Vitamin C/Stearic Acid Hybrid Monolayer Adsorption at Air–Water and Air–Solid Interfaces

Ikbal Ahmed,†‡ Anamul Haque,† Shreya Bhattacharyya,† Prasun Patra,‡ Jasper R. Plaisier,§ Fabio Perissinotto,§ and Jayanta Kumar Bal†§*

†Centre for Research in Nanoscience and Nanotechnology, University of Calcutta, Technology Campus, Block JD2, Sector III, Salt Lake, Kolkata 700098, India
‡Amity Institute of Biotechnology, Amity University, Kolkata 700135, India
§Elettra—Sincrotrone Trieste S.C.p.A., S.S. 14 km 163.5 in Area Science Park, Basovizza, 34149 Trieste, Italy
§Department of Physics, Abhedananda Mahavidyalaya, University of Burdwan, Sainthia, Birbhum 731234, West Bengal, India

Supporting Information

ABSTRACT: Because of the antioxidant activity of vitamin C (Vit C) polar heads, they can be used as a protective agent for fatty acids. Hence, the study on the growth of Vit C/stearic acid (SA) mixed binary films at air–water interface (known as Langmuir monolayer) and air–solid interface (known as Langmuir–Blodgett films) is of paramount interest. Although Vit C is situated at subsurface beneath SA molecules and interacts via hydrogen bonding between the hydroxyl groups of Vit C and SA, several Vit C molecules may infiltrate within SA two-dimensional matrix at the air–water interface. The increased mole fraction of Vit C (0.125–0.5) and the reduction of temperature (from 22 to 10 °C) of the subphase water result in an increase in the amount of adsorbed Vit C at the air–water interface. The surface pressure (σ)–area (A) isotherms illustrate that such inclusion of Vit C provokes a spreading out of Vit C/SA binary monolayers, which lead to an alteration of different physicochemical parameters such as elasticity, Gibbs free energy of mixing, enthalpy, entropy, interaction energy parameter, and activity coefficient. However, being polar in nature, the transfer of pure Vit C on substrates gets affected. It can be transferred onto substrate by mixing suitably with SA as confirmed by infrared spectra. Their structures, extracted X-ray reflectivity, and atomic force microscopy (topography and phase imaging) are found to be strongly dependent on the nature of the substrate (hydrophilic and hydrophobic).

INTRODUCTION

In animal body, vitamin C (Vit C) acts as an important nutrient.1 Besides, it plays a vital role in the production of collagen, an important cellular component of tissue, muscles, teeth, bones, and skin. Being an antioxidant, Vit C inhibits skin impairment triggered by free radicals. In addition, Vit C has a detoxifying effect on liver.2 It also endorses healthy cell development, proper calcium absorption, and cell restoration. However, it is well known that Vit C is extremely unstable.1 For example, it is highly sensitive to heat and degrades on illumination of light, storage in unsuitable containers, and in the presence of preservatives.3 Degradation of Vit C was recognized by Yuan and Chen.4 Vit C is destroyed throughout the food engineering and storage processes.5 To stop this degradation, Vit C is conjugated with biocompatible fatty acids (e.g., stearic acid (SA)).1 Corvis et al. proposed that stearic acid (SA) can protect Vit C from chemical reactions and thermal degradation.6 Hence, exploring the mechanism of binding Vit C with SA is a lucrative topic of research. However, a proper and reliable model of these interactions is not well established.

Molecules mixing at air–water interface experienced in a Langmuir–Blodgett (LB) trough provides a model system to explore the interactions involved within the binary mixture.6 Notably, Vit C is water-soluble, and hence, addition of hydrocarbon chains to the ring results in amphiphilic molecules that show strong surface activity.7−10 Several works were performed on the interactions between Vit C derivatives and various biomolecules.7,10−14 Mottola et al. reported an enhanced adsorption of ascorbyl palmitate at air–water interface in the presence of a lipid monolayer.15 Zhang et al. reported the electrocatalytic activity of self-assembled multi-wall carbon nanotubes toward Vit C and dopamine.16 Vit C was found advantageous for selective and sensitive determi-
nation of dopamine. It is established that pure Vit C cannot make a stable monolayer at the air–water interface, but its derivative of amphiphilic structure can produce a stable monolayer and penetrate lipid membrane. Surprisingly, the interaction of pure Vit C with fatty acid is less reported. Although Vit C is surface-inactive at the air–water interface, our study suggests that in the presence of fatty acids (such as SA), it forms a stable monolayer at the air–water interface. Therefore, we are interested to study the interaction involved in this interesting phenomena and surface orientation of Vit C/SA mixture during the process.

In this paper, we explored the interactions of Vit C with SA at the air–water interface by studying the pressure–area (π–A) isotherms and their thermal stability. This study is accomplished with a special focus on how Vit C can alter the conformation of the SA Langmuir monolayer at various temperatures. For a better understanding of temperature effect, we calculated the physicochemical parameters such as elasticity, Gibbs free energy of mixing, enthalpy, entropy, interaction energy parameter, and activity coefficient of Vit C and SA, which have not been reported so far. In addition, we reported the structure of Vit C/SA binary mixture after transferring onto hydrophilic and hydrophobic substrates forming LB films. The structures of these binary films are determined using X-ray reflectivity (XRR) and atomic force microscopy (AFM).

