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Suppressing Interfacial Water Signals to Assist Peak Assignment of the N^+-H Stretching Mode in Sum Frequency Generation Vibrational Spectroscopy (SFG)

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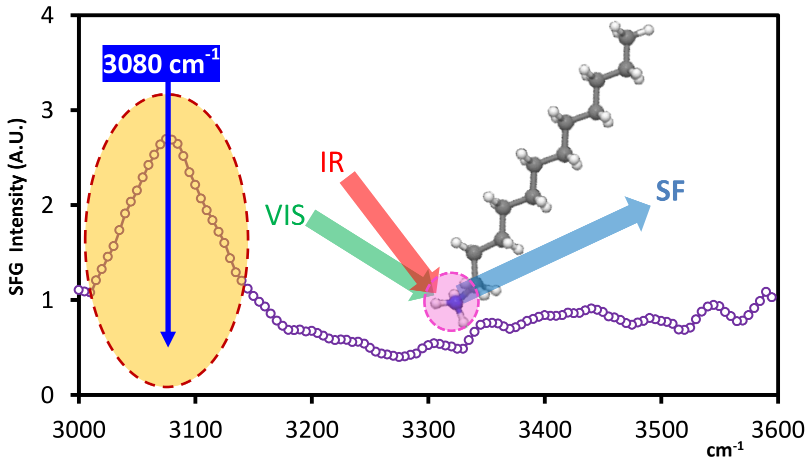
ABSTRACT

Amine is one of the common functional groups of interest due to its presence in natural abundance of proteins, surfactants and other chemicals. However, its accurate spectral assignment of vibrational modes, critical to interpreting SFG signals for characterizing various bio-interfaces such as protein-membrane interaction and surfactant adsorption, still remains elusive. Here we present a systematic study to identify and justify the correct peak assignment of the N^+-H stretching mode at the air-water interface. We used three special surfactants: hexadecylamine (a primary amine without counterions), dodecylamine hydrochloride (a primary amine with counterions) and hexadecyltrimethylammonium bromide as a control (the N^+-H stretching mode is absent in this quarternary amine). We suppressed the SFG interfacial water signals using saturated NaCl solutions. Our designed experiments resolved the current controversy and concluded that the 3080 cm^{-1} peak is from the N^+-H vibrations, while the 3330 cm^{-1} peak is not due ammonium species but rather originates from the interfacial water vibrational modes or the backbone amide modes.



KEYWORDS: sum frequency generation, amine, amide A, saturated brine solution.

1. Introduction

Over the past fifteen years, SFG has been extensively utilized to characterize the surface activities of proteins and surfactants at various interfaces. The modes of action of such macromolecules have been elucidated via their SFG active vibrational modes such as C-H stretching modes (methyl, methylene and phenyl),[1-2] SO_3 stretch,[3-4] backbone C=O stretch (amide I band),[5-6] C-N stretch and N-H bend (amide III band),[7] N-H stretch,[8-11] and O-H stretching modes.[12] Although the SFG peak assignments for such stretching modes have been generally agreed upon within the SFG community, the origin of the 3300 cm^{-1} peak remains under debate. Specifically, this peak has been assigned as the amide A band by some research groups[9, 13-14] and as the amine N-H stretch by others.[8, 10, 15] In particular, this 3300 cm^{-1} SFG peak was first observed and assigned to the hydrophilic side chains of the lysine and arginine residues by Cremer et al.[15] Due to the lack of solid spectroscopic evidence, Somorjai et al. later only attributed this peak to either the ammonium side chain lysine residues or the helical backbone amide A mode.[16] By observing the direct time dependent correlation between the helical backbone amide I and this 3300 cm^{-1} peak, Chen et al. then suggested that this 3300 cm^{-1} peak to originate from the backbone amide A mode of the protein, not from the side chain ammonium groups. Further studies of an isosequential peptide (LK_{14}) by Somorjai et al. using SFG and quartz crystal microbalance seemingly lead to the conclusion that this 3300 cm^{-1} peak is contributed by the amide A mode of the peptide backbone. However, Castner et al. attempted to "put this controversy to rest" by studying the ^{15}N isotopically labeled 14-amino acid amphiphilic model peptides LKα14 using SFG.[10] In their study, they observed a spectral shift of the 3300 cm^{-1} peak, which was attributed to the fact that the peptide is isotopically labelled. They thus concluded that the 3300 cm^{-1} SFG peak originates from the vibrational modes of the side chain terminal amine group of lysine residues. This controversy turned out to be still under debate when Yan et al. recently reported the chirality of the structure that gives rise to the SFG peak at 3300 cm^{-1}, which thereby was suggested to originate from the amide A mode from the protein backbone.

