THE VIBRATIONAL SPECTRUM OF WATER
IN LIQUID ALKANES

MORGAN P. CONRAD AND HERBERT L. STRAUSS
Department of Chemistry, University of California, Berkeley, California 94720

ABSTRACT   The water wire hypothesis of hydrogen-ion transport in lipid bilayers has prompted a search for water aggregates in bulk hydrocarbons. The asymmetric stretching vibration of the water dissolved in *n*-decane and in a number of other alkanes and alkenes has been observed. The water band in the alkanes is very wide and fits to the results of a J-diffusion calculation for the water rotation. This implies that the water is freely rotating between collisions with the solvent and certainly not hydrogen bonded to anything. The existence of water aggregates is thus most unlikely. In contrast, water in an alkene is hydrogen bonded to the solvent molecules (although not to other water molecules) and shows an entirely different spectrum.

INTRODUCTION

The surprisingly high proton permeability (~ 1 μm/s) reported for both artificial and natural lipids has led to suggestions of a special mechanism for proton transport (1–5). Nichols and Deamer (1–4) have proposed the existence of chains of water molecules that serve as proton wires. The protons presumably move along the chains in a manner similar to the way they move through pure water or ice. This model is supported by noting that the activation energy for proton transport is ~ 20 kcal/mol (5, 6) about that in ice. If one assumes that all the water exists in chains and that the conductivity of a chain is the same as that of ice, then one can account for the observed permeability (7). The chain mechanism implies, of course, that the water in a lipid is aggregated into dimers or larger clusters, and these aggregates should show up in the vibrational spectra. Here we present the results of a vibrational study of water dissolved in a variety of liquid alkanes and alkenes. We establish the nature of the water by matching the observed band shape of the water with that calculated from a model. The similarity between a liquid alkane and the interior of a lipid membrane as well as other evidence concerning the occurrence of aggregates in dissolved water is considered in the Discussion section. We first review the vibrational spectra to the extent necessary to understand our arguments, and then present our results and analysis.

SPECTROSCOPY OF WATER

Vibrations

There has been extensive work on the vibrational spectroscopy of water, much of it for the purpose of determining

Dr. Conrad's current address is Becton-Dickinson Monoclonal Center, 2375 Garcia Ave., Mountain View, CA 94303

and understanding the effect of hydrogen bonding on the spectrum and on intermolecular structure (8, 9). Water is an asymmetric top molecule with C_{2v} symmetry and three vibrational modes, which are active in both the infrared and Raman. In the gas phase, the symmetric stretch ν_1, the symmetric bend ν_2, and the asymmetric stretch ν_3 are observed at 3,657, 1,595, and 3,756 cm^{-1}, respectively. The ν_2 and ν_3 vibrations give rise to strong absorption in the infrared, and the pure rotational motion gives rise to strong absorption in the far infrared.

The numerous studies of the vibrational spectra of water in various solvents (9–12) agree that, as the extent of hydrogen bonding increases, both the ν_1 and ν_3 bands move to lower frequencies, with an approximately linear correlation between the shift and the solvent basicity as measured by its pK_a value. There is also a linear correlation between the $0 \cdots 0$ distance and the observed OH stretching frequency in a wide variety of systems (13). Extensive tables of vibrational frequencies observed for the water dissolved in various solvents have been presented (9).

Both the ν_1 and ν_3 band intensities increase as the bands move to lower frequencies. This is most notable for the ν_1 band, which is extremely weak in an inert solvent such as CCl$_4$, yet almost as strong as ν_3 in tetrahydrofuran (THF). The ratio of the area of the ν_1 band to the area of the ν_3 band is proportional to the red shift (11). The width of the infrared bands also increases with the shift to lower frequency.

When strong hydrogen bonds are formed, the normal vibrational modes change, and the description of the stretching modes as symmetric and antisymmetric is no longer applicable. Instead, one must discuss the motion in terms of free and bonded OH stretches. Recent model calculations have ascertained many possible configurations of small (2–8 molecules) water clusters (14). The predicted vibrational spectra all have large, broad bands at $\sim 3,400$

wavenumbers due to bonded OH stretches, as well as less shifted bands attributed to free OH stretches, and are in agreement with experimental spectra of water clusters in molecular beams [15].

Therefore, if water clusters do exist in alkanes or alkenes, their infrared spectra would be expected to have two broad bands of roughly equal intensity, a bonded OH stretch near 3,400, and a free OH stretch around 3,600 wavenumbers. If, instead, there are no clusters, the spectra would have two bands around 3,600 and 3,700 wavenumbers. In addition, however, the actual spectrum of water in weakly hydrogen bonded solvents often shows wings attributed to rotational motion [16, 17], and so it is necessary to consider the possible effect of rotational motion of the spectra.

