Hydration of Oligo(ethylene glycol) Self-Assembled Monolayers Studied Using Polarization Modulation Infrared Spectroscopy

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The interaction with water of protein-resistant monolayers (SAMs), self-assembled from (triethylene glycol) terminated thiol $HS(CH_2)_{11}(OCH_2CH_2)_3OMe$ solutions, was studied using in and ex situ polarization-modulated Fourier transform infrared spectroscopy. In particular, shifts in the position of the characteristic C−O−C stretching vibration were observed after the monolayers had been exposed to water. The shift in frequency increased when the SAM was observed in direct contact with a thin layer of water. It was found that the magnitude of the shift also depended on the surface coverage of the SAM. These findings suggest a rather strong interaction of oligo(ethylene glycol) SAMs with water and indicate the penetration of water into the upper region of the monolayer.

Introduction

Oligo(ethylene glycol) (OEG) and poly(ethylene glycol) (PEG) are materials of tremendous importance to biotechnological applications, such as biosensing[1,2] and model membranes.[3,4] In particular, it has been found that OEG-coated (and PEG-coated) surfaces are resistant to protein adsorption,[5] although the underlying mechanisms of this resistance have yet to be fully established. Several mechanisms have been discussed, and this subject is still the focus of research and a matter of debate. It is clear, however, that the understanding of the water−OEG interaction is a vital step required to elucidate the mechanism of protein resistance. Recent findings[6,7] suggest that a variation of the surface density impacts the protein resistance. Theoretical work by Wang et al.[8] implies that OEG conformers that have gauche rotations in opposite directions around neighboring ethylene glycol (EG) units are penetrated by water because on these structures water can form double and triple hydrogen bonds with up to three oxygen atoms along the (EG) molecule.[9] In the same context, electrostatic, repulsive forces have been found with fibrinogen-functionalized AFM tips on approach to OEG-grafted gold substrates.[10] It has been suggested that this repulsive potential arises from a tightly bound layer of hydroxide ions that preferably penetrate into the self-assembled monolayers (SAMs) and create a net negative electrostatic potential,[11] which then acts against the negatively charged protein molecules.

However, it is still not clear how these findings are related to the actual mechanism leading to protein resistance on a molecular level. A further impediment is the observation that helical and all-trans conformations seem to coexist in one given OEG SAM and that the ratio between the conformations can vary from sample to sample.[9]

Another fundamental issue on a more general level is the structure of water in contact with organic matter, and this problem has received significant attention.[12−15] Hence, structural deviations from the bulk properties of the water layer adjacent to an OEG-functionalized interface are of significant general interest. There are indications based on scattering experiments that the water phase close to the OEG SAM can exhibit a change in density.[15,16]

In this letter, we present a polarization modulation infrared reflection absorption spectroscopy (PM-IRRAS) study of the influence of exposure to water of tri(ethylene glycol) (EG3)-terminated alkanethiol self-assembled monolayers. By means of PM-IRRAS, it was possible to observe in situ (i.e., in the presence of a water layer) subtle changes in the characteristic ether vibrations originating from the region of the SAM that is in direct contact with the solvent. By contrast, unlike scattering that measures average bulk behavior, this study offers insight into local interactions between the water and the OEG molecules.

Experimental Methods

Materials. Methoxy-tri(ethoxy)undecanethiol (EG3-OMe) was obtained from ProChimia, Poland. Self-assembled monolayers were grown from 500 μM solutions in ethanol (puriss., absolute from Riedel-de Haën). We used standard MilliQ water at 18.2 MΩ cm (Millipore "Synthesis").

SAM Preparation. SAMs were grown on prime-grade silicon wafers (Compart Technolgy Ltd.), which had been previously coated

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with 5 nm of chromium and 100 nm of gold by thermal evaporation. The gold-coated wafers were cleaned in an ozone-producing reactor for 1 h and were then rinsed thoroughly with ethanol, blow dried in a nitrogen stream, and immediately immersed in the EG3-OMe solution. After the desired immersion time, the wafer was taken out, rinsed with ethanol and blow dried with nitrogen. The SAM-coated wafer was then stored under argon until the measurement was performed. The protein resistance of the samples was tested after the PMIRRAS experiments by immersing the samples in a 100 mg/mL BSA/H_2O solution for ca. 20 h. Results relating protein resistance and surface coverage will be reported in ref 17.

