Self-assembly and molecular packing in cholesteryl esters at interfaces

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To understand the self-assembly and molecular packing in cholesteryl esters relevant to biological processes, we have studied them at the air-water and air-solid interfaces. Our phase and thickness studies employing imaging ellipsometry and atomic force microscopy along with surface manometry show that the molecular packing of cholesteryl esters at interfaces can be related to Craven's model of packing, given for bulk. At the air-water interface, following Craven's model, cholesteryl nonanoate and cholesteryl laurate exhibit a fluidic bilayer phase. Interestingly, we find the fluidic bilayer phase of cholesteryl laurate to be unstable and it switches to a crystalline bilayer phase. However, according to Craven, only cholesteryl esters with longer chain lengths starting from cholesteryl tridecanoate should show the crystalline bilayer phase. The thickness behavior of different phases was also studied by transferring the films onto a silicon substrate by using the Langmuir-Blodgett technique. Texture studies show that cholesterol, cholesteryl acetate, cholesteryl nonanoate, cholesteryl laurate, and cholesteryl myristate exhibit homogeneous films with large size domains, whereas cholesteryl palmitate and cholesteryl stearate exhibit less homogeneous films with smaller size domains. We suggest that such an assembly of molecules can be related to their molecular structures. Simulation studies may confirm such a relation. *Published by AIP Publishing.* [<http://dx.doi.org/10.1063/1.4984119>]

I. INTRODUCTION

The self-assembly of cholesteryl esters (CEs) at the interfaces has relevance to many biological processes.^{1,2} Cholesterol (Ch) and CEs are found in low density lipoprotein and pathological lesions of atherosclerosis.^{3,4} Recently it has been reported that CEs accumulate excessively in pancreatic cancer cells.⁵ There have been extensive studies on the bulk properties of CEs, but comparatively very few studies have been reported on their two-dimensional (2D) behavior. As Ch and CEs are known to form Langmuir films at the air-water (A-W) interface,^{6–8} they can be studied as ideal 2D model systems relevant to biomembranes.^{9,10} Further, a Langmuir film can be transferred onto a solid substrate by using the Langmuir-Blodgett (LB) technique to get an LB film.¹¹ In the LB film, one can control the various parameters like film thickness, homogeneity, and molecular orientation that are useful in device fabrication.

The thickness along with the surface pressure (π) measurements as a function of the surface density (i.e., equal to inverse of area per molecule) gives useful information on the organization of molecules. Lafont *et al.* have reported the variation in thickness of a Ch film along with the surface pressure-area per molecule (π - A_m) measurements.¹² However, for CEs, the thickness variation as a function of area per molecule (A_m) has not been reported. Further, it is known that the A-W interface can facilitate the formation of well-defined thin films of soft matter.¹³ At the A-W interface, studies on CEs have shown that short chain esters form stable monolayers; higher homologues form stable or unstable

fluidic bilayers, crystalline bilayers, and three-dimensional (3D) structures.^{6,7,14} These studies were based on surface manometry, Brewster angle microscopy, reflection, and epifluorescence microscopy. However, reports on the direct thickness measurements of the phases for CEs at the A-W and air-solid (A-S) interfaces are very rare. To get an insight into the molecular packing in a given phase at the interfaces, one needs to know the exact thickness of the film in the corresponding phase. In this context, imaging ellipsometry (IE) is a useful technique to visualize different phases and measure their thicknesses.^{15,16} In the literature, though there have been some reports on the LB film of Ch,^{12,17} not much attention has been given to the thickness measurement of the LB films of CEs.

There have been some attempts to draw an analogy in the packing of CEs at the A-W interface with that in the bulk.⁶ In the bulk, Craven and co-workers from their extensive X-ray crystal structure studies of CEs have given a model^{18,19} based on the interactions of cholesteryl-cholesteryl (m-i), cholesteryl-chain (m-ii), and chain-chain (crystalline bilayer) parts of the molecule (Fig. 1).