Rotational Motion and Lineshape

The infrared spectrum depends on vibrational dipoles. Since molecular rotation changes the direction in space of these dipoles, rotational motion contributes to the vibrational spectrum. Gordon [18, 19] analyzed the vibrational band shape in terms of correlation functions and obtained

$$I(\nu) = \frac{1}{2\pi} \int_{-\infty}^{\infty} e^{-i2\pi\nu t} \langle \mu(0) \cdot \mu(t) \rangle \, dt. \qquad (1)$$

Here $I(\nu)$ is the normalized intensity as a function of frequency ν, μ a normalized transition dipole moment, and the brackets represent an equilibrium statistical average. In this formula, the origin of frequency is taken at the center of the band being considered. Conversely, one can calculate the correlation function from the observed band shape

$$\langle \mu(0) \cdot \mu(t) \rangle = \int_{-\infty}^{\infty} I(\nu) \, e^{i2\pi\nu t} \, 2\pi \, d\nu. \qquad (2)$$

With the neglect of the collision-induced components and vibration-rotation coupling effects,[1] the correlation function can be factored into a purely vibrational part depending only on the time correlation between the length $M(t)$ of the dipole vector

$$G_v(t) = \langle M(0)M(t) \rangle \qquad (3)$$

and a purely rotational part depending only on the orientation $\hat{\mu}(t)$ of the dipole [21, 22]

$$G_R(t) = \langle \hat{\mu}(t) \cdot \hat{\mu}(0) \rangle . \qquad (4)$$

At room temperature, the rotational motion of molecules in solution is essentially classical and several classical models for rotation of motion in liquids have been proposed [23]. From these come classical correlation functions that yield classical spectra. A first-order quantum mechanical correction is obtained by multiplying the spectrum by

[1]Most authors assume these assumptions to be good or at least adopt them as working hypotheses. For a contrary view see reference 20.

$e^{-h\nu/2kT}$, where ν is again measured from the band center frequency [24].

One set of these models consists of diffusional, or stochastic, models. These models are most appropriate when there is a strong interaction between the molecules, and describes the motion as a series of jumps between fixed states or positions. Such models might be appropriate for describing the motion of a water molecule that jumps between different partners in an aggregate. The jumps occur infrequently; that is, the time spent between jumps is much longer than the actual time spent in the jump. Angular momentum is ignored, so there is no correlation between consecutive jumps, and the process is Markovian. Within this overall framework, the models differ as to the allowed axes and angles of rotation. The most commonly used model of this type is that an anisotropic small step diffusion (ASSD), which entails the calculation of a rotational diffusion tensor $\mathbf{R}$, which in turn depends on a friction constant tensor. The calculated value for the friction depends on whether "stick" or "slip" boundary conditions are applied [25].

At the opposite extreme from the rotational diffusion models is the free rotation model, which assumes no solvent-solute interaction and complete memory of angular momentum with time. The molecule's motion is described by Euler's equations of motion in the absence of any external torques or friction. For a few cases such as symmetric tops, the classical solutions to Euler's equations are straight forward, but for other systems, such as the asymmetric top water, the solutions are complicated combinations of theta functions, and must be numerically integrated. Leickman et al. have presented lineshape equations and results for many of the soluble cases [21, 22].

Recently, new techniques for the numerical solution of these equations using trajectory calculations have been developed [26]. The molecule's orientation is expressed not in terms of Euler angles, but in terms of a Cartesian rotation matrix $\mathbf{D}(t)$, whose components are given by

$$D_{ia}(t) = \hat{e}_i \, \hat{b}_a(t) \qquad (5)$$

where $\hat{e}_i$, $i = 1, 2, 3$, are the space-fixed unit vectors and $\hat{b}_a(t)$, the body-fixed unit vectors. The resulting equations of motion for $\mathbf{D}$ and the angular velocity ω can be readily treated on a computer and used to obtain G_R [26, 27]. The model has been applied to water vapor and the calculated spectrum compares well with experiment [26, 27].

Intermediate between the free rotation and the diffusion models lie the extended diffusion models, first developed by Gordon [28] for linear molecules and later extended to symmetric [29, 30] and asymmetric [31, 32] top molecules. In the J-diffusion version of these models, the molecule freely rotates with a correlation time t_ω, then undergoes an instantaneous collision, keeping its old orientation but getting a new random angular momentum. One can view the model as a random walk in angular momentum space, with step frequency $B = 1/t_\omega$ and no

correlation between successive steps. t_ω is the angular momentum correlation time.[2]

EXPERIMENT

The infrared spectrum of water in an alkane has apparently not been observed previously. The most obvious difficulty comes from the low solubility of water [77 p.p.m. by weight in n-octane (33)]. However, this is still 28% of the solubility in CCl_4, where the ν_3 absorbance of H_2O is moderately strong (an absorbance of ~0.07 in a 2 mm cell) and has been observed for many years (16, 17).

One would think that someone would have observed the spectrum, and indeed Jacob et al. have tried (34). It turns out that a number of other factors, discussed below, add to the difficulty of observing the water spectrum.

The alkane solvents, of 99% stated purity, were purchased from either Aldrich (Aldrich Chemical Co., Inc., Milwaukee, WI) or MCB (MCB Mfg. Chemists, Inc., Los Angeles, CA) and were used straight from the bottle. The water was standard laboratory distilled water. The 1-heptene was Aldrich "gold label" of 99%+ purity, but the various decenes (1,5-cis and 5-trans) (K & K Laboratories, Inc., Irvine, CA) were of considerably lower stated purity. These were dried over molecular sieves, treated with potassium metal, and run through a salicylic acid column, which was then flushed with 99%+ pentane to recover all the alkene. The pentane was boiled off. In many cases a bright green coating of unknown composition formed on the potassium.

