IR Study on the Relaxation of the Phosphate Group of 5′-dCMP in ²H₂O and H₂O Solutions

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The analysis of the IR $\nu_s(PO_3{}^{2-})$ band shape of disodium deoxycytidine 5′-monophosphate, 5′-dCMP, in ²H₂O and H₂O solutions at different concentrations, 0.005−0.90 mol dm⁻³, and temperatures, 7−70 °C, provides information on the relaxation and dynamics of the phosphate group. Self-association of this mononucleotide is detected at concentrations higher than ∼0.28 mol dm⁻³. The relaxation, dominantly vibrational, is ∼0.31 ps faster in H₂O than in ²H₂O and ∼70 fs faster than for the mononucleotide 5′-CMP. Kubo−Rothschild's and Oxtoby's models for vibrational dephasing describe the IR time-correlation functions up to ∼3 ps. The stretched exponential model takes values for the dispersion parameter, α, close to 1, i.e., approaches the Markovian Kubo−Rothschild's model, and random fluctuations prevail at concentrations ≤0.28 mol dm⁻³. The modulation parameters resulting from these models indicate that the $\nu_s(PO_3{}^{2-})$ oscillator is under a fast modulation regime at low concentration but is slowing to an intermediate modulation regime as concentration approaches ∼0.3 mol dm⁻³.

Introduction

Vibrational relaxation[1−6] plays a crucial role in many aspects of chemistry, physics, and biology, e.g., reaction dynamics,[7−11] thermal chemistry,[12,13] electron transfer,[14] photochemistry,[15−17] excimer formation,[18] and photobiological processes[19−22] such as vision and photosynthesis. The dynamics and vibrational relaxation processes of biomolecules in aqueous solutions have motivated several studies.[20,23−27] Nucleosides and nucleotides are particularly attractive molecules.[28] The charged phosphate group in nucleotides and nucleic acids is responsible for interactions with counterions and important features of the reactivity of these molecules; this has encouraged different spectroscopic studies on the phosphodiester group.[29−32] For this reason, the present work is devoted to obtaining information on the dynamics and relaxation of the $PO_3{}^{2-}$ group of a deoxymononucleotide in aqueous solution, i.e., on how this group relaxes its vibrational energy in a milieu approaching physiological conditions. The symmetric stretching mode of the phosphate group, $\nu_s(PO_3{}^{2-})$, of mononucleotides is very localized. It gives an intense band in IR spectroscopy at ∼980 cm⁻¹ in aqueous solutions but unfortunately is very weak in the isotropic Raman spectra, I_{iso}, and especially at concentrations below ∼0.3 mol dm⁻³ used to avoid mononucleotide self-association.[33−39] Thus, the profile of this IR band may be used as a probe to provide information on the relaxation and dynamics of the phosphate group.

For large molecules, vibrational relaxation dominates the time-correlation function, CF.[40,41] Additionally in aqueous solutions Coulombic and dipolar forces[42] promote vibrational relaxation of anions,[43,44] and the hydrogen bonding of the water[44−48] inhibits anion reorientation. Early studies[33,34] on the relaxation of the $\nu_s(PO_3{}^{2-})$ mode of the nucleotide 5′-GMP in

²H₂O showed that the vibrational relaxation time, τ_v, obtained from the Raman I_{iso} spectra takes values similar to those obtained for the infrared relaxation time, τ_{ir}. All these arguments indicate that the infrared CF for this mode of nucleotides in aqueous solutions is basically of vibrational type.[36,37] Among the different vibrational relaxation pathways, vibrational dephasing[49−52] appears as the most important for polyatomic anions in water.[53−55] Different stochastic theories have been developed for vibrational dephasing.[51,56−61] The applications of these models may give information on the dynamics of the phosphate group of nucleotides and in particular about the modulation of the $\nu_s(PO_3{}^{2-})$ oscillator in water solutions.

Previous results on the mononucleotide cytidine 5′-monophosphate, 5′-CMP, were obtained,[36,37] and now we are interested in studying the possible differences in the dynamics of the molecule deoxycytidine 5′-monophosphate, 5′-dCMP. Does the absence of the O2′H group in the ribose moiety affect the dynamics of the phosphate group? In other words, are there significant differences in the dynamics of the phosphate group of mononucleotides and deoxymononucleotides? Other questions are also raised. Deuterium oxide is a commonly used solvent in IR spectroscopy of biomolecules because it does not absorb IR radiation in relevant spectral regions but is the behavior of these molecules similar in ²H₂O and H₂O? What type of events modulate the $\nu_s(PO_3{}^{2-})$ oscillator in aqueous solution? Could we speak about a certain local order surrounding the phosphate group?

Crystallographic data on 5′-dCMP·Na₂ heptahydrate[62] and monohydrate[63] indicate that the compound is orthorhombic, space group $P2_12_12_1$, showing an unusual gauche−trans conformation around the C4′−C5′ bond, and accompanied, however, by a normal C3′-exo sugar pucker. In relation to the conformational state of the 5′-ribonucleotides or 5′-deoxyribonucleotides, it was clearly established by ¹H NMR studies[64,65] that the conformational preferences of the phosphate backbone are maintained when going from the crystal to the aqueous

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solution. Some other conformational studies of 5′-dCMP by ^{13}C chemical shifts and [13]C−[31]P coupling constants as a function of pH in aqueous solution[66] point to a preference (70−80%) for the trans orientation of the P5′−O5′−C5′−C4′ fragment, with little dependence on the nature of the sugar. Consequently, the effect of conformational changes on the phosphate oscillator may be overlooked.

