

Vibrational characterization of a planar-supported model bilayer system utilizing surface-enhanced Raman scattering (SERS) and infrared reflection–absorption spectroscopy (IRRAS)

Chad L. Leverette^{a,*}, Richard A. Dluhy^b

^a Department of Chemistry and Physics, University of South Carolina Aiken, Aiken, SC 29801-6399, USA

^b Department of Chemistry, University of Georgia, Athens, Georgia 30602-2556, USA

Received 28 October 2003; accepted 13 May 2004

Available online 28 July 2004

Abstract

A combined spectroscopic method utilizing surface-enhanced Raman scattering (SERS) and infrared reflection–absorption spectroscopy (IRRAS) has been employed to study the structural complexities of a model bilayer system. These two vibrational spectroscopic techniques offer complementary information, and when used in concert, present an extremely powerful tool for the structural elucidation of ultrathin monomolecular assemblies. The success of this analysis approach is based on the use of a unique metal film planar-support that has a total thickness that is amenable for IRRAS analysis while providing an ideal surface conducive for surface-enhanced Raman scattering experiments. Therefore, the same thin film system can be monitored by both techniques. In this study, these metal planar-supports, composed of optimized vapor-deposited Ag films, were prepared and monitored following a procedure previously developed [J. Phys. Chem. B, 106, 2002, 8747]. Monitoring these films is essential to ensure a reproducible rough surface morphology that gives rise to the greatest surface enhancements for SERS. The model bilayer systems studied are comprised of (1) an inner monolayer composed of long hydrocarbon chain alkanethiols, and (2) an outer monolayer composed of the phospholipid, 1,2-[d₆₂]-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC-d₆₂). Two types of monomolecular architecture techniques are employed to form these bilayers—self-assembly and Langmuir–Blodgett transfer. The first step in the preparation of these films involves the self-assembly of the alkanethiol onto the Ag surface. The formation of this stable chemisorbed monolayer produces a hydrophobic surface that acts as the driving force for the organization of the lipid monolayer. The hydrophobic surface comprised of the long alkyl chains of the alkanethiol forces the lipid to transfer alkyl chains down leaving the polar headgroup portion of the lipid molecule exposed. Deuteration of one of the phospholipid layer is performed to allow each monomolecular layer of the bilayer to be spectroscopically observed separately. The Raman experiments utilize a unique fiber-optic interface that provides high collection efficiency and sensitivity for monolayer samples. From the dual spectroscopic analysis performed on this model bilayer system, it appears that the inner 1-dodecanethiol monolayer undergoes a slight disordering upon addition of the outer phospholipid monolayer. This disordering is observed with spectral intensity changes for the $\nu(\text{C-S})$ gauche (G) and trans (T) bands at 633 and 705 cm^{-1} , respectively, the $\nu(\text{C-C})$ stretches between 1060 and 1130 cm^{-1} , and the vibrations associated with the terminal methyl group, $\nu(\text{CH}_3)$, based on the SERS and IRRAS analysis. Even though the inner layer does undergo this slight conformational change, it appears to maintain its structure as an overall ordered assembly. The outer DPPC-d₆₂ monolayer was transferred in the gel state to the alkanethiol surface and maintains its high degree of conformational order after the completion of the transfer. Spectral data as well as calculated spectral band intensity ratios are presented that show the power of this dual spectroscopic approach to the structural determination of a model bilayer system.

© 2004 Elsevier B.V. All rights reserved.

Keywords: Bilayer; Self-assembly; Langmuir–Blodgett; Surface enhanced raman scattering; Infrared reflection absorption spectroscopy

1. Introduction

Biophysical monomolecular films represent a fundamental area of research in the fields of biological, physical, and materials science. Applications based on ultrathin (≥ 2.5 nm) film systems are numerous and have been observed in the

* Corresponding author. Tel.: +1-803-641-3291;
fax: +1-803-641-3382.
E-mail address: chadl@usca.edu (C.L. Leverette).

areas of lubrication and corrosion inhibition, fabrication of semiconductors, novel sensor technology, electronics, bioprocessing, programmed drug delivery, catalytic interfaces, and stable cytomimetic biomaterials [2–6]. One particularly useful thin film geometry is a bilayer system consisting of two monomolecular films. A model bilayer mimics complex biological membranes and provides an architecture to study the complexities of these physiologically relevant systems [7]. Due to the vast applications provided by thin films systems from both the biological and material science perspective, development of techniques capable of elucidating the structural complexities of these systems as well as gaining insight into the relationship between structure and function is very desirable.

For production of a model bilayer system, preparation includes the use of monolayer techniques (i.e. Langmuir–Blodgett (L–B), self-assembly, and vesicle fusion) [2]. These techniques produce model bilayers that are adsorbed onto solid supports. The overall significance of the use of these architectural design techniques lies in the ability to engineer surfaces where the components that constitute the surface are easily controlled, modified, and assembled. By simply varying conditions, the researcher has the ability to preferentially choose the composition and physical state of the molecules in the monolayer. This capability allows the experimenter to tailor a surface for a specific purpose. Membrane architecture comprised of monomolecular films can be formed that mimics physiologically relevant biomembranes. In addition, the planar nature of these model systems is extremely important and allows for the investigation of soluble components within the bilayer model system, which is not an option when studying colloidal dispersions [8]. Current examples of planar-supported bilayer surfaces include assembled bilayers prepared by L–B transfer [9,10] and vesicle fusion [4,11–13] onto hydrophilic and hydrophobic surfaces as well as the fabrication of phospholipid-containing bilayers on gold through the use of thiol-terminated supporting layers [5,14–20].

Although biophysical monolayers have been extensively used as models for native biomembranes and interfacial systems, much work still remains to structurally characterize the detailed organization and function of the monolayers present in these systems. One particular analysis methodology that has established itself as an important tool to study order/disorder processes in thin films systems is vibrational spectroscopy [21]. Specifically, the use of IR and Raman techniques for the characterization of monolayer systems is appreciable due to the excellent sensitivity these techniques provide for monitoring conformational order and organization [8]. Because of its ease of experimental design and manipulation of experimental variables, IR spectroscopy has been more widely utilized to investigate the complexities of thin film surfaces and interfaces [8]. However, Raman spectroscopy is equally powerful when used to analyze thin films due to its sensitivity to internal molecular structure, the chemical nature of the molecule, and the local molecular

environment [22]. In addition, due to the different selection rules that govern IR and Raman spectroscopy, these techniques are complementary providing insight into the various polar and non-polar moieties of a given molecule not observed solely with one vibrational technique.

One of the most widely utilized forms of vibrational spectroscopy applied to planar-supported thin film systems is infrared reflection–absorption spectroscopy (IRRAS). IRRAS has been successfully applied to numerous studies monitoring the conformational change of monolayer systems on metal substrates [23]. Due to the fact that IRRAS is most efficient on reflective surfaces, it was believed that this technique could only be used to analyze thin film systems on metallic substrates; however, due to advances in instrumentation and experimental geometries, applications of IRRAS for monitoring samples on dielectric surfaces are appearing in the literature [24]. When using metallic surfaces, the surface selection rule of IRRAS, which states that only vibrational dipole moments oriented perpendicular to the surface will be observed, uniquely separates this technique as a powerful tool for the analysis of functional group orientation in supported monolayer films.