Both the n-alkanes and alkenes have significant combination band structure in the vicinity of the water OH stretching bands and since the water bands are expected to be weak, an accurate reference spectrum of the dry solvent must be obtained. This proves to be difficult because the solvents absorb atmospheric water vapor surprisingly quickly. Reference samples were prepared by drying each solvent over a 4-Å molecule sieve in a small vial, which was then placed in a transfer chamber that was evacuated (with some boiling of the solvent) and sealed. This chamber was transferred into a homemade drybox containing a Helium atmosphere continuously circulated over an inert gas purifier and estimated to contain ~1 ppm H_2O. In the dry box, the solvent was further dried over potassium metal for ~15 min while the sample cell was assembled. To ensure absolute dryness of the sample when taking the spectra, a small piece of potassium was placed inside the cell. No change with time in the spectral region of interest was observed despite the long scanning times (a few hours) sometimes used.

The wet sample was prepared by starting with some dry solvent, adding a few drops of distilled water, shaking, and allowing the mixture to settle for a few hours. The cell and windows were washed with isopropanol to remove the still active potassium, then rinsed with hexane and dried before inserting the wet sample. We first used KBr windows and found that irreversibly absorbed H_2O from the wet sample gave a broad absorption around 3,250 cm^{-1} that was unchanged on addition of a relatively dry alkane. Thereafter, CaF_2 windows were used and no such phenomenon was observed.

The spectrometer used was a vacuum Fourier transform infrared (FTIR) spectrometer (model 8000; Nicolet Instrument Corp., Madison, WI) with the entire optical path except a small sample tube evacuated to better than 1 Torr. The tub, which included ~8 cm of optical path, was purged with house nitrogen passed through a 4-ft drying tube filled with calcium sulfate. Despite these measures, on the sensitive absorbance scale used in our experiments, some sharp bands of atmospheric water do appear and must be subtracted from the spectra. We used a standard globar source in conjunction with a KBr beamsplitter and a liquid-nitrogen–cooled MCT-InSb sandwich detector (E771; Infrared Associates, Inc., New Brunswick, NJ) set to the high frequency mode (InSb,

sensitive to light <1,800 cm^{-1}). This detector is roughly thirty times more sensitive than a standard TGS detector. All spectra (unless noted) were run at a nominal 2 cm^{-1} resolution, typically with several thousand scans obtained in 2 h. The beam diameter at the sample was roughly 12 mm. The liquid cell (Harrick Scientific Corp, Ossining, N.Y.) held 2 mm teflon spaces between 1-in–diameter CaF_2 windows, sealed with Viton O-rings. The 2 mm thickness was a compromise between the wish to get as much H_2O absorbance as possible and the need to get significant radiation through the solvent.

To obtain the spectra of the dissolved water, without solvent interference, three transmission spectra were obtained. One was of the empty cell, another of the cell containing dry solvent, and the last that of the cell full of wet solvent. The second and third were both ratioed against the first to obtain absorbance spectra. The ratioed spectra were then subtracted from each other to obtain the absorption difference spectra presented here. To compensate for minute differences in cell length, etc., the dry spectrum could be multiplied by a correction factor before subtraction. This factor was always between 0.99 and 1.01. In most cases an appropriately scaled spectrum of water vapor was also subtracted.

A final experimental difficulty arises from the unexpectedly large width of the water band (see Results). The large width, compared with that in CCl_4, for example, means that the peak height estimated from the relative solubilities of water in CCl_4 and in an alkane gives a value that is considerably too high. Furthermore, CCl_4 has very little absorption in the OH stretching region, whereas the hydrocarbons show considerable absorption due to combination bands. It is particularly difficult to find a weak broad absorption against an appreciable background, and this accounts for the failure of previous workers to find the spectrum.

RESULTS

The spectra of the fundamental water stretches in the normal alkane solvents n-octane, n-decane, n-undecane, and n-tetradecane are presented in Figs. 1–4. Due to the greatly expanded absorbance scale, it proved difficult to completely subtract some small sharp bands caused by
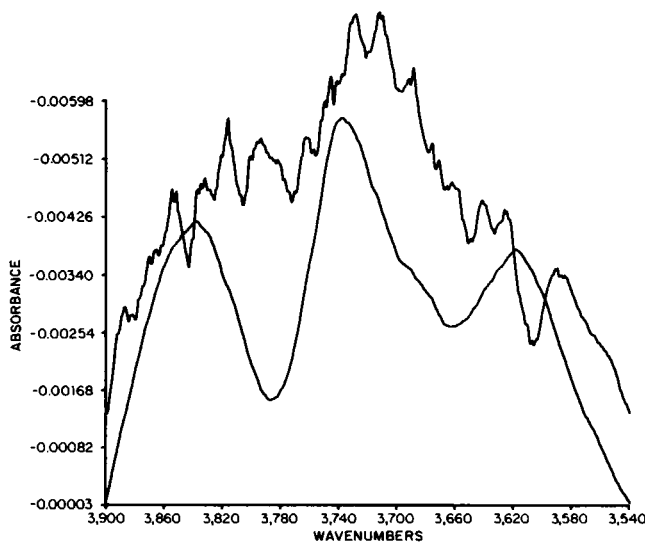

FIGURE 1 The infrared spectrum of water dissolved in n-octane in the OH stretching region. The top curve shows the difference between the spectra of wet and dry octane. Both spectra were taken in a cell with a 2 mm path length. Note the expanded absorbance scale. The bottom curve is a spectrum of water vapor (in air) that has been broadened by convoluting the observed spectrum with a 50-cm^{-1}–wide Lorentz function. The absorbance scale of the vapor spectrum is arbitrary.