In this study, a systematic approach has been applied to directly investigate the peak assignment of the N^+-H stretch using a set of surfactants possessing relevant headgroups. Since the electrostatic field of the adsorbed surfactant layer may be strong enough to orient several interfacial water layers,[17] leading to a strong enhancement of the SFG interfacial water O-H signals, the N-H signals in this regime may be dominated by such strong water signals and thus hard to observe. To minimize the interference of the interfacial water O-H stretching modes, we minimized the Debye–Hückel screening length by using supersaturated NaCl brine solution to maximize the bulk ionic strength as previously demonstrated.[18]

2. Experimental Section

In this study, three types of surfactant were used: hexadecylamine (HAD, 98%), Hexadecyltrimethylammonium bromide (CTAB, >99%) and dodecylamine hydrochloride (DAH, >99%) purchased from Sigma Aldrich and used as received (Fig. 1), and NaCl (Sigma Aldrich, >99.99%) which was roasted at 800 ^oC overnight to remove possible organic impurities.[19] Freshly purified water (Ultrapure Milli-Q unit from Millipore, USA) with a resistivity of 18.2 MΩ.cm was used to make all solutions in the experiments. In the SFG measurements, a specific volume of the concentrated surfactant aqueous stock solution (10 mM) was injected into a neat water/salt solution reservoir of 20 mL to achieve the desired concentration. A magnetic micro-stirrer was used for 10 seconds to ensure a homogeneous concentration distribution of the added surfactant molecules. All experiments were carried out at room temperature (~23^oC).

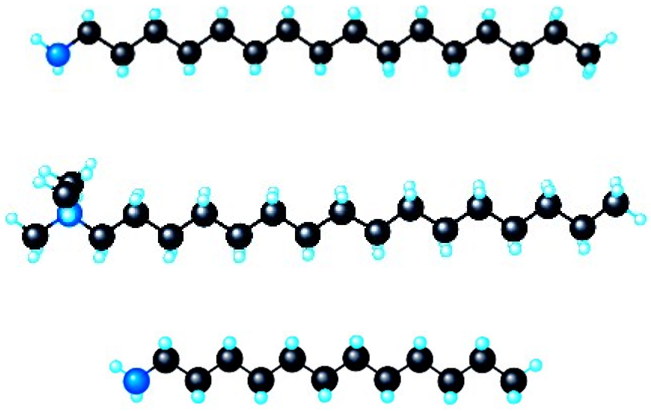


Figure 1. Molecular structures of HAD (top), CTAB (middle) and DAH (bottom). The counter-ions are omitted.

The SFG setup has previously been described.[20-21] Briefly, the visible and the tunable IR beams were spatially and temporally overlapped on the solution interface. The visible beam was generated by frequency-doubling the fundamental output pulses (1064 nm, 10 Hz) of 36 ps pulse-width from an EKSPLA solid state Nd:YAG laser (PL2241). The tunable IR beam was generated by an EKSPLA optical parametric generation/ amplification and difference frequency system based on LBO and $AgGaS_2$ crystals. Fluctuations in the beam energies were only 3% standard deviation in the tunable IR beam and 1.5 % in the visible beam. In our SFG measurements, the incident angle for the visible beam was $\alpha_{vis} = 60^o$ and for the IR beam, $\alpha_{IR} = 54^o$. We used s, s, and p polarizations, respectively for the SF output, visible input and IR input. We found that very small changes in the height of the air-water interface, as caused by inevitable evaporation during the experiments, could significantly impact the quality and sensitivity of the SFG signals collected. To overcome this experimental issue we used a home built He-Ne laser leveller to monitor and control the interface height.