As previously discussed, Raman spectroscopy is also very sensitive to conformational order/disorder processes in biophysical monolayer systems. The primary limiting factor to the widespread application of Raman spectroscopy to the analysis of thin films is the ability to detect the weak Raman scattering generated from thin film systems [8]. To circumvent this limitation, the spectroscopic sensitivity of the technique can be enhanced through surface strategies (surface-enhanced Raman scattering, SERS), resonance conditions (resonance Raman spectroscopy, RRS), or waveguide sampling geometries (waveguide Raman spectroscopy, WRS). Surface-enhanced Raman scattering offers routine enhancements of 10^4 – 10^6 for thin film analysis making this approach attractive for thin film analysis. The primary drawbacks of SERS for thin film analysis involve producing reproducible planar-supports composed of oblate metal particles that produce the needed enhancements as well as the difficulties that can be found in the interpretation of SERS spectra. Although the interpretation can be more difficult, SERS also has a surface selection rule that states that vibrations with tensor components oriented along the vertical axis of the metal particles on the surface will be enhanced the most. Therefore, specific information regarding the packing and orientation of biophysical molecules on the surface of the metal particles is available through the utilization of SERS. A great deal of pioneering work in the use of SERS for thin film analysis has been reported by Aroca and co-workers [25–28]. The most recent SERS work by this group involves spatially mapping mixed L–B films physically adsorbed onto silver island films [27].

In this study, a model planar-supported bilayer is formed and composed of an inner layer of 1-dodecanethiol self-assembled onto a Ag surface and a L–B transferred 1,2-[d₆₂]-dipalmitoyl-sn-glycero-3-phosphocholine

(DPPC-d₆₂) phospholipid outer layer. This model bilayer system has a relevant architecture similar to thin films systems used in a variety of applications. This model bilayer is characterized using SERS and IRRAS on the same film. This approach provides a detailed study on the conformational stability of the inner monolayer upon transfer of the outer layer as well as information regarding the structure of the individual monolayers upon formation of a bilayer system. Conformational stability is defined as the preservation of the preferred conformation of the inner, self-assembled, 1-dodecanethiol monolayer upon transfer of the outer, phospholipid layer. In this study, a stable bilayer surface was produced where the properties of the films were easily controlled. The stability arises from the use of a well-ordered self-assembled alkanethiol monolayer chemisorbed onto the Ag surface. This surface acts as the driving force for the formation and organization of the phospholipid. In this experiment, the Ag substrate has been optimized for SERS experiments [1] and offers a non-traditional thick metal surface that can be easily used to obtain infrared spectra of thin films using IRRAS. The advantage of this metal film over previous studies is that the same metal support can be used for difficult thin film analysis utilizing both spectroscopies. This combination of spectroscopies on the same sample allows for detailed characterization of the supported model bilayer.

2. Materials and methods

2.1. Reagents

Water was deionized, distilled and filtered with a Barnstead Nanopure reverse osmosis/deionization system to a resistance of 18.2 M Ω and was used throughout. A Piranha solution consisting of 1:4 30% H₂O₂/A.C.S. reagent grade concentrated H₂SO₄ (J. T. Baker) was used to clean all glassware. (3-Mercaptopropyl)trimethoxysilane (97%) (MPS) (Alfa Aesar, Ward Hill, MA) and HPLC grade 2-propanol (J. T. Baker) were used for the pretreatment of the glass substrate. Silver (100% purity) wire was obtained from SPI (West Chester, PA). 1-dodecanethiol (98%) (Aldrich) and 200 proof ethanol (Pharmco, Brookfield, CT) were purchased for the self-assembly of molecular monolayers. 1,2-[d₆₂]-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC-d₆₂) (Avanti Polar Lipids, Alabaster, AL) was purchased and transferred by the Langmuir–Blodgett approach onto the hydrophobic alkylated metal film. All reagents were used as received.

2.2. Substrate preparation and the formation of vapor-deposited silver island films (AgIF) metal substrates

The preparation and vapor-deposition of the metal substrate surface used for experiments followed a procedure previously developed [1]. Briefly, glass microscope slides

(VWR Scientific; cut to dimensions of 1.5 cm \times 1.5 cm) were pretreated with the coupling reagent, MPS, and were directly used for the vapor-deposition of the AgIF. The reproducibility of these metal surfaces was monitored with atomic force microscopy (AFM) and Ellipsometry (data not shown). AFM monitors the surface morphology, while Ellipsometry monitors the overall Ag thickness. Typical substrate total thickness was ca. 70 nm with a surface roughness predominantly composed of fairly isolated, oblate Ag particles with diameters between 30–40 nm. Both of these characteristics of the Ag film are of extreme importance to ensure an optimal surface that gives rise to the largest enhancements in SERS. Once the metal film was formed, the surface was immediately used as the support for the model bilayer system.

2.3. Self-Assembly of 1-dodecanethiol

The AgIF surfaces were immersed in a 15 mM 1-dodecanethiol solution in 100% ethanol for at least 12 h to allow the self-assembled monolayers to form. These surfaces were then removed from the solution, rinsed carefully with copious amounts of 100% ethanol, and allowed to completely air dry before analysis of the surface took place. Both SERS and IRRAS analysis were conducted on this surface prior to the L–B transfer of the outer DPPC-d₆₂ layer.

2.4. Langmuir–Blodgett transfer of a DPPC-d₆₂ monolayer

DPPC-d₆₂ was dissolved in chloroform at a final concentration of 1 mg/mL. A single Langmuir–Blodgett (L–B) monolayer of DPPC-d₆₂ was deposited onto the Ag metal surface made hydrophobic by the self-assembled monolayer of 1-dodecanethiol using a Joyce–Loebl (Gateshead, England) Langmuir–Blodgett trough. The alkylated metal substrate was immersed into the subphase, after which an aliquot of the DPPC-d₆₂/chloroform stock solution was spread on the water surface. The surface was then allowed to equilibrate for at least 10 min before the start of film compression. The L–B trough consisted of a pure water subphase made from the Barnstead Nanopure system, and was thermostatted to 22 °C. After the equilibration period, the monolayer was compressed to the desired final surface pressure of 45 mN m⁻¹ at a rate of 0.5 cm² min⁻¹. The substrate was then vertically raised through the compressed monolayer film at a rate of 20 mm min⁻¹, while the surface was held constant (estimated deviation from the set pressure, $\leq \pm 1$ mN m⁻¹), and the monolayer was deposited in a single pass through the interface. Deposition of the single monolayer was assured by monitoring the transfer ratio for each substrate. After the L–B deposition, the substrate consisting of an inner layer of 1-dodecanethiol and an outer layer of DPPC-d₆₂ was spectroscopically analyzed using both SERS and IRRAS techniques on the same sample.

2.5. Surface-enhanced Raman scattering

The Raman system used for the collection of the surface enhanced Raman spectra is described in detail elsewhere [22]. The excitation was incident on the sample at an angle of 60° and was produced by the 514.5 nm line of a Coherent Radiation Innova 300 Series Ar⁺ laser. The typical laser power at the sample was 150 mW with an average spot size of 30 μm in diameter. The scattered radiation was collected at the surface normal through the optical interface designed in our lab [22]. Integration times were typically 60 s with five coadds. The spectrometer is an ISA 500M f/4 spectrometer equipped with a Spectrum One CCD2000 charge-couple device detector (Instruments SA, Edison, NJ). The CCD chip (SITe ST-005A) is 2000×800 pixels in size, back-illuminated, with an individual pixel size of 15 μm . The detector is liquid nitrogen cooled to a temperature of -140°C and is back thinned giving a quantum efficiency of 85% at 550 nm. The grating used in the spectrometer had 1200 grooves/mm with a blaze wavelength of 750 nm. The slit widths used in the experiments were typically 0.30 mm. Wavelength calibration was performed using a second order polynomial fit to Ne atomic emission lines [29] using a grams-based program written in our laboratory; wavenumber precision is estimated at better than $\pm 1 \text{ cm}^{-1}$.