[2]That is, the probability of the angular momentum not changing by time t is given by $1/t_\omega\, e^{-t/t_\omega}$, where t is measured from the time of the previous collision.
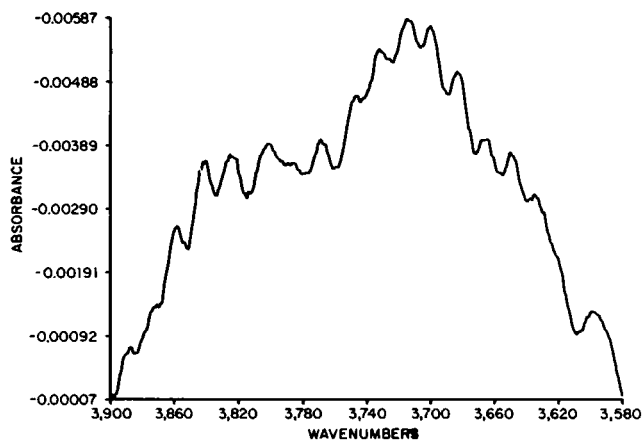
FIGURE 2 The infrared spectrum of the OH stretching region of water dissolved in n-decane taken under the same conditions as that of Fig. 1. The slightly lower absorbance of this spectrum corresponds to the lower solubility of water in n-decane as compared to n-octane.

FIGURE 3 The infrared spectrum of the OH stretching region of water dissolved in n-undecane taken under the same conditions as that of Fig. 1. The absorbance scale in the figure has been multiplied by 10.

traces of atmospheric water, and the bands are somewhat noisy. However, all still show a broad band central peak around $3,715$ cm^{-1}, and side bands, the most noticeable of those around $3,810$ cm^{-1}. The spectra of water in the different alkanes look slightly different from one another, with apparently more side band structure for the longer alkanes (Figs 1–4). However, the solubility of water decreases as the length of the alkane increases and consequently the water spectrum gets weaker. Note that the absorbance scale for octane (Fig. 1) goes to ~0.006 absorbance units whereas that for Fig. 4 goes only to 0.003. The major systematic error in the spectra comes from the subtraction of the dry spectrum from the wet spectrum, and it is difficult to know whether or not the differences among the spectra are real. We will not attempt to interpret these possible differences.

Fig. 1 includes a spectrum of water vapor that has been artificially broadened by convolution with a 50 cm^{-1} Lorentzian. The similarity between the two leads us to assign the side bands in the alkane solvent spectra to rational P and R branches.

The small downward shift of the frequency ($3,715$ cm^{-1} vs. $3,756$ cm^{-1} in the vapor) is easily explained on the basis of the Kirkwood-Bauer-Magat model (9, 35). This model accounts for the shifts of vibrational bands of a solute due to the dielectric effects of the solvent. The shift of the frequency may be taken to be characteristic of the monomer, if it fits that calculated by the KBM theory, and indeed our observed shift does (Conrad, M. P., and H. L. Strauss, manuscript submitted for publication; 27). The integrated area of the observed band compared with the alkene bands (shown below) is roughly that expected from the relative solubilities. No lower frequency bands appear in the region where hydrogen bonded complexes would be. We immediately conclude that most, if not all of the water is monomeric and that the observed band is ν_3. We also infer that, in the alkane, the water can rotate nearly as

freely as in the gas phase, a statement made more precise in the next section.

One sample was prepared with D_2O instead of normal water. The spectra showed no peaks in the OH stretching region, indicating that the lines are not somehow caused by changes in the solvent interacting with the dissolved water. Unfortunately, the actual D_2O bands are buried under various intense solvent bands and could not be observed.

For comparison, the spectra of water in 1-decene, and 5-cis, and 5-trans decene are presented in Fig. 5. The difference between these spectra and those of water in the alkanes is striking. Two bands due to the antisymmetric and symmetric stretches are seen and are somewhat more equal in intensity and lower in frequency than are those of water in CCl_4 (Conrad, M. P., and H. L. Strauss, manuscript submitted for publication; 27), indicating a greater degree of solute-solvent interaction in the alkene. This