3. Results and Discussion

3.1 SFG O-H stretch spectra of CTAB and DAH solution at the air/water interface in the 3000-3600 cm^{-1} regime.

Possessing the ammonium headgroups and halide counter-ions (chloride and bromide), DAH and CTAB are both positively charged and their adsorption layer at the air/water interface creates a strong electrostatic field that affects multiple interfacial water layers, leading to a strong SFG water signal enhancement.[11, 17, 21] However, unlike DAH, the ammonium group of CTAB is quarternary thus it should not produce any N^+-H stretch signature observed by SFG. CTAB is therefore an excellent control species in the determination of the N^+-H spectroscopic signature. It is worth emphasizing that although HAD does not contain any counter-ions, it carries a primary ammonium group with a pK_b value of 3.4, implying that its headgroup remains mostly in the form of ammonium ions (positively charged) at neutral pH. Because the interaction among the interfacial water molecules is rather complex,[22-26] we chose not to perform data fitting on the SFG O-H broad band (3000- 3600 cm^{-1}) in this study.

We first obtained the SFG signals in the 3000-3600 cm^{-1} regime of the air/aqueous interface of the 1 mM DAH and 1 mM CTAB solutions (Fig. 2). Since the electric fields resulted from the positively charged ammonium headgroups of the surfactants, it was expected that the signals would be similar as their dominating electrostatic influences on the interfacial water layers should not cause significant spectral differences detectable by SFG. However, the two spectra exhibited noticeable differences which unambiguously stem from the N-H bonds of the ammonium headgroups. The most prominent spectral difference was the existence of an extra peak at around 3100 cm^{-1} observed with DAH (Fig. 2).

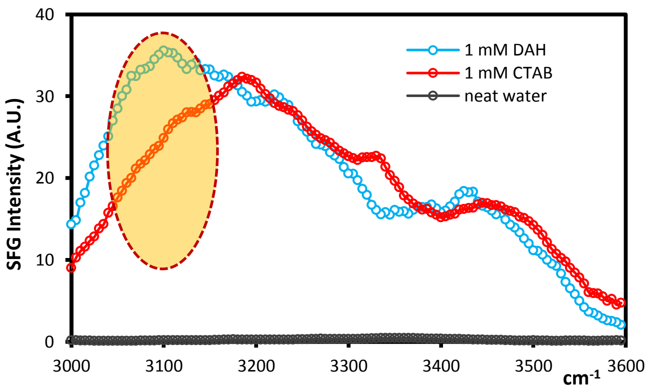


Figure 2. ssp SFG interfacial water signals of 1 mM DAH and CTAB solutions. To assist the SFG band comparison, the SFG signals for CTAB were divided by 2 to make it comparable to that of the DAH.

3.2 SFG O-H stretch spectra of CTAB and DAH solution at the air/saturated salt solution interface in the 3000-3600 cm^{-1} regime.

It has been reported previously by non-linear spectroscopy that the ionic strength of the solution directly affects the effective electrostatic length of the adsorption layer field (known as the Debye length, l_D).[18] In particular Tahara and co-workers [27] show that an excess salt concentration dramatically reduces the SFG intensity by reducing the thickness of the probed water layer. The Debye length was found to decrease with increasing electrolyte (surfactant) concentration, or equivalently, the solution ionic strength, I. For 1:1 electrolyte solutions, we have[28]

$$l_D = \sqrt{\frac{\varepsilon\varepsilon_0 k_B T}{2e^2 N_A I}} \qquad (1)$$

where ε_o is the permittivity of free space, ε is the dielectric constant, k_B is the Boltzmann constant, T is the absolute temperature, N_A is the Avogadro number and e is the elementary charge. The shorter Debye length means that fewer interfacial water layers are affected by the electric field, leading to weaker water SFG signals.