According to the model, cholesteryl caproate (number of carbon in alkyl chain $n = 6$) to cholesteryl octanoate ($n = 8$) should have m-i packing, cholesteryl nonanoate ($n = 9$) to cholesteryl laurate ($n = 12$) should have m-ii packing, and cholesteryl esters, cholesteryl tridecanoate ($n = 13$) onwards should have crystalline bilayer packing. It will be interesting to study the packing of CEs at the A-W and A-S interfaces based on Craven's model.

In this article, we report our measurements of the variation of film thickness as a function of A_m (d - A_m curve) carried out simultaneously with the π - A_m measurements for Ch and CEs. Using IE, we have carried out a comparative study of the

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FIG. 1. The schematic representation of the molecular packing in the bulk for cholesteryl esters.

texture and thickness of the different phases of Ch and CEs at the A-W and A-S interfaces. We have also used atomic force microscopy to study the morphology of the LB films. Based on our studies, we suggest a relationship between the structure of a molecule and its assembly at the A-W and A-S interfaces.

II. EXPERIMENTAL

A. Materials

The materials, cholesterol (Ch), cholesteryl acetate (ChA), cholesteryl nonanoate (ChN), cholesteryl laurate (ChL), cholesteryl myristate (ChM), cholesteryl palmitate (ChP), and cholesteryl stearate (ChS) were obtained from Sigma-Aldrich. Their molecular structures are shown in Fig. 2. The purity of the materials was confirmed by checking their

FIG. 2. A schematic representation of the molecular structure of cholesterol (top) and cholesteryl esters ($n = 2$, ChA; $n = 9$, ChN; $n = 12$, ChL; $n = 14$, ChM; $n = 16$, ChP; $n = 18$, ChS).

FIG. 3. Variations of surface pressure (π) and thickness (d) as a function of area per molecule (A_m) at the A-W interface for films: (a) ChA, (b) ChN, (c) ChL, (d) ChM, (e) ChP, and (f) ChS. The thicknesses were measured in a selected region of area $386 \times 485 \mu\text{m}^2$.

melting points under a polarizing optical microscope. The melting points agree with those reported in the literature.

B. Surface manometry

The surface pressure (π) as a function of the area per molecule (A_m), i.e., π - A_m isotherm gives information on phase transitions and molecular arrangements at the A-W interface. For our experiments, stock solutions of concentration 1 mg/ml were prepared in chloroform (HPLC grade). Surface manometry studies were performed using a commercially obtained Langmuir trough (KSV Instruments). Ultrapure de-ionized water (Millipore water, Milli-Q) of resistivity greater than 18 M Ω cm was used as the subphase (pH 5.7). The barrier speed for the compression of the film was 1.5 cm²/min. All our experiments were carried out at room temperature ($\sim 26^\circ\text{C}$).

C. Langmuir-Blodgett film deposition

The LB films were prepared by transferring Langmuir films onto a hydrophilic silicon substrate. The silicon substrate was made hydrophilic by treating it with a piranha solution (concentrated H₂SO₄ and H₂O₂ in 4:1 v/v ratio). The transfer of the Langmuir films was carried out during the upstroke of the silicon substrate at a rate of 2 mm/min at a π value of ~ 1 mN/m. The transfer ratio for all the cases was about 1.

D. Brewster angle microscopy

A Brewster angle microscope (BAM) enables direct visualization of the textures of the Langmuir films to characterize different phases.²⁰ We have used an imaging ellipsometer (EP3, Accurion) with a laser source of wavelength 532.1 nm and a charge-coupled device (CCD) to grab the images with an in-plane resolution of 2 μm . The angle of incidence was set close to the Brewster angle for the A-W interface ($\sim 53^\circ$).