■ RESULTS AND DISCUSSION

Interaction at Air–Water Interface. Surface Pressure–Area Isotherm of Pure Vitamin C and Stearic Acid. Figure 1

![Figure 1. π–A isotherm of pure vitamin C (Vit C) and pure stearic acid (SA).](image)

depicts the typical surface pressure (π)–area (A) isotherm of pure SA and Vit C monolayers. SA provides a typical condensed monolayer with a limit area (A_{lim}) of 22 Å²/ molecule and a threshold area of the condensation (A_0) (the area per molecule at π = 1.5 mN/m) of 28 Å², both values in agreement with the values reported by Seoane et al. and Parra-Barraza et al. The monolayer of SA displays an appreciable change in slope at π = 24 mN/m, manifesting a transition from the liquid condensed state to the solid state. We recorded the isotherm beyond collapse pressure (π_c). The collapse pressure of SA is found to be π_c = 59 mN/m. The surface pressure of pure Vit C starts to increase after a long compression. It implies that, because of its solubility in water, Vit C exhibits a very small surface activity. With many Vit C molecules immersed in subphase water, the apparent area per molecule (<0.016 nm²) is found bizarre. To check the reproducibility of the isotherm of pure Vit C, the π–A isotherm measurements were taken three times, which show identical behavior.

Surface Pressure–Area Isotherm of Vit C/SA Binary Monolayer. To study the adsorption behavior of Vit C with SA monolayer at the air–water interface, we examined the π–A isotherms of SA by mixing different amounts of Vit C (X_{Vit C} = 0.125, 0.25, and 0.50) at 22 °C, which are depicted in Figure 2a. Phase transitions from gas phase to liquid phase and liquid phase to condensed phase at π_{L→S} = 1–1.5 mN/m and π_{S→C} = 20–24 mN/m, respectively, are observed. The collapse pressure π_c decreases appreciably with increase of X_{Vit C} (see Figure 2b). This might be ascribed to the adsorption of Vit C with SA monolayer, where Vit C propagates from the subphase water to the air–water interface. Wydro et al. reported similar results. Likewise, Gargallo et al. stated that π_c of poly(maleic anhydride-alt-stearoyl) methacrylate reduced from 52 to 31 mN/m with the addition of chitosan (3 g/L) in acetic acid solution (0.3 mol/L). But unsaturated acids (e.g., oleic acid, linoleic acid, etc.) display the opposite behavior. The drop of the slope of isotherms (see Figure 2) with Vit C mole fraction indicates that SA becomes more fluidic or flexible with the incorporation of Vit C. This can be attributed to the alteration of the orientation of SA monolayers from perpendicular to lean as a result of a strong interaction with Vit C. Such alteration provokes early collapse. Additionally enhanced adsorption of Vit C with SA due to rise in mole fraction from 0 to 0.5 initiates shifting of isotherm toward greater molecular areas and inferior surface pressure values. Such expansion ratifies that Vit C is proficient enough to absorb onto an SA monolayer. Vit C is not completely ejected to the bulk water, rather it interacts with SA polar heads at the subphase. However, several Vit C molecules may attach with the polar carboxyl (−COOH) group of SA, which modifies isotherm characteristics. A similar phenomenon was encountered in our previous study on chitosan–fatty acid mixed monolayer.

Excess area and excess Gibbs free energy at certain surface pressures are calculated to explore the interactions between Vit C and SA molecules in the mixed monolayer. The plot of ideal area/molecule, A_{id}, and the experimentally determined area/molecule, A, with increasing mole fraction of Vit C in SA is shown in Figure 3a. There is a considerable deviation of A from the ideal case. The excess area can be deduced from the difference between the experimentally determined area/molecule (A) and the ideal area/molecule (A_{id}),

\[ A_{ex} = A - A_{id} \] (1)

where

\[ A_{id} = A_{SS}X_{SA} + A_{Vit C}X_{Vit C} \]

Molecular interactions between two constituent molecules within a binary system can be realized by studying the departure from the ideal mixing line. Usually, A_{ex} can be used for elucidating different molecular interaction and lateral packing as an important physical parameter as well. Figure 3 shows the molecular area as a function of Vit C mole fraction for binary Vit C/SA monolayers on water subphase at representative surface pressure. The solid lines show that the
two components are mixed with effective states. The dash-dotted lines present that the two kinds of components are "ideal mixtures" with theoretical area or immiscible. As linearity between experimental molecular area and mole fraction does not hold, Vit C/SA monolayers are considered to be miscible and interact with each other, the nonideal monolayers formed at the interface. The excess area $A_{\text{exc}}$ takes its highest value for 0.5 mole fraction of Vit C (shown in Figure 3a). It varies from 24 to 26 Å$^2$ with increase in pressure from 1 to 5 mN/m. However, the excess Gibbs free energy, $\Delta G_{\text{exc}}$, for the mixed monolayer at a certain surface pressure is evaluated by integrating the total excess area over pressure as follows:

$$\Delta G_{\text{exc}} = N_a \int_0^\pi A_{\text{exc}} \, d\pi$$

(2)

where $N_a$ is Avogadro’s number. A minus sign of $\Delta G_{\text{exc}}$ for a mixed monolayer suggests that the interactions are attractive in nature, whereas a positive value corresponds to repulsive interactions. Figure 3b illustrates $\Delta G_{\text{exc}}$ for various mole fractions of Vit C. The mixed monolayer exhibits positive values for all of these cases with maximum at $X_{\text{Vit C}} = 0.5$. It implies that repulsive interactions are dominant among Vit C and SA in the mixed monolayer. Hence, these two components may find difficulty in mixing homogeneously. The extent of interaction increases with the increase of mole fraction of Vit C.