Immersion in Water. After the spectrum was taken, the wafer with the freshly grown SAM was immersed for 18 h in MilliQ water. The wafer was then taken out and dried thoroughly with nitrogen and stored under argon.

PM-FTIRRAS Measurements. Two PMIR systems were employed in our study.

a. Ex Situ Spectra. Ex-situ spectra were taken using a dry-air-purged Bio-Rad FTS-6000 Fourier transform IR spectrometer. In addition, a polarization modulator (PM) was employed,[18] which was controlled by a Hinds Instruments PEM-90 photoelastic modulator control unit. The half-wave retardation was set at 37 kHz. Data were collected at 20 Hz at a resolution of 8 cm^{-1}, and an undersampling ratio (UDR) of 4 was used to improve the speed of data acquisition. To avoid aliasing, we placed a UDR filter into the optical path of the IR beam, which prevents radiation at higher wavenumbers from reaching the detector. The detector was fixed at an angle of 75.8°, and the sample holder was rotated to give the maximum interferogram signal, which was achieved at a sample angle of 82.9°.

b. In Situ Spectra. The in-situ spectra were recorded using a dry-air-purged Bruker Vertex 70 spectrometer with a PEM 50 polarization modulation unit. The resolution was 4 cm^{-1}, and the measuring time was 4 min, corresponding to 256 co-added scans. The incident angle was adjusted as described in the Results and Discussion section.

The detector signal is electronically split into two parts, one containing the (low-frequency) component from the moving mirror and the second being the modulation of the initially p-polarized light by the photoelastic modulator. The second signal oscillates continuously between p and s polarization. The time-dependent state of polarization can be described by the phase difference ϕ between the constituent field components. The time dependence of the signal can be obtained by Fourier expansion of the signal, which introduces zeroth- and second-order Bessel functions (J_0 and J_2). The polarization modulated reflectivity R is then given by[19]

$$R = C \frac{J_2(\phi_0)(R_p - R_s)}{(R_p + R_s) + J_0(\phi_0)(R_p - R_s)} \tag{1}$$

where R_p and R_s are the reflectivities for p and s polarization, respectively, and C is a constant describing the attenuation in the optical setup and the detector efficiency. The ex situ spectra thus obtained were corrected for the PEM response functions and background using a method described in detail by Zamlynny.[20] Because the spectra were taken using polarization modulation, the use of a reference sample was not required.

For the in situ measurements, we used a liquid cell filled with water, similar to the one described in ref 18, with one difference being that a BaF_2 half-cylinder was used instead of a triangular prism, which allowed for variation of the incident angle and led to an improved signal.[17]

Because of the strong water absorption bands, the above-mentioned correction for the PEM response function was not possible for the in situ spectra. Instead, a reference sample was measured under the same conditions, and its spectrum was subtracted from the sample
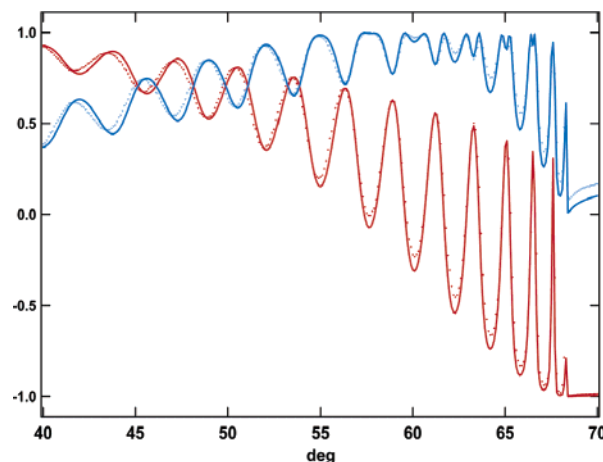

Figure 1. Ellipsometric functions x (solid blue) and y (solid red) simulated (dots) using a model consisting of the window material BaF2, water, and gold in order to determine the thickness of the water layer. This example shows a sample with a thickness of 4.33 μm.

spectrum. The reference sample was a gold wafer with no SAM that was UV cleaned immediately before the measurement. This correction method is described in more detail in refs 20 and 21.

