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### Dynamics of water interacting with biomolecules

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## 9 WATER IN TRIGLYCERIDE OILS: THE C=O STRETCH VIBRATION

In the previous chapter, we studied the hydrogen-bond configuration of water molecules confined in triglyceride oil and identified several stable hydrogen-bonded water species. Here we investigate the water-triglyceride interaction in more detail, by studying the transient response of the carbonyl stretch vibration of the triglycerides with and without added water. We find that hydrogen-bond formation between a water molecule and the carbonyl group of the triglyceride leads to a frequency downshift of the absorption of the carbonyl stretch vibration from  $\sim 1745 \text{ cm}^{-1}$  to  $\sim 1728 \text{ cm}^{-1}$ . This hydrogen-bond formation affects the transient spectral response of the carbonyl stretch vibration, and speeds up the vibrational relaxation, which we find to be strongly inhomogeneous. We further observe that the anisotropy of excited carbonyl stretch vibrations decays via vibrational (Förster) energy transfer between carbonyl stretch vibrations, and determine the Förster radii to be  $2.6 \pm 0.1 \text{ \AA}$  for triacetin and  $2.5 \pm 0.1 \text{ \AA}$  for tributyrin, independent of the water content.

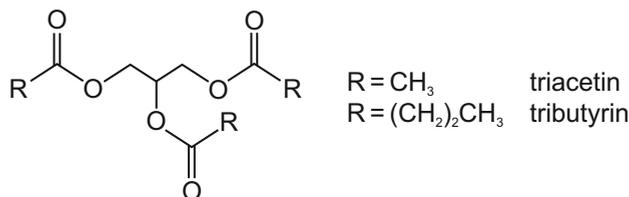


FIGURE 9.1. Triglycerides used in this study.

## 9.1 INTRODUCTION

Water plays an active role in many biological systems, and is known to influence the structure and functioning of for example proteins<sup>19,20,24,173,174</sup>, DNA<sup>175,176</sup> and phospholipids<sup>21,177</sup>. One major class of biological molecules that has hardly been studied so far in terms of its interaction with water is the triglycerides. Triglycerides are important for metabolism, serving as a source of energy, and are also commonly used in daily life in, for example, foods, pharmaceuticals and cosmetics<sup>22</sup>. Even though triglycerides are mostly hydrophobic, small amounts of water dissolve in triglyceride oil, which can have considerable influence on triglyceride properties<sup>183–186</sup>.

In chapter 8, we studied the hydrogen-bond structure and dynamics of water molecules in triglyceride oils, by probing the (transient) infrared absorption of the water OD stretch vibration. We identified water molecules forming a single strong hydrogen bond to the triglyceride, water molecules forming two weaker hydrogen bonds to the triglycerides, and, in the case of the triglyceride triacetin, water clusters. Based on the OD stretch vibrational frequency and the molecular structure of the triglycerides, we hypothesized that the singly- and doubly-hydrogen-bonded water molecules form hydrogen bonds with the carbonyl groups of the triglyceride. To confirm this, and to obtain more detailed information on the interaction between water molecules and triglycerides, we study the response of the carbonyl stretch vibration of the triglycerides.

In this chapter, we study the carbonyl stretch vibrations of triacetin and tributyrin (fig. 9.1), both with and without added water, using linear infrared and polarization-resolved femtosecond infrared spectroscopy. Both triglycerides have been extensively characterized in the OD spectral region in chapter 8.

## 9.2 EXPERIMENTAL

Triacetin and tributyrin are purchased from Sigma-Aldrich (purity >99%), and carefully dried. Even though the triglyceride oils are mostly hydrophobic, they do absorb small amounts of water ( $\sim 0.5$  ml/l) under atmospheric conditions. This water is removed using 4 Å molecular sieves (Sigma-Aldrich). After drying, part of the triglyceride oil is hydrated again by adding an excess amount of heavy water ( $\text{D}_2\text{O}$ ). To ensure a homogeneous distribution of water in the oil

phase, the mixtures are left to equilibrate for at least five days. The final water concentrations in hydrated triglyceride oils are  $57 \pm 2$  ml and  $6.8 \pm 1$  ml for triacetin and tributyrin, respectively, which corresponds to molar water to lipid ratios of 1:1.7 and 1:9 (see fig. 8.2).