FIGURE 4 The infrared spectrum of the OH stretching region of water in n-tetradecane taken under the same conditions as that of Fig. 1. The absorbance scale has been multiplied by 10. The solvent has a weak band at ~3,630 and the band seen at about this position of our water spectrum may result from imperfect subtraction of the solvent band.
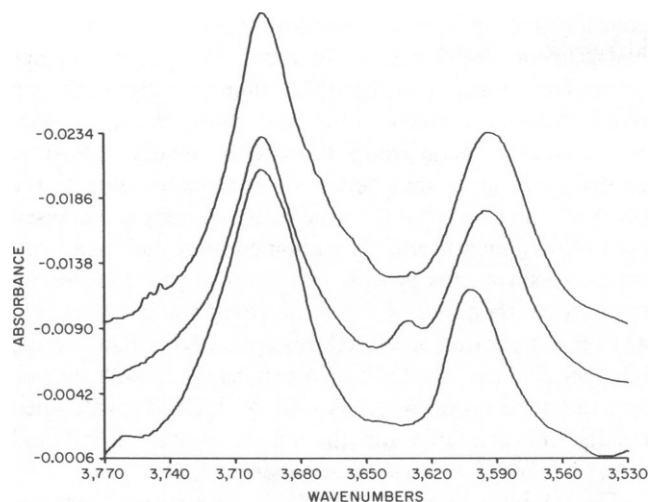
FIGURE 5   The infrared spectra of the OH stretching region of water in a number of decenes: *top, cis* 5-decane; *middle, trans* 5-decane; *bottom,* 1-decene. Compare to Figs. 1–4. Note that the peak absorbance is much larger, the signal-to-noise ratio is much better, and the band shapes are completely different from those in Figs. 1–4. The high frequency band is due to the asymmetric stretch, the low frequency band is due to the symmetric stretch.

interaction also accounts for the simple band shape and the lack of rotational structure in the spectra. The bands are what is expected for slightly hydrogen bonded water molecules and there is no evidence for water cluster formation in the alkenes.

Model of Water Rotation

To help interpret the spectra, a computer program was written to generate the J-diffusion model correlation functions and spectra. The trajectory approach of Fredkin et al. (26) for free rotors was extended to allow randomization of the angular momentum during collisions. The details have been discussed elsewhere (Conrad, M. P., and H. L. Strauss, manuscript submitted for publication; 28). To check our procedures, we calculated the results for free water, and found that they compare favorably with those in the literature (26).

Classical time-correlation functions (C.F.s) of the extended diffusion ("J") model applied to water were calculated for collision time constants t_w of 0.3, 0.2, 0.1, 0.07, 0.04, and 0.02 ps along with their Fourier transforms (Eq. 1). Fig. 6 compares the actual experimental spectrum for water in decane with the spectra derived from the above correlation functions. We observe a reasonable fit for $t_w = 0.07$ ps, and conclude that the value for t_w should lie between 0.07 and 0.1 ps. Likewise, we compare the computed correlation functions with that obtained by Fourier transforming the spectra (Eq. 2) in Fig. 7. We again observe a fit for values of t_w around 0.08 ps.

DISCUSSION

The hypothesis that there exist chain mechanisms for transport in membranes is a relatively old one (7, 36, 37) and has been suggested both to account for ion permeability and to account for neutral water permeability. However, it is equally possible to account for the water transport on the basis of a solubility-diffusion model (7) that assumes that monomeric water dissolves in the hydrophobic region of a lipid bilayer and then diffuses across.
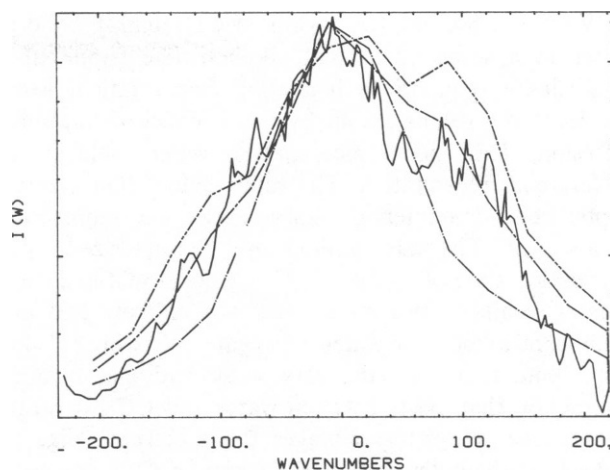

FIGURE 6   The experimental spectrum of *n*-decane (solid curve) compared to the spectra calculated from the J diffusion model for interrupted free rotation. The curves are for various correlation times. 0.1 ps, — - - —; 0.07 ps, — - —; 0.04 ps, —·—.

The high values found for the hydrogen ion permeability (1–5) have intensified the search for a special mechanism of H^+ transport and have brought up the water wire hypothesis in a new form.

The hydrophobic region of a bilayer has often been considered as similar to bulk hydrocarbon.[3] It is known that both the H_2O and H^+ permeabilities of lipids increase dramatically above the main lipid phase transition (6, 7) where the hydrophobic regions are more liquidlike. Therefore liquid bulk hydrocarbons such as we have studied should, if anything, give an overestimate of any special mechanism that aids transport.