Experimental Section

Materials and Instrumentation. Disodium deoxycytidine 5′-monophosphate, 5′-dCMP, from Sigma Chemical Co., purity 98%, was desiccated, and 0.005−0.9 mol dm^{−3} solutions in distilled water and deuterium oxide from Scharlau, deuteration degree >99.8% checked by its IR spectrum, were prepared. Their IR spectra were recorded using a Specac Ltd. vacuum-tight heatable cell with BaF_2 windows and a 48-μm path length, measured from its interference fringes. A vacuum system for circulating water, thermostatic control, and temperature monitoring designed by the authors[36] was used. The temperature stability inside the cell window was ±0.05 °C.

The spectra were recorded between 7 and 70 °C, in a vacuum, pressure ≤ 133.3 Pa, using a Bomem DA3 FTIR spectrometer with a high-detectivity MCT detector. A nominal resolution of 2 cm^{−1} was selected, the corresponding effective spectral resolution after Hamming's apodization being $s = 1.77$ cm[−1]. The spectra were recorded with a digital resolution of 0.964 cm^{−1}. To obtain a high signal−noise ratio, S/N, 1000 inter-ferograms were co-added for each spectrum of 0.9−0.3 mol dm^{−3} solutions, 2000 for 0.3−0.2 mol dm^{−3}, 4000 for 0.2−0.07 mol dm^{−3}, 6000 for 0.07−0.04 mol dm^{−3}, 7500 for 0.04−0.01 mol dm^{−3}, and 15000 for 0.01−0.005 mol dm^{−3}, this last concentration being the detection limit of the spectrometer.

Data Treatment. The lower absorbance points of the $\nu_s(PO_3^{2-})$ band were used for a linear baseline correction, ∼1015−912 cm^{−1}. This integration range is ∼8.6 full widths at half height, fwhh. Peak position and fwhh of the band were determined using GRAMS/AI 7.00 software.[67] The ratio of the spectral resolution to fwhh of the band was below 0.15. Despite this low ratio, the instrumental line-shape distortion on the observed band was corrected applying Dijkman's equation[68]

$$\Delta\nu_{1/2}^t = \Delta\nu_{1/2}^a[1 - 2(s/\Delta\nu_{1/2}^a)^2]^{1/2} \tag{1}$$

where $\Delta\nu_{1/2}^t$ is the true fwhh and $\Delta\nu_{1/2}^a$ the observed or apparent fwhh. Measurements of the band moments, CFs, as well as fitting to Kubo−Rothschild's,[51,56−58] Oxtoby's,[59] and stretched exponential[60,61,69,70] models were performed with programs developed by the authors. The Fourier transformation was computed by direct numerical integration of the transform integral at each time point. The spectral range considered for second moment and CF evaluation was in the order of 7 times the fwhh of the band. This spectral range involves an average time resolution for the CFs of 0.40[71] or 0.6 ps.[72] According to the spectral resolution used, 1.77 cm^{−1}, the obtained CFs are reliable[71] up to 9 ps. Nevertheless, the use of a finite spectral range of integration produces numerical fluctuations in the resulting CFs for times larger than 3 ps. This effect is particularly important for the spectra in H_2O at low concentration of the nucleotide. Hence the relaxation time, τ_{ir}, was evaluated by integration of the CF over the time range from 0 to 3 ps, where the CF approaches zero. The modulation time, τ_c, results from the fitting of Kubo−Rothschild's and Oxtoby's models to the experimental CF. Regarding the dielectric field effect on the CF[73−75] and according to the results of Ikawa et al.,[76] this effect
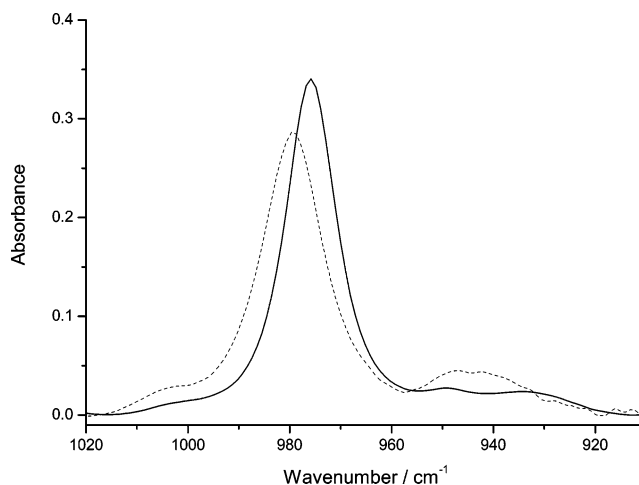

Figure 1. IR $\nu_s(PO_3^{2-})$ band of 5′-dCMP 0.1 mol dm^{−3} at 26 °C in [2]H_2O (solid line) and in H_2O (dashed line). The spectra have been baseline corrected.
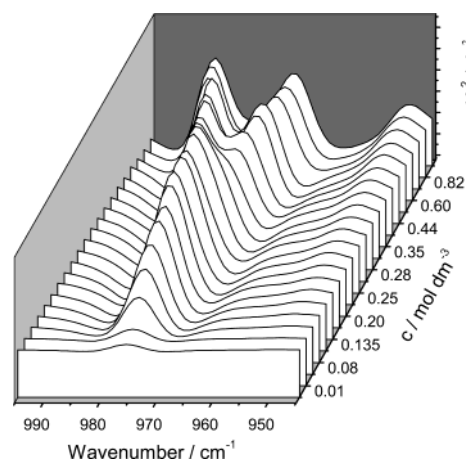