We thus suppressed the SFG water signals of the O-H stretching band using a saturated NaCl solution in a similar manner to the work of Tahara and co-workers [27] and found that the SFG broadband of the O-H stretch in the 3000-3600 cm^{-1} decreased (Fig. 3). The spectral differences between DAH and CTAB thus became much clearer; most notably, a strong peak at around 3080 cm^{-1} was observed with 0.01 mM DAH, but not with 0.01 mM CTAB. Since DAH is capable of forming hydrogen bonds with interfacial water molecules, one would expect to observe spectral differences in the O-H broad band in the 3100-3600 cm^{-1} region. Indeed, the two commonly reported peaks[22, 29] at 3200 cm^{-1} and 3400 cm^{-1} are farther separated in the case of DAH than with CTAB (Fig. 3). The difference is due to the more directed hydrogen bonding scheme between DAH and those water molecules that can narrow down the peaks, leading to a larger separation between them. To gain a greater understanding of the origin of this observation requires more accurate spectral assignments of these peaks achievable through heterodyne SFG detection, which is beyond the scope of the current study.

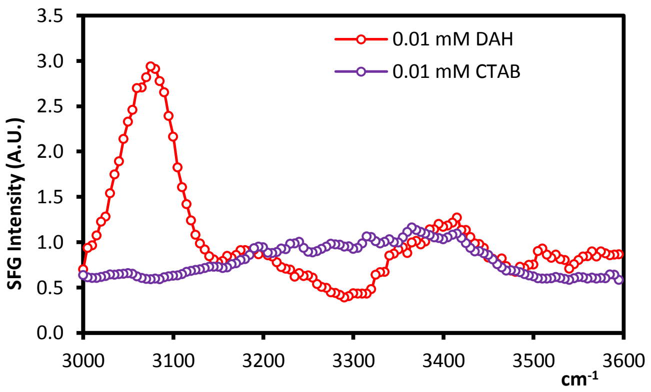


Figure 3. ssp SFG signals in the 3000-3600 cm^{-1} of 0.01 mM DAH and CTAB in saturated NaCl solutions.

It is worth noting that the peak at 3080 cm^{-1} has been experimentally observed and tentatively assigned to the N$^+$-H stretching modes by Richmond et al., which aligns well with the IR and Raman data previously reported on the alkyl ammonium group.[11, 30] On the other hand, by ^{15}N isotope labelling the ε-amino group of LKα14 peptide, Castner et al.[10] assigned the N$^+$-H stretching mode to be at 3300 cm^{-1}. Their peak assignment argument was primarily based on the spectral shift of the 3300 cm^{-1} peak, which is resulted from the ^{15}N labelling. In their study, a reference peak shift of around 8 wavenumbers (only slightly larger than the resolution of the SFG spectrometer of ~5 cm^{-1}) was used, despite the fact that this value was previously reported for ^{15}N labeled aniline, a benzene derivative.[31-32] In fact, it is rather doubtful that an amine group bonded to an alkyl with a chain length of 5, would behave similarly to one bonded to a π-bonded conjugated phenyl group. According to previous Raman and IR studies on the ammonium group,[33-36] all of the N$^+$-H stretching modes have been observed around 3000-3200 cm^{-1}, leaving the spectral peaks beyond 3200 cm^{-1} exclusively to the water's O-H stretching or the backbone amide vibrational modes. Furthermore, the data of Castner et al.[10] showed a visible peak at 3080 cm^{-1}, although it appeared to be much weaker than the peak at 3300 cm^{-1}. Interestingly, they concluded, without explanation, that the peptide adsorption occurred at the positively charged CaF_2 surface. This conclusion was based on the occurrence of the 3300 cm^{-1} peak of which origin was being investigated and despite the fact that such a small peptide as LKα14 attains a high net charge of +6 at neutral pH. Furthermore, peptide adsorption at the positively charged CaF_2 surface is also unlikely to be proven by the amide I band because the charge distribution of LKα14 should drive the peptide to favor the horizontal orientation on charged surfaces, which would lead to no SFG amide I signals detectible in neither ssp nor ppp polarization combinations.[6, 37]