We measure linear and time-resolved infrared spectra of the carbonyl stretch vibrations of the triglycerides. Since the absorption cross section of the carbonyl stretch vibration is quite high, we need to work with ultrathin samples ( $\sim 1 \mu\text{m}$ ) to maintain sufficient light intensity at the detector. Ultrathin samples are prepared by sandwiching a small volume of triglyceride oil ( $\sim 2 \mu\text{l}$ ) between two  $\text{CaF}_2$  windows, and wrapping the window edges with parafilm, which prevents oil from leaking out and  $\text{H}_2\text{O}$  from leaking in, for at least a day.

The linear infrared spectra described in this chapter are recorded with a FTIR spectrometer (Bruker Vertex 80v). The time-resolved spectra are acquired with the dual-color pump-probe setup described in ref. 196. The pump and probe pulses are centered around  $1745 \text{ cm}^{-1}$ , in resonance with the carbonyl stretch vibrations of the triglycerides.

## 9.3 RESULTS

### 9.3.1 LINEAR SPECTRA

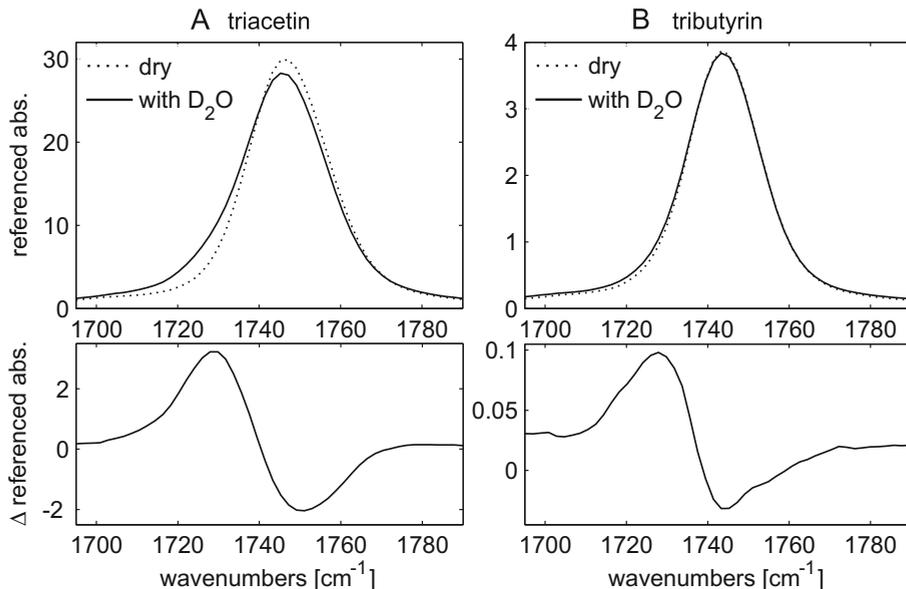


FIGURE 9.2. Linear absorption spectra of triacetin (A) and tributyrin (B), with and without added  $\text{D}_2\text{O}$ . The spectra are referenced to the intensity of the CH stretch modes around  $2960 \text{ cm}^{-1}$ . The bottom row shows the absorption difference between the referenced spectra with and without added  $\text{D}_2\text{O}$ .

Figure 9.2 presents the linear absorption spectra of triacetin and tributyrin with and without added water, in the spectral region of the carbonyl stretch vibration. In the absence of water, the absorption of the carbonyl stretch vibration is centered around  $1746\text{ cm}^{-1}$  or  $1743\text{ cm}^{-1}$ , for triacetin and tributyrin, respectively. Addition of water leads to a decrease of the amplitude of the absorption band and the appearance of an additional absorption feature around  $1728\text{ cm}^{-1}$ . This additional feature arises from the formation of hydrogen bonds between water molecules and the carbonyl groups of the triglyceride. Since hydrogen-bond formation weakens the covalent bond between the carbon and oxygen atoms of the carbonyl group, the carbonyl stretch vibrational frequency is lowered. The vibrational frequency we observe for the hydrogen-bonded carbonyl groups corresponds very well to what has been previously observed for the carbonyl groups of methyl acetate forming a single hydrogen bond to a water molecule<sup>197,198</sup>. Carbonyl groups of methyl acetate that were hydrogen-bonded to two water molecules were found to absorb at  $1703\text{ cm}^{-1}$ .<sup>197,198</sup> This finding indicates that the carbonyl groups absorbing at  $1728\text{ cm}^{-1}$  are hydrogen-bonded to a single water molecule, which agrees with the fact that the water concentration is low.