To characterize and elucidate the details of the influence of Vit C on the physical state of fatty acid monolayer, the compression modulus $C_s^{-1}$, which is inverse of the compressibility ($C_s$), of the binary films was calculated. From $\pi - A$ isotherms, we obtained $C_s^{-1}$, which is defined as

$$C_s^{-1} = -A \left( \frac{\partial^2 \pi}{\partial A^2} \right)_T$$

(3)

It is also known as equilibrium in-plane elasticity. $C_\text{r}^{-1}$ is obtained by numerical calculation of the slope of the $\pi - A$ isotherms$^{23,24}$ and plotted against area $A$ (see Figure 4a) and surface pressure $\pi$ (see Figure 4b). The modulus is zero, corresponding to clean air–water interface, and increases with the mole fraction of Vit C present at the interface. In addition, the value of compression modulus depends on the state of the film, being larger for more condensed monolayers. The presence of Vit C is found to affect the elasticity/compressibility of SA monolayers, as depicted in Figure 4. The pure SA monolayer yields a large elasticity with compression. But a radical drop of elasticity after mixing with Vit C is encountered. Elasticity attains its maximum value around 30–50 mN/m for pure SA, whereas in binary monolayer, the maximum is observed in the pressure range of 25–50 mN/m. At low surface pressures (i.e., large area/molecule), the elasticity is similar for pure and mixed monolayers, whereas at high pressure, they differ from each other significantly. Therefore, the effect of Vit C on the in-

Figure 2. (a) $\pi - A$ isotherm of Vit C/stearic acid (SA) as a function of mole fraction. (b) Evolution of collapse pressure ($\pi_c$) with Vit C mole fraction at temperature $T = 22$ °C.

Figure 3. (a) Progression of area per molecule measured experimentally $A_{12}$ (solid lines with symbols) and calculated ideal area per molecule $A_{\text{id}}$ (dash-dotted line) with mole fraction of Vit C in Vit C/SA binary system at $\pi = 1, 3, \text{and} 5$ mN/m. (b) Corresponding excess Gibbs free energy, $\Delta G_{\text{exc}}$.
plane elasticity of Vit C/SA mixed layer is substantial. So, an effective interaction of Vit C with fatty acid is encountered at the air–water interface even though Vit C has less surface activity. Furthermore, hysteresis (i.e., compression–decompression cycle) measurements (see Section S1, Supporting Information) were performed to explore the reversibility. A very small hysteresis is detected for the monolayer having a higher concentration of Vit C. It implies that the monolayers are irreversible to some extent.

Temperature-Dependent Isotherm of Mixed Monolayer. Isotherms of Vit C/SA mixed monolayer are measured with varying temperature from 22 to 10 °C keeping the other experimental parameters same as shown in Figure 5a. The depression of the temperature of subphase water makes a very little expansion of area/molecule. The limit area \( A_{\text{lim}} \) is increased from 23.4 to 24.3 Å²/molecule, and \( \pi \) decreases from 53 to 48 mN/m. The elasticity (shown in Figure 5b) is found to drop from 4800 to 3202 mN/m, indicating the incorporation of additional soft Vit C molecules. However, the incorporation of Vit C facilitates the subtle expansion of binary monolayer (of 1 Å² only) and the small reduction of \( \pi \) and \( C_s^{-1} \). This expansion can be attributed to the change in the solubility of Vit C with temperature. A similar expansion along with enhanced surface activity and elasticity of pure Vit C is observed (see Figure S2, Supporting Information). An analogous phenomenon was encountered by Neto et al. and Shalmashi et al. They proposed that the mole fraction solubility \( N \) of Vit C varies with temperature \( T \) in kelvin as follows: \( \ln N = A + BT \), where \( A \) and \( B \) are the parameters dependent on solvent nature. For water subphase, it was found that the values of \( A \) and \( B \) are \( \approx -11.7 \) and \( \approx 0.028 \), respectively. Therefore, substituting these values, one can extract the values of \( N \) at different temperatures. The calculated values of solubility are found to be \( \approx 0.032, 0.027, \) and \( 0.023 \) at \( T = 22, 16, \) and \( 10 \) °C, respectively. This implies that the cooling down of subphase water minimizes the solubility of Vit C in water. Essentially, Vit C molecules are expelled from thebulk and reside near the air–water interface to take part in the evolution of the surface pressure–area isotherm.

For spreading of monolayers, the Gibbs free energy of spreading, \( \Delta G_s \) (kJ/mol), and surface entropy, \( \Delta S_s \) (kJ/(mol K)), can be calculated according to the formulas

\[
\Delta G_s = N_f \int_0^{\pi_0} \pi \, dA
\]

\[
\Delta S_s = A \left( \frac{\partial \pi}{\partial T} \right)_A
\]

where \( \pi_0 \) is the maximum pressure at which the integral is calculated. Furthermore, if \( \Delta G_s \) and \( \Delta S_s \) are known, then surface enthalpy \( \Delta H_s \) (kJ/mol) can be easily determined as

\[
\Delta H_s = \Delta G_s + T \Delta S_s
\]

The calculated values of \( \Delta G_s \), \( \Delta S_s \), and \( \Delta H_s \) for mixed binary monolayers are tabulated in Table 1 for three different values of \( \pi \) and \( A \). Notably, \( \Delta G_s \) values are always positive irrespective of temperature and surface pressure, mimicking repulsive

Figure 4. In-plane elasticity \( C_s^{-1} \) of Vit C/SA binary mixture as a function of (a) area/molecule and (b) surface pressure at different mole fractions of Vit C.

Figure 5. (a) Isotherm study of Vit C/SA \( (X_{\text{Vit C}} = 0.125) \) mixture with variation in temperature. (b) Temperature-dependent in-plane elasticity of Vit C/SA mixed monolayer.
Table 1. Physicochemical Parameters for Monolayers of Vit C and SA Binary System at the Air/Water Interface as a Function of Temperature

| Temperature (°C) | ΔG_s (kJ/mol) | ΔS_s (kJ/(mol K)) | ΔH_s (kJ/mol) |
|------------------|---------------|-------------------|---------------|
| 22               | 3.31          | 14.11             | 38.38         |
| 16               | 3.82          | 14.86             | 38.38         |
| 10               | 4.02          | 16.42             | 38.38         |

Figure 6. (a) Interaction energy parameter (ζ) and (b) activity coefficient (γC, γSA) as a function of Vit C mole fraction XVC in the binary monolayer system. The solid and open symbols correspond to γSA and γVC.

