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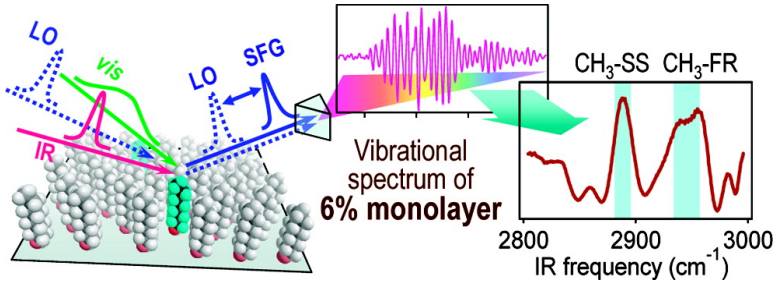

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Heterodyne-Detected Vibrational Sum Frequency Generation Spectroscopy

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Abstract: We present a new technique of broad-band heterodyne-detected sum frequency generation (HD-SFG) spectroscopy and demonstrate its high sensitivity allowing surface-selective measurements of vibrational spectra at submonolayer surface coverage, as low as a few percent of a monolayer. This was achieved without the help of surface enhancement phenomena, on a transparent dielectric substrate (water), and without introducing fluorescent labels, in fact, without utilizing any electronic resonances. Only the intrinsic vibrational transitions were employed for the detection of the analyte molecules (1-octanol). Unlike conventional (homodyne-detected) SFG spectroscopy, where the signal intensity decreases quadratically with decreasing surface coverage, in HD-SFG, the scaling is linear, and the signal is amplified by interference with a reference beam, significantly improving sensitivity and detection limits. At the same time, HD-SFG provides the phase as well as the amplitude of the signal and thus allows accurate subtraction of the non-resonant background—a common problem for surfaces with low concentrations of analyte molecules (i.e., weak resonant signals).

Introduction

Detection of adsorbed molecules at interfaces is of paramount importance in many areas of chemistry, physics, and biology, ranging from surface functionalization chemistry, electrochemistry, and heterogeneous catalysis to semiconductor passivation, biofouling, and cell membrane biology. In the past decade, vibrational sum frequency generation (SFG) spectroscopy has emerged as one of the main tools for characterization of the molecular structure and dynamics at interfaces.[1−3] Its advantages are (1) the richness of the molecular-level information (e.g., orientation and conformation) available from the fingerprint mid-IR spectra and (2) the surface selectivity that allows monolayer sensitivity without introducing fluorescent labels or resorting to surface enhancement techniques that typically require a metal surface.

However, improving the detection limits of SFG below a single monolayer coverage has proven to be a challenge. The main reason is that in its conventional homodyne-detected implementation, the intensity of the coherent second-order non-linear SFG signal scales unfavorably (quadratically) with the surface coverage N of the analyte molecules

$$I_{\mathrm{SFG}} \propto |E_{\mathrm{SFG}}|^2 \propto |\chi^{(2)}|^2 = N^2|\langle\beta^{(2)}\rangle|^2 \qquad (1)$$

where $\chi^{(2)} = N\langle\beta^{(2)}\rangle$ is the macroscopic non-linear susceptibility of the surface expressed through the molecular hyperpolarizability $\beta^{(2)}$ averaged over the orientational distribution at the interface. This implies that detection of 10% of a monolayer entails a factor of 100 decrease in the SFG signal intensity as compared to a close-packed monolayer. Since the acquisition time scales quadratically with the inverse of the signal-to-noise ratio, such a decrease of the signal level would require a prohibitively long acquisition time for the conventional vibrational SFG spectroscopy of samples significantly below monolayer coverage.