As also discussed in refs 20 and 21, the incident angle has a significant impact on the observed peak intensity. For our experiments, incident angles of about 70−80° were found to yield a good signal-to-noise ratio. Note that these angles are not necessarily the optimized angles obtained from electrodynamic calculations[22] (e.g., after Abeles' matrix method) as a result of geometric effects and the surface selection rule on metallic substrates and that even above the angle of total reflection a good signal-to-noise ratio may be achieved if the evanescent wave penetrates through only a thin layer of liquid.

The obvious and principal problem for the application of IR spectroscopy to the study of solid−liquid interfaces is the absorption of infrared radiation by the liquid, particularly water. To maximize the signal-to-noise ratio of the spectra, the liquid layer has to be as thin as possible. However, maybe in contrast to some electrochemical "atomic-scale" studies of ions near the interface, the liquid layer has to have a certain minimum thickness in order to mimic bulk solution or semi-infinite solution behavior, in particular for aqueous solutions of biological macromolecules. The optimization and control of the liquid layer thickness is therefore crucial. Variable-angle ellipsometric measurements of the thickness of the water layer in the cell were performed on a Picometer ellipsometer (Beaglehole Instruments, Wellington, New Zealand) equipped with a HeNe laser at 632.8 nm. This phase-modulated instrument measures x and y, which are related to the real (Re) and imaginary (Im) parts of the reflectivity by

$$x = \text{Re}(r)\frac{2}{1 + \text{Re}(r)^2 + \text{Im}(r)^2} \qquad y = \text{Im}(r)\frac{2}{1 + \text{Re}(r)^2 + \text{Im}(r)^2}$$

The thickness of the water layer was determined for representative samples by fitting a simple three-layer model (BaF_2, H_2O, Au) to the measured ellipsometric parameters as shown in Figure 1. The fringes in the simulated data set are of slightly larger amplitude than those in the data. This can be attributed to the noncommensurate long-range roughness of the substrate and prism. The measured thicknesses ranged between ∼1 and ∼5 μm. Because of the relatively strong background from water in solution, even in PMIRRAS, it is

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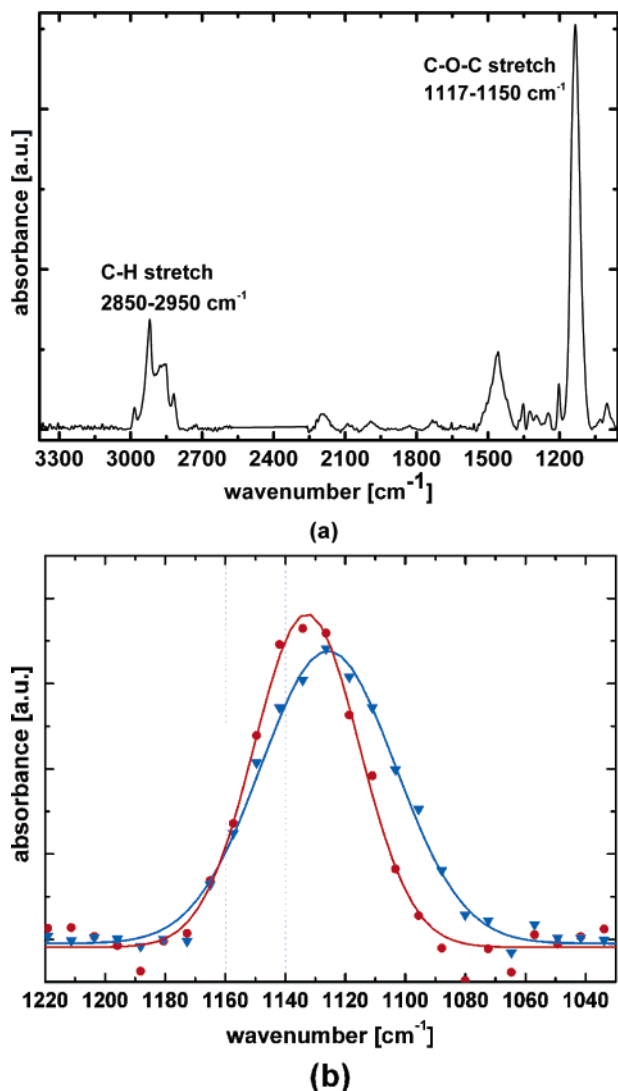(22) Popenoe, D. D.; Stole, S. M.; Porter, M. D. *Appl. Spectrosc.* **1992**, *46*, 79−87.