The interaction energy parameter ζ and activity coefficient γi (i = Vit C, SA) of Vit C and SA in the binary monolayer can be evaluated as well from the values of ΔGexc. Both ζ and γi are two important parameters for quantitative analysis of the thermodynamic properties and stability of monolayers. According to Kodama et al., ζ can be obtained using the following equation

$$\xi = \frac{\Delta G_{\text{exc}}}{RTX_{\text{Vit C}}X_{\text{SA}}} \quad (7)$$

whereas activity coefficient γi can be evaluated using the Margules equations for given binary monolayer systems as follows

$$\ln \gamma_{\text{Vit C}} = \xi X_{\text{SA}}^2$$
$$\ln \gamma_{\text{SA}} = \xi X_{\text{Vit C}}^2 \quad (8)$$

where ζ is the interaction parameter, which is ascribed to cohesive interaction between dissimilar molecules. The negative sign of ζ indicates that the molecular interactions of the films are more attractive than those of single component monolayer, whereas the positive sign of ζ means phase separation and repulsion force between SA and Vit C. The calculated values of interaction parameter ζ for the binary systems are depicted in Figure 5. The ζ values are found to be positive as ΔGexc values are positive. Generally, the interaction parameters increase with surface pressures at all mole fractions, which implies the intermolecular interactions between constituent molecules reinforced with the improvement of surface pressure. Specifically, the higher value of ζ corresponds to the stronger interaction, and its composition dependence is related to the packing of one molecule surrounded by other molecules. The interaction energy parameter ζ values at 5 mN/m progressively increase with XVC indicating that the increase of Vit C molecules produces a strong repulsive interaction with SA molecules.

In thermodynamics, activity coefficient is used to determine the nonideal behavior of a mixture or departure from ideality as predicted by Raoult’s law. Physically, it describes the escaping tendency of the constituent molecules from the mixture. So, if the activity coefficient is less than 1.0, it indicates that the molecules have strong attractive force, and therefore, a high energy is required to isolate them. Conversely, when the activity coefficient is greater than 1.0, it implies that the molecules have a strong repelling force. In this case, lesser energy is needed to separate the constituent molecules. However, it is evident from Figure 6 that γSA increases with...
along depth. By knowing this structure, one can understand the growth mechanism and find the way of relocating Vit C on solid surfaces of different kinds. XRR data and analyzed curves for three binary LB films deposited on hydrophobic and hydrophilic surfaces are shown in Figure 7. A layered structure is evident for all of the films on both the surfaces, but their height and coverage differ drastically. However, to get the quantitative information about the layering, all of the XRR data were fitted by considering a truthful model structure and the corresponding EDPs are given in their insets. For the fitting, each film is divided into different boxes or layers. Each box or layer has a constant density, and roughness is incorporated at each interface.

EDP indicates a drastic density variation along depth, which leads to an overall two-layered structure in all of the LB films. The bottom layer (designated as L_bot) adjacent to the interface is found denser than the top overlayer (designated as L_top). The density profile illustrates that the electron densities of the bottom layer ($\rho_{\text{bot}}$) and the top layer ($\rho_{\text{top}}$) of binary films grown on hydrophilized Si vary around 0.40 and 0.30 e/Å$^3$, respectively. The high density of L_bot can be attributed to the presence of a largely dense Vit C (mass density $\approx 1.65$ g/cm$^3$) compared to SA (mass density $\approx 0.94$ g/cm$^3$). These layered structures are not expected from pure SA on hydrophilic Si in one stroke (up) at $\pi = 30$ mN/m, rather a homogeneous monolayer is likely to be formed.7 Although bilayer structures

Figure 7. XRR data (different symbols) and analyzed curves (solid line) of Vit C/SA hybrid LB films on (a) hydrophilic and (b) hydrophobic Si(100) substrates, deposited by one and two strokes (shown by arrow), respectively, at the surface pressure $\pi = 30$ mN/m (the curves are shifted vertically for clarity). Inset: corresponding EDPs showing possible model structures of LB films.

Figure 8. AFM images (scan area = 1500 × 1500 nm$^2$) of Vit C/SA hybrid LB films on hydrophobic (a–c) and hydrophilic (d–f) Si(100) substrates deposited by one stroke (up) and two strokes (down–up), respectively, at high pressure $\pi = 30$ mN/m. The pair of images (a, d), (b, e), and (c, f) obtained from hybrid films, where $X_{\text{Vit C}} = 0.5, 0.25,$ and $0.125$, respectively.
of pure SA are expected to be formed on hydrophobic Si in two strokes, their densities would not differ much and the quality would be good. Hence, in the present study, the interaction between Vit C and SA provokes the bilayer formation. On the other hand, it proves the adsorption of Vit C within the Langmuir monolayer and LB films. However, the thickness of high density of \( L_{\text{bot}} \) is \( \approx 1.0 \) nm, whereas the thickness of \( L_{\text{top}} \) is \( \approx 1.5-2.0 \) nm. By looking at the thickness of \( L_{\text{bot}} \) it is evident that it does not correspond to a layer solely, rather it represents a part of it. It is well known that the thickness of an LB monolayer film made of pure SA is around 2.5 nm.\(^{34}\) The total thickness of the film, \( L_{\text{bot}} + L_{\text{top}} \) is itself around 2.5–3.0 nm. Essentially, these two apparent layers are residing adjacent to each other, otherwise the thickness of the film would be greater than 3.0 nm. Probably, \( L_{\text{bot}} \) corresponds to both Vit C and SA, whereas \( L_{\text{top}} \) corresponds to only SA. Notably, the excess density (i.e., \( \rho_{\text{top}} - \rho_{\text{bot}} = 0.1 \text{ e/Å}^3 \)) appears due to the incorporation of Vit C within the SA monolayer. It suggests that the thickness of \( L_{\text{bot}} \) is determined by the transferred Vit C molecules on Si surfaces. On the other hand, a completely different EDP is encountered for the LB film deposited on hydrophobic Si in two strokes (down–up sequence). The thickness and density of \( L_{\text{bot}} \) are found to be \( \approx 2.5 \) nm and 0.34 e/Å\(^3\), respectively, whereas the thickness and density of \( L_{\text{top}} \) are \( \approx 2.5–3.5 \) nm and 0.3–0.8 e/Å\(^3\), respectively.