### 9.3.2 TIME-RESOLVED SPECTRA

Figure 9.3 presents the isotropic transient absorption spectra at different delay times for triacetin and tributyrin, after excitation of the carbonyl stretch vibration. At early delay times, we observe a bleach of the fundamental transition of the carbonyl stretch vibration, centered around  $1748\text{ cm}^{-1}$ , and an induced absorption around  $1725\text{ cm}^{-1}$ , corresponding to the excited state absorption associated with the  $1\rightarrow 2$  vibrational transition. These signals decay on a picosecond timescale to a thermal difference spectrum, which reflects the heating of the sample following the dissipation of the energy of the vibrational excitation. For all triglyceride samples, we observe the vibrational relaxation of the carbonyl stretch vibration to be quite inhomogeneous. This can be seen clearly in figure 9.4, which presents the isotropic absorption change for dry triacetin at different probe frequencies. At low frequencies, the vibrations decay much faster than at high frequencies.

To analyze the inhomogeneous vibrational relaxation in more detail, we fit the transient spectra to a spectral decomposition model that includes three spectral components that decay independently to the thermalized ground state:

$$S(\omega, t) = \sum_{i=1}^3 \sigma_{CO,i}(\omega) N_i(0) e^{-t/T_{1,i}} + \sigma_{end}(\omega) \left( 1 - \sum_{i=1}^3 N_i(0) e^{-t/T_{1,i}} \right) \quad (9.1)$$

Here the vibrational lifetimes of each component,  $T_{1,i}$ , are the primary fit parameters, while the spectral components  $\sigma_{CO,i}$  are calculated using singular value decomposition. The spectrum  $\sigma_{end}$  of the thermalized ground state is taken directly from the transient spectrum at 220 picoseconds. We use three components as we found this to be the minimum amount of components that is needed to accurately describe the inhomogeneous vibrational decay; two components

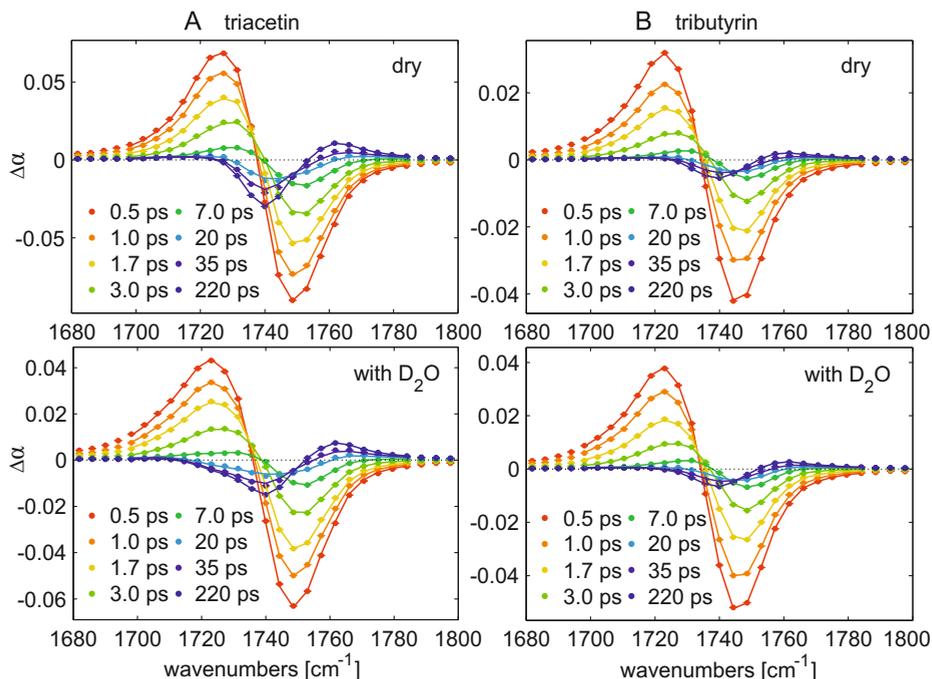


FIGURE 9.3. Isotropic absorption change as a function of frequency for different picosecond delay times, after excitation of the carbonyl stretch vibrations of triacetin (A) and tributyrin (B) with and without added  $D_2O$ . The solid lines represent fits using the model described in the text.