(a)

(b)

Figure 2. (a) Typical spectrum (resolution 8 cm^{-1}) obtained from an EG3-OMe SAM. The characteristic features are found in the CH stretching region (2800−3000 cm^{-1}) and in the C−O−C stretching region around 1130 cm^{-1}. (b) Typical shift of the position of the C−O−C stretching vibration to lower wavenumbers. The peak of the freshly prepared EG3-OMe SAM was at 1132.8 cm^{-1} (red dots) and shifted to 1125.7 cm^{-1} after 24 h of immersion in H$_2$O (blue triangles).

impossible in this setup to distinguish the water bands of bulk water from interfacial water.

Results and Discussion

Oligo(ethylene glycol) C−O−C Region (Peak Shifts Ex Situ). The first evidence for peak shifts in the C−O−C stretching band comes from ex situ measurements after the SAM has been exposed to water. Figure 2a shows a typical spectrum of an EG3-OMe SAM on gold in the mid-infrared spectral region. Characteristic C−H vibrations are visible in the range around 2900 cm^{-1}. The main feature is the sharp band at ∼1132 cm^{-1} arising from asymmetric C−O−C stretching vibrations, consistent with earlier work.[23] Figure 2b is an enlargement of the C−O−C region with a comparison of spectra taken before (red dots) and after (blue triangles) immersion in water. The difference between these signals was investigated for many samples and was found to be significant. Whereas the absolute position of the C−O−C
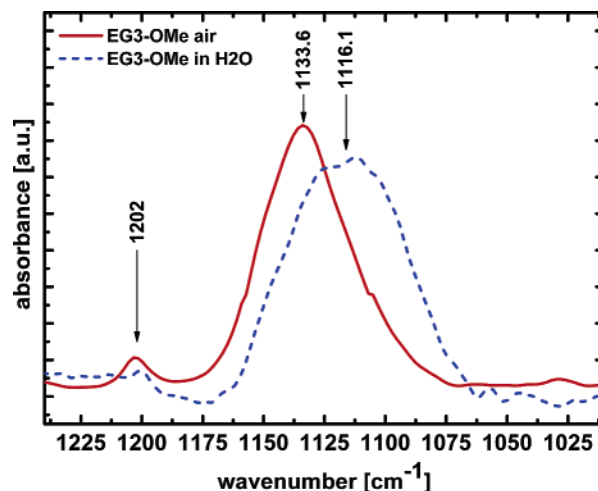
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Figure 3. C−O−C stretching region of an EG3-OMe SAM observed by PM-IRRAS modulated at 1500 cm^{-1} (resolution 4 cm^{-1}). The band position measured in situ is shifted to lower wavenumbers by 17.5 cm^{-1}, and the in-situ peak is broadened by 28.4 cm^{-1} (from 47.2 to 75.6 cm^{-1}).

vibration varied by 1 or 2 cm^{-1} from sample to sample, for a given sample a consistent shift in the peak position by up to 7 cm^{-1} could be observed upon exposure to water.

The peak positions from the collected data were determined by a Lorentz fit to the C−O−C peak. An analysis of a set of 32 samples, which were prepared under identical conditions, showed the following results. The mean peak position of the freshly prepared samples was found at (1131.99 ± 1.34) cm^{-1}.

After immersion in H$_2$O, the average peak position shifted to (1126.56 ± 2.00) cm^{-1}. This results in an average red shift of (5.43 ± 2.40) cm^{-1}, where the given error represents the standard deviation calculated from the peak positions and shifts of the set of samples.