**Atomic Force Microscopy Study.** These structural information are also in agreement with the AFM results. Two different features having different heights constituting the bilayer structure are evident in the surface topography images (shown in Figure 8a–f), although their morphologies are different on polar and apolar surfaces. But these morphologies do not alter appreciably with the mole fraction of Vit C. The basic difference between the structures on hydrophilic and hydrophobic surfaces is that the two features reside side by side and one by one vertically on the former (Figure 8d–f) and the latter ones (Figure 8a–c), respectively. The phase images (see Figure S3, Supporting Information) confirm these structures. Two distinct phase contrasts manifesting Vit C and SA are observed in all of the films deposited on hydrophilic Si. Heights and coverages obtained from AFM images (height as well as phase images) are in good agreement with the EDP of the films. Nevertheless, coverage of the films or layers is calculated from the bearing plot of AFM images using Windows Scanning x Microscope (WSxM) software. These results obtained from XRR and AFM eventually lead to the model structure shown schematically in Figure 9.

**Infrared Spectroscopy Study.** To confirm the existence of Vit C within binary films, Fourier transform infrared (FTIR) study was carried out using Jasco FT/IR-6300 in the wavelength range of 350–4000 cm\(^{-1}\). Pure Vit C (drop-cast), pure SA, and Vit C/SA (\( X_{\text{Vit C}} = 0.5 \)) mixed LB films on Si substrates displayed FTIR spectra (shown in Figure 10) featuring typical bands of SA and Vit C. The assigned FTIR adsorption bands derived from Figure 10 are tabulated in Table 2. In the normalized transmission spectrum of Vit C, the absorption bands appearing at 1762 and 1675 cm\(^{-1}\) are related to C=O stretching of the five-membered lactone ring and C=C stretching vibrations combined with the neighboring vibrations. Other vibrational bands are detected in the wavelength range 1200–1500 cm\(^{-1}\), which originate from CH\(_2\) scissoring, twisting, and wagging and the C–H deformation modes, which are similar to those reported by Panicker et al.\(^{35}\) Particularly, the weak band at 1463 cm\(^{-1}\) is attributed to CH\(_2\) scissoring. The band at 1777 cm\(^{-1}\) is generated from C–O–C stretching. In addition, very strong C–O–C stretches are visible at 1142, 1113, and 1077 cm\(^{-1}\). Also, the C–O–H band probably has contribution to the bands in the region 1046–1081 cm\(^{-1}\). The bands in the range 990–1027 cm\(^{-1}\) correspond to the deformation of lactone ring. The peaks at 3526 and 3411 cm\(^{-1}\) are due to O–H stretching. The strong peak at 3031 cm\(^{-1}\) is due to C–H stretching. Intense bands in the region 3216–3529 cm\(^{-1}\) are assigned to O–H stretches due to the presence of humidity in the sample.\(^{36}\) Two strong bands at 1706 and 721 cm\(^{-1}\) appearing in the transmission spectra of SA are attributed to stretching vibration of C=O and the bending vibration of the hydrogen bond (OH···H) of carboxylic acid. Both bands are characteristic bands of carboxylic acid.

In the transmission FTIR spectra of Vit C/SA mixture, the transmittance dip gets broader in the range of 3153–2623 cm\(^{-1}\) in comparison to pure SA, probably due to the effect of strong bands of O–H in the region 3216–3526 cm\(^{-1}\) range of Vit C.

![Figure 9. Schematic diagram of (a) pure Vit C and (b) Vit C/SA hybrid system at air–water interface. LB films of Vit C/SA binary mixture deposited on (c) hydrophilic Si (by one upstroke) and (d) hydrophobic Si (by two strokes) at \( \pi = 30 \text{ mN/m} \).](image1)

![Figure 10. Normalized FTIR spectra for pure Vit C, pure SA, and hybrid Vit C/SA films. The curves are shifted vertically for clarity.](image2)
Table 2. Assignment of FTIR Spectra of Pure SA, Pure Vit C, and Their Hybrid Films Deduced from Figure 10