3.3 SFG O-H stretch spectra of HAD solution at the air/solution interface in the 3000-3600 cm^{-1} regime.

To better clarify the spectral assignment of the 3080 cm^{-1} peak, we further reduced the electrostatic effect of the electrical double layer formed by the ionic surfactant and the counter ions. We then measured the SFG spectrum of the HAD adsorption layer in the 3000-3600 cm^{-1} regime. Due to the absence of the counter ions, the interfacial water layers did not experience any increase in orderliness due to the electrical double layer, thus no drastic SFG interfacial signal jumps were observed (Fig. 4). Again the peak at around 3080 cm^{-1} appeared when HAD was adsorbed at both the air/water and the air/saturated NaCl interfaces. Interestingly, two strong peaks at around 3150 cm^{-1} and 3335 cm^{-1} were observed when HAD was adsorbed at the air/water interface. These two peaks were significantly suppressed when water was replaced by saturated NaCl solutions, implying that the peaks originate from the vibrational modes of the interfacial water layers, not from the ammonium headgroups. Since HAD can form hydrogen bonds with the interfacial water molecules, its adsorption at the air/water interface likely alter the hydrogen bonding scheme of the interfacial water layer, which can be spectroscopically detected. Because the surfactant species being studied possess no secondary structure, we believe that the highly suppressible peaks at around 3150 cm^{-1} and 3330 cm^{-1} originate from the interfacial water molecules rather than the ammonium groups. Therefore, in the case of LKα14, the N^+-H stretch of the ε-amino group is expected to be at around 3080 cm^{-1}, and the peak at 3330 cm^{-1} can be attributed to either the interfacial water molecules or the backbone amide A of the peptide. It is worth mentioning that the existence and the exact peak location of the 3300 cm^{-1} peak was reported to be entirely dependent on the secondary conformation of the peptides,[14, 38] which is unlikely if it indeed originates from the sidechain ammonium groups. Lastly, if the 3300 cm^{-1} peak indeed originate from the interfacial water molecules, it is entirely plausible that the ^{15}N labelled ε-amino groups of LKα14 affected the interfacial water molecules differently than the unlabeled peptide, leading to the reported peak shift of 8 cm^{-1} reported by Castner et al.[10]

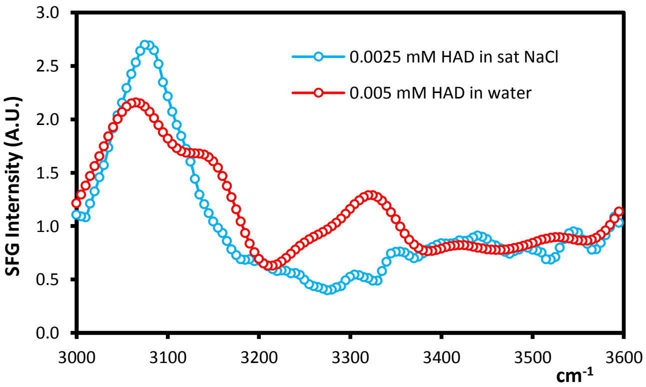


Figure 4. ssp SFG signals in the 3000-3600 cm^{-1} of HAD in water and saturated NaCl solutions.

Our present study is complementary to a recent study by Yan et al.[14], of which major short coming is the assumption that the amide backbone N-H stretching modes of an α-helical structure possess some chirality. We strongly believe that our study provides unprecedentedly direct experimental evidence for the spectral assignment of the 3080 cm^{-1} SFG peak.

4. Conclusions

In this study, we have presented a systematic SFG data set to shed light on the SFG peak assignment of the N$^+$-H stretching mode. We believe that our data on CTAB provides solid evidence that the peak at 3080 cm^{-1} originates from the N$^+$-H vibrations, in line with the existing IR and Raman data in the literature.[30] In addition, the suppressibility of the 3330 cm^{-1} peak obtained with HAD clearly demonstrates its affiliation with the vibrational modes of the interfacial water molecules. The present study was primarily aimed at clarifying the origin of the 3080 cm^{-1} and 3300 cm^{-1} peaks of ammonium species in SFG spectroscopy, further studies into the interfacial water structures are desirable using more advanced surface sensitive techniques.

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Notes

The authors declare no competing financial interests.

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