The result of a red shift was consistently obtained for all samples studied, even though the magnitude varied in the given range. One of the sources of experimental uncertainty may be due to a certain variation of the SAM from sample to sample, as also observed in similar studies.[9]

We also note that there was a consistent broadening (width w of the Lorentz peak) of the C−O−C peaks upon exposure to water by up to 34 cm^{-1} (the average broadening was $\langle w_{\text{before}} - w_{\text{after}} \rangle = 13.3$ cm^{-1}). One reason may be a certain increased level of disorder and that only a certain fraction of the EG units is associated with water.

Oligo(ethylene glycol) C−O−C Region (Peak Shifts In Situ). Further and more direct evidence for water penetration into the EG section of the SAM and the subsequent disordering is provided by in situ PM-IRRAS studies of the CH and C−O−C regions. The observed alkyl CH$_2$ asymmetric stretch is at 2924 cm^{-1}. Values above 2918 cm^{-1} indicate an increase in the gauche-rich disordered state.[11] EG3-OMe already exhibits significant gauche defects ex situ, and a less significant change in the CH$_2$ peak position was observed in situ by comparison to the C−O−C antisymmetric stretching vibration.

The red shift of the C−O−C antisymmetric stretching vibration from the ethylene glycol units at the surface of the SAM observed upon immersion in water was considerably increased when observed in situ (i.e., in contact with water, see Figure 3). The peak is red shifted by 17.5 cm^{-1} and is significantly broadened from $w_{\text{before}} = 47.2$ to $w_{\text{after}} = 75.6$ cm^{-1}. Note that the broadening is significantly larger than the line shift, suggesting that it is not
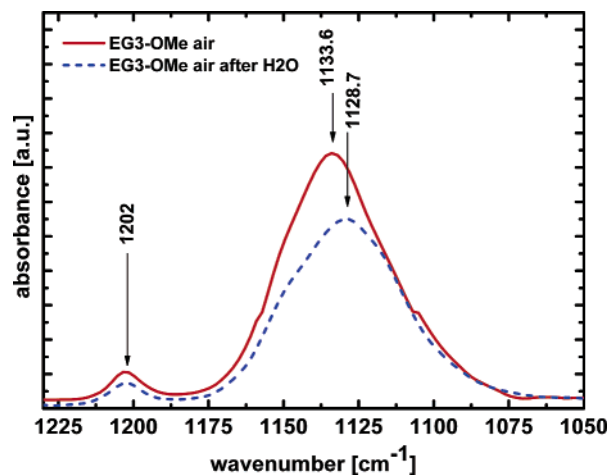
Figure 4. C−O−C stretching region of an EG3-OMe SAM observed by PM-IRRAS modulated at 1500 cm^{-1} (resolution 4 cm^{-1}) and measured in air (ex situ) before (fresh wafer from thiol solution) and after exposure to water. The band position is shifted to lower wavenumbers by 4.9 cm^{-1}.
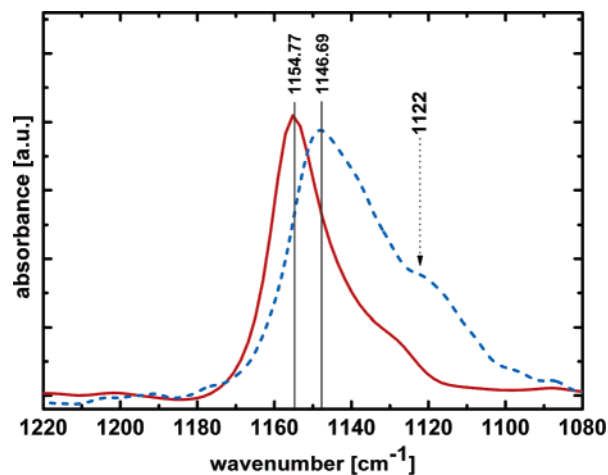

Figure 5. C−O−C stretching region of a mixed (74% deuterated/ 26% protonated) EG3-OMe SAM observed by PM-IRRAS modulated at 1500 cm^{-1} (resolution 4 cm^{-1}). In air (ex situ, solid line), the peak position is at 1155 cm^{-1}, and in H$_2$O (in situ, dashed line), it is at 1147 cm^{-1}. The band position observed in H$_2$O is shifted to lower wavenumbers by 8.1 cm^{-1}. A more pronounced shoulder appears at about 1122 cm^{-1}.

simply an effect of the inhomogeneity of the system (i.e., some OEG units are associated with water but others are not, which would cause line broadening of the order of the peak shift) but a "new" or additional relaxation channel of the C−O−C modes when the atoms are associated with water. However, this interpretation would assume that only a single absorption line is observed. Although the data do not resolve any substructure, we note that in the literature[24,25] and our own experiments the C−O−C band was shown to consist of at least three lines. Thus, the line broadening may also be due to differential line shifts.