E. Imaging ellipsometry

An ellipsometer measures the change in polarization states in terms of the ellipsometric angles delta (Δ) and psi (Ψ).²¹ Here Δ is the relative phase shift, and $\tan(\Psi)$ is the relative amplitude ratio of the Fresnel reflection coefficients R_P and R_S given by the relation,

$$\tan(\Psi) \exp(i\Delta) = \frac{R_P}{R_S} = f(\theta_0, \lambda, n_s, n_0, n_j, k_j, d_j),$$

where θ_0 is the angle of incidence, λ is the wavelength of the incident light, n_s and n_0 are the optical constants of the substrate and the ambient medium respectively, n_j and k_j are the optical constants of the films, and d_j is the film thickness with j being the number of layers.¹⁵

We have used an imaging ellipsometer for thickness and texture studies. At the A-W interface, for the thickness measurement as a function of A_m (d - A_m curve) simultaneously

TABLE I. The film thickness of different phases exhibited by the materials at the A-W and A-S interfaces. The symbols used are as follows: n = number of carbon in alkyl chain, A_0 = limiting area per molecule, π_c = collapse pressure, d_{CW} = thickness estimated from the d - A_m curve, d_{EW} = thickness measured at the A-W interface using IE, d_{ES} = thickness measured at the A-S interface using IE, d_{AS} = thickness measured at the A-S interface using AFM, a = L₂ phase, b = fluidic bilayer phase, and c = crystalline bilayer phase. The symbol in the parentheses indicates the particular phase.

Material (n)	A_0 ($\text{\AA}^2$)	π_c (mN/m)	Phase sequence in Langmuir films at A-W interface	d_{CW} (nm)	d_{EW} (nm)	d_{ES} (nm)	d_{AS} (nm)
Ch	41.00	44.00	(G + L ₂), L ₂ , (L ₂ + 3D crystallites)	1.70	1.70 (a)	1.70 (a)	1.75 (a)
ChA (2)	43.00	17.00	(G + L ₂), L ₂ , (L ₂ + 2D crystalline + 3D crystallites)	2.50	2.40 (a)	2.50 (a)	2.20 (a)
ChN (9)	21.50	3.20	(G + fluidic bilayer), fluidic bilayer, (fluidic bilayer + bright dots), (fluidic bilayer + circular domains + 3D structures)	4.50	4.00 (b)	3.50 (b)	4.00 (b)
ChL (12)	17.00	...	[G + fluidic bilayer (unstable)], (G + crystalline bilayer + 3D structures)	8.00	5.50 (c)	6.00 (c)	5.70 (c)
ChM (14)	23.00	...	G + crystalline bilayer + 3D structures	10.00	5.60 (c)	6.30 (c)	5.00 (c)
ChP (16)	24.00	...	(G + crystalline bilayer), (G + crystalline bilayer + 3D crystallites)	5.00	5.00 (c)	4.50 (c)	5.00 (c)
ChS (18)	21.00	...	G + crystalline bilayer + 3D structures	8.50	7.00 (c)	5.10 (c)	5.80 (c)

with the π - A_m measurements (Fig. 3), the incident angle was set to 50° . This thickness measurement was carried out in a selected region of area $386 \times 485 \mu\text{m}^2$. The thickness value (d_{CW}) was obtained (Table I) from the d - A_m curve, corresponding to a particular π value. It was the same π value at which the film was transferred onto a silicon substrate for the preparation of the LB film. For the measurement of thickness of a particular phase at the A-W interface (d_{EW}) and at the A-S interface (d_{ES}), we have chosen the angle of incidence to be around 50° and 60° , respectively. For the d_{EW} and d_{ES} measurements, we have selected a smaller region of area $35 \times 40 \mu\text{m}^2$. For the optical modeling of the films at the A-W interface, we assumed a system of air–film–water. Corresponding to the A-S interface, we have assumed a system of air–film–silicon dioxide–silicon. Experimentally Δ and Ψ were obtained and then the EP4 modeling software was used for the thickness calculation. For Ch and some CEs, Alonso *et al.* and Lafont *et al.* have used optical constants for similar kinds of measurements at the A-W interface.^{7,12} For our calculation, we have used their values. The calculations yield the thickness value with an error of ± 0.10 nm.

