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Vibrational sum-frequency generation at protein modified air–water interfaces: Effects of molecular structure and surface charging

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ABSTRACT

Protein and surfactant modified air–water interfaces are an important model system for colloid science as many applications for example aqueous foams in food products rely on our knowledge and ability to tune molecular structures at these interfaces. That is because interfaces are a fundamental building block in the hierarchical structure of foam, where in fact the molecular level can determine properties on larger length scales. For that reason it is of great importance to increase our ability to study air–water interfaces with molecular level probes and to obtain not only information on coverage but also direct information on interfacial composition, molecular order, orientations as well as information on the charged state of an interface. Vibrational sum-frequency generation (SFG) is a powerful tool that can help to address these issues and is inherently surface sensitive. In this contribution we will review recent developments in the use of SFG for studies of biomolecules at aqueous interfaces and discuss current issues with the interpretation of SFG spectra from electrified interfaces. In order to guide interpretations from interface spectroscopy we invoke the use of complementary methods such as ellipsometry and zetapotential measurements of bulk molecules.

1. Introduction

In-situ studies of protein modified surfaces and interfaces on a molecular level are of great importance in various fields of research. In fact many macroscopic material properties such as the stability of aqueous foam can be controlled by interfacial layers of proteins. For that reason, tuning foam properties must start at the interface were intermolecular forces such as steric, electrostatic and van der Waals forces between protein, surfactant and solvent molecules determine the molecular structure of interfacial layers. Consequently, it is of great importance to address adsorption phenomena on a molecular scale and to reveal interfacial structures of surface adsorbed proteins, surfactants or mixtures of the latter. In many cases, interfaces that are being used in foam stabilization are electrified. Here, interfacial charging, arrangement and charge screening by H_2O and ions at the interface can dramatically influence intermolecular interactions. Addressing these issues is, therefore, at the very heart of current research not only in foams [1–5] but also for emulsions [6–8]. A molecular level understanding of aqueous interfaces has, however, much wider implications as it is equally important for other biomolecule interfaces e.g. in membrane proteins [9,10] or in catalysis [11]. Addressing the molecular structure of buried interfaces under relevant conditions is, however, a major challenge. Most experimental methods that are applicable under these conditions lack interface selectivity or do not have the sensitivity for low number densities of surface adsorbed molecules which are small compared to the number of bulk molecules. For that reason, spectroscopic signatures of interfacial molecules are overwhelmed by the bulk signal. Facing the latter two challenges requires both an increase in surface selectivity and sensitivity. Over the last decade second-order nonlinear optical (NLO) spectroscopy and in particular vibrational sum-frequency generation (SFG) as well as second-harmonic generation (SHG) have matured to highly versatile tools that can address many interfacial properties from molecular composition [12,13], orientation [14–16], molecular order [17], electronic structure [18] to surface charge and even molecular dynamics on ultra-short time scales [19–22]. As NLO spectroscopy provides useful information on these interfacial properties and is not limited to certain material properties e.g. surface roughness or noble metals like in SERS or SEIRAS, it has been employed to a large variety of buried interfaces such as solid–liquid [23], liquid–liquid [7], and liquid–gas interfaces at ambient conditions. Furthermore, NLO spectroscopy is not necessarily limited to planar interfaces, but can be used to address colloidal interfaces [24,25,7] as well. Here, additional information on particle size, charging and interfacial adsorption can be easily obtained.

Considerable progress has been made by introducing phase-sensitive SFG (PS-SFG) [26••], where the phase of vibrational bands in SFG spectra can be unambiguously measured and attributed to net molecular orientations of interfacial species that could either point up or downward with respect to the interfacial plane. The phase is

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therefore a direct measure of molecular orientation and such an explicit determination is only possible due to the coherent process of sum-frequency or second-harmonic generation (see Theoretical description of SFG at electrified interfaces section). This important trend in SFG spectra analysis is highlighted by the steadily increasing number of groups that employ phase sensitive SFG spectroscopy. A direct comparison of PS-SFG with conventional SFG can be found in a recent paper from the Bonn group [27••]. However, many studies have been reported that make use of relative changes in the phase between two vibrational bands to monitor e.g. charge reversal at electrified interfaces with a conventional SFG setup [3,28–31].

In this article we will first address recent developments in surface vibrational sum-frequency generation of biomolecules at electrolyte–air interfaces and provide an overview on strategies on how to resolve surface charging at protein and surfactant modified air–water interfaces.

2. Principles of broadband sum-frequency generation (SFG)

2.1. Theoretical description of SFG at electrified interfaces

The interaction of light with matter is in the case of high electric field strength not necessarily only linear, but can have strong nonlinear components which lead to a change in frequency and direction. In dipole approximation the polarization that is induced by an external electric field can be written as series of linear and nonlinear contributions:

$$P_\alpha\left(\vec{r},\omega\right) = \varepsilon_0 \sum_{\beta} \chi^{(1)}_{\alpha\beta} E_\beta + \varepsilon_0 \sum_{\beta\gamma} \chi^{(2)}_{\alpha\beta\gamma} E_\beta E_\gamma + \varepsilon_0 \sum_{\beta\gamma\delta} \chi^{(3)}_{\alpha\beta\gamma\delta} E_\beta E_\gamma E_\delta + \cdots \tag{1}$$

Here, $\chi^{(1)}_{\alpha\beta}$ and $\chi^{(2)}_{\alpha\beta\gamma}$ denote the linear and second-order electric susceptibility, respectively. Accordingly, the second-order polarization $P^{(2)}_\alpha$ is directly proportional to the second-order susceptibility which is strongly dependent on materials symmetry. In materials with centrosymmetry which can be also found in amorphous materials, liquids and gases in a time average $\chi^{(2)}_{\alpha\beta\gamma}$ vanishes in the bulk due to symmetry reasons (see below). Surfaces and interfaces, however, are a break in bulk symmetry and thus give rise to additional contributions to $\chi^{(2)}_{\alpha\beta\gamma}$ that are not equal to zero and are dominated by interfacial molecules and their nonlinear optical responses.