---

[3]For example, a comparison of the phase properties of lipid bilayers and those of bulk hydrocarbon may be found in (7, 38).
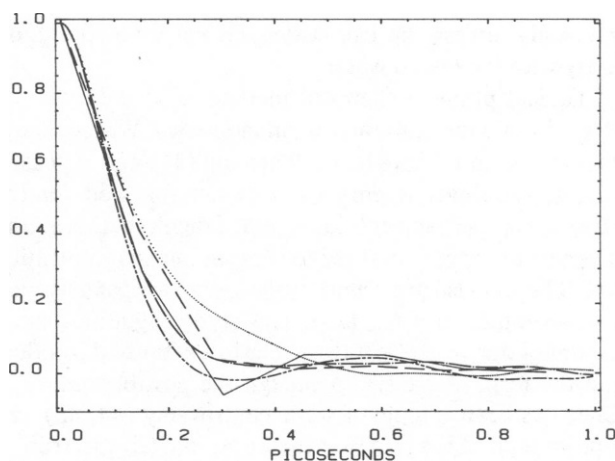

FIGURE 7   The rotational correlation functions of water. The experimental correlation functions obtained by Fourier transforming the spectrum (— and — — represent the transforms of the two halves of the spectrum). The functions calculated from the J-diffusion model with different values of t_w are — - - —, 0.1 ps; — - —, 0.07 ps; —·—, 0.04 ps.

We have observed the asymmetric stretching band of water in a series of normal alkanes. The band has a surprisingly large width indicating free rotation. More precisely, the results are fit by the J model of rotational diffusion. This model pictures the water molecule as undergoing free rotation. The rotational motion is interrupted at random intervals, and a new angular momentum is assumed. The interruptions are characterized by a correlation time of ~0.08 ps. The good fit of the experimental results to this model makes it unlikely that any significant amount of water aggregate exists in the solution. Note that even the very weak hydrogen-bonding interaction that exists between water and CCl_4 or water and alkene completely changes the spectrum (Fig. 5). Indeed, we have tried to fit the water in CCl_4 spectrum using the J-model without success (Conrad, M. P., and H. L. Strauss, manuscript submitted for publication; 27). Other workers have also been unable to fit the spectrum in this way (Leickman, J. C., and J. Soussen-Jacob, unpublished results). Furthermore, the state of water in an alkane is just the same as that of similar solutes such as NH_3. We have found that NH_3 in n-decane shows vibrational bands which also fit the J model with $t_\omega = 0.1$ ps (Conrad, M. P., and H. L. Strauss, manuscript submitted for publication; 27). A variety of other studies have given similar correlation times for the rotation of both HF and water in cyclohexane (39, 40).

The experimental spectra we observe are noisy due to a variety of experimental difficulties. This leads to the question of whether a second bound water spectrum could be hidden under the spectrum due to free water. The bound water spectrum would have a low-frequency band at ~3,400 cm^{-1} that is as intense as the higher-frequency band. We ought to be able to observe such a lower frequency band if any bound water were present. We emphasize that even if a band could be hidden under our noisy spectrum, the free band we do observe would change drastically, unless the free water did not interact significantly with the bound water.

Classical physical chemical methods also indicate that water in alkane solvents is monomeric. Water obeys Henry's law in CCl_4 (41), cyclohexane (41) and n-hexane (42). Interestingly, Henry's law is not followed for the water in complex solvents such as 1,2 dichloroethane, and so dimers may occur in these solvents at high concentration (41). There is also one report of the spectroscopic detection of water dimers in CCl_4 (43). This study suggests a small amount of dimer, 3.6% of the water in a saturated solution, is in the form of dimers. Although the possible nature of dimer has been a topic of some controversy (44, 45), the important point is again that the water in CCl_4 spectrum is very different than the one we observe for water in alkanes.

Of course, our studies only address the state of water in the hydrophobic interior of a lipid membrane. There is direct evidence that the water associated with a lipid is concentrated in the head group layer and leaves the hydrocarbon region relatively water free. This evidence comes from x-ray, neutron diffraction, and dielectric and related studies. Neutron diffraction shows the water concentrated in the head group region with a nearly water-free hydrocarbon layer that decreases in thickness due to the tilting of the alkyl chains as the water content is increased (46). X-ray measurements combined with dielectric constant measurements provide estimates of the thickness of the hydrocarbon and of the head group water layers (47, 48). For hydrated bacterial phosphatidyl ethanolamine bilayers, for example, the hydrocarbon layer is ~28 Å thick and the head group water is ~25 Å thick (48). Detailed results are available for the effect of cholesterol and n-alkanes additives on these numbers (47, 48).

The solubility of water in n-alkanes goes up with temperature and is expected to be very much greater in liquid alkanes than in the crystalline ones.[4] We have, therefore, examined the state of water in the phase in which its concentration is at maximum. Of course, the hydrocarbon in a lipid is not the same as in the neat liquid. The hydrocarbon in a lipid is closer to a liquid crystal and so, in some respects, intermediate in state between the liquid and the crystal. The solubility of water in such a phase should be lower than that in the liquid. The hydrocarbon in a lipid is bound by the head groups, which partly determine the arrangement of the hydrocarbon chains and which can interact strongly with water. Whether the interaction with the head groups can drive the water into the hydrocarbon layer is a question that remains to be answered, but the neutron-diffraction and x-ray results show that not much water can be so driven.