2.6. Infrared reflection–absorption spectroscopy (IRRAS)

The infrared spectra were collected on a Bio-Rad Fourier Transform Infrared Spectrometer FTS-60 (Bio-Rad, Digilab Division, Cambridge, MA) equipped with an external reflection accessory and a narrow-band, liquid N₂ cooled HgCdTe detector (Infrared Associates, Orlando, FL). The radiation was p-polarized at an angle of incidence equal to 80° on the Ag metal surface. Typically, 1024 scans were recorded per spectrum at 4 wavenumber (cm^{-1}) resolution using a triangular apodization and one level zero filling.

2.7. Spectral data analysis

Post-processing (i.e. baseline correction, normalization, and CCD spike removal) of all data was performed using the Grams/32 software package (Galactic Industries, Nashua, NH). The baseline correction consisted of linear fit of the data in the C–C, C–H, and C–D stretching spectral regions. Normalization of the data was performed by dividing by the band height intensity of particular spectral bands insensitive to the changes occurring during film formation. The bands chosen for normalization varied for the FTIR and Raman analysis. Descriptions and validation of the bands used for normalization is provided in the results section. For determination of frequency position and band height intensity as well as calculation of the spectral band intensity ratios, a Grams-based program was used to calculate the peak heights and peak positions of the Raman and FTIR spectral bands.

3. Results and discussion

A dual-layer, thick (ca. 70 nm), vapor-deposited silver substrate was used as the support for the model bilayer system. Although the metal film has a total thickness of 70 nm, the outermost surface of the film mimics a traditional, thin, vapor-deposited silver film that is conducive for SERS analysis. In addition, the thick nature of the substrate allows for analysis utilizing IRRAS. The success of this study is based on the unique properties of this non-traditional metal substrate. The details of this unique substrate geometry have been described previously [1]. Basically, the substrate consists of an overlayer/underlayer Ag structure vapor-deposited on glass. After formation of the ideal silver island film geometry, the substrate was removed from vacuum and immediately used for the self-assembly of the alkanethiol inner monolayer. After the inner monolayer was self-assembled on the metal substrate and characterized via SERS and IRRAS, a second outer monolayer composed of the phospholipid, DPPC-d₆₂, was transferred via the Langmuir–Blodgett technique onto the substrate. From the vibrational analysis, the momentary dip of the substrate through the subphase of the Langmuir film did not perturb the metal surface appreciably as determined by spectral band intensities. It should be noted, however, that it was observed that prolonged contact of the metal substrate with the subphase of a Langmuir film would alter the properties of the metal film. Stability of the substrates with prolonged deposition time is being investigated and will be reported at a later date. In general, after prolonged exposure, the water subphase appears to remove the silver island films from the metal surface, thus completely changing the characteristics of the substrate. Immersion time of the metal substrate in the subphase should be brief in order to maintain the integrity of the metal surface. In these experiments, a single monolayer was transferred onto the substrate; therefore, the time the substrate interacted with the subphase was minimal.

Figs. 1 and 2 show representative SERS spectra of a self-assembled monolayer (SAM) of 1-dodecanethiol chemisorbed to the Ag metal substrate before and after the L–B deposition of a DPPC-d₆₂ monolayer in the $\nu(\text{C–H})$ region as well as the low wavenumber region. Fig. 1(a) is a spectrum of a self-assembled monolayer of 1-dodecanethiol chemisorbed onto the Ag metal substrate in the $\nu(\text{C–H})$ stretching region before the addition of the DPPC-d₆₂ layer. Fig. 2(a) is a spectrum of the same film as in Fig. 1(a) except in the $\nu(\text{C–C})$ region. Fig. 3 is the IRRAS spectra ($\nu(\text{C–H})$ stretching region) of a self-assembled monolayer of 1-dodecanethiol before and after the L–B deposition of the deuterated lipid. Fig. 3(a) represents the self-assembled alkanethiol monolayer prior to the L–B deposition of the outer layer. Table 1 lists the vibrational bands and their relative frequencies for 1-dodecanethiol chemisorbed onto a Ag metal surface for both the SERS and IRRAS spectra.

In previous work by Bryant and Pemberton [30], a possible orientation of 1-dodecanethiol is given for a

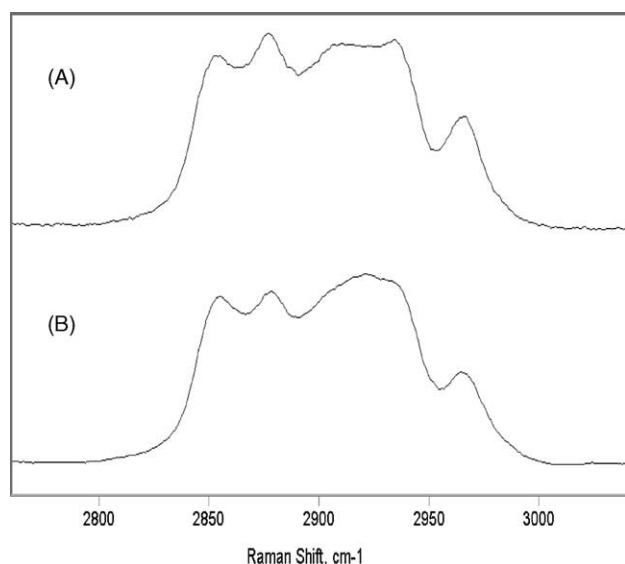


Fig. 1. (A) A representative Raman spectrum of a self-assembled molecular monolayer of 1-dodecanethiol chemisorbed onto the Ag metal substrate in the $\nu(\text{C-H})$ stretching region before the addition of the DPPC- d_{62} outer layer. (B) A representative Raman spectrum of a self-assembled molecular monolayer of 1-dodecanethiol chemisorbed onto the Ag metal substrate in the $\nu(\text{C-H})$ stretching region after the addition of the DPPC- d_{62} outer layer.

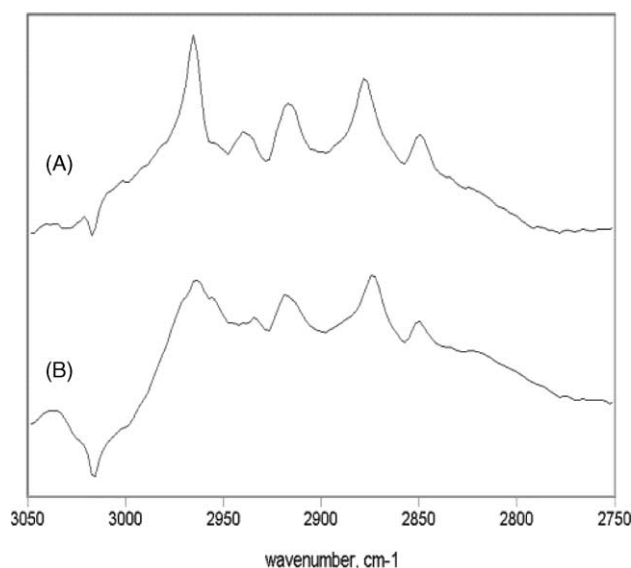


Fig. 3. (A) A representative IRRAS spectrum of a self-assembled molecular monolayer of 1-dodecanethiol chemisorbed onto the Ag metal substrate before the addition of the DPPC- d_{62} outer layer. (B) A representative IRRAS spectrum of a self-assembled molecular monolayer of 1-dodecanethiol chemisorbed onto the Ag metal substrate after the addition of the DPPC- d_{62} outer layer.