F. Atomic force microscopy

An atomic force microscope (AFM) (Agilent 5500) was employed in the acoustic alternate current (AAC) mode to study the surface morphology and the thickness (d_{AS}) of LB films. A cantilever tip (Silicon, NSC15/Al BS, Mikromasch) with a spring constant in the range of 20–80 N/m and resonance frequency in the range of 265–410 kHz was used to scan the film surface. The AFM images were analyzed using the WSxM software.

III. RESULTS AND DISCUSSION

A. Simultaneous π - A_m and d - A_m measurements

The simultaneous measurements of the thickness-area per molecule (d - A_m) and the surface pressure-area per molecule (π - A_m) carried out for CEs at the A-W interface are shown in Fig. 3 (for Ch see Fig. S1 of the [supplementary material](#)). The π - A_m isotherms for Ch, ChA, and ChN show stable Langmuir films with well-defined collapse pressure values of 44 mN/m, 17 mN/m, and 3.2 mN/m, respectively. For ChL, ChM, ChP, and ChS, the isotherms show a continuous increase in π without any sharp change in the slope. Such a behavior for ChM has been reported by Nishimura *et al.*²² However, in some cases, the π - A_m curve shows a slight change in the slope. In this region, our BAM studies did not show any significant changes in the texture of the film. This change in the slope may be due to the reorganization of domains in the film. The compression-decompression experiments show the Langmuir films to be stable even after repeated cycles. The loops for the first cycle for CEs are shown in Fig. S2 of the [supplementary material](#). The limiting area per molecule (A_0) values (obtained by extrapolation to zero π value at the steep region of the isotherm) are shown in Table I. The A_0 values suggest a monolayer film for Ch and ChA. The compressibility modulus calculation suggests the monolayer film to be in a high density liquid or condensed (L_2) phase. For ChN,

ChL, ChM, ChP, and ChS, the A_0 values suggest a bilayer film.

Figure 3 and Fig. S1 of the [supplementary material](#) show that in general, the thickness starts increasing sharply even before the rise in π and then shows a gradual increase. We have tabulated the thickness value (d_{CW}) obtained from the d - A_m curve (Table I). In general, the d - A_m curve follows the π - A_m isotherm.

B. Switching of molecular packing in ChL

The BAM studies for ChL at the A-W interface show the coexisting G (dark region) and fluidic (grey) phases at large A_m [Fig. 4(a)]. The fluidic phase can be seen with mobile circular boundaries. It was very unstable and hence it was not possible to measure the thickness. The fluidic phase of ChL was similar in nature to that of the stable fluidic phase seen for ChN. The measured thickness indicated the fluidic phase of ChN to have an m-ii packing. We find that the unstable fluidic phase of ChL transforms to a crystalline phase [Fig. 4(b)] immediately. The thickness (d_{EW}) of the crystalline phase measured at the A-W interface yields a value of 5.5 nm, which is close to the

FIG. 4. The BAM images of ChL at the A-W interface: (a) Coexistence of G and fluidic bilayer (unstable) phases at $A_m = 28 \text{ \AA}^2$; (b) coexistence of G and crystalline bilayer ($d_{EW} = 5.5$ nm) phases at $A_m = 28 \text{ \AA}^2$. (c) The ellipsometric contrast image of ChL on the silicon substrate ($d_{ES} = 6$ nm). The box (size $\sim 35 \times 40 \mu\text{m}^2$) indicates the region where the thickness was measured. The dimension of the images [(a)-(c)] is $386 \times 485 \mu\text{m}^2$. (d) The AFM image of the ChL film on the silicon substrate yields a height of 5.7 nm (d_{AS}). The switching of the molecular packing of ChL from (e) m-ii type to (f) crystalline bilayer type ($X = 1.74$ nm, length of main skeleton; $Y = 1.40$ nm, length of chain).

estimated (ChemDraw software, version 12.0) thickness of 4.88 nm for the crystalline bilayer packing [Fig. 4(f)]. Neglecting the slight difference to arise from the molecular rearrangement, we conclude that ChL has a crystalline bilayer packing corresponding to the crystalline bilayer phase. The IE [Fig. 4(c)] and AFM [Fig. 4(d)] studies further confirm the crystalline bilayer packing for ChL at the A-S interface. In bulk, according to Craven, ChL should have only an m-ii type packing. But our study suggests that at the A-W interface, the m-ii packing switches to a crystalline bilayer packing that is known for the next higher homologue, cholesterol tridecanoate (ChT) onwards.^{6,18,19} Recently, it has been reported that temperature can induce a switching in the shape of the self-assemblies (micelles to vesicles) in the bulk.²³ In our case, the interface induces switching in the molecular packing of ChL from m-ii to the crystalline bilayer type.