Optical heterodyne detection has been demonstrated to enhance the sensitivity of many coherent spectroscopies for bulk-phase studies.[4−6,20] Here, we present heterodyne-detected (HD) SFG spectroscopy that overcomes the limitations of the conventional (homodyne-detected) SFG technique by linearizing the spectroscopic signal using interference of the SFG signal optical field E_{SFG} with a reference beam (referred to as the local oscillator, LO), E_{LO}. The total signal intensity in the heterodyne-detection scheme is

$$I_{\mathrm{HD-SFG}} \propto |E_{\mathrm{SFG}} + E_{\mathrm{LO}}|^2 = |E_{\mathrm{SFG}}|^2 + |E_{\mathrm{LO}}|^2 + 2\mathrm{Re}[E_{\mathrm{SFG}}E_{\mathrm{LO}}^*] \qquad (2)$$

By using the LO beam that is much stronger than the SFG signal, $E_{\mathrm{LO}} \gg E_{\mathrm{SFG}}$, the intensity of the cross-term (last term in eq 2) is greatly enhanced. After subtracting the independently measured LO intensity (second term), the extracted cross-term of the heterodyne signal (the so-called

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spectral interferogram, SI) is linear in the SFG field (i.e., in the surface coverage)

$$I_{SI} \propto 2\mathrm{Re}[E_{SFG}E^*_{LO}] \propto N\langle\beta^{(2)}\rangle \quad (3)$$

HD-SFG thus enables a significant improvement of the signal-to-noise ratio by amplifying the weak SFG signal through interference with the strong LO reference beam. This allows us to obtain vibrational spectra at a few percent monolayer coverage. In addition, heterodyne detection yields both amplitude and phase of the signal. This not only provides additional information about the molecular structure of the interface[7,8] but also allows correct subtraction of the background signal[4] of the neat interface (the non-resonant electronic contribution as well as vibrations of impurities), a ubiquitous problem in situations when one is interested in detecting a low concentration of analyte molecules at a surface or interface (i.e., samples with weak resonant signals).

Experimental Procedures

The broad-band vibrational SFG spectroscopy setup, described in detail elsewhere,[9,10] is based on a high-power amplified femtosecond Ti-sapphire laser system (Spectra Physics Spitfire sub-50 fs HP). One-half of the 2 mJ fundamental output pulse (800 nm, fwhm 35 fs) was used to pump an optical parametric amplifier (OPA) followed by the signal− idler re-timing with a manual delay stage and difference frequency mixing in a 0.5 mm thick $AgGaS_2$ crystal producing 4−5 μJ IR pulses centered at 2900 cm^{-1}, temporal fwhm ∼80 fs. A broad-band SFG scheme was employed that uses spectrally broad (fwhm ∼250 cm^{-1}) IR and narrow-band visible pulses (fwhm 15 cm^{-1}) obtained using a high-power deposited Etalon (TecOptics). The laser power at the sample was 2−3 μJ/pulse for IR and up to 10−15 μJ/pulse for the visible at a 1 kHz repetition rate. The SFG signal was collimated after the sample with a lens, focused onto a monochromator entrance slit, then frequency-dispersed through the 300 mm monochromator (Acton Spectra-Pro 300i), and detected using a liquid nitrogen cooled CCD (Princeton Instruments Spec-10:100B, 100 × 1340 pixels). We used SSP polarizations for the SFG, visible, and IR beams, respectively, in all measurements.

The reference LO beam in our HD-SFG setup (Figure 1) was generated by sum frequency mixing of small portions of the visible and IR beams (∼1% of the visible and ∼5% of the IR) in a 1 mm thick $KNbO_3$ crystal. The phase matching in the crystal limited the spectral bandwidth of the LO to ∼120 cm^{-1} and its time width to ∼250 fs. Intensity of LO beam was adjusted using a variable density filter to optimize detection of the cross-term. The LO beam was recombined with the visible beam using a dichroic beam splitter. IR, visible, and LO beams were spatially overlapped at the sample surface by a 3 in. diameter, 45 cm focal length on-axis parabolic mirror focusing all beams into a ∼230 μm diameter spot at the sample with a 65° incidence angle from surface normal. The LO beam was aligned such that after reflection off the sample surface, it propagated collinearly with the SFG signal generated at the sample surface (Figure 1).