self-assembled monolayer on roughened Ag surfaces. The proposed orientation of 1-dodecanethiol describes the C-S bond and the C-C backbone of 1-dodecanethiol to be predominately perpendicular to the surface with the plane of

the backbone slightly tilted ca. 15° . The methylene groups are estimated to be parallel to the surface in this orientation. The authors also suggest that the molecule is rotated about the chain axis ca. 45° . This projected orientation

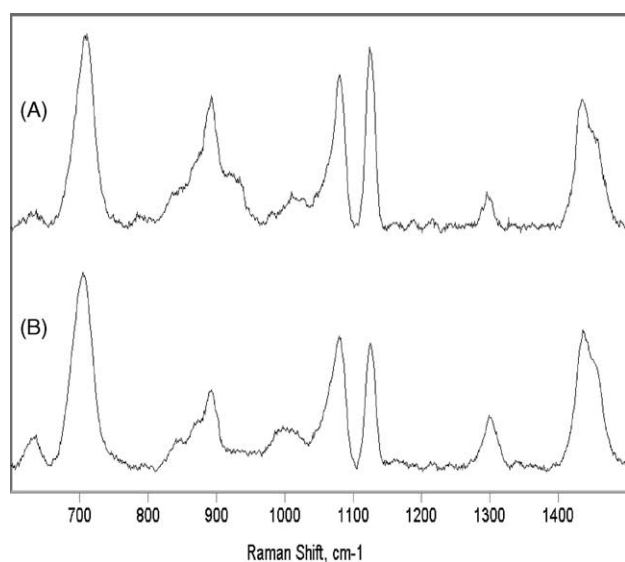


Fig. 2. (A) A representative SERS spectrum of a self-assembled molecular monolayer of 1-dodecanethiol chemisorbed onto the Ag metal substrate in the $\nu(\text{C-C})$ stretching region before the addition of the DPPC- d_{62} outer layer. (B) A representative SERS spectrum of a self-assembled molecular monolayer of 1-dodecanethiol chemisorbed onto the Ag metal substrate in the $\nu(\text{C-C})$ stretching region after the addition of the DPPC- d_{62} outer layer.

Table 1

The vibrational bands and their relative frequencies for the SAMs of 1-dodecanethiol chemisorbed onto the Ag metal surfaces for both the SERS and IRRAS spectra

| Band assignments ^a for SAMs of 1-dodecanethiol | SERS (cm^{-1}) (observed) | IRRAS (cm^{-1}) (observed) |
|---|--------------------------------------|---------------------------------------|
| $\nu(\text{C-S})_G$ | 633 | |
| $\nu(\text{C-S})_T$ | 705 | |
| CH_2 rock | 789 | |
| | 843 | |
| | 870 | |
| CH_3 rock _T | 893 | |
| | 930 | |
| | 1015 | |
| $\nu(\text{C-C})_T$ | 1064 | |
| $\nu(\text{C-C})_G$ | 1080 | |
| $\nu(\text{C-C})_T$ | 1126 | |
| CH_2 twist | 1298 | |
| δCH_2 bend | 1430–1450 | |
| $\nu_s(\text{CH}_2)$ | 2852 | 2849 |
| $\nu_s(\text{CH}_3)$ | 2877 | 2878 |
| $\nu_a(\text{CH}_2)$ | 2904 | 2916 |
| $\nu_s(\text{CH}_2, \text{FR})$ | 2921 | |
| $\nu_s(\text{CH}_3, \text{FR})$ | 2936 | 2939 |
| $\nu_a(\text{CH}_3, \text{op})$ | | 2956 |
| $\nu_a(\text{CH}_3, \text{ip})$ | 2965 | 2965 |

G, gauche; T, trans; ν_a , antisymmetric vibration; ν_s , symmetric vibration.

^a Assignments taken from ref. 30–32, and 42.

allows the terminal methyl group to become primarily parallel to the surface of the film. Due to the surface selection rules of SERS, orientation of adsorbed molecules can be determined. The surface selection rule for a SERS active Ag surface with excitation red of the surface plasmon is that vibrations of the molecules with components along the surface normal (z axis) will have greater enhancement than vibrations without a z tensor component. Bryant and Pemberton [30] showed from their data that the orientation of the adsorbed molecules of 1-dodecanethiol must be along the surface normal because of the intense bands observed for the $\nu(\text{C-S})$ headgroup, $\nu(\text{C-C})$ backbone, and the $\nu(\text{CH}_3)$ methyl group. The methylene groups of each alkyl chain have their vibrational components orthogonal to the C–C backbone and hence to the surface normal and will only be weakly enhanced. The conclusion that the terminal methyl group is oriented parallel to the surface was determined because the antisymmetric methyl in plane vibration, $\nu_a(\text{CH}_3, \text{ip})$ and the symmetric methyl vibration, $\nu_s(\text{CH}_3)$, are both observed in the spectra of 1-dodecanthiol adsorbed onto the Ag surface. These modes are orthogonal to each other, indicating that their respective tensor components are in two separate planes. Therefore, if both modes are observed, then the methyl group must be tilted in such away as to have a portion of each of the tensor components for these two bands to be partially aligned with the surface normal. In fact, the greater intensity for the $\nu_a(\text{CH}_3, \text{ip})$ mode relative to the $\nu_s(\text{CH}_3)$ mode suggests that the methyl group is oriented more parallel to the surface. In comparison, it has been reported that tilted long alkyl chain thiol compounds that bond to the surface in a similar manner through the S atom, will have a preferential orientation of the terminal methyl group depending on the number of carbons in the backbone [31,32]. For an even number of carbons, like in 1-dodecanthiol, if the Ag–S–C bond angle is ca. 110° with the Ag–S bond primarily perpendicular to the surface, the terminal methyl group will have a symmetric methyl stretch with a transition dipole that is perpendicular to the surface. This implies that the terminal methyl group would be aligned perpendicular to the surface. In this geometry, the orthogonal in plane and out of plane methyl vibrations will not be observed due to the fact that their transition dipoles are oriented parallel to the surface. In the odd number of carbons case, given the same bonding geometry at the metal surface, the in plane and out of plane methyl vibrations will be observed because the orientation of the terminal methyl group will be more parallel to the surface. Therefore, sensitive orientation information utilizing vibrational spectroscopy can be gleaned from observation of the methyl vibrations. For an alkanethiol compound perfectly perpendicular to the surface of a Ag film, with a Ag–S–C bond angle equal to 145° , the terminal methyl group would be slightly tilted towards the surface allowing tensor components for the $\nu_a(\text{CH}_3, \text{ip})$, $\nu_a(\text{CH}_3, \text{op})$, and the $\nu_s(\text{CH}_3)$ vibrations to be observed [2]. The spectral band intensities and frequency positions observed from Figs. 1(a) and 2(a)

agree with the proposed orientation derived by Bryant and Pemberton [30].

In addition, the authors propose a highly ordered packing arrangement for different chain length alkanethiols on the Ag surface including monolayers of 1-dodecanethiol. Due to the fact that 1-dodecanethiol ($\text{CH}_3-(\text{CH}_2)_n\text{-SH}$ with $n = 11$) as well as other longer chain alkanethiols bond through the S atom, the alkyl chain of a given molecule is allowed to interact with adjacent alkyl chains of neighboring molecules through van der Waals forces. Minimization of the free energy of the monolayer governs the ordering of the alkanethiol molecules into a dense packing arrangement [30]. The primary difference between SAMs of 1-dodecanethiol and SAMs of other longer chain alkanethiols is that the SAMs of 1-dodecanethiol possess a higher degree of gauche conformers as compared to the SAMs of the other longer chain alkanethiols. The spectral data shown in Figs. 1(a) and 2 (a) are indicative of a well-ordered packing arrangement.