C. Phase behavior and lateral assembly

The phase sequences of Ch, ChA, ChN, ChL, and ChM at the A-W interface are shown in Table I. We find that Ch and ChA exhibit the L_2 phase; ChN exhibits the fluidic bilayer phase; ChL exhibits the unstable fluidic bilayer phase that switches to crystalline bilayer phase, and ChM exhibits the crystalline bilayer phase. Further, ChA and ChN exhibit some new phases that are shown in the [supplementary material](#) (see Fig. S3). The BAM images of ChA, ChN, ChL, and ChM are shown in Figs. 5, 6, 4, and 7, respectively (for Ch see Fig. S4 of the [supplementary material](#)). They exhibit homogeneous films with large size domains ($>100\ \mu\text{m}$). The LB films of Ch, ChA, ChL, and ChM show similar texture as that at the A-W interface. In the case of ChN, the texture at the A-S interface [Fig. 6(b)] was slightly different due to dewetting of the fluidic

FIG. 5. (a) The BAM image of ChA at the A-W interface: The uniform L_2 phase ($d_{EW} = 2.4\ \text{nm}$) at $A_m = 41\ \text{\AA}^2$. (b) The ellipsometric contrast image of ChA on silicon substrates ($d_{ES} = 2.5\ \text{nm}$). The box (size $\sim 35 \times 40\ \mu\text{m}^2$) indicates the region where the thickness was measured. The dimension of the images [(a) and (b)] is $386 \times 485\ \mu\text{m}^2$. (c) The AFM image of the ChA film on the silicon substrate yields a height of 2.2 nm (d_{AS}).

FIG. 6. (a) The BAM image of ChN at the A-W interface: A uniform bilayer ($d_{EW} = 4\ \text{nm}$) phase at $A_m = 20.5\ \text{\AA}^2$. (b) The ellipsometric contrast image of the ChN on the silicon substrate ($d_{ES} = 3.5\ \text{nm}$). The box (size $\sim 35 \times 40\ \mu\text{m}^2$) indicates the region where the thickness was measured. The dimension of the images [(a) and (b)] is $386 \times 485\ \mu\text{m}^2$. (c) The AFM image of the ChN film on the silicon substrate yields a height of 4 nm (d_{AS}).

phase. Further, the phases of Ch, ChA, ChN, ChL, and ChM were confirmed by the AFM.

The phase sequences of ChP and ChS are shown in Table I. They exhibit the crystalline bilayer phase. The BAM images of ChP and ChS at the A-W interface are shown in Figs. 8 and 9, respectively. They show less homogeneous films with smaller size domains. The LB films of ChP and ChS as seen in the IE

FIG. 7. (a) The BAM image of ChM at the A-W interface: Coexistence of G and the crystalline bilayer ($d_{EW} = 5.6\ \text{nm}$) phases with 3D structures at $A_m = 35\ \text{\AA}^2$. (b) The ellipsometric contrast image of ChM on silicon substrates ($d_{ES} = 6.3\ \text{nm}$). The box (size $\sim 35 \times 40\ \mu\text{m}^2$) indicates the region where the thickness was measured. The dimension of the images [(a) and (b)] is $386 \times 485\ \mu\text{m}^2$. (c) The AFM image of the ChM film on the silicon substrate yields a height of 5 nm (d_{AS}).