Heterodyne detection was performed using spectral interferometry[5,6] with a time-delayed (τ = ∼3 ps, introduced by a manual delay stage) LO pulse. In the frequency domain (i.e., in the spectra recorded on the CCD after the monochromator), this resulted in a characteristic fringe pattern $\propto e^{i\omega\tau}$ in the cross-term $I_{SI}(\omega)$ referred to as a SI (Figure 1).[6]

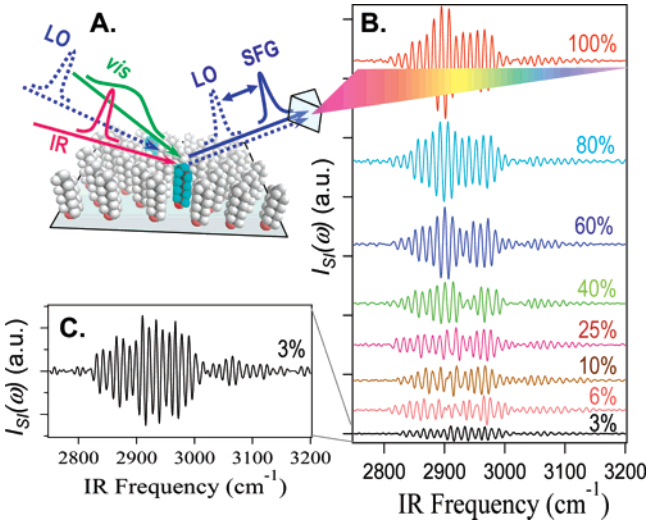

Figure 1. (A) Schematic representation of the broad-band heterodyne-detected HD-SFG experiment. (B) SI (real part shown) for samples of varying surface coverage of 1-octanol, from 100 to 3% monolayer. (C) Enlargement of the signal from the 3% monolayer sample.

This allows us to utilize the broad-band SFG scheme[11,12] and to take advantage of multiplex detection with a CCD chip. The τ = ∼3 ps delay yielded ∼10 cm^{-1} fringe spacing, compatible with the spectrometer resolution (∼2 cm^{-1}) such that there was no under-sampling. The fringe pattern was also used to compensate for the phase drift between acquisitions using the phasing procedure described next.

The SI were recorded at the full resolution of our CCD (1340 pixels) without binning. The overall HD signal level was adjusted by tuning the intensity of the LO beam and was limited only by the dynamic range of the CCD detector (65535 counts/pixel). At 100% 1-octanol monolayer coverage (1.0 mM bulk concentration), the total HD-SFG-detected signal I_{HD-SFG}, eq 2, was typically ∼22 000 counts per pixel, and the fringe depth of the SI (i.e., the magnitude of the cross-term I_{SI}, eq 3) around the CH_3 symmetric stretch peak was 2400 counts, while the homodyne SFG signal level I_{SFG}, eq 1, was ∼200 counts per pixel for a 100 s exposure time. The LO field was therefore ∼10 times stronger than that of the SFG signal field for the 100% 1-octanol monolayer sample, and the E_{LO}/E_{SFG} field ratio increased accordingly with decreasing surface coverage. The heterodyne setup was covered to eliminate air currents, allowing a phase stability of $\lambda/4$ over 10 min. Thus, the depth of the spectral fringes was not affected by the phase drifts over the 100 s CCD collection times used in all measurements.

We demonstrated the HD-SFG technique on a model system: mixed monolayers of 1-octanol/deuterated 1-octanol at the air/water interface. The samples were prepared using double-distilled water. 1-Octanol ($C_8H_{18}O$, Fisher Scientific, >99%) and deuterated 1-octanol (C_8D_{17}-OH, Cambridge Isotope Laboratories, 98%) were used as received. The overall concentration was kept constant at 1.0 mM, corresponding to a saturated Gibbs monolayer at the air/water interface, according to literature reports.[13] A total of 10 min was allowed for the monolayer to form at the surface before the SFG measurements. Evaporation, and the associated lowering of the sample surface, was controlled by covering the sample dish with a plastic film with two holes for beam access.