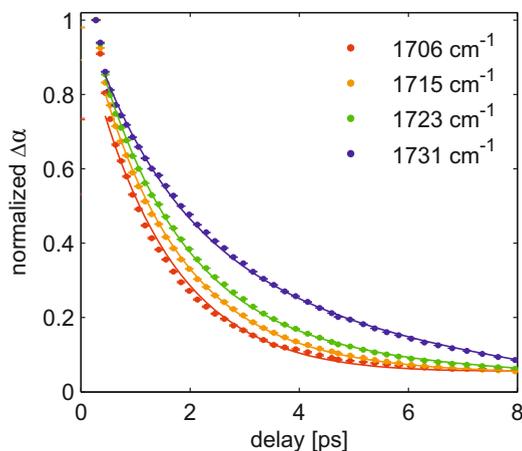


FIGURE 9.4. Isotropic absorption change after excitation of the carbonyl stretch vibrations of dry triacetin, as a function of delay time for different probe frequencies (normalized at 0.3 picoseconds). The solid lines represent fits using the model described in the text.

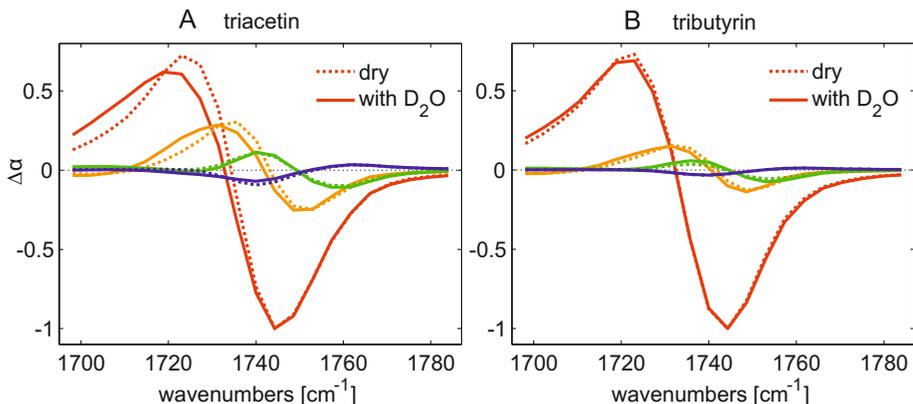


FIGURE 9.5. Spectral components that describe the isotropic absorption change (fig. 9.3) for triacetin (A) and tributyrin (B) with and without  $D_2O$ . The components are normalized on the intensity of the first component (shown in red).

fail to fully capture the dynamics beyond 15 picoseconds after the excitation, while introducing a fourth component does not significantly reduce the least-squares error of the fit. The result of the triple component fit is displayed with solid lines in figure 9.3 and 9.4, and is in good agreement with the data at all frequencies and delay times.

The spectral components resulting from the fit are displayed in figure 9.5. For all samples, each consecutive spectral component increases in center frequency, with the first and main component exhibiting a bleaching signal centered around  $\sim 1745 \text{ cm}^{-1}$ , the second component around  $\sim 1750 \text{ cm}^{-1}$  and the third around  $\sim 1760 \text{ cm}^{-1}$  or  $\sim 1755 \text{ cm}^{-1}$ , for triacetin and tributyrin, respectively. The lifetimes associated with each component are presented in table I, and range from about 1.5 picoseconds for the lowest-frequency component to about 40 picoseconds for the highest-frequency component. The large range of lifetimes directly reflects the strong inhomogeneity of the vibrational relaxation of the carbonyl vibrations.

Addition of water to the triglycerides affects the isotropic transient spectrum, as can be seen most clearly by inspection of the spectral components (fig. 9.5). For the triglycerides with water, all spectral components show a redshift,

TABLE I. Lifetimes of the spectral components (fig. 9.5) that describe the isotropic absorption change for triacetin and tributyrin with and without added  $D_2O$ .