Figure 2. Variation of the second derivative (sign changed) of the $\nu_s(PO_3^{2-})$ band of 5′-dCMP in [2]H_2O with concentration (26 °C). The spectra have been baseline corrected.

could be neglected if the product of the concentration of the solute molecule in mol dm^{−3} and the extinction coefficient in mol^{−1} dm[3] cm^{−1} of the absorption band concerned is smaller than ∼2000 cm^{−1}. This effect would be important for wide and intense bands in the low-frequency range of the spectrum (far IR). For the $\nu_s(PO_3^{2-})$ band of 5′-dCMP in [2]H_2O and H_2O using very small cell path length, 48 μm, low concentrations, 0.005−0.28 mol dm^{−3}, and a band that appears at ∼980 cm^{−1}, an insignificant effect would be expected, the resulting values of the indicated product being less than 180 cm^{−1}. Considering the isotope shifts,[74] phosphorus has only one nuclide, and the isotopic abundance of [16]O is 99.762% ([18]O 0.200% and [17]O 0.038%, respectively),[77] then frequency shifts due to isotopes are also negligible.

Results and Discussion

Band Parameters. Very weak absorptions appear in the low-wavenumber wing of the $\nu_s(PO_3^{2-})$ band between 950 and 930 cm^{−1}, see Figure 1. Moreover, the second derivative of the band, Figures 2 and 3, show new components in the low-wavenumber side of the band at concentrations higher than ∼0.3 mol dm^{−3}. The appearance of different components in the bands of mononucleotides in aqueous solution has been related to self-association of these molecules forming aggregates at high concentration.[33,36,38,39] For these reasons, only the high-wavenumber
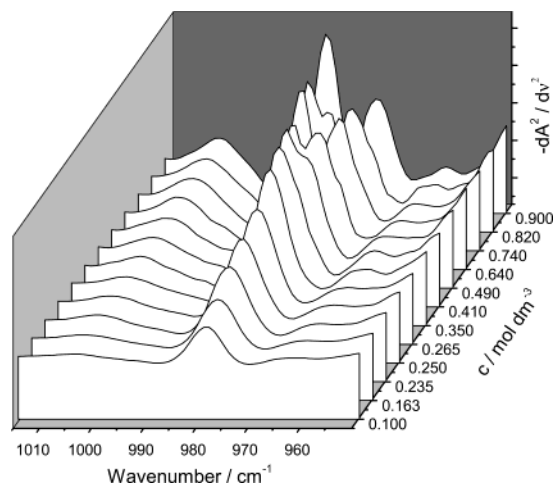
Figure 3. Variation of the second derivative (sign changed) of the $\nu_s(PO_3^{2-})$ band of 5′-dCMP in H_2O with concentration (26 °C). The spectra have been baseline corrected.
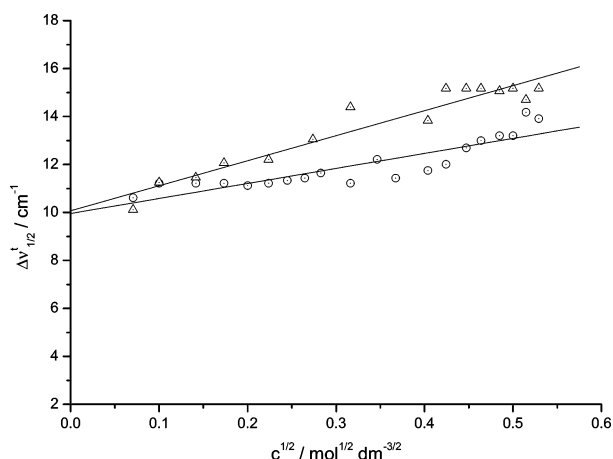

Figure 4. True fwhh of the $\nu_s(PO_3^{2-})$ band vs square root of 5′-dCMP concentration (26 °C) in (△) H_2O and (⊙) ^2H_2O. Linear regressions, $\Delta\nu^t_{1/2} = A + Bc^{1/2}$, in H_2O $A = 10.07$, sd(A) = 0.31, $B = 10.44$, sd(B) = 0.83, sd($\Delta\nu^t_{1/2}$) = 0.50, $N = 15$; and in ^2H_2O $A = 9.96$, sd(A) = 0.27, $B = 6.26$, sd(B) = 0.78, sd($\Delta\nu^t_{1/2}$) = 0.49, $N = 20$.

side of the band was considered for the calculations in order to minimize possible line broadening due to the formation of aggregates; the other side was generated by symmetry.[78–84] Similarly, the band-shape analysis was applied to concentrations lower than 0.3 mol dm^{-3}.