Results and Discussion

We monitored the CH_3-stretch modes in the 2800−3000 cm^{-1} region while varying the mol fraction of 1-octanol, thus changing

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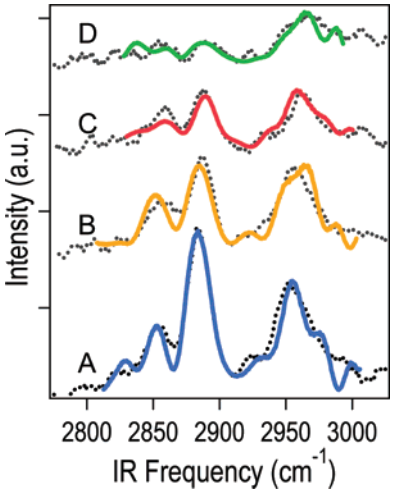

Figure 2. Comparison of the power spectra of 1-octanol CH-stretch vibrations obtained from the heterodyne-detected spectral interferograms (thick colored lines) with the conventional (homodyne-detected) SFG spectra (black dashed lines) for surface coverages of (A) 100%, (B) 80%, (C) 60%, and (D) 40% monolayer.

the surface coverage N of the CH_3 groups without the potential complications of changing the molecular orientation and intermolecular packing of the alkane chains. For comparison, we present both heterodyne- and homodyne-detected SFG spectra obtained using the same signal acquisition time on the CCD chip, 100 s. The two main transitions observed, marked by cyan shadows in Figure 4, are the CH_3 symmetric stretch ($\sim$2880 cm^{-1}) and Fermi resonance ($\sim$2940 cm^{-1}), in agreement with the previously reported measurements for SSP polarization.[13]

The broad-band HD-SFG SI were obtained by recording the total heterodyne-detected intensity spectrum I_{HD-SFG}, eq 2, and then subtracting the LO intensity spectrum $|E_{LO}|^2$ (second term in eq 2) to reveal the cross-term, eq 3. The LO intensity spectrum was recorded on the same CCD detector, in exactly the same experimental configuration, by simply blocking the IR beam such that the SFG signal from the sample was not generated. After subtraction of the LO, we performed inverse Fourier transform into the time-domain to filter out the remaining homodyne contribution centered at $\tau = 0$ delay (center of LO pulse) since the desired cross-term, eq 3, was centered around $\tau = \sim$3 ps delay between the LO and the SFG pulses. Fourier transforming back into the frequency domain yielded the cleaned-up SI shown in Figure 1B (real part shown), with the LO spectral envelope completely removed. Clean SI can be recorded using the 100 s CCD acquisition time for samples ranging from 100% 1-octanol in the monolayer to a fully deuterated monolayer, whose signal is referred to next as the background signal of the neat interface. Figure 1C shows an enlargement of the spectral interferogram for the 3% 1-octanol monolayer sample, demonstrating the signal-to-noise level achievable in this technique. In fact, interferograms for samples below the 1% octanol monolayer could be recorded with similar signal-to-noise ratios, but the analysis of the spectra is restricted due to the purity of *d*-octanol (vide infra).

The absolute value squared of the thus obtained interferograms, corrected for the spectrum of the local oscillator, accurately reproduces the homodyne-detected SFG spectra as shown in Figure 2, providing validation of the HD-SFG measurements. However, the comparison can be made only for

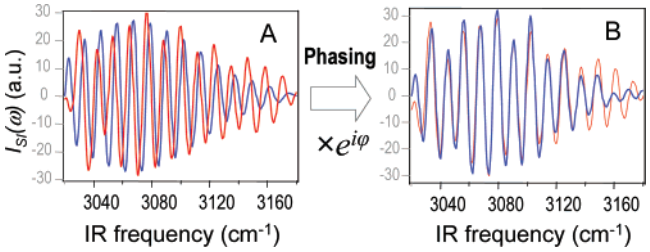

Figure 3. Phasing procedure allowing us to lock the phase of the SI to that of the background signal of the neat (fully deuterated) interface. (A) Real parts of the SI from 10% octanol monolayer sample (red line) and neat interface (blue line) before phasing in the spectral region around 3100 cm^{-1}. (B) Same two SI after phasing (red interferogram multiplied by $e^{i\varphi_{adj}}$).

samples close to monolayer coverage. Below $\sim$40% monolayer, the homodyne-detected SFG does not produce usable spectra for the chosen 100 s acquisition time. The two main reasons for this are (1) the resonant part of the homodyne SFG signal decreases quadratically with the surface coverage N, eq 1, quickly reducing the resonant 1-octanol signal below the noise level and (2) at low coverage, the background part of the response (non-resonant electronic contribution as well as impurities and the broad red-tail of the water OH-stretch band evident in Figure 4A) interferes with and masks the weak resonant CH-stretch transitions of 1-octanol.