For sum-frequency generation the effective susceptibility $\chi^{(2)}$ of an interface can be written in the following form:

$$\chi^{(2)} = \chi^{(2)}_{\mathrm{NR}} + \sum\nolimits_q \frac{A_q e^{i\theta}}{\omega_q - \omega + i\Gamma_q} \quad . \tag{2}$$

Here, $\chi^{(2)}_{NR}$ is the non-resonant part and the sum term is the part of $\chi^{(2)}$ which is resonant with either of the two fundamental frequencies or with the radiated sum frequency wave. For SHG, two photons with identical frequencies ω are mixed at the interface and generate a third photon with a frequency of 2ω, while in vibrational SFG a photon with IR frequencies ω_{IR} and a photon with a frequency in the visible region ω_{vis} are mixed to generate the sum frequency $\omega_{SF} = \omega_{IR} + \omega_{vis}$. In general the amplitude or oscillator strength A_q is a function of net molecular orientations at an interface. Assuming, that $A_q = N\beta_q$ is the oscillator strength of an individual molecule with a hyper-polarizability β_q and the interfacial number density N, A_q can be expressed by an integral over all possible molecular orientations at the interface

$$A_q = N \int f(\Omega)\beta_q(\Omega)\, d\Omega. \tag{3}$$

In Eq. (3), $f(\Omega)$ reflects the orientation distribution of molecules that contribute to the qth vibrational mode and necessarily reflects the molecular order at an interface. Therefore, random orientations of molecules or molecular arrangements with centrosymmetrical structures in bulk liquids or gases will lead in dipole approximation to negligible intensities because the integral in Eq. (3) vanishes in this case. A perfectly ordered adsorption layer with well aligned molecules results, however, into the highest amplitude and SFG intensity. In case of a strong interfacial electric field, additional contributions due to an electric field induced break of inversion symmetry can be expected and particular those molecules which are most strongly affected by the exponentially decaying static field become SFG or SHG active. This effect can be expressed in terms of an effective second-order contribution and an additional third-order contribution to the second-order polarization:

$$P^{(2)}_{\omega_{SF}} = \varepsilon_0 \chi^{(2)} E_{\omega_1} E_{\omega_2} + \varepsilon_0 \chi^{(3)} E_{\omega_1} E_{\omega_2} \int_0^\infty E^{dc}(z)\, dz \tag{4}$$

where the static electric field $E^{dc}(z)$ of the electrified interface is averaged in the z direction perpendicular to the interface. Integration of Eq. (3) for $z = 0$ to the point where the interfacial field has decayed to zero leads to

$$P^{(2)}_{\omega_{SF}} = \varepsilon_0 \chi^{(2)} E_{\omega_1} E_{\omega_2} + \varepsilon_0 \chi^{(3)} E_{\omega_1} E_{\omega_2} \Phi(0) \tag{5}$$

with $\Phi(0)$ being the surface potential. Eq. (5) directly implies that electric field induced effects at charged interfaces can dramatically affect second-order signals. This can be the result of pure isotropic $\chi^{(3)}$ contributions as shown in Eqs. (4) and (5) which are direct measures of interfacial electric fields. Field induced reorientation of molecular dipoles such as H_2O at the interface can also lead to substantial changes in $\chi^{(2)}$. These originate from interfacial molecules that become increasingly aligned within the interfacial electric field and result in an increase in orientational average (Eq. 3). Such an orientation polarization due field alignment of interfacial dipoles is only possible at an electric field strength $\gg 10^8$ V/cm because otherwise kT at room temperature largely exceeds the energy that is gained by such a field induced realignment. Field strength necessary for these effects to occur can be, however, only found within the first hydration shell of small ions. In any case, it is rather challenging for the experimenter to retrieve information on the actual origin of this field induced contribution and certainly needs to be addressed in future experimental and theoretical works. However, independent on its molecular origin, electric field induced effects are restricted to molecules near the interface where the electric field is strong. Otherwise both field induced orientation and isotropic $\chi^{(3)}$ effects become negligibly small. Thus, independent of their origin they can serve as indicators of the charging behavior at interfaces.

2.2. Experimental details of SFG

Below we will discuss the most commonly used variants of current SFG spectrometers which are conventional broadband SFG and phase-sensitive SFG. Narrowband SFG where narrowband visible and IR pulses are used to generate the SFG beam, is also in use by many groups. Here, a comparatively inexpensive setup can be of advantage, while rapid acquisition of SFG spectra is often hampered due to low repetition rates and low peak intensities. There is also considerable effort to extend the capabilities of SFG to a two-dimensional spectroscope [32•,20•] where molecular dynamics on ultra-fast time scales can be addressed and to achieve a high resolution of <1 cm^{-1} [33]. As these topics are outside the scope of this paper we refer to excellent descriptions of the latter in the references noted above.

2.2.1. Conventional broadband SFG

One of the most advanced techniques is broadband sum-frequency generation which typically uses an amplified Ti:sapphire femtosecond laser. The amplifier system in our lab consists of a femtosecond oscillator (MaiTai, Spectra Physics) that produces 100 fs pulses at a rate of 80 MHz and several nJ pulse energy. The oscillator is used as a seed laser for a Spitfire XP (Spectra Physics) chirped pulse amplifier (CPA)