The wavenumber of the band maximum shifts only 1 cm^{-1} on increasing concentration, 975.8–976.8 cm^{-1} (0.005–0.280 mol dm^{-3}) in ^2H_2O and 978.7–979.7 cm^{-1} (0.005–0.265 mol dm^{-3}) in H_2O. The Cauchy-Gauss index, CG, of the band shape evaluated from the behavior of the truncated second band moment, M_2, with the integration interval[85] is between 0.50 and 0.68, i.e., inhomogeneous and homogeneous contributions to the fwhh should be expected.[86] Similar values were obtained for 5′-CMP.[36] The second band moment, M_2, shows a very small increase with concentration, e.g., from 85 to 89 cm^{-2} at 0.1 and 0.28 mol dm^{-3} in ^2H_2O, respectively. The observed increase of the true fwhh with concentration, Figure 4, suggests that the main contribution to the IR CF is of vibrational type.[87,88] Linear approximations for the increase of the true fwhh with $(c)^{1/2}$ give deviations smaller than the spectral resolution, Figure 4. The extrapolated values of the true fwhh as $(c)^{1/2} \rightarrow 0$ are 9.96 and 10.07 cm^{-1} in ^2H_2O and H_2O, respectively. Since the average value of the fwhh is ~12 cm^{-1}, vibrational dephasing may be

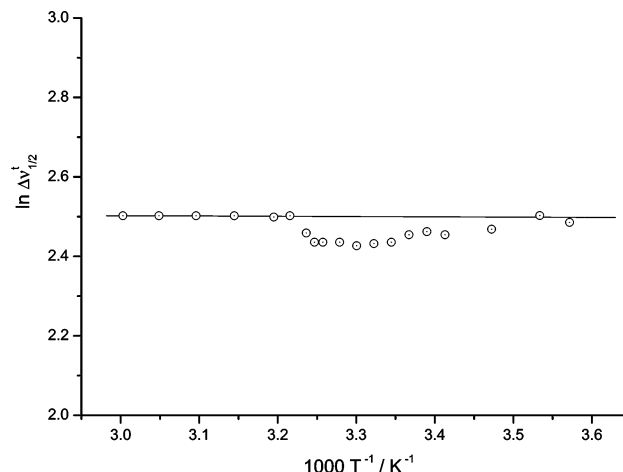**Figure 5.** Natural logarithm of the true fwhh of the $\nu_s(PO_3^{2-})$ band of 5′-dCMP in ^2H_2O vs the inverse of temperature. Linear regression, $\ln \Delta\nu^t_{1/2} = A + B/T$, $A = 2.525$, sd(A) = 0.023, $B = -7.5$, sd(B) = 7.3, sd($\ln \Delta\nu^t_{1/2}$) = 0.0014, $N = 6$.

considered the most important pathway for vibrational relaxation.[89] The change of solvent from ^2H_2O to H_2O tends to produce a band broadening. This was also observed for 5′-CMP,[37] the C–H stretching of different organic molecules,[90] the antisymmetric stretching mode of azide anion,[91] and the stretching of the cyanide anion.[92] This broadening tends to increase with concentration, Figure 4. The fwhhs obtained for 5′-dCMP and the slopes of their increase with $(c)^{1/2}$ are higher than those for 5′-CMP,[37] indicating a higher efficiency of the relaxation mechanisms for 5′-dCMP.

The effect of temperature on the band shape was studied at a concentration of 0.2 mol dm^{-3}, i.e., at a concentration at which only one band component is observed and spectra with a high S/N ratio may easily be recorded. The wavenumber of the band maximum decreases only 2 cm^{-1} between 7 and 60 °C in ^2H_2O. The integrated intensity decreases also from 9.77 to 7.56 absorbance cm^{-1}. A very small increase of the fwhh is observed with temperature. Rakov's method[6,93] assumes the following temperature dependence of the IR fwhh

$$(\Delta\nu_{1/2})_{ir} = (\Delta\nu_{1/2})_{vib} + Ae^{-U/kT} \qquad (2)$$

where $(\Delta\nu_{1/2})_{vib}$ is the vibrational width and the rotational dependence for the rotational width is of Arrhenius type. For the $\nu_s(PO_3^{2-})$ band, a plateau is observed above 38 °C, Figure 5, and small deviations are apparent below this temperature. The very small decrease of $\ln \Delta\nu^t_{1/2}$ vs $1/T$ suggests that the contribution of the exponential term in eq 2 is small. Instead a constant factor seems to be dominant, which is to be expected according to eq 2 for a dominant vibrational relaxation mechanism. The use of an "activation energy" to describe a possible temperature dependence of $(\Delta\nu_{1/2})_{vib}$ would be unphysical considering Figure 5.

Correlation Functions and Relaxation Times. The IR CFs, $G_{ir}(t)$, of the $\nu_s(PO_3^{2-})$ mode have been determined from the recorded FTIR spectra, Figure 6. The experimental CFs are between the corresponding Gaussian and Cauchy's profiles, in agreement with the values for the CG index indicated previously. They have an initial Gaussian decay, i.e., a parabolic profile of its natural logarithm, below 1 ps, Figure 7, and a subsequent slow, exponential-like decay, i.e., a straight line, in Figure 7. The decay of $G_{ir}(t)$ becomes faster when concentration increases, Figure 6. This was also observed for other oscillators.[33,37,81,88] The infrared relaxation time, τ_{ir}, obtained by integrating $G_{ir}(t)$
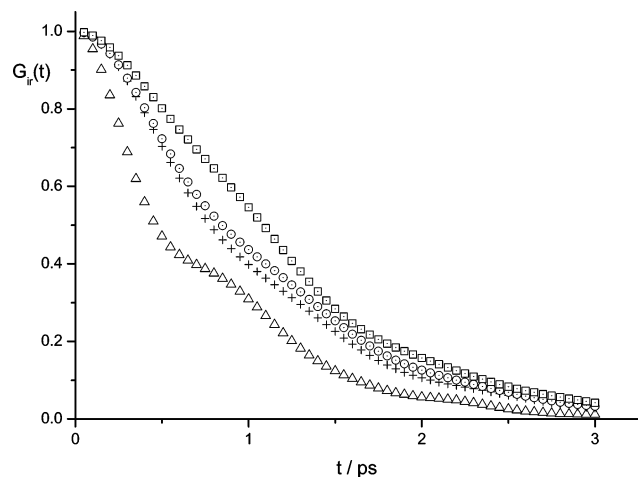
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Figure 6. Infrared correlation function for the $\nu_s(PO_3^{2-})$ band of 5′-dCMP (26 °C) in ^2H_2O: (□) 0.005 mol dm^{-3}, (⊙) 0.1 mol dm^{-3}, (+) 5′-dCMP 0.28 mol dm^{-3}, and H_2O (△) 0.200 mol dm^{-3}.