FIG. 8. (a) The BAM image of ChP at the A-W interface: Coexistence of G and the crystalline bilayer ($d_{EW} = 5$ nm) phases at $A_m = 25 \text{ \AA}^2$. (b) The ellipsometric contrast image of ChP on the silicon substrate ($d_{ES} = 4.5$ nm). The box (size $\sim 35 \times 40 \mu\text{m}^2$) indicates the region where the thickness was measured. The dimension of the images [(a) and (b)] is $386 \times 485 \mu\text{m}^2$. (c) The AFM image of the ChP film on the silicon substrate yields a height of 5 nm (d_{AS}).

exhibit similar textures at the A-S interface as that at the A-W interface. The AFM images show less homogeneous films (compared to Ch, ChA, ChN, ChL, and ChM) with smaller size domains ($\sim 10 \mu\text{m}$).

FIG. 9. (a) The BAM image of ChS at the A-W interface: Coexistence of G and crystalline bilayer ($d_{EW} = 7$ nm) phases with 3D structures at $A_m = 30 \text{ \AA}^2$. (b) The ellipsometric contrast image of ChS on the silicon substrate ($d_{ES} = 5.1$ nm). The box (size $\sim 35 \times 40 \mu\text{m}^2$) indicates the region where the thickness was measured. The dimension of the images [(a) and (b)] is $386 \times 485 \mu\text{m}^2$. (c) The AFM image of the ChS film on the silicon substrate yields a height of 5.8 nm (d_{AS}).

TABLE II. Calculated lengths (ChemDraw) of main skeleton (X) and chain (Y) for cholesteryl esters, n = number of carbon in the alkyl chain.

Molecules (n)	X (nm)	Y (nm)	X-Y (nm)
ChA (2)	1.74	0.15	1.59
ChN (9)	1.74	1.01	0.73
ChL (12)	1.74	1.40	0.34
ChM (14)	1.74	1.65	0.09
ChP (16)	1.74	1.91	-0.17
ChS (18)	1.74	2.16	-0.42

We suggest that the assembly of molecules in a film can be related to the length of the main skeleton (X) with that of the chain (Y). The X and Y values for CEs estimated from ChemDraw are given in Table II. We find that for positive values of (X-Y), the film tends to be homogeneous with large size domains. For negative values of (X-Y), the film tends to be less homogeneous with smaller size domains. According to this relation, we expect ChT which lies between ChL and ChM to exhibit homogeneous films with large size domains. For ChT, Alonso *et al.* have reported an ordered phase based on their grazing incidence X-ray diffraction (GIXD) studies.⁷ Hence we may infer that the homogeneous film with large size domains can be related to the ordered phase. Further, for ChP and ChS, we observed less homogeneous films with smaller size domains that can be related to the less-ordered phase as reported by Alonso *et al.*⁷ As a follow up, we suggest that ChM should also have an ordered phase as compared to ChP and ChS. It will be interesting to investigate it by a GIXD study. Fractal analysis of the BAM images²⁴ may also reveal a correlation between the domain size and the ordered/less-ordered phase. Recently, there have been some simulation studies on the domain formation in biomimetic membranes.²⁵ Our studies on cholesteryl esters along with the simulation studies may give a better understanding of the domain formation.

D. Film thickness at A-W and A-S interfaces

The thicknesses measured using the IE and AFM are shown in Table I. The d_{EW} and d_{ES} values give the thicknesses measured by the IE at the A-W and A-S interfaces, respectively. The d_{AS} value gives the thickness measured by AFM at the A-S interface. We find that in general, the thicknesses d_{EW} , d_{ES} , and d_{AS} agree reasonably well. It may be noted that the d_{EW} and d_{ES} values were obtained for a smaller, well defined region indicated by a box (size $\sim 35 \times 40 \mu\text{m}^2$) in a particular phase of our interest.