Heterodyne detection overcomes both of these problems. First, the use of the strong LO beam amplifies the overall signal, improving the signal-to-noise ratio. Second, it provides linear (as opposed to quadratic) scaling with decreasing surface coverage. Third, the knowledge of the phase of the HD-SFG signal with respect to the background signal from the neat interface (100% deuterated 1-octanol monolayer) allows correct subtraction of the background contribution to reveal the resonant 1-octanol signal.[4,14]

The value of the phase in the HD-SFG spectra cannot be preserved from experiment to experiment, due to long-term drift and especially when samples are changed. To lock the phases in all measurements relative to the background signal from the neat interface (100% deuterated 1-octanol monolayer at the air/water interface), we developed the following phasing procedure. Neat interfaces (in this case, 100% deuterated monolayer) are often characterized by a predominantly non-resonant response leading to a broad SFG signal spectrum. The spectral region around 3100 cm^{-1} is outside the CH_3 vibrational transitions of interest. The neat interface SFG background is non-zero in this region (Figure 1B), resulting possibly from the broad red-tail of the water OH-stretch band.[3,7,15,16] The SFG signal in this region does not depend on the 1-octanol surface coverage ranging from 0 to 100%.

Figure 3A shows magnified HD-SFG spectral interferograms around 3100 cm^{-1} for the neat interface ($N = 0\%$) and for a sample with $N = 10\%$ 1-octanol surface coverage. The shapes of the spectral interferograms for both concentrations are similar in this spectral region, but the phases differ. By simply adding a phase φ_{adj} to the complex-valued HD-SFG spectral interferogram for the 10% sample (i.e., multiplying it by an $e^{i\varphi_{adj}}$ factor),

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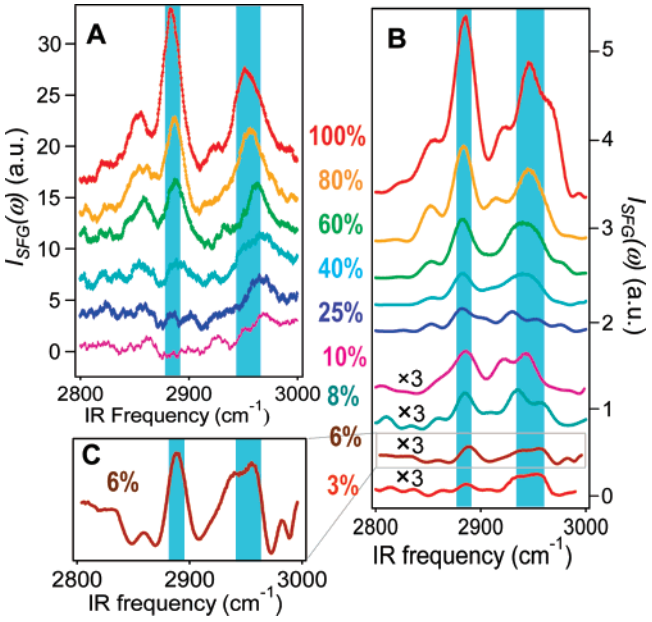

Figure 4. (A) Homodyne-detected SFG spectra of CH-stretch of saturated octanol/*d*-octanol monolayers (octanol mol fraction indicated) at different surface coverages obtained using a 100 s acquisition time. (B) Heterodyne-detected power spectra extracted from the SI after subtracting out the background signal from the neat interface, 100 s acquisition time. (C) Enlargement of the heterodyne-detected spectrum of the 6% monolayer. Spectra are vertically offset for clarity: (A) homodyne spectra offsets are 0, 3, 6, 9, 12, and 15 au for 10, 25, 40, 60, 80, and 100% samples and (B) heterodyne spectra offsets are 0, 0.4, 0.8, 1.2, 1.9, 2.2, 2.5, 2.9, and 3.3 au for 1, 3, 6, 8, 10, 25, 40, 60, 80, and 100% samples, respectively.