Figure 8. Infrared correlation time, τ_{ir}, obtained by the integration of the infrared correlation function of the $\nu_s(PO_3^{2-})$ band vs nucleotide concentration at 26 °C for (●) 5′-CMP in ^2H_2O, (⊙) 5′-dCMP in ^2H_2O, and (△) 5′-dCMP in H_2O.
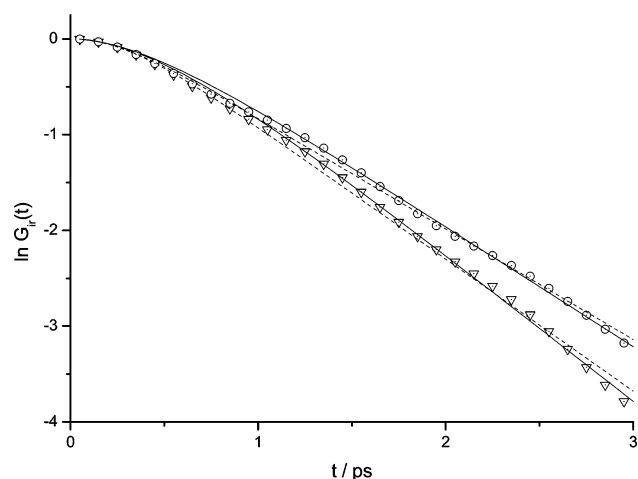

Figure 7. Natural logarithm of $G_{ir}(t)$ for the $\nu_s(PO_3^{2-})$ band of 5′-dCMP 0.2 mol dm^{-3} in ^2H_2O at 7 °C (⊙) and 60 °C (▽). Kubo's (solid lines) and Oxtoby's (dashed lines) fitted equations. Kubo's and stretched exponential functions coincide (α ~1).

Figure 9. Variation of τ_{ir} with temperature for 5′-dCMP 0.2 mol dm^{-3} in ^2H_2O. Linear regression, $\tau_{ir} = A + Bt$, $A = 1.0431$, sd(A) = 0.0068, $B = -0.00108$, sd(B) = 1.87×10^{-4}, sd(τ_{ir}) = 0.0125, $N = 20$.

also shows this trend, Figure 8. The truncation of the integration interval is more severe for the spectra in H_2O. This produces oscillations of the CFs that make the evaluation of τ_{ir} below 0.1 mol dm^{-3} difficult. Between 0.1 and 0.28 mol dm^{-3}, the relaxation may be considered ~0.31 ps faster in H_2O than in ^2H_2O. This decrease in the relaxation time in H_2O was also observed in 5′-CMP,[37] azide anion,[91] and cyanide anion.[92] Several causes have been proposed[91,92] to interpret this isotopic effect as well as the associated band broadening previously mentioned; diverse couplings with two-phonon processes of the solvent modes[92] and different solvent-coupling strength.[91,94] The shift of the $v = 0 \rightarrow v = 1$ transition of $\nu_s(PO_3^{2-})$ between H_2O and ^2H_2O is only 3 cm^{-1}. Since this solvent shift is related to quadratic terms of the solvent−solute potential of the $\nu_s(PO_3^{2-})$ mode,[43] no relevant differences in solvent coupling strength would be expected. Direct resonance interactions of the $\nu_s(PO_3^{2-})$ mode with the vibrational modes of the solvent do not seem evident. The vibrational frequencies of the isolated solvent molecules H_2O (^2H_2O) are relatively far from the $\nu_s(PO_3^{2-})$ frequency, 1595 (1179), 3651 (2666), and 3756 (2789) cm^{-1} for bending, symmetric stretching, and antisymmetric stretching modes, respectively.[94] Nevertheless, different type of coupling between the $\nu_s(PO_3^{2-})$ mode and multiphonon processes of the bath in the two solvents would involve differences
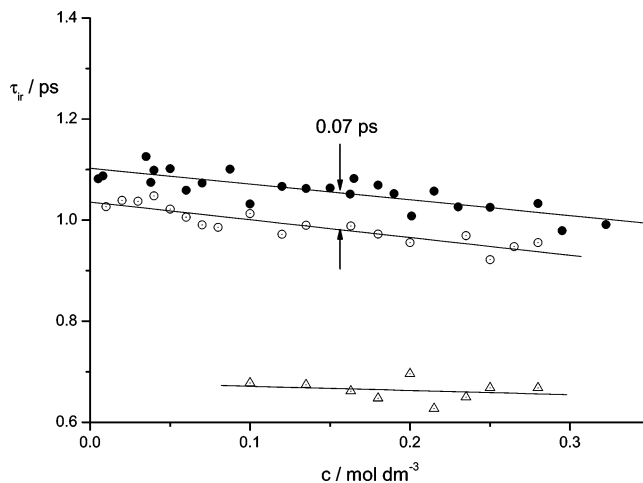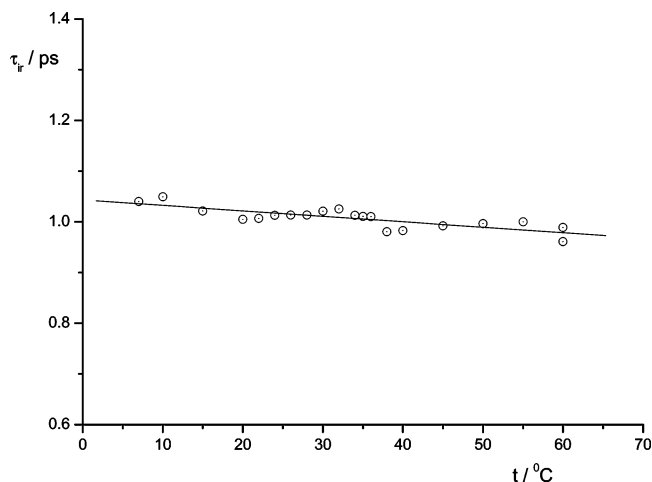
in the relaxation time.[95,96] The bath-bending mode and bath rotational libration modes would participate in this interaction.[23,92,97−99] A comparison with the mononucleotide 5′-CMP in ^2H_2O indicates that the $\nu_s(PO_3^{2-})$ oscillator relaxes about 70 fs faster in 5′-dCMP.[36] The sugar moiety of 5′-dCMP, d-ribose, does not have the O2′H group of the ribose in 5′-CMP, and the resulting decrease of H-bond interactions with the solvent and the corresponding increase of molecular mobility could give rise to a more efficient relaxation.