| sample   | IR band (cm\(^{-1}\)) | description* |
|----------|------------------------|--------------|
| Vit C    | 3526, 3410, and 3318   | ν(O−H)       |
|          | 3031                   | ν(C−H)       |
|          | 1762                   | ν(C=O) of five-membered lactone ring system |
|          | 1675                   | ν(C=O) coupled with neighboring vibrations along conjugated system |
|          | 1498                   | ν(C=C)       |
|          | 1463                   | CH\(_3\) scissoring |
|          | 1321                   | ν(C=OH)      |
|          | 1277                   | ν(C=C−O−C)   |
|          | 1198                   | O−H in-plane vibration |
|          | 1200−1500              | νs, νt, and ν of CH\(_2\) and the C−H deformation modes |
|          | 1142, 1113, and 1077   | ν(C−O−C)     |
|          | 1023                   | C−H in-plane bending vibration |
|          | 989 and 869            | C−H out-of-plane bending vibrations |
|          | 821, 757, and 721      | C−C in-plane bending vibration |
|          | 682 and 629            | O−H out-of-plane bending vibrations |
|          | 472 and 448            | C−C out-of-plane bending vibrations |
| SA       | 2920                   | ν\(_s\)(C−H)CH\(_3\) |
|          | 2851                   | ν\(_s\)(C−H)CH\(_2\) |
|          | 1705                   | ν(C=O) secondary amide |
|          | 1465                   | CH\(_3\) scissoring |
|          | 1122                   | ν(C=C−C)     |
|          | 934                    | bending vibration of H bond (OH−H) |
|          | 721                    | ν(C=O)       |
| Vit C/SA | 3526, 3410, and 3318   | ν(O−H)       |
|          | 2920                   | ν\(_s\)(C−H)CH\(_3\) |
|          | 2851                   | ν\(_s\)(C−H)CH\(_2\) |
|          | 1463                   | CH\(_3\) scissoring |
|          | 1321                   | ν(C=OH)      |
|          | 1113                   | ν(C=C−O−C)   |
|          | 1023                   | C−H in-plane bending vibration |
|          | 934                    | bending vibration of H bond (OH−H) |
|          | 721                    | ν(C=O)       |

*ν = stretching vibration; ν\(_s\) = symmetric stretching vibration; ν\(_a\) = asymmetric stretching vibration; ω = wagging; s = scissoring; t = twisting.

In conclusion, we have demonstrated that when Vit C ratio increases in Vit C/SA mixture, it employs an in-plane compression-induced lateral displacement of the SA molecules at air–water interface. This incorporation leads to an expanded state that exhibits less compressibility and collapse pressure. Existing literature only reports on the structure and the mechanical properties of mixed monolayers encompassing Vit C derivatives in the vicinity of room temperature. The present study prolongs these properties of pure Vit C toward low temperatures (down to 10 °C). Suppression of temperature of subphase permits added number of Vit C molecules to populate the air–water interface. Accordingly, a small increment in the average area per molecule is observed. Additionally, the temperature drop leads to a rise in entropy, enthalpy, and Gibbs free energy. On the other hand, thorough structural information of transferred films on hydrophobic and hydrophilic Si(100) surfaces obtained from XRR and AFM shows that a bilayer film is formed on the former substrate, whereas a monolayer is formed on the latter surface. Although no appreciable difference in the structure and morphology of monolayer films is observed with Vit C mole fraction, in the case of bilayer films, the coverage of the top layer tends to decrease with the increase of Vit C. Perhaps its random orientation in the first monolayer of SA grown at the time of downstroke inhibits the further attachment of SA molecules at the time of upstroke. The presence of Vit C within binary LB films is confirmed from IR study. However, the structures of films at several conditions are important to replicate the ordering of the predeposited films at the air–water interface, which essentially have insinuations in the biological applications of human body.

### EXPERIMENTAL SECTION

Stearic acid (SA) ([CH\(_3\)(CH\(_2\))\(_{17}\)COOH, Sigma-Aldrich Co., 99%) and Vit C ([l-ascorbic acid C\(_{6}\)H\(_8\)O\(_6\), Acros Organics, 99%, molecular weight ∼176.12 g/mol) solutions of concentration 50 mM were prepared in ethanol, whereas Vit C/SA mixed solutions were prepared by mixing SA with Vit C solutions homogeneously at different mole fractions of Vit C (X\(_{Vit\ C}\) = 0.125, 0.25, and 0.50). Langmuir monolayer and LB film were prepared in a double-barrier Langmuir–Blodgett trough (Apex Instruments, model no.: LBXD-NT). In the center of the trough, a Wilhelmy plate was partially immersed in aqueous phase to measure the change in surface tension with surface pressure. Before proceeding any kind of experiment in an LB trough, it was thoroughly cleaned with ethanol, followed by Milli-Q water (resistivity ~18.6 MΩ cm) to make the trough totally dirt-free. For hybrid monolayer study, three homogeneously mixed solutions having different mole fractions of Vit C were spread dropwise using a Hamilton syringe (precision of 2.5 μL). After pressure stabilization, surface pressure (mN/m) versus area per molecule (nm\(^2\)) (π−A) isotherms were recorded beyond collapse pressure (πc) at a constant compression speed of 10 mm/min. For thermal stability study, the temperature of subphase water was decreased to 10 °C from room temperature using a chiller (FirstSource Company). For comparison, the measurements were also performed on pure SA and Vit C solutions. Furthermore, LB films were transferred at a constant pressure π = 30 mN/m on hydrophilic and hydrophobic Si(100) substrates in a single stroke (upstroke) and double strokes.
(down—upstroke), respectively. During film deposition, the barrier compression speed and the dipping/lifting speed of substrates were maintained at 8 and 5 mm/min, respectively.

