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Study The Influence Of Interaction Of Ascorbic Acid Isoforms With 1, 2 Dioctanoyl-Sn-Glycero-3-Phosphocholine Lipid Monolayer As A Cell Membrane Model At Different Conditions.

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ABSTRACT

Recently, molecular films of biomolecules have received increased attention because of their potential applications in biological sensors, bioreactors and other applications in materials science. Phospholipids are possible to create compatible interfaces between traditional materials and biomaterials for constructing biosensors or biochemical probes. The properties of the films can be easily varied by changing the structure of the amphiphilic molecule, the composition of the subphase and the surface pressure. In this investigation an attempt to study the influence of interaction of *L*- and *D*-ascorbic acid biomolecule isoforms: *L*-AscA and *D*-AscA with DOPC monolayer on Langmuir- monolayer formation and obtain a thin film of DOPC surfactant on a modified subphase with biologically and optically-active acids *L*-AscA and *D*-AscA, as an object that simulates cellular membranes. The results showed significant differences in interactions between the synthetic, natural AscA biomolecule isoforms and DOPC monolayer in different concentration 10^{-3}, $10^{-2}$and 10^{-1} Mat temperatures 25, 37 and 41°Cat a fixed pressure were presented.

Keywords: Langmuir-Blodgett films, Lipid monolayer, DOPC, Ascorbic acid, Fluidity, Pressure-Area isotherm

Abbreviations:

PC: Phosphatidylcholine; DOPC (C_8): 1,2dioctanoyl-sn-glycero-3-phosphocholine; AscA: Ascorbic acid; (π-A): Pressure- Area isotherms; π_{coll}: Pressure collapse; L: Liquid phase; LE: Liquid Expand phase LC: Liquid Condense phase; C: Solid phase; S: Solid phase; G: Gas phase

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INTRODUCTION

In the last years, Langmuir monolayers have been used as extremely versatile and easy to handle model systems allowing the investigation of interactions with different species of biochemical compounds (Ions, Drugs, DNA, Vitamin, Peptides, Hormones, Polymers, and NPs….etc.) spread on the surface or dissolved in the subphase [1-4]. Amphiphilic molecules forming the monolayers are trapped at the air/water interface and give indirect information about interactions with the other components via changes detected by highly surface sensitive techniques [5-8]. Phosphatidylcholine (PC) phospholipids with short chain (1,2dioctanoyl-sn-glycero-3-phosphocholine DOPC(C_8)) have been widely studied with the Langmuir technique, because they are amphiphilic compounds, ideal for forming ordered and compact monolayers, and for their potential applications in biological sensors and other applications in materials science [9-12].Ascorbic acid AscA (Vitamin C) was intensively studied biomolecules. AscA cannot be synthesized by humane body and therefore the presence of these compounds in human organism is dependent on the foods. Within this group, the investigations are focused mainly on AscA isoforms *L*-AscA and *D*-AscA. The *L*-AscA, which can come in natural (found in foods) and synthetic isoforms (found in most other supplements) while, *D*-AscA isoforms, meanwhile, does not exist in nature and though chemically identical to its counterparts are molecularly different. AscA possess antioxidant properties, are precursors of vitamin A and have beneficial influence on human organism through prevent coronary vascular diseases, cancers, positively affect the immune system and prevent eye diseases [12-18]. In this investigation an attempt to study the influence of interaction of *L*- and *D*-AscA isoforms with DOPC monolayer as cell membrane model and obtain a thin film of DOPC surfactant on a modified subphase with biologically and optically-active acids (*L*-AscA and *D*-AscA), as an object that simulates cellular membranes.

MATERIALS AND EXPERIMENTAL METHODS

Materials

Phospholipid used in this study was 1,2dioctanoyl-sn-glycerol-3-phosphocholine DOPC(C_8), obtained from Sigma Aldrich. *L*- and *D*-AscA received from (Meligen corp., RF and Khimreaktiv corp., RF, respectively) .Their chemical structures are in figure 1;they all had purities of 99% and were used as received. Spectroscopic grade chloroform (Aldrich) was used as the spreading solvent. Water was prepared using a Millipore Milli-Q system, which had a resistivity of >18.2 MΩ×cm.

Experimental Methods

*Langmuir-Blodgett technique*an automatically controlled Langmuir film balance KSV3000 (KSV Instruments Ltd., Finland) equipped with a platinum Wilhelmy plate, was used to obtain the surface pressure-area (π-A) isotherms of monolayers at the air/water interface. For all experiments, The DOPC was dissolved in chloroform in concentration 10^{-3} M. DOPC was sprayed on the trough was filled with: first on thesurface of purified water subphase, second on the surface of*L*- and *D*-AscA subphase in concentration 10^{-3} , 10^{-2} and 10^{-1} M. The temperature was maintained by external water bath circulation 25, 37 and 41°C. The air/water interface could be compressed and expanded symmetrically with two teflon barriers at a desired rate. The cleanliness of the trough and subphase was ensured before each experiment by aspirating the surface of subphase. When the surface pressure fluctuation was found to be less than 0.1mN/m during the compression cycle, DOPC sample was spread on the subphase surface by using a microsyringe (Hamilton Co., USA). Five minutes were allowed for solvent evaporation and monolayer equilibration before an experiment was started. After allowing for the solvent to evaporate, the monolayer at the air/water interface was continuously compressed at a rate of 15 cm^2/min to obtain the(π-A) isotherms.

