct.,* **242,** 333–342 (1991).
70. J. Pilet and J. Brahms, "Investigation of DNA structural changes by infrared spectroscopy," *Biopolymers,* **12,** 387–403 (1973).
71. J. L. R. Arrondo, F. M. Goni, and J. M. Macarulla, "Infrared spectroscopy of phosphatidylcholines in aqueous suspension—A study of the phosphate group vibrations," *Biochim. Biophys. Acta,* **794,** 165–168 (1984).
72. J. H. Crowe, L. M. Crowe, and D. Chapman, "Infrared spectroscopic studies on interactions of water and carbohydrates with biological membranes," *Arch. Biochem. Biophys.,* **232,** 400–407 (1984).
73. W. Pohle, C. Selle, H. Fritzsche, and M. Bohl, "Comparative FTIR spectroscopic study upon the hydration of lecithins and cephalins," *J. Mol. Struct.,* **408/409,** 273–277 (1997).
74. A. Blume, W. Hübner, and G. Messner, "Fourier transform infrared spectroscopy of ^{13}C═O labeled phospholipids. Hydrogen bonding to carbonyl groups," *Biochemistry,* **27,** 8239–8249 (1988).