Before LB deposition, Si(100) substrates were cleaned extensively following Radio Corporation of America (RCA) and hydrofluoric acid (HF) treatment methods.37 In the RCA treatment, Si surfaces (each piece size ≈22 × 12 mm²) were boiled in a mixture of ammonium hydroxide (NH₄OH, Sigma-Aldrich, 25%), hydrogen peroxide (H₂O₂, Acros Organics, 39%), and Milli-Q water (H₂O/NH₄OH/H₂O₂ = 2:1:1, by volume) for 10 min at 100 °C, which makes the surfaces hydrophilic due to the incorporation of hydroxyl group (−OH) in the Si dangling. In HF treatment, the RCA-cleaned dry Si were vertically dipped into 10% HF solution (every time volume was kept constant at 50 mL) for 3 min. The substrates were washed immediately in Milli-Q water for removing the residues (if any) and dried by spinning them at a high rpm (5000) in a spin coater. The qualities of hydrophilicity and hydrophobicity were tested by water contact angle measurements, which yield ~14 and 79°, respectively.38

XRR measurements of all prepared films were performed at the beamline MCX38 at Elettra-Sincrotrone Trieste (selected energy = 8 keV and wavelength λ = 1.54 Å). The beamline is equipped with a high-resolution four-circle diffractometer (θ − 2θ) and a three (X, Y, and Z) translational stage. Scattered intensities were detected using a scintillator detector. XRR data were recorded in a specular condition, that is, the incident intensities were detected using a scintillator detector. XRR data were imaged by an atomic force microscope (Asylum Research MFP-3D) in noncontact mode, and scans (different scan ranges) were executed at different positions on the films’ surface for good statistical information. A sharp needlelike tip residing at the edge of a silicon cantilever (Olympus RC-800 PSA, spring constant = 0.76 N/m, resonant frequency = 71 kHz) was used for scanning. For image processing and analysis, Windows Scanning x Microscope (WSxM, where x is force, tunneling, near-optical, etc.) software39 and Gwyddion software were used. The presence of Vit C in binary LB films was confirmed by transmission Fourier transform infrared (FTIR) spectroscopy measurements with a Jasco FT/IR-6300 spectrometer.

**ASSOCIATED CONTENT**

*Supporting Information*
The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsomega.8b02235.

Hysteresis study (compression–decompression cycle) of Vit C/SA mixed monolayers at air–water interface for three different mole fractions of Vit C (Section S1); temperature-dependent isotherm and elasticity of pure Vit C (Section S2); and phase and topography imaging of Vit C/SA bilayer LB film (Section S3) (PDF)

**AUTHOR INFORMATION**

**Corresponding Author**
*E-mail: jayanta.bal@gmail.com.*

**ORCID®**
Ilkal Ahmed: 0000-0003-0969-788X
Anamul Haque: 0000-0003-4244-9271
Jayanta Kumar Bal: 0000-0002-1814-1018

**Present Address**
^1^Aliah University, Newtown, Kolkata, West Bengal, 700160, India

**Notes**
The authors declare no competing financial interest.

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**REFERENCES**

(1) Johnson, L. E.; Mergens, W. J. Added Ascorbates and Tocopherols as Antioxidants and Food Improvers. In *Nutrient Additions to Food, Nutritional, Technological and Regulatory Aspects*; Bauernfeind, J. C., Lachance, P. A., Eds.; Food & Nutrition Press, 1991; pp 433–458.
(2) Lewin, S. *Vitamin C: Its Molecular Biology and Medical Potential*; Academic Press: London, 1976.
(3) Shan, L. W. The Application of Vitamin C—From Food and Beverages to Cosmetic Products. *Sci. Hong Kong* 2008.
(4) Yuan, J.-P.; Chen, F. Degradation of Ascorbic Acid in Aqueous Solution. *J. Agric. Food Chem.* 1998, 46, 5078–5082.
(5) Corvis, Y.; Menet, M.-C.; Négrier, P.; Lazerges, M.; Espeau, P. The Role of Stearic Acid in Ascorbic Acid Protection from Degradation: A Heterogeneous System for Homogeneous Thermodynamic Data. *New J. Chem.* 2013, 37, 761.
(6) Ahmed, I.; Dildar, L.; Haque, A.; Patra, P.; Mukhopadhyay, M.; Hazra, S.; Kulkarni, M.; Thomas, S.; Plaisier, J. R.; Dutta, S. B.; et al. Chitosan-Fatty Acid Interaction Mediated Growth of Langmuir Monolayer and Langmuir-Blodgett Films. *J. Colloid Interface Sci.* 2018, 514, 433–442.
(7) Capuzzi, G.; Lo Nostro, P.; Kulkarni, K.; Fernandez, J. E. Mixtures of Stearoyl-6-O-Ascorbic Acid and α-Tocopherol: A Monolayer Study at the Gas/Water Interface. *Langmuir* 1996, 12, 3957–3963.
(8) Benedini, L.; Fanani, M. L.; Maggio, B.; Wilke, N.; Messina, P.; Palma, S.; Schulz, P. Surface Phase Behavior and Domain Topography of Ascorbyl Palmitate Monolayers. *Langmuir* 2011, 27, 10914–10919.
(9) Zulueta Diaz, Y. d. I. M.; Mottola, M.; Vico, R. V.; Wilke, N.; Fanani, M. L. The Rheological Properties of Lipid Monolayers Modulate the Incorporation of L-Ascorbic Acid Alkyl Esters. *Langmuir* 2016, 32, 587–595.
(10) Mottola, M.; Wilke, N.; Benedini, L.; Oliveira, R. G.; Fanani, M. L. Ascorbyl Palmitate Interaction with Phospholipid Monolayers: Electrostatic and Rheological Preponderancy. *Biochim. Biophys. Acta, Biomembr.* 2013, 1828, 2496–2505.
(11) Zhang, M.; Gong, K.; Zhang, H.; Mao, L. Layer-by-Layer Assembled Carbon Nanotubes for Selective Determination of Dopamine in the Presence of Ascorbic Acid. *Biosens. Bioelectron.* 2005, 20, 1270–1276.
(12) Takahashi, M.; Komuro, E.; Niki, E.; Tanaka, K. Action of Fatty Acid Esters of α-Ascorbic Acid as Antioxidants in Phosphatidylcholine Liposomal Membranes. *Bull. Chem. Soc. Jpn.* 1992, 65, 679–684.
(13) Capuzzi, G.; Lo Nostro, P.; Kulkarni, K.; Fernandez, J. E.; V incieri, F. F. Interactions of 6-O-Stearoylascorbic Acid and Vitamin K 1 in Mixed Langmuir Films at the Gas/Water Interface. *Langmuir* 1996, 12, 5413–5418.
Influence of Concentration, Temperature, and Role of Intermolecular Interactions.