As was expected from the behavior of the fwhh with temperature, Figure 5, a very small negative slope is obtained in the dependence of the infrared relaxation time, τ_{ir}, vs temperature, Figure 9.

Models for Vibrational Dephasing. The main processes leading to vibrational relaxation of molecules and ions in the liquid phase are[52,86] energy relaxation, resonant energy exchange, and vibrational dephasing. The principal channel for energy relaxation is intraionic repartition of energy between vibrational modes. However, the large frequency gap (≥ 110 cm^{-1}) between the $\nu_s(PO_3^{2-})$ mode and the closest mode makes the vibrational energy transfer to other vibrational degrees of freedom rather unlikely. Resonant energy exchange is important in pure liquids. However, it should be irrelevant in diluted solutions in H_2O and ^2H_2O, with normal vibrations far from the $\nu_s(PO_3^{2-})$

vibration of nucleotides, considering the small value of the normal coordinate derivative of the P−O bond dipole moment.[53,100] The behavior of the fwhh with concentration, Figure 4, the dilution of mononucleotide solutions, and the results for other anions in aqueous solutions[53,80,101−103] indicate that vibrational dephasing should be the most efficient mechanism in the relaxation of the $\nu_s(PO_3^{2-})$ mode. Its contribution to the average fwhh, $\sim$12 cm^{-1}, estimated as stated previously, Figure 4, is $\sim$10 cm^{-1}. Thus vibration phase relaxation should be regarded as the main broadening process.[104−106]

Three main types of modulation events are responsible for vibrational dephasing, viz., Gaussian−Markovian,[51,56−58] non-Markovian,[60,61,69,70,107,108] and purely discrete Markovian.[109−112] The simple Gaussian−Markovian theory for vibrational dephasing has proven its adequacy to describe CFs for the symmetric stretching mode, $\nu_s(PO_3^{2-})$, of different nucleotides in aqueous solution.[34,36,37,113] Nevertheless, in aqueous solutions the hydrogen bonding of the water inhibits anion reorientation, and the simple Gaussian−Markovian theory for vibrational dephasing may not be appropriate,[80] inhomogeneous broadening could be important in these systems. A non-Markovian modeling function, the stretched exponential, has been proposed.[60,61,69,70,107,108] The application of this model could reveal if some kind of local order[61,114] is around the phosphate group of nucleotides in aqueous solutions. The purely discrete Markovian models would be more appropriate for molten salts[115] and quasispherical anions,[116] but this does not apply in this case.

Different models have been developed for vibrational dephasing based on the simple Gaussian−Markovian theory. According to Kubo−Rothschild's model,[51,56−58] the logarithm of the vibrational CF is expressed by

$$\ln G_v(t) = -\langle\omega^2\rangle\{\tau_c^2[\exp(-t/\tau_c) - 1] + \tau_c t\} \qquad (3)$$

where τ_c is the modulation time, which may be roughly considered as the time between collisions in the solvent, and $\langle\omega^2\rangle = 4\pi c^2 M_2$ is the mean-squared frequency displacement about the band center caused by the different environments "seen" by the oscillator; its magnitude is determined by the strength of the coupling between the oscillator and its environment; hence, it is called modulation amplitude. Oxtoby[59] proposed another equation for this CF