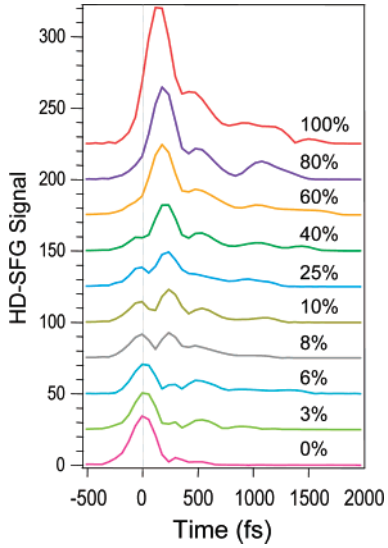

Figure 5. Time-domain vibrational free induction decays at various 1-octanol mol fractions in the monolayer, showing vibrational quantum beats of the CH-stretch modes of 1-octanol and the background response of the neat (0%, fully deuterated) interface consisting of both non-resonant components peaking at $t = 0$ and resonant vibrations of impurities (≤2% level).

we can achieve nearly perfect overlap in this spectral region (Figure 3B), thus locking the phase of the 10% sample to the 0% (neat interface) sample (background). The accuracy of the obtained phase φ_{adj} is better than ±5°. Using this phasing procedure, we ensure that the spectral phases for all measured samples are locked relative to the spectral phase of the reference sample (neat interface, 100% deuterated 1-octanol). The ability to retrieve the phase for each measured spectrum with respect to a chosen standard signal (e.g., neat interface background) using simple phasing of the spectral interferograms is a consequence of the phase being locked across the spectrum of the LO pulse, a unique advantage of the broad-band spectral interferometry approach not available, for example, in the scanning phase-sensitive SFG detection.[7,8]

After the phasing procedure, the background signal of the neat interface (100% deuterated 1-octanol) can be subtracted to reveal the spectral signatures of the analyte (1-octanol), which are otherwise masked by the interference with the background, especially at low concentrations. We also note that, in the special case of absorbing substrates, the SFG signals contain an additional phase contribution form the complex Fresnel factors.[17] However, the same additional phase is also contained in the background reference signal of the neat interface and is therefore subtracted after the phasing procedure.

The extracted background-free HD-SFG power spectra shown in Figure 4B demonstrate that this technique enables vibrational spectroscopy of surfaces at coverages as low as a few percent monolayer, by far exceeding the sensitivity limits of the conventional SFG spectroscopy (Figure 4A). As an example, the HD-SFG spectrum for the 6% monolayer sample (Figure 4C) exhibits the same two main transitions, the CH_3 symmetric stretch and Fermi resonance, as in the higher concentration samples. The increased noise level on the wings of the spectrum results from the limited bandwidth of the LO pulse in our current setup (only ∼120 cm^{-1} due to phase matching in the $KNbO_3$ crystal).

The time-domain representation of the HD-SFG spectra (Figure 5), obtained by simply Fourier transforming the SI in Figure 1, naturally separates out the mostly non-resonant (i.e., instantaneous) background signal from the resonant part of the response—the free induction decay (FID), which shows the characteristic vibrational quantum beats.[10] The neat interface background signal (Figure 5, bottom trace) was measured by performing HD-SFG on a fully deuterated monolayer. The clearly discernible non-instantaneous component in the time-dependent signal from the neat interface sample demonstrates our ability to detect impurities in the deuterated 1-octanol (2% or below, according to the manufacturer). We note that the knowledge of the spectral phase with respect to a well-defined reference sample (neat interface) allows us to subtract this neat interface signal (i.e., essentially eliminate the impurities contaminating the resonant 1-octanol signal at low concentrations). For intermediate concentrations (8−25%), the FID curves show destructive interference between the background FID signal peaked around $\tau = 0$ and the resonant octanol signal, resulting in an apparent dip in the overall FID.