A C−O−C peak at 1116 cm^{-1} (together with a peak at 1348 cm^{-1}) has been attributed to a more helical conformation of the ethylene glycol moieties, whereas peaks around 1140 cm^{-1} indicate an all-trans conformation.[23,25] The red shift of the main C−O−C peak, as observed in water, can be seen as an indication of an intensity enhancement of the helical peaks when in contact with water. This is in agreement with the calculation of Wang et al.[8] that the adsorption of water on isolated (EG)$_3$ molecules is energetically most favorable on the conformers that have gauche rotations. However, solvation of the SAM also leads to amorphization of the OEG moieties, as described by Zolk et al.[9]

A subsequent measurement in air after the in-situ measurement shows as before a red shift of about 5 cm^{-1} (Figure 4).

Figure 5 illustrates the care that must be taken to make the correct band assignments. Recent articles[25,26] attribute peak frequencies to at least two factors: the molecular conformation and the degree of ordering in the SAM. More specifically, frequencies might shift if, in the case of OEG SAMs, the conformation changes from all-trans to helical or if the SAM contains more or less defects. The issue becomes even more complex because all-trans and helical phases seem to coexist in any given OEG SAM and the exact determination of the surface coverage is very difficult.

We note that the observation of changes in the O−H stretching and H−O−H bending modes of hydration water inside the SAM was not possible. In situ spectra show the bending mode at ~1620 cm^{-1}; however, this band originates most likely from bulk-like

water in the vicinity of the SAM. However, no water bands were observed in ex situ spectra. This may be due to the fact that ex situ there is a smaller number of molecules associated with each OEG moiety (smaller than the estimated three to four in ref 27), which is in agreement with the smaller red shift of ex situ measurements.

Environmental effects on vibrational spectra have also been observed for other systems.[28,29] They represent an important piece of evidence for the interaction of molecules in SAMs with solvent molecules. For instance, Ong and co-workers,[30] using sum-frequency spectroscopy, have shown that H-bonding to C$_{16}$ monolayers terminated with MeO, EtO, PrO, and BuO resulted in in situ blue shifts in the CH stretching bands. This blue shift is thought to arise from hydrogen bonding between water molecules and the oxygen of the ether. This observation and argument applied to the C−O stretching mode results in a red shift of this mode because the formation of the hydrogen bond to the oxygen lone pair would reduce the extent of electron donation to the C−H antibonding orbitals. This would consequently lead to a strengthening of the C−H bonds and therefore a weakening of the C−O bond, which would explain the observed red shift. Generally, Ong et al. conclude that the hydrogen bonding between the oxygen atoms and water may induce increased disorder near the surface of the monolayer. One may speculate that this association causes either a weakening in the C−O bond or an increase in the effective mass of the vibrating entity attributed to C−O−C. Both of these would lead to a decrease in frequency.

Coverage Dependence. There are indications that the ability of OEG-coated surfaces to withstand protein adsorption is related to the surface coverage of the oligomer molecules.[6,27] The C−O−C peak shift was therefore studied for different SAM coverages. The coverage was controlled by interrupting the SAM formation after a given period of time. On the basis of our own growth kinetics studies calibrated by PMIRRAS and in agreement