The spectrum of water in the alkenes is dramatically different from that in the alkanes. It is comparable to that of water in CCl_4. There is some evidence that water is indeed associated with CCl_4 and solubility studies suggest that $\chi_{ws}/\chi_w\chi_s = ~1$ where the χ's represent the mole fractions of the water-solvent dimer, of the water and of the solvent respectively (49, 43). We emphasize that the water in an alkene may be similarly complexed to the double bond, but that our spectra show no evidence of water associating with other water molecules. This statement is not as conclusive as the statement that the water in an alkane is free since there exists no detailed theory for the band shape in an alkene.

Finally, we note that a few of the permeability studies of H^+ transport in membranes give rather lower values and this suggests that some of the observed transport may be due to artifacts in the membrane (50, 51). Of course, our studies only address the state of water in the hydrophobic interior of the membrane and offer no evidence on the possible state of water near the head groups. Obtaining such evidence will require future study.

_____

[4]The solubility is three times less in liquid octane at 0°C than at 25°C (33).

REFERENCES

1. Nichols, J. W., and D. W. Deamer. 1980. Net proton-hydroxyl permeability of large unilamellar liposomes measured by an acid-base titration technique. *Proc. Natl. Acad. Sci. USA.* 77:2038–42.
2. Nichols, J. W., M. W. Hill, A. D. Bangham, and D. W. Deamer. 1980. Measurement of net proton-hydroxyl permeability of large unilamellar liposomes with the fluorescent pH probe 9-amino acridine. *Biochim. Biophys. Acta.* 596:393–403.
3. Deamer, D. W., and G. L. Barchfeld. 1984. Proton-hydroxide permeability of liposome membranes. *In* Hydrogen-Ion Transport in Epithelia. J. G. Forte, D. G. Warnock, and F. C. Rector, editors. John Wiley & Sons, Inc., New York. 13–19.
4. Deamer, D. W., and J. W. Nichols. 1983. Proton-hydroxide permeability of liposomes. *Proc. Natl. Acad. Sci. USA.* 80:165–8.
5. Rossignol, M., P. Thomas, and C. Grignon. 1982. Proton permeability of liposomes from natural phospholipid mixtures. *Biochem. Biophys. Acta.* 684:195–9.
6. Elamrani, K., and A. Blume. 1983. Effect of the lipid phase transition on the kinetics of H^+/OH^- diffusion across phosphatidic acid bilayers. *Biochem. Biophys. Acta.* 727:22–30.
7. Fettiplace, R., and D. A. Haydon. 1980. Water permeability of lipid membranes. *Physiol. Rev.* 60:510–49.
8. Pimentel, G. C., and A. L. McClellan. 1960. The Hydrogen Bond. Freeman Publications, San Francisco. 475 pp.
9. Scherer, James R. 1978. The vibrational spectroscopy of water. *Adv. Infrared Raman Spectrosc.* 5:149–216.
10. Mohr, S., W. D. Wilk, and G. M. Barrow. 1965. The association of water with bases and anions in an inert solvent. *J. Am. Chem. Soc.* 87:3048–52.
11. Glew, D. N., and N. S. Rath. 1970. H_2O, HDO, and CH_3OH infrared spectra and correlation with solvent basicity and hydrogen bonding. *Can. J. Chem.* 49:837–856.
12. Burneau, A., and J. Corset. 1972. Spectres d'absorption, entre 1000 et 11000 cm^{-1}, des molécules H_2O, D_2O et HOD libres et en interaction avec des bases, en solutions diluées. *J. Chim. Phys.* 69:142–52.
13. Novak, A. 1974. Hydrogen bonding in solids. *Struct. Bonding.* 18:177–216.
14. Reimers, J. R., and R. O. Watts. 1984. The structure and vibrational spectra of small clusters of water molecules. *Chem. Phys.* 85:83–112.
15. Vernon, M. F., D. J. Krajnovich, H. S. Kwok, J. M. Lisy, Y. R. Shen, and Y. T. Lee, 1982. Infrared vibrational predissociation spectroscopy of water clusters by the crossed laser-molecular beam technique. *J. Chem. Phys.* 77:47–57.
16. David, J. G. 1972. Comment on possible motional effects on the vibrational spectrum of water in the fundamental stretching region. *Spectrochimica Acta.* 28A:977–985.
17. Fox, J. J., and A. E. Martin. 1940. Investigations of infrared spectra: (2.5–7.5μ) absorption of water. *Proc. R. Soc. Lond. B. Biol. Sci.* 174:235–262.
18. Gordon, R. G. 1965. Molecular motion in infrared and Raman spectra. *J. Chem. Phys.* 43:1307–1312.
19. Gordon, R. G. 1968. Correlation functions for molecular motion. *Adv. Mag. Reson.* 3:1–42.
20. Van Woerkam, P. C. M., J. De Bleys, M. De Zwart, and J. C. Leyte. 1974. Intermolecular vibrational relaxation in liquids. *Chem. Phys.* 4:236–248.
21. Leickman, J. C., Y. Guissani, and S. Bratos. 1978. Infrared study of diluted solutions of polyatomic molecules. I. Band shape of fundamentals associated with nondegenerate normal modes. *J. Chem. Phys.* 66:3380–3390.
22. Aguado-Gómez, M., and J. C. Leickman. 1985. Rotational relaxation of asymmetric-top molecules: Vectorial correlation functions. In press.
23. Steele, W. A. 1976. The rotation of molecules in dense phases. *Adv. Chem. Phys.* 34:1–104.
24. Steele, W. A. 1979. Rotational spectral band shapes in dense fluids. *In* Vibrational Spectroscopy of Molecular Liquids and Solids. S. Bratos and R. M. Pick, editors. *NATO Advanced Study Institute Series B.* 56:61–89.
25. Hu, C.-M., and R. Zwanzig. 1974. Rotational friction coefficients for spheroids with the slipping boundary condition. *J. Chem. Phys.* 60:4354–4357.
26. Fredkin, D. R., A. Komornicki, S. R. White, and K. R. Wilson. 1983. Ab initio infrared and Raman spectra. *J. Chem. Phys.* 78:7077–7092.
27. Conrad, M. P. 1984. Infrared studies of low interaction binary systems. Ph.D. dissertation, University of California, Berkeley.
28. Gordon, R. G. 1966. On the rotational diffusion of molecules. *J. Chem. Phys.* 44:1830–1836.
29. McClung, R. E. D. 1972. Rotational diffusion of symmetric top molecules in liquids. *J. Chem. Phys.* 57:5478–5491.
30. St. Pierre, A. G., and W. A. Steele. 1972. Collisional effects upon rotational correlations of symmetric top molecules. *J. Chem. Phys.* 57:4638–4646.
31. Leickman, J. C., and Y. Guissani. 1981. On extended diffusion models for asymmetric top molecules in liquids. *Mol. Phys.* 42:1105–1120.
32. Leickman, J. C. 1980. Rotational correlation functions for asymmetric-top molecules in extended-diffusion models. *Phys. Rev. A.* 22:2286–2289.
33. Polak, J., and C. Y. Lu. 1973. Mutual solubilities of hydrocarbons and water at 0 and 25°C. *Can. J. Chem.* 51:4018–4023.
34. Jacob, J., J. Leclerc, and J. Vincent-Geisse. 1969. Etude des mouvements moléculaires en milieu liquide au moyen de la largeur des bandes de vibration dans l'infrarouge. *J. Chim. Phys.* 66:970–977.
35. Turrell, G. 1974. Solute-solvent interactions in liquids. *Chem. Phys.* 3:433–481.
36. Hodgkin, A. L., and R. D. Keynes. 1955. The K^+ permeability of a giant nerve fiber. *J. Phys. London.* 128:61–88.
37. Huang, C., and T. E. Thompson. Properties of lipid bilayer membranes separating two aqueous phases: water permeability. *J. Mol. Biol.* 15:539–554.
38. Nagle, J. F. 1982. Theory of the main lipid bilayer phase transition. *Annu. Rev. Phys. Chem.* 31:157–195.
39. Rothschild, W. G. 1968. Rotational motion in solution: hydrogen halides in cyclohexane. *J. Chem. Phys.* 49:2250–2257.
40. Evans, M. W. 1976. Reorientation of water molecules free from hydrogen bonding. *J. Chem. Soc. Far. Trans. II.* 72:2138–2146.
41. Christian, S. D., H. E. Affsprung, and J. R. Johnson. 1963. The molecular complexity of water as a solute in benzene. *J. Chem. Soc.* 1896–1898.
42. Roddy, J. W., and C. F. Coleman. 1968. Solubility of water in hydrocarbons as a function of water activity. *Talanta.* 15:1281–1286.
43. Magnusson, L. B. 1970. Infrared absorbance by water dimer in carbon tetrachloride solution. *J. Phys. Chem.* 74:4221–4228.
44. Kollman, P. A., and A. D. Buckingham. 1971. The structure of the water dimer. *Mol. Phys.* 3:567–70.