Further insight into the orientation of the self-assembled monolayer of 1-dodecanethiol can be obtained by employing the technique of infrared reflection–absorption spectroscopy (IRRAS). Only limited infrared vibrational information is obtained for this particular thin film due to IR's inherently weak sensitivity to bonds that do not exhibit a large dipole moment. In general, IR is sensitive to bonds that have an appreciable dipole (O–H, N–H), whereas Raman is stronger for bonds that do not (C–C, C–S) [33]. For the inner alkanethiol layer, Raman spectroscopy characterizes most of the moieties of the molecule; however, using IRRAS, only the C–H stretches of the alkanethiol can be observed. Nevertheless, orientation information can be obtained from the $\nu(\text{C-H})$ stretching region. In external reflection infrared spectroscopy, certain selection rules do exist for an adsorbate on a metal surface. The magnitude of the absorbance is proportional to the mean-square electric field and to the dipole moment derivative of the vibrational mode with respect to the normal coordinate of the vibration. This relationship is expressed in the following equation:

$$A \propto |M \times E|^2 \quad (1)$$

At highly reflective surfaces (i.e. Ag, Au, etc.), the mean-square electric field is anisotropic. This condition leads to the ability of determining functional group orientations for thin organic films adsorbed onto highly reflective surfaces [34]. Maximum detection at a highly reflective surface occurs when p-polarized light is used at near-grazing angles of incidence ($\sim 80^\circ$). Under these conditions, the electric field vector is oriented perpendicular to the surface (i.e. in the z -direction). Therefore, equation 1 can be rewritten into the form:

$$A \propto |M \times z|^2 \propto \cos^2 \theta \quad (2)$$

where θ is the angle of orientation of the vibrational mode with respect to the surface normal. Therefore, the most strongly excited vibrations will be those with dipole transitions that are oriented perpendicular to the surface [34].

Fig. 3 (a) and (b) are representative IRRAS spectra of a self-assembled monolayer of 1-dodecanethiol chemisorbed onto the same Ag surface before and after the addition of the outer DPPC-d₆₂ layer in the $\nu(\text{C-H})$ stretching region. It is important to mention that, in Fig. 3 (a) the antisymmetric in plane methyl vibration, $\nu_a(\text{CH}_3)$, at 2965 cm^{-1} and the symmetric methyl stretch, $\nu_s(\text{CH}_3)$, at 2878 cm^{-1} are much more intense than the 2916 cm^{-1} antisymmetric methylene stretch, $\nu_a(\text{CH}_2)$, and the 2850 cm^{-1} symmetric methylene stretch. Based on the surface selection rule for IRRAS on highly reflective metal surfaces, the $\nu_a(\text{CH}_3, \text{ip})$ is stronger in intensity than the $\nu_s(\text{CH}_3)$ mode further supporting the fact that the terminal methyl group is oriented somewhat parallel to the surface. The methylene stretches are almost orthogonal to the surface normal and, therefore, only weakly observed.

The combined evidence from the SERS and IR-RAS data suggests that the self-assembled monolayer of 1-dodecanethiol exists in a well-ordered, nearly all trans configuration prior to the adsorption of the outer layer of DPPC-d₆₂. This data further confirms previous studies that the self-assembly of alkanethiols on metal surfaces produces well-ordered, well-defined hydrophobic surfaces [1,2,30–32,35–42].

A DPPC-d₆₂ Langmuir monolayer was transferred to the surface of the planar-supported monolayer of 1-dodecanethiol. The alkanethiol surface acts as the driving force for the formation and organization of the outer phospholipid film. This organization results in a planar-supported bilayer with the headgroups of the deuterated phospholipid exposed.

Since both layers of the bilayer system contain alkyl chains, it is impossible to spectroscopically separate the orientation information obtained from the $\nu(\text{C-H})$ stretching region, if both layers are composed of hydrogenated alkyl chains. A common tool employed in analyzing bilayer systems is to deuterate the alkyl chains of one of the layers. Due to the mass differences between ^1H and ^2H (sometimes written as ^2D), the vibrational modes for the C–H moiety appear at different frequencies than those for C–D, therefore enabling the structural changes in both layers to be studied. The substitution of ^1H by ^2H causes negligible changes to the structure and function of the molecules in the respective layer [7]. Deuteration of the phospholipid DPPC was chosen due to the commercial availability of this compound. Future studies will include the deuteration of the alkanethiol (inner layer) and the L–B transfer of a hydrogenated phospholipid.

Figs. 1(b) and 2(b) are representative of the vibrational bands of 1-dodecanethiol after the addition of the outer DPPC-d₆₂ layer. As the phospholipid layer adsorbs onto the surface, it is proposed that the alkanethiol molecules undergo a conformational change that is observed as increased gauche conformations within the carbon backbone, sulfur metal headgroup, and the terminal methyl. Several observable differences are identified for certain vibrational bands of the alkanethiol inner layer after the addition of the outer

layer. Most of the differences appear in the vibrational bands associated with the Ag–S–C bond and the terminal methyl group of the 1-dodecanethiol self-assembled monolayers. The terminal methyl group is oriented in an ‘umbrella’ configuration that has vibrational tensor components partially aligned along the surface normal. Due to the SERS selection rule previously discussed, it is clear that methyl bands will be some of the most enhanced in the SERS spectra due to their orientation even though this moiety exists farther away from the Ag surface. This is seen in Fig. 1 (a) in the $\nu(\text{C-H})$ stretching region. In this spectrum, the symmetric methyl band, $\nu_s(\text{CH}_3)$, at 2876 cm^{-1} and the antisymmetric in plane methyl band, $\nu_a(\text{CH}_3, \text{ip})$, are dominant over the methylene vibrations. The $\nu_s(\text{CH}_3, \text{FR})$ at 2936 cm^{-1} is also a dominant band for a self-assembled monolayer of 1-dodecanethiol on a Ag surface. A decrease in the intensity of these bands upon the addition of the outer DPPC-d₆₂ layer, as shown in Fig. 1 (b), is indicative of a disordering of the alkyl chains of the monolayer of 1-dodecanethiol. The shifting away from the surface normal leads to a decrease in the intensity of these bands. Evidence for this decrease in intensity is observed in Fig. 1 (b) for the methyl vibrational bands. More specifically, the symmetric stretch methyl band at 2876 cm^{-1} and the fermi resonance band associated with the methyl group at 2936 cm^{-1} slightly decrease upon addition of the outer layer. The antisymmetric in plane methyl vibration at 2964 cm^{-1} also decreases upon addition of the outer layer. A ratio of the symmetric methyl vibration and the antisymmetric in plane methyl vibration was calculated for several samples to verify the change in conformation for the terminal methyl group. This ratio changed from 1.76 ± 0.06 for the alkanethiol films without a transferred outer layer to 1.89 ± 0.04 for the alkanethiol films after transfer of the outer lipid. This ratio suggests that there is a greater decrease in the intensity of the antisymmetric in plane methyl vibration compared to the symmetric methyl vibration. Furthermore, this can be understood that the terminal methyl must be undergoing an orientational change away from being parallel to the surface upon addition of the outer lipid monolayer. Further evidence for the increased disorder of the inner 1-dodecanethiol layer is observed in Fig. 2 (b). This spectral region has been baselined and normalized to the CH₂ bending modes. The methylene bending modes should be least affected by changes in orientation of the alkanethiol molecules since the methylene groups are orthogonal to the surface normal. Fig. 2(b) shows a strong decrease in the trans methyl rock band, $\nu(\text{CH}_3)\text{ rock}_T$, at 893 cm^{-1} that is also attributed to the methyl group tensor moving away from the surface normal. It is unclear why the shape of this band has changed upon addition of the phospholipid outer layer. Further evidence can be observed for the $\nu(\text{C-C})_G$ gauche band at 1080 cm^{-1} and the $\nu(\text{C-C})_T$ band at 1126 cm^{-1} . A small increase in the $\nu(\text{C-C})_G$ band and a slight decrease in the $\nu(\text{C-C})_T$ band upon the addition of the DPPC-d₆₂ outer layer also indicate that a slight disordering of the