We may compare the thickness d_{EW} with the d_{CW} values, both measured at the A-W interface. We find a reasonably good agreement in the d_{EW} and d_{CW} values for Ch, ChA, ChN, and ChP. For the cases of ChL, ChM, and ChS, the thicknesses d_{CW} were of higher values when compared to d_{EW} . We would like to point out that the thickness d_{EW} was measured for a particular phase (as indicated in parentheses in Table I). Here, the barriers were at rest and the Langmuir film was static. The value d_{CW} was estimated from the d - A_m curve (Fig. 3) corresponding to a particular π value. In this case, the Langmuir film was undergoing a dynamic change due to the continuous

compression, and the average thickness was measured in a much larger region of area $386 \times 485 \mu\text{m}^2$ (about 130 times larger compared to the area used for the measurement of d_{EW} at the A-W interface). For ChL, ChM, and ChS, such a large region contains some 3D structures along with other phases (as observed in BAM images) resulting in higher d_{CW} values.

Thickness measurements confirmed a monolayer phase for Ch and ChA. For ChN, the measured thickness values confirmed the fluidic phase to be bilayer. To understand the packing in the light of Craven's model, we have calculated (ChemDraw) the thickness of ChN for m-ii, and the crystalline bilayer packing, which was found to be 3.48 nm and 4.50 nm, respectively. Our measured values are closer to 3.48 nm suggesting m-ii packing for ChN. Further, for ChM, ChP, and ChS, our measured thickness values suggest a crystalline bilayer packing. For ChP and ChS, Alonso *et al.* have reported the thickness values to be 5.8 nm and 6.47 nm, respectively, based on their ellipsometry studies at the A-W interface.⁷ Their values are comparable to our values measured by IE for ChP and ChS in the crystalline bilayer phase at the A-W interface.

IV. CONCLUSIONS

Our direct thickness measurements show that the Craven's model^{18,19} of molecular packing proposed for bulk can be extended to the packing at the A-W interface. Accordingly, we find that cholesteryl nonanoate forms a stable fluidic bilayer phase and cholesteryl myristate, cholesteryl palmitate, and cholesteryl stearate form a crystalline bilayer phase. Interestingly, in cholesteryl laurate we find a switching behavior. Cholesteryl laurate forms a fluidic bilayer phase as suggested by Craven, but it immediately transforms to a crystalline bilayer phase. We suggest that cholesteryl laurate with a chain length just at the transition point^{18,19} has an m-ii type packing in the beginning and then it switches to crystalline bilayer packing. The crystalline bilayer phase should be shown only by cholesteryl esters with a longer chain length, starting from cholesteryl tridecanoate. Switching of the packing in cholesteryl laurate can be attributed to the dominant role played by the A-W interface. We have measured the variation in thickness of the film as a function of area per molecule (d - A_{m} curve) for cholesterol and cholesteryl esters using imaging ellipsometry along with the surface manometry measurements. The thickness of a particular phase of a film at the A-W interface measured by imaging ellipsometry (d_{EW}) agrees well with that at the A-S interface measured by the imaging ellipsometry (d_{ES}) and atomic force microscopy (d_{AS}). In general, the films of cholesteryl esters exhibit similar texture at the A-W and A-S interfaces indicating a controlled transfer onto the solid substrate that is useful for device applications. We find that cholesterol, cholesteryl acetate, cholesteryl nonanoate, cholesteryl laurate, and cholesteryl myristate exhibit homogeneous films with large size domains, whereas cholesteryl palmitate and cholesteryl stearate exhibit less homogeneous films with smaller size domains. We can relate the

homogeneity and size of the domain to the molecular order of the film reported by Alonso *et al.* based on their GIXD studies.⁷ We suggest that in general, the assembly of the molecules in the films at the A-W and A-S interfaces can be related to the structure of the molecule. This gives scope for the modeling and simulation studies for a better understanding of the assembly of cholesteryl esters at interfaces.

SUPPLEMENTARY MATERIAL

See [supplementary material](#) for variations of surface pressure (π) and thickness (d) as a function of area per molecule (A_{m}) at the air-water interface for cholesterol (Fig. S1); the π - A_{m} compression-decompression cycle for cholesteryl esters (Fig. S2); Brewster angle microscope images of cholesteryl acetate and cholesteryl nonanoate (Fig. S3); Brewster angle microscope, ellipsometric contrast, and atomic force microscope images for cholesterol (Fig. S4).

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