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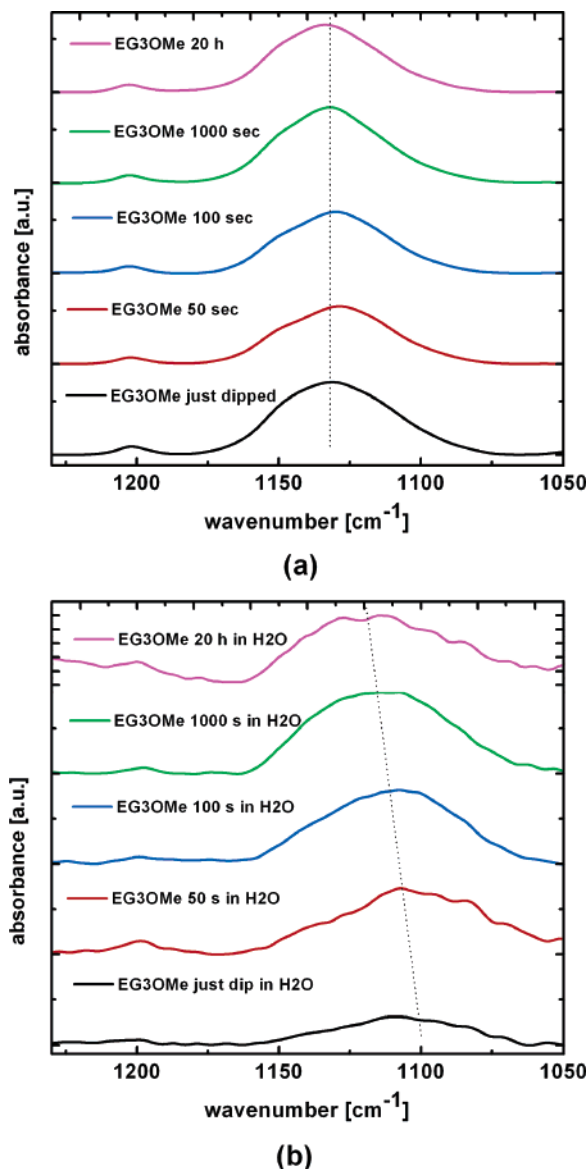(31) Schreiber, F. *J. Phys.: Condens. Matter* **2004**, *16*, R881−R900.

Figure 6. C−O−C peaks (resolution 4 cm⁻¹) for different surface coverages (immersion times) in (a) air and (b) H₂O. SAM formation was interrupted after 50, 100, and 1000 s and 20 h (from bottom to top, respectively). For short immersion times (i.e., low surface coverage), the peak shift is larger.

with previous studies on SAM growth,[6,32−34] we estimate that under the present experimental conditions (room temperature,

500 μM ethanolic thiol solution) after $\sim$8 min the surface coverage amounted to $\sim$50% and after 1000 min a coverage >90% was achieved.

Within this range of coverage, no significant changes in the average red shift could be observed in the ex situ measurements (Figure 6a), although the intermediate coverage regime is usually associated with a higher level of disorder.[6]

In situ measurements, however, show a pronounced dependence of the peak shift as a function of immersion time (surface coverage). The peak shifts in contact with water are altogether larger than those measured ex situ, and the magnitude of the shift decreases with increasing immersion time (surface coverage) (Figure 6b).

Summary and Conclusions

By means of (polarization-modulated) infrared reflection adsorption spectroscopy, we measured peak positions of the ether vibrational mode in methoxy-tri(ethoxy)undecanethiol self-assembled monolayers before and after immersion in water as well as in situ, under a water layer of $\sim$4 μm.

We observe a shift of this peak to lower wavenumbers after 18 h of exposure to water. The peak shifts on average are about 5 cm⁻¹, but those of individual samples varied. In situ measurements on samples in direct contact with water show a peak shift of about 17 cm⁻¹, along with a peak broadening.

In situ data taken for samples with increasing surface coverage (achieved by an increase in immersion time in the OEG solution during SAM formation) indicate a decrease in the peak shift upon immersion in water.

As an explanation of these findings, we suggest the penetration of water molecules into the ethylene glycol region of the SAM, resulting in the formation of hydrogen bonds with the oxygen atoms and thereby causing a lowering of the vibrational frequency of the ether band. This is in good agreement with the literature.[8,9]

In conclusion, we have provided in situ evidence for significant hydration effects of triethylene glycol-terminated thiol SAMs, which are thought to be relevant to interactions with proteins in aqueous solutions.

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