carbon backbone in the inner layer has transpired while the overall well-ordered packing arrangement is maintained. The $\nu(\text{C-S})_{\text{T}}$ band decreases slightly after addition of the outer layer; while the $\nu(\text{C-S})_{\text{G}}$ band at 633 cm^{-1} does increase in intensity upon addition of the outer layer indicating increased disorder. The ratio of the intensities of the $\nu(\text{C-S})_{\text{T}}$ to the $\nu(\text{C-S})_{\text{G}}$ is indicative of the degree of ordering within the area surrounding the C–S bond in these systems [30]. For the alkanethiol monolayers produced, the intensity ratio of the trans $\nu(\text{C-S})$ bond to the gauche $\nu(\text{C-S})$ bond is 13.46 ± 3.2 for inner layers without the transferred outer phospholipid layer compared to 6.94 ± 1.7 for inner layers after the transferred outer phospholipid layer. The higher variance in the alkanethiol monolayers prior to transfer of the outer phospholipid is due to the increased difficulty in the calculation due to a reduction in the signal to noise ratio (SNR) for the gauche $\nu(\text{C-S})$ spectral band. The decrease in this ratio implies that the gauche $\nu(\text{C-S})$ vibration ca. 633 cm^{-1} has increased in intensity implying localized disorder around the Ag–S–C bond. Although it appears that a conformational change has occurred upon transfer of the outer phospholipid monolayer, the presence of the relatively intense $\nu(\text{C-C})_{\text{T}}$ band at 1126 cm^{-1} , the band positions and intensity for the methylene bending modes ca. 1440 cm^{-1} , and the position and intensity for the symmetric methylene stretch at 2850 cm^{-1} verifies that even though a slight disordering has occurred, the inner alkanethiol layer still exists in a highly ordered state.

Further characterization of the self-assembled monolayer of 1-dodecanethiol after the addition of the deuterated lipid is provided from the IRRAS analysis. As the outer layer of DPPC-d₆₂ is transferred onto this inner layer, drastic changes in the IR vibrational bands of the 1-dodecanethiol inner layer are observed (Fig. 3(b)). The most obvious differences are associated with the bands assigned to the methyl groups. The antisymmetric in plane methyl stretch, $\nu_{\text{a}}(\text{CH}_3, \text{ip})$, at 2965 cm^{-1} has decreased in intensity and exhibits considerable band broadening. The symmetric methyl stretch, $\nu_{\text{s}}(\text{CH}_3)$, at 2878 cm^{-1} has also decreased slightly in intensity. In addition, the symmetric methyl band at 2878 cm^{-1} shifts to 2874 cm^{-1} upon addition of the outer layer. This is further support of the SERS data that the terminal methyl group is experiencing a conformational change upon addition of the outer lipid layer. In contrast, the band positions for the methylene stretches do not change appreciably. The antisymmetric methylene stretch at 2916 cm^{-1} slightly shifts to 2917 cm^{-1} , which may indicate slight disordering; although, the frequency position at 2917 cm^{-1} is still an indication of a highly ordered assembly [7].

From the dual spectroscopic analysis performed on this model bilayer system, it appears that the inner alkanethiol monolayer undergoes a slight disordering upon addition of the outer phospholipid monolayer. Even though the inner layer does undergo this slight conformational change, it is still believed to be an overall ordered assembly.

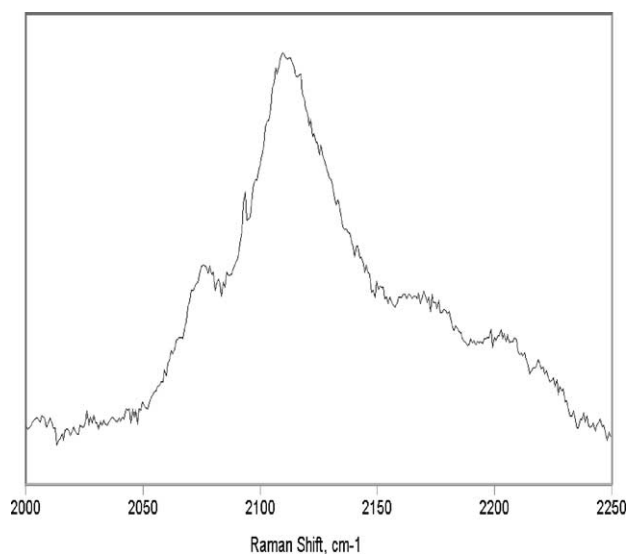


Fig. 4. A representative SERS spectrum of the $\nu(\text{C-D})$ vibrational bands in the region of $2000\text{--}2250\text{ cm}^{-1}$ for the DPPC-d₆₂ outer monolayer adsorbed on top of the self-assembled monolayer of 1-dodecanethiol.

From the spectral evidence presented thus far (Figs. 1–3), it does appear, that compared to IRRAS, SERS is most sensitive to the inner 1-dodecanthiol layer in this model bilayer system. This sensitivity of SERS to the first layer is due to the distance dependence properties outlined in the electromagnetic theory that defines the mechanism for the large enhancements observed [43]. The adsorbate closest to the metal surface will have the greatest enhancement. This does not mean that the SERS vibrational information from the second transferred layer is completely lost. Limited vibrational bands attributed to the DPPC-d₆₂ molecules can be seen in the Raman spectrum regardless of their weaker intensities. Fig. 4 represents the $\nu(\text{C-D})$ vibrational bands in the region of $2000\text{--}2250\text{ cm}^{-1}$ for the DPPC-d₆₂ molecules adsorbed on top of the self-assembled monolayer of 1-dodecanethiol. From this figure, five broad bands of various intensities can be observed that represent the CD₂ and CD₃ moieties of the DPPC-d₆₂ molecules. Table 2 lists the vibrational assignments for these C–D bands and their relative frequencies for both SERS and IRRAS methods. It has been well documented that the intensities

Table 2

The vibrational bands and their relative frequencies for the L–B transferred outer monolayer of DPPC-d₆₂ for both the SERS and IRRAS spectra

| Band assignments ^b for L–B film of DPPC-d ₆₂ | SERS (cm ⁻¹) (observed) | IRRAS (cm ⁻¹) (observed) |
|--|-------------------------------------|--------------------------------------|
| Headgroup vibrations | | 820–1400 |
| $\nu_{\text{s}}(\text{C-N})$ | | 1487 |
| C=O stretch | | 1730 |
| $\nu_{\text{s}}(\text{CD}_3)$ | 2073 | 2070 |
| $\nu_{\text{s}}(\text{CD}_2)$ | 2106 | 2088 |
| $\nu_{\text{a}}(\text{CD}_2)$ | 2175, 2200 | 2194 |
| $\nu_{\text{a}}(\text{CD}_3)$ | 2217 | 2219 |