	$T_{1,1}$ [ps]	$T_{1,2}$ [ps]	$T_{1,3}$ [ps]
Triacetin	$1.6 \pm 0.1$	$7.4 \pm 0.5$	$43 \pm 3$
Triacetin with $D_2O$	$1.6 \pm 0.1$	$7.3 \pm 0.5$	$32 \pm 3$
Tributyrin	$1.4 \pm 0.1$	$6.8 \pm 0.5$	$40 \pm 4$
Tributyrin with $D_2O$	$1.3 \pm 0.1$	$6.2 \pm 0.5$	$32 \pm 5$

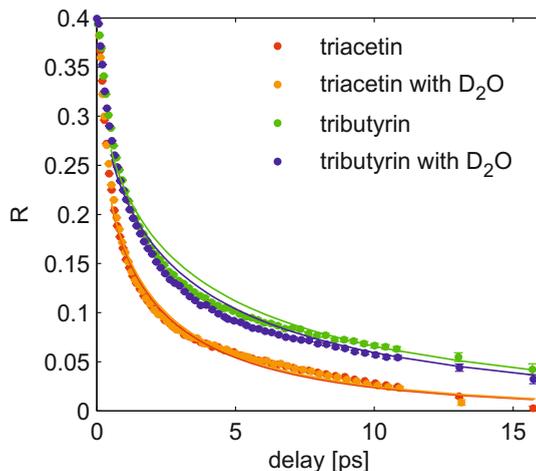


FIGURE 9.6. Anisotropy decay of the carbonyl stretch vibrations of triacetin and tributyrin with and without added  $D_2O$ , averaged over the frequency range  $1700\text{--}1785\text{ cm}^{-1}$ . The solid lines represent fits to a Förster transfer formula (eq. 9.2).

as was observed for the linear absorption spectra. This redshift results from the response of carbonyl groups that are hydrogen-bonded to water and that absorb at lower frequencies.

The addition of water lowers the vibrational lifetime. The lifetime decreases most strongly for the third component, which has an associated lifetime of  $43 \pm 3$  or  $40 \pm 4$  ps for dry triacetin and tributyrin, respectively, and a lifetime of  $32 \pm 5$  ps for triacetin and tributyrin with water. This indicates that the vibrational lifetime of carbonyl groups that are hydrogen-bonded to water is on average shorter than the vibrational lifetime of non-hydrogen-bonded carbonyl groups.

### 9.3.3 ANISOTROPIC RESPONSE

To further characterize the interaction between water molecules and the triglycerides, we measured the anisotropic response of the carbonyl stretch vibrations. The anisotropy is directly proportional to the second-order rotational correlation function, and decays due to molecular reorientation and vibrational (Förster) energy transfer. Figure 9.6 presents the anisotropy decay for triacetin and tributyrin with and without added water (the anisotropy is corrected for the isotropic heating contribution). For both triglycerides, the anisotropy decays almost to zero in about 15 picoseconds. Since triglyceride molecules are expected to reorient on a much slower timescale, considering their large size, and librations would lead only to partial anisotropy decay, the anisotropy likely decays as a result of vibrational energy transfer between carbonyl groups. The anisotropy decay as a result of energy transfer between the same type of oscil-

lators can be described by<sup>66,199</sup>.

$$R(t) = \frac{2}{5} e^{-\frac{4}{3} \pi^{3/2} c \sqrt{r_o t / T_1}} \quad (9.2)$$

where  $c$  is the concentration of oscillators, in this case carbonyl groups,  $T_1$  is the vibrational lifetime and  $r_o$  the Förster radius. The Förster radius denotes the distance between oscillators at which the rate of energy transfer between them is 50% within the vibrational lifetime. This distance depends on the transition dipole moment of the vibration and the homogeneous versus inhomogeneous linewidth (see eq. 2.34). In writing eq. 9.2, it is assumed that the distance between the oscillators is statistically distributed, and that the orientation of oscillators is independent of this distance<sup>199</sup>. This is not the case for the carbonyl groups of triglycerides, where the three carbonyl groups of each molecule sample a limited amount of distances and orientations with respect to each other. Nonetheless, we observe that the anisotropy decay of the carbonyl stretch vibration is quite well described by eq. 9.2, as indicated by the solid lines in figure 9.6, which are the result of a least-squares fit to this equation. For tributyrin, the measured anisotropy initially decays somewhat faster than the Förster formula can capture, most likely due to faster-than-average energy transfer between carbonyl groups located on the same molecule, which does not lead to a full decay of the anisotropy, because the direction of these carbonyl groups is correlated. This is less important for triacetin, as the molecules, and thus the carbonyl groups located on different molecules, are closer together.