(14) Capuzzi, G.; Kulkarni, K.; Fernandez, J. E.; Vinci, F. F.; Lo Nostro, P. Mixtures of Ascorbyl-Stearate and Vitamin D3: A Monolayer Study at the Gas/Water Interface. J. Colloid Interface Sci. 1997, 186, 271–279.

(15) Mottola, M.; Wilke, N.; Benedini, L.; Oliveira, R. G.; Fanani, M. L. Ascorbyl Palmitate Interaction with Phospholipid Monolayers: Electrostatic and Rheological Preponderance. Biochim. Biophys. Acta. Biomembr. 2013, 1828, 2496–2505.

(16) Seoane, R.; Muñones, J.; Conde, O.; Muñones, J.; Casas, M.; Inbarnegaray, E. Thermodynamic and Brewster Angle Microscopy Studies of Fatty Acid/Cholesterol Mixtures at the Air/Water Interface. J. Phys. Chem. B 2000, 7735–7744.

(17) Parra-Barraza, H.; Burboa, M. G.; Sánchez-Vázquez, M.; Juárez, J.; Goycoolea, F. M.; Valdez, M. A. Chitosan-Cholesterol and Chitosan-Stearic Acid Interactions at the Air-Water Interface. Biomacromolecules 2005, 6, 2416–2426.

(18) Wydro, P.; Krajewska, B.; Hac-Wydro, K. Chitosan as a Lipid Binder: A Langmuir Monolayer Study of Chitosan–Lipid Interactions. Biomacromolecules 2007, 8, 2611–2617.

(19) Gargallo, L.; Leiva, A.; Urriza, M.; Alegria, L.; Miranda, B.; Ràdic, D. Modification of the Air-Water Interface by a Chitosan Adsorption Process. Effect on an Amphiphilic Polymer Monolayer. Polym. Int. 2004, 53, 1652–1657.

(20) Gains, G. L., Jr. Insoluble Monolayers at Liquid–Gas Interfaces; Wiley Interscience: New York, 1966.

(21) Frey, S. L.; Chi, E. Y.; Arrattia, C.; Majewski, K.; Kjaer, K.; Lee, K. Y. C. Condensing and Fluidizing Effects of Ganglioside GM1 on Phospholipid Films. Biophys. J. 2008, 94, 3047–3064.

(22) Goodrich, F. C. In Molecular Interaction in Mixed Monolayers, Proceedings of the 2nd International Congress of Surface Activity; Butterworth: London, 1957; Vol. I, pp 85–91.

(23) Decher, G. Langmuir-Blodgett films: An introduction. By M. C. Petty, xviii, 234 pp., Cambridge University Press, Cambridge 1996, Softcover, e16.95, ISBN 0-521-42450-X. Adv. Mater. 1997, 9, 843–844.

(24) Desbrieres, J. Viscosity of Semiflexible Chitosan Solutions: Influence of Concentration, Temperature, and Role of Intermolecular Interactions. Biomacromolecules 2002, 3, 342–349.

(25) Neto, A. C. R.; Pires, R. F.; Malagoni, R. A.; Franco, M. R. Solubility of Vitamin C in Water, Ethanol, Propan-1-OL, Water + Ethanol, and Water + Propan-1-Ol at (298.15 and 308.15) K. J. Chem. Eng. Data 2010, 55, 1718–1721.

(26) Shalnashi, A.; Eliass, A. Solubility of l-(+)-Ascorbic Acid in Water, Ethanol, Methanol, Propan-2-OI, Acetone, Acetonitrile, Ethyl Acetate, and Tetrahydrofuran from (293 to 323) K. J. Chem. Eng. Data 2008, 53, 1332–1334.

(27) Kodama, M.; Shibata, O.; Nakamura, S.; Lee, S.; Sugihara, G. A Monolayer Study on Three Binary Mixed Systems of Dipalmitoyl Phosphatidyl Choline with Cholesterol, Cholesterol and Stigmasterol. Colloids Surf., B 2004, 33, 211–226.

(28) Chen, Y.; Sun, R.; Wang, B. Monolayer Behavior of Binary Systems of Betulinic Acid and Cardiolipin: Thermodynamic Analyses of Langmuir Monolayers and AFM Study of Langmuir–Blodgett Monolayers. J. Colloid Interface Sci. 2011, 353, 234–240.

(29) Lawrie, G.; Gentle, I.; Barnes, G. The Structure of Mixed Monolayer Films of DPPC and Hexadecanol. Colloids Surf., A 2000, 171, 217–224.

(30) Muzzarelli, R. A. A.; Frega, N.; Miliani, M.; Muzzarelli, C.; Cartolari, M. Interactions of Chitin, Chitosan, N-Lauryl Chitosan and N-Dimethylaminopropyl Chitosan with Olive Oil. Carbohydr. Polym. 2000, 43, 263–268.

(31) Nagadome, S.; Suzuki, N. S.; Mine, Y.; Yamaguchi, T.; Nakahara, H.; Shibata, O.; Chang, C.-H.; Sugihara, G. Langmuir Films (Langmuir Films) Behavior of Multi-Component Systems Composed of a Bile Acid with Different Sterols and with Their 1:1 Mixtures. Colloids Surf., B 2007, 58, 121–136.

(32) Raoul, F. Loi Generale Des Tensions de Vapeur Des Dissolvants. C. R. Acad. Sci. 1887, 104, 1430–1453.