^b Assignments taken from ref. 44 and 45.

and peak positions of the vibrations present in the Raman $\nu(\text{C-H})$ stretching region are complicated by both interchain lateral packing and intrachain conformational order in hydrocarbons [22]. Although complicated, intensity ratios and band parameters derived from the Raman $\nu(\text{C-H})$ vibrational modes can be used to determine information about molecular structure in hydrocarbon and membrane assemblies [7]. One example of this is a Raman band parameter, R_1 , that compares the ratio between the band intensity of the 2882 cm^{-1} $\nu_a(\text{CH}_2)$ and the 2850 cm^{-1} $\nu_s(\text{CH}_2)$. This ratio, defined as

$$R_1 = \frac{I(\nu_a\text{CH}_2)}{I(\nu_s\text{CH}_2)} \quad (3)$$

reflects both intermolecular lateral chain interactions as well as intramolecular (i.e. conformational) chain order. A relative decrease of the $\nu_a(\text{CH}_2)$ band with respect to the $\nu_s(\text{CH}_2)$ band is indicative of intramolecular disordering of the monolayer [22]. Similar order/disorder parameters have been derived for the $\nu(\text{C-D})$ stretching region that also gives insight into the lateral interactions and conformational changes that occur in deuterated phospholipid monolayer assemblies [44,45]. Two comparable ratios to R_1 can be given that compare the antisymmetric methylene, $\nu_a(\text{CD}_2)$, bands to the symmetric methylene, $\nu_s(\text{CD}_2)$, vibrational stretch at 2106 cm^{-1} . There exists two antisymmetric methylene stretches: (1) 2175 cm^{-1} $\nu_a(\text{CD}_2)$, which is the mode for carbons C-7 to C-15, and (2) 2197 cm^{-1} $\nu_a(\text{CD}_2)$, which is assigned to the C-D bonds between the head-group-alkyl chain interface and the sixth carbon of the alkyl chain of DPPC- d_{62} [45]. Ratios for both of these antisymmetric stretches to the symmetric stretch are required to gain an overall understanding of the order/disorder processes that is comparable to the conclusions gained from the ratio R_1 . From literature, the C-D ratios range from 0.2 for an ordered assembly (gel-like properties) to 0.5 for a disordered system (liquid-crystalline properties) [44,45]. From the SERS data in Fig. 4, these C-D ratios were calculated. For the C-D ratio that compared the band heights of the antisymmetric methylene stretch at 2197 cm^{-1} to the symmetric methylene stretch at 2106 cm^{-1} , a value equal to 0.34 ± 0.05 was obtained. For the C-D ratio that compared the band heights of the antisymmetric methylene stretch at 2175 cm^{-1} to the symmetric methylene stretch at 2106 cm^{-1} , a value equal to 0.24 ± 0.04 was obtained. Comparing these values to those obtained in literature [44,45] for a monolayer of DPPC- d_{62} , we concluded that our L-B transferred layer exists in a gel-like ordered state on top of the self-assembled monolayer of 1-dodecanethiol. This result verifies expectations that the single DPPC- d_{62} transfers and maintains its structure as on ordered assembly. The transfer was conducted at a high surface pressure on the isotherm for DPPC- d_{62} . At high surface pressures, the DPPC- d_{62} molecules on the surface of the water sub-phase are arranged in a gel-like packing arrangement. If the transfer is conducted at this high surface pressure, the transferred monolayer will also exist in a gel-like state on the

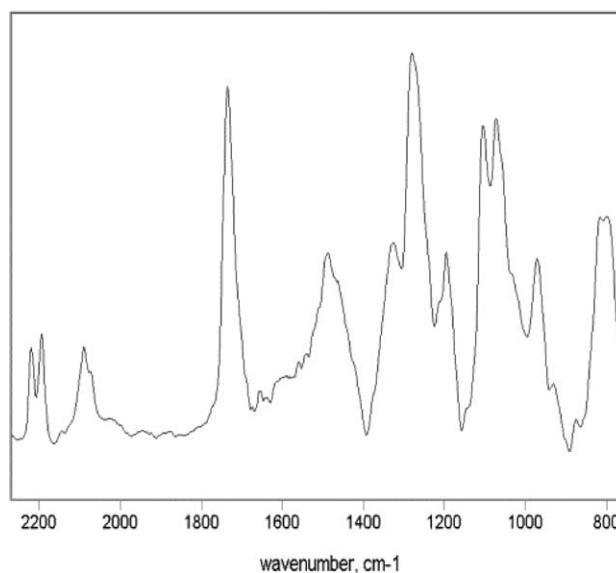


Fig. 5. A representative IRRAS spectrum of the DPPC- d_{62} outer monolayer adsorbed on top of the self-assembled monolayer of 1-dodecanethiol.

substrate. The L-B technique allows transfers to be made at any surface pressure. This flexibility allows the physical state of the transferred monolayer to be determined before the transfer has even begun. Because it was the goal of this initial study for the bilayer system to be comprised of an outer highly ordered DPPC- d_{62} monolayer, a high surface pressure for transfer that would guarantee the gel-like physical state of the phospholipid was chosen.

This outer, gel-like, phospholipid layer can also be examined by IRRAS. Fig. 5 represents the vibrational bands of the deuterated phospholipid DPPC- d_{62} using the IRRAS technique. The band assignments and band frequencies are also listed in Table 2. IRRAS is much more sensitive to the phospholipid monolayer as compared to the inner 1-dodecanethiol layer. This is due to the nature of the moieties in the phospholipid. Not only is the $\nu(\text{C-D})$ stretching region accessible in the IR, but also observation of the head-group vibrations composed of the carbonyl stretch (C=O), PO_2^- , and the C-N stretch at 708 cm^{-1} is also feasible. The positions and intensities of the spectral bands associated with the headgroups are similar to those observed for bulk deuterated DPPC. The key determination of the structural state of the phospholipid can be obtained from the band positions of the C-D stretches [44]. The frequency (2088 cm^{-1}) of the symmetric methylene, $\nu_s(\text{CD}_2)$, band for the transferred L-B film is indicative of a monolayer with gel-like characteristics. These values can be as high as 2094 cm^{-1} for a disordered assembly. Also, the observed frequency (2194 cm^{-1}) for the antisymmetric methylene, $\nu_a(\text{CD}_2)$, vibration is also indicative of a highly ordered assembly. This value can vary up to a frequency of 2196 cm^{-1} for a monolayer that exists in a liquid-crystalline state. These band positions further validate the conclusions gathered from the SERS data for the same DPPC- d_{62} monolayer film.

4. Conclusions

A preliminary model bilayer system comprised of an inner self-assembled monolayer of 1-dodecanethiol and an outer L–B transferred monolayer of DPPC-d₆₂ has been formed and completely characterized using surface-enhanced raman scattering and infrared reflection–absorption spectroscopy. This approach was successful due to the use of a unique metal film planar-support that is thick enough for IRRAS analysis while providing an ideal surface conducive for surface-enhanced raman scattering experiments. This dual spectroscopic approach utilizing SERS and IRRAS on the same sample is very powerful for obtaining information from both monolayers of the bilayer assembly. In addition, even though each vibrational technique is most sensitive to only one of the monolayers, limited spectroscopic information from the other respective monolayer is observable. Therefore, once structural conclusions have been made using the vibrational technique that is most sensitive for that monolayer, verification of those conclusions is possible utilizing the other vibrational spectroscopic technique.