From the fit, we obtain Förster radii of  $2.6 \pm 0.1 \text{ \AA}$  and  $2.5 \pm 0.1 \text{ \AA}$  for triacetin and tributyrin, respectively, independent of the water content. As expected, the Förster radii for triacetin and tributyrin are similar, because the transition dipole moment and spectral width of the carbonyl stretch vibration are similar for these triglycerides (see fig. 9.2). The overall decay of the anisotropy is slower for tributyrin, since the average distance between carbonyl groups is larger for this triglyceride.

## 9.4 DISCUSSION

We observe that the vibrational relaxation of the carbonyl stretch vibrations of triacetin and tributyrin is very inhomogeneous. The vibrational relaxation can be described well with three spectral components, with associated lifetimes of  $\sim 1.5$  ps,  $\sim 7$  ps and  $\sim 40$  ps. These spectral components probably do not represent specific species of carbonyl vibrations, but rather serve to describe a continuous distribution of vibrational relaxation times across the inhomogeneously broadened carbonyl absorption band. This distribution likely arises from different local conformations of the glycerol backbone and fatty acid chains of the triglyceride. Below the melting point, triglycerides adopt different polycrystalline phases that are thought to persist to some extent in the liquid<sup>22</sup>, and that give rise to distinct C=O stretch vibrational frequencies<sup>200,201</sup>.

We further observe that adding water to the triglycerides leads to a broadening of the transient C=O vibrational response towards the low-frequency side

and a decrease in vibrational lifetime, due to the response of carbonyl groups that are hydrogen-bonded to water. A similar reduction in vibrational lifetime upon hydrogen-bond formation has been observed for the carbonyl vibrations of methyl and ethyl acetate<sup>202–204</sup>, and was explained by the increase in the vibrational density of states (VDOS) as a result of the low-frequency vibrations of the intermolecular hydrogen bond<sup>203</sup>. An alternative explanation is that the lifetime of the carbonyl stretch vibration decreases with decreasing frequency, due to better spectral overlap with a lower-frequency vibrational mode to which the carbonyl stretch vibration relaxes.

We observed that the anisotropy of excited carbonyl stretch vibrations decays via vibrational (Förster) energy transfer between carbonyl stretch vibrations, and that the water content does not influence the anisotropy decay. The insensitivity of the anisotropy decay to the presence of water is likely due to the low water concentration. Since most carbonyl groups are not hydrogen-bonded to a water molecule, the carbonyl groups that are hydrogen-bonded to water are on average located far away from each other, meaning that the anisotropy decay of hydrogen-bonded carbonyl groups is likely dominated by energy transfer to non-hydrogen-bonded carbonyl groups.

## 9.5 CONCLUSIONS

We studied the effect of water on the carbonyl stretch vibration of triacetin and tributyrin, using linear infrared and polarization-resolved femtosecond infrared spectroscopy. We observe that hydrogen-bond formation between a water molecule and the carbonyl group of the triglyceride leads to a frequency downshift of the absorption of the carbonyl stretch vibration from  $\sim 1745\text{ cm}^{-1}$  to  $\sim 1728\text{ cm}^{-1}$ .

Excitation of the carbonyl stretch vibration induces a transient absorption response that can be described with three spectral components with increasing frequency and associated decay times ranging from 1.3 to 43 picoseconds, reflecting a strongly inhomogeneous vibrational relaxation. This inhomogeneity is present for both the dry triglycerides and the triglycerides with added water. Adding water leads to a broadening of the spectral components and a slight speedup of the vibrational relaxation.

The anisotropy of excited carbonyl stretch vibrations decays via vibrational (Förster) energy transfer between carbonyl stretch vibrations. We find a Förster radius of  $2.6 \pm 0.1\text{ \AA}$  for triacetin and  $2.5 \pm 0.1\text{ \AA}$  for tributyrin, independent of the water content.