It must be emphasized that, while we chose to show only real parts of the SI in Figures 1 and 3 and power spectra (absolute value squared) in Figures 2 and 4 for direct comparison with the conventional homodyne-detected SFG spectra, the HD-SFG measurement provides both real and imaginary parts of the vibrational response of the surface sample. The knowledge of the SFG phase in many cases contains valuable molecular-level information,[7,8] for example, absolute orientation (up vs down) of molecules at the interface.[18,19]

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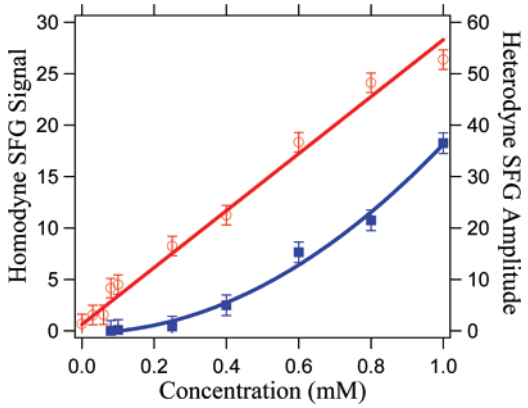

Figure 6. Scaling of the homodyne-detected SFG intensity (blue squares) and HD-SFG signal amplitude (red circles) with the surface coverage (1-octanol mol fraction in the monolayer) for the CH_3 symmetric stretch mode.

The linear scaling of the resonant (background-free) CH-stretch HD-SFG signal with the octanol mol fraction is demonstrated in Figure 6, which shows the peak amplitude of the most prominent vibrational mode, the CH_3 symmetric stretch resonance (2880 cm^{-1}) extracted from the background-free HD-SFG spectra shown in Figure 4B by multi-Lorentzian fit commonly used in fitting SFG spectra.[8,10,13] This allows extension of the HD-SFG spectroscopy to samples with surface coverage significantly below a single monolayer. In addition, the signal amplitude is much larger for the HD-SFG, which alleviates the problem of the electronic read-out noise. On the contrary, the homodyne-detected SFG intensity of the same transition, extracted from similar multi-Lorentzian fits of the conventional SFG spectra in Figure 4A, follows the expected quadratic scaling with the surface coverage. Note that homodyne SFG signal is zero within the signal-to-noise ratio for surface coverages 25% monolayer and below.

The signal-to-noise ratio in the SI and the extracted spectra (Figures 1 and 4) for lower coverage samples allow us to suggest that HD-SFG may enable the obtainment of vibrational spectra for samples at or below 1% monolayer coverage. In fact, several SI were recorded for samples with a 1-octanol mol fraction of 1, 0.5, and 0.1% (not shown), containing mostly the background signal, with a signal-to-noise ratio similar to that in Figure 1. However, the isotopic purity of the deuterated 1-octanol provided by the supplier (Cambridge Isotope Laboratories) is 98%. While the CH-stretch transitions of the isotopic impurities ($\leq$2% level) could be readily detected in these samples, for the chosen octanol/*d*-octanol model, system testing of the HD-SFG technique at low surface coverage is limited by the chemical purity of the samples rather than by the sensitivity of the spectroscopic detection.

Conclusion

We developed a new technique of heterodyne-detected SFG spectroscopy and demonstrated its high sensitivity allowing measurements of vibrational spectra of submonolayers, at surface coverages as low as a few percent of a monolayer. This was achieved on a transparent dielectric substrate (water), without resorting to the total internal reflection geometry, and without the help of surface enhancement phenomena. Unlike ultrasensitive fluorescence detection, which requires utilizing electronic transitions and/or introducing fluorescent labels, only the intrinsic vibrational resonances of the analyte (1-octanol) molecule were employed in the detection. The technique may find applications in the ultrasensitive spectroscopic detection of molecules at surfaces/interfaces in chemistry and biology. The improved sensitivity could potentially broaden the range of applications of the non-linear surface-selective vibrational spectroscopy, in particular, to biological systems (e.g., probing surfaces of biomembranes).

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