Calculations of vibrational band intensity ratios and the identification of spectral frequencies for both layers were determined that aid in the elucidation of the molecular structure of each layer as the bilayer assembly was formed. From the dual spectroscopic analysis performed on this model bilayer system, it appears that the inner 1-dodecanethiol monolayer undergoes a slight disordering upon addition of the outer phospholipid monolayer. This disorder has been determined to occur throughout the self-assembled alkanethiol molecules based on the SERS and IRRAS data. Even though the inner layer does undergo this slight conformational change, it appears to maintain its structure as an overall ordered assembly. The outer DPPC-d₆₂ monolayer was transferred in the gel state to the alkanethiol surface and maintains its conformational order after the completion of the transfer. Through the marriage of these two complementary vibrational techniques applied to the same sample, detailed structural characterization of this model bilayer system was achieved. This approach represents a powerful analysis scheme for the investigation of model bilayer systems.

Acknowledgements

We would like to thank Dr. Douglas L. Elmore for many helpful discussions. The research presented here was supported by the National Institutes of Health grant EB001956 (R.A.D.).

References

- [1] C.L. Leverette, V.A. Shubert, T.L. Wade, K. Varazo, R.A. Dluhy, *J. Phys. Chem. B* 106 (2002) 8747.

- [2] A. Ulman, *An Introduction to Ultrathin Organic Thin Films, From Langmuir–Blodgett to Self Assembly*, Academic Press, San Diego, CA, 1991.
- [3] A. Ulman, *Characterization of organic thin films*, Butterworth–Heinemann, Stoneham, MA, 1995.
- [4] C.A. Keller, B. Kasemo, *Biophys. J.* 75 (1998) 1397.
- [5] C.W. Meuse, G. Niaura, M.L. Lewis, A.L. Plant, *Langmuir* 14 (1998) 1604.
- [6] K.G. Marra, T.M. Winger, S.R. Hanson, E.L. Chaikof, *Macromolecules* 30 (1997) 6483.
- [7] I.W. Levin, *Vibrational spectroscopy of membrane assemblies*, Wiley, Heyden, 1984.
- [8] R.A. Dluhy, S.M. Stephens, S. Widayati, A.D. Williams, *Spectrochim. Acta* 51A (1995) 1413.
- [9] L.K. Tamm, H.M. McConnell, *Biophys. J.* 47 (1985) 105.
- [10] H.T. Tien, *Adv. Mater.* 2 (1990) 316.
- [11] R. Rapuano, A.M. Carmona-Rebeiro, *J. Colloid Interface Sci.* 193 (1987) 104.
- [12] F. Picard, T. Buffeteau, B. Desbat, M. Auger, M. Pezolet, *Biophys. J.* 76 (1999) 539.
- [13] E. Kalb, S. Frey, L.K. Tamm, *Biochim. Biophys. Acta* 1103 (1992) 307.
- [14] H. Lang, C. Duschl, M. Gratzel, H. Vogel, *Thin Solid Films* 210–211 (1992) 818.
- [15] J. Spinke, J. Yang, H. Wolf, M. Liley, H. Ringsdorf, W. Knoll, *Biophys. J.* 63 (1992) 1667.
- [16] E.-L. Florin, H.E. Gaub, *Biophys. J.* 64 (1993) 375.
- [17] M. Stelzle, G. Weissmuller, E. Sackman, *J. Phys. Chem.* 97 (1993) 2974.
- [18] C. Duschl, M. Liley, G. Corradin, H. Vogel, *Biophys. J.* 67 (1994) 1229.
- [19] R. Naumann, A. Jonczyk, R. Kopp, J. van Esch, H. Ringsdorf, W. Knoll, P. Graber, *Angew. Chem. Int. Ed. Engl.* 34 (1995) 2056.
- [20] C. Duschl, M. Liley, H. Lang, A. Ghandi, S.M. Zakeeruddin, H. Stahlberg, J. Dubochet, A. Nemetz, W. Knoll, H. Vogel, *Mater. Sci. Eng. C4* (1996) 7.
- [21] R.A. Dluhy, in: A. Baszkin, W. Norde (Eds.), *Physical Chemistry of Biological Interfaces*, Marcel Dekker Inc., New York, 2000, p. 711.
- [22] C.L. Leverette, R.A. Dluhy, *Langmuir* 16 (2000) 3977.
- [23] J.D. Swalen, J.F. Rabolt, in: J.R. Ferraro, L.J. Basile (Eds.), *Fourier Transform Infrared Spectroscopy*, Academic Press, Orlando, 1985, pp. 283–314.
- [24] H. Brunner, U. Mayer, H. Hoffman, *Appl. Spectrosc.* 51 (1997) 209.
- [25] R. Aroca, C. Jennings, G.J. Kovacs, R.O. Loutfy, P.S. Vincent, *J. Phys. Chem.* 89 (1985) 4051.
- [26] R. Aroca, D. Battisti, *Langmuir* 6 (1990) 250.
- [27] R. Aroca, L.J.L. Constantino, *Langmuir* 16 (2000) 5425.
- [28] G.J. Kovacs, R.O. Loutfy, P.S. Vincent, C. Jennings, R. Aroca, *Langmuir* 2 (1986) 689.
- [29] C.-H. Tseng, J.F. Ford, C.K. Mann, T.J. Vickers, *Appl. Spectrosc.* 47 (1993) 1808.
- [30] M.A. Bryant, J.E. Pemberton, *J. Am. Chem. Soc.* 113 (1991) 3629.
- [31] R.G. Nuzzo, L.H. Dubois, D.L. Allara, *J. Am. Chem. Soc.* 112 (1990) 558.
- [32] L.H. Dubois, B.R. Zegarski, R.G. Nuzzo, *J. Am. Chem. Soc.* 112 (1990) 570.
- [33] J.R. Ferraro, K. Nakamoto, *Introductory Raman Spectroscopy*, Academic Press, San Diego, CA, 1994.
- [34] R.J. Lipert, B.D. Lamp, M.D. Porter, in: F.M. Mirabella (Ed.), *Modern Techniques in Applied Molecular Spectroscopy*, Wiley, New York, 1998, p. 83.
- [35] R.G. Nuzzo, F.A. Fusco, D.L. Allara, *J. Am. Chem. Soc.* 109 (1987) 2358.
- [36] M.D. Porter, T.B. Bright, D.L. Allara, C.E.D. Chidsey, *J. Am. Chem. Soc.* 109 (1987) 3559.
- [37] C.D. Bain, E.B. Troughton, Y.-T. Tao, J. Evall, G.M. Whitesides, R.G. Nuzzo, *J. Am. Chem. Soc.* 111 (1989) 321.

- [38] M.A. Bryant, J.E. Pemberton, *J. Am. Chem. Soc.* 113 (1991) 546.
- [39] J.E. Pemberton, in: A. Ulman (Ed.), *Characterization of Organic Thin Films*, Butterworth–Heinemann, Boston, MA, 1995, p. 87.
- [40] R.G. Nuzzo, E.M. Korenic, L.H. Dubois, *J. Chem. Phys.* 93 (1990) 767.
- [41] L.H. Dubois, B.R. Zegariski, R.G. Nuzzo, *J. Chem. Phys.* 98 (1993) 678.
- [42] D.D. Popenoe, S.M. Stole, M.D. Porter, *Appl. Spectrosc.* 46 (1992) 79.
- [43] J. Gersten, A. Nitzan, *J. Chem. Phys.* 73 (1980) 3023.
- [44] S. Sunder, D. Cameron, H.H. Mantsch, H.J. Bernstein, *Can. J. Chem.* 56 (1978) 2121.
- [45] M.T. Devlin, I.W. Levin, *J. Raman Spectrosc.* 21 (1990) 441.