$$\ln G_v(t) = -\langle\omega^2\rangle\tau_c^2 \ln\cosh(-t/\tau_c) \qquad (4)$$

Both equations behave at long times as straight lines with slopes $-\langle\omega^2\rangle\tau_c$. These equations have been fitted to the experimental CFs, $G_{ir}(t)$, obtained from the real part of the Fourier transform of the infrared band profiles, Figure 7. The observed values of M_2 have been used to evaluate $\langle\omega^2\rangle$. The modulation time, τ_c, and the quantity $\langle\omega^2\rangle^{1/2}\tau_c$, corresponding to the best fit to each model, were evaluated. Both models fit $G_{ir}(t)$ well, but Kubo−Rothschild's function fits $G_{ir}(t)$ better than Oxtoby's function from 1 ps, whereas at very short times, <1 ps, Oxtoby's function is somewhat better, Figure 7. The modulation time and modulation amplitude tend to increase slightly with concentration, Figures 10 and 11. A similar trend was observed for 5′-CMP.[36,37] According to the results for τ_{ir}, the modulation time is 0.20−0.30 ps faster in H_2O than in ^2H_2O, Figure 10. The quantity $\langle\omega^2\rangle^{1/2}\tau_c$ is in all cases less than unity, Figure 11 and Table 1, indicating that the collision frequency, τ_c^{-1}, is larger than the root-mean-square frequency displacement. Then the modulation is considered to be fast, and slowing to an intermediate modulation regime as concentration approaches $\sim$0.3 mol dm^{-3}, i.e., nucleotide self-association, begins to be
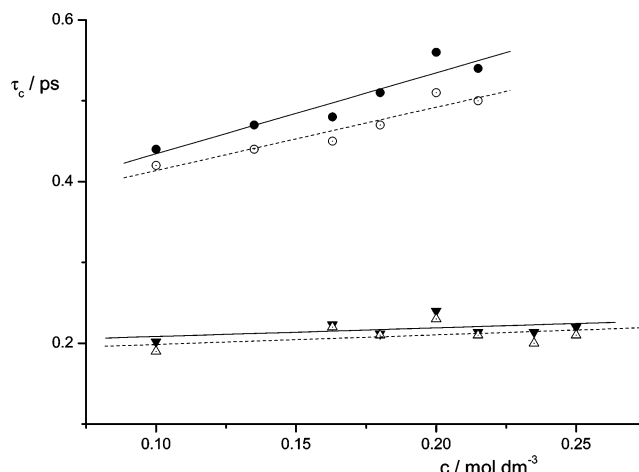

Figure 10. Modulation time, τ_c, for the $\nu_s(PO_3^{2-})$ oscillator of 5′-dCMP vs concentration in ^2H_2O (26 °C) obtained from Kubo's (●, solid line) and Oxtoby's (☉, dashed line) models. Results in H_2O from Kubo's (▼, solid line) and Oxtoby's (△, dashed line) models.

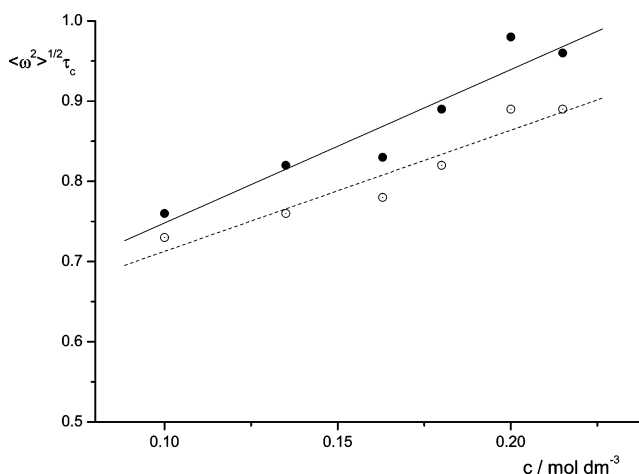**Figure 11.** Modulation parameter $\langle\omega^2\rangle^{1/2}\tau_c$ for the $\nu_s(PO_3^{2-})$ oscillator of 5′-dCMP vs concentration in ^2H_2O (26 °C) obtained from Kubo's (●, solid line) and Oxtoby's (☉, dashed line) models. Linear regressions, $\langle\omega^2\rangle^{1/2}\tau_c = A + Bc$, for Kubo's model: $A = 0.56$, sd(A) = 0.05, $B = 1.91$, sd(B) = 0.31, sd($\langle\omega^2\rangle^{1/2}\tau_c$) = 0.029; for Oxtoby's model: $A = 0.56$, sd(A) = 0.040, $B = 1.51$, sd(B) = 0.23, sd($\langle\omega^2\rangle^{1/2}\tau_c$) = 0.022.

important. In other words, homogeneous broadening[86] produced by the solvent, probably librational modes of water,[97] is significant at low concentration, but inhomogeneous broadening[86] produced by the formation of long-lived static structures or complexes with the oscillator (such as aggregates by nucleotide self-association and H bonds with water molecules) begin to be important at $\sim$0.3 mol dm^{-3}. The modulation dynamics of the $\nu_s(PO_3^{2-})$ mode in aqueous solution results from an interplay between short-lived repulsive forces and long-lived attractive forces. The first forces prevail at low concentration, and the others become relevant from $\sim$0.3 mol dm^{-3}. At 0.2 mol dm^{-3}, a very small increase of τ_c and $\langle\omega^2\rangle^{1/2}\tau_c$ with temperature is observed, Table 1. This indicates that pure dephasing by homogeneous broadening mechanisms receives additional contributions from inhomogeneous broadening mechanisms.