45. Magnusson, L. B. 1971. The structure of water dimer. *Mol. Phys.* 3:571.

46. Worcester, D. L. 1976. Neutron beam studies of biological membranes and membrane components. *Biol. Membr.* 3:1–46.

47. Simon, S. A., McIntosh, T. J., and Latorre, R. 1982. Influence of cholesterol on water penetration into bilayers. *Science (Wash. DC).* 216:65–6.

48. McIntosh, T. J., Simon, S. A., and MacDonald, R. C. 1980. The organization of n-alkanes in lipid bilayers. *Biochim. Biophys. Acta.* 597:445–463.

49. Kirchnerova, J., and G. C. B. Cave. 1976. The solubility of water in low dielectric solvents. *Can. J. Chem.* 54:3909–3916.

50. Gutknect, J. 1984. Proton/hydroxide permeabilities of lipid bilayer membranes. *In* Hydrogen Ion Transport in Epithelia. J. G. Forte, D. G. Warnock, and F. C. Rector, Jr., editors. John Wiley & Sons, Inc., New York. 3–12.

51. Cafiso, D. C., and W. L. Hubbell. 1983. Electrogenic H^+/OH^- movement across phospholipid vesicles measured by spin-labeled hydrophobic ions. *Biophys. J.* 44:49–57.