To describe non-Markovian dynamics, Rothschild et al.[60,61] proposed the use of a fractional, or stretched, exponential for the vibrational CF

$$\ln G_v(t) = -M_2 T^2 \sum_{n=0}^{\infty} \frac{(-1)^n (t/T)^{2+n\alpha}}{n!(1 + n\alpha)(2 + n\alpha)} \qquad (5)$$

Relaxation of Phosphate Groups

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TABLE 1: Modulation Parameters for the $\nu_s(PO_3^{2-})$ Oscillator of 5′-dCMP 0.2 mol dm^{-3} in ^2H_2O at Different Temperatures Fitting Kubo−Rothschild's and Oxtoby's Models to the Experimental CFs

		Kubo−Rothschild		Oxtoby	
$T/°C$	M_2/cm^{-2}	τ_c/ps	$\langle\omega^2\rangle^{1/2}\tau_c$	τ_c/ps	$\langle\omega^2\rangle^{1/2}\tau_c$
7	80.02	0.44	0.74	0.41	0.69
10	77.95	0.45	0.75	0.41	0.68
15	74.58	0.55	0.89	0.49	0.79
20	76.58	0.54	0.89	0.49	0.80
22	77.24	0.53	0.87	0.47	0.78
24	74.55	0.55	0.89	0.49	0.80
26	75.58	0.53	0.87	0.48	0.78
28	76.15	0.52	0.86	0.47	0.77
30	74.53	0.53	0.86	0.48	0.77
32	73.72	0.53	0.86	0.48	0.77
34	77.44	0.50	0.82	0.45	0.75
35	77.87	0.49	0.82	0.45	0.74
36	78.38	0.49	0.81	0.44	0.74
38	89.04	0.42	0.74	0.39	0.69
40	88.88	0.42	0.74	0.39	0.68
45	86.26	0.42	0.74	0.39	0.74
50	84.52	0.43	0.75	0.40	0.69
55	83.03	0.43	0.75	0.40	0.69
62.5	78.05	0.45	0.75	0.42	0.69
65	79.29	0.55	0.92	0.49	0.82
67.5	78.11	0.55	0.92	0.49	0.82
70	78.45	0.56	0.94	0.50	0.84

where T is a time constant and α the dispersion or shape parameter, $0 < \alpha \leq 1$. When $\alpha = 1$, eq 5 reduces to eq 3;[70] i.e., the stretched exponential model coincides with Kubo−Rothschild's model, and random fluctuations dominate the vibrational dephasing of the oscillator. However, when α diminishes being less than 1, nonrandom fluctuations gain significance and we can speak about some local order around the oscillator.[114] This is not the case for our system. The values of α resulting from the best fits of eq 5 to the infrared CFs[70] are very close to 1. This indicates that the $\nu_s(PO_3^{2-})$ mode is under a Markovian modulation, an expected result considering the small wavenumber shift, ∼1 cm^{-1}, observed with concentration. That is, in relation to the phosphate group, the 5′-dCMP solutions between 0.005 and 0.28 mol dm^{-3} may not be considered as partially ordered systems.[117]

As stated previously, the values of $\Delta\nu^t_{1/2}$ and τ_{ir} indicate that the relaxation mechanisms for 5′-dCMP are more efficient than for 5′-CMP. The values obtained for the modulation parameters τ_c and $\langle\omega^2\rangle^{1/2}\tau_c$ in 5′-dCMP, Figures 10 and 11 and Table 1, are smaller than in 5′-CMP.[36,37] Thus the modulation also follows a faster regime in the deoxynucleotide.

Conclusions

A band-shape analysis of the IR $\nu_s(PO_3^{2-})$ band of 5′-dCMP in ^2H_2O and H_2O has been performed in relation to the relaxation processes of this vibrational mode. The second derivative spectra reveal the presence of 5′-dCMP aggregates when concentration reaches ∼0.28 mol dm^{-3}. A similar self-association process was observed for the mononucleotide 5′-CMP. The relaxation of this mode in aqueous solutions is mainly governed by vibrational relaxation processes. Among these, vibrational dephasing seems to be the main relaxation pathway. Good results have been obtained applying Kubo−Rothschild's and Oxtoby's models for vibrational dephasing. Nevertheless, Oxtoby's equation fits the initial part, <1 ps, of the experimental CFs better, whereas Kubo−Rothschild's equation fits the long time, >1 ps, behavior of the CFs better. The modulation is fast (homogeneous broadening), but inhomogeneous broadening processes slow the

modulation to an intermediate regime as concentration approaches ∼0.3 mol dm^{-3}. The stretched exponential model coincides with the Kubo−Rothschild's model in all cases ($\alpha \sim 1$), indicating that Markovian modulation is dominant for the $\nu_s(PO_3^{2-})$ mode.

The relaxation is about 70 fs faster for 5′-dCMP than for 5′-CMP. The modulation parameters for the $\nu_s(PO_3^{2-})$ oscillator indicate that the deoxynucleotide is under a faster modulation regime. The absence of the O2′H group, the reduction of H-bond interaction with the solvent, and the increase of the molecular mobility can cause a more efficient relaxation. From the point of view of the molecular dynamics of nucleotide anions, ^2H_2O and H_2O cannot be considered similar solvents. A band broadening is observed in H_2O, the relaxation time is about 0.31 ps faster in this solvent, and the modulation time 0.20−0.30 ps faster than in ^2H_2O.

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