The principal characteristics of Langmuir monolayers from which data on the molecular organization of the monolayers and molecular interactions therein can be drawn include the course, shape, and the location of the (π-A) isotherms. First, the molecular area (A°) that corresponds to the surface area occupied by one molecule in a highly compressed monolayer is estimated, and this is done by extrapolation of the steep linear part of the (π-A) isotherms to zero surface pressure. Second, the collapse pressure (π_{coll}), that is a pressure at which a monolayer breaks, is determined from the point where an isotherm halts to rise upon monolayer compression. Third, to characterize the state of the monolayers and the phase transitions, the compression modulus (C_S^{-1}) is calculated. The compression modulus is defined as:

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$$C_S^{-1} = -A\,(d\pi/dA) \ldots\ldots\ldots (1)$$

Where *A* is area per molecule and π is surface pressure. C_S^{-1} coefficient gives the information on the phase, in which monolayer occurs. The states of monolayers are classified on the basis of the maximal values of C_S^{-1} in the plots of C_S^{-1} versus π in the following way: $C_{S,max}^{-1}$ =12.5 -50 mN/m liquid phase (L); $C_{S,max}^{-1}$ = 50-100 mN/m liquid expand phase (LE); $C_{S,max}^{-1}$ = 100-250 mN/m liquid condense phase (LC); $C_{S,max}^{-1}$>250 mN/ m solid phase (S). The minima in the plots of C_S^{-1} versus π on the other hand, correspond to the phase transitions.

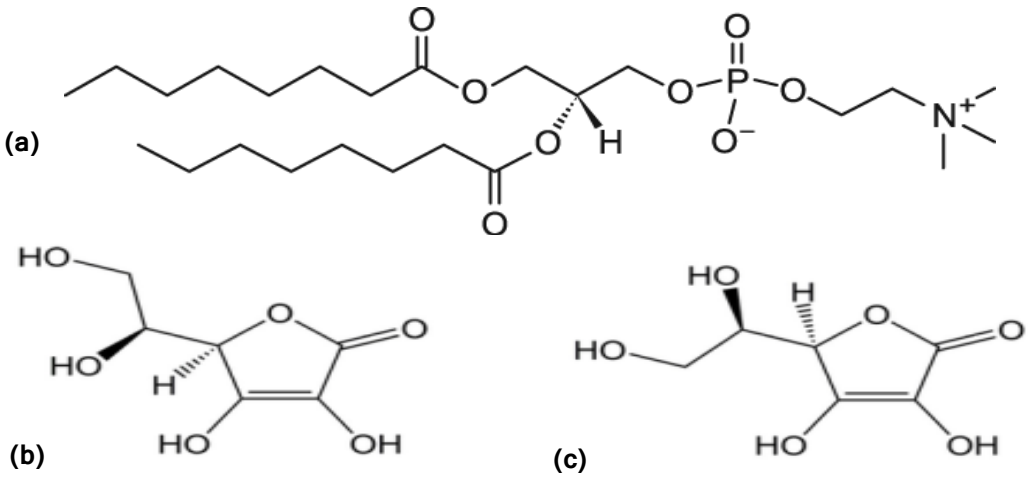

Figure 1: The chemical structures of (a) DOPC, (b) *L*-AscA and (c) *D* -AscA

RESULTS AND DISCUSSION

The (π-A) isotherms of DOPC monolayerat different temperatures

The (π-A) isotherms show that PCs with short alkyl chain have smooth increase of surface pressure, which could be explained as the monolayer fluidity of DOPC, because they are dominated by the weak order of its relatively short hydrocarbon chains. Short chains of DOPC make relatively flexible and weak interaction between the chains and these facts agree in figure 2, by increasing temperature from 25 to 41°C, the surface pressure and the monolayer C_S^{-1}decreases. At area 225/molecule, the surface pressure values have 47 mN/m, C_S^{-1}173mN/m for 25°C, in 233/molecule 45mN/m,C_S^{-1}187mN/m for 37 °C, and in 256/molecule 43mN/m,C_S^{-1}133mN/m for 41°C respectively. It explains that, the (π-A) isotherms are dominated by the combined effect of hydrophobicity of alkyl chains and interactions between hydrophilic part and aqueous subphase. The isotherm of DOPC is dominated by the interactions between the hydrophilic part and the aqueous surface because of relatively short alkyl chain. So, by increasing temperature, hydrogen bonding between head group and aqueous phase is broken, and the surface pressure through full range is reduced. So, by increasing temperature, the thermal motion of alkyl chains is increased, and the surface pressure is increased. By all accounts, with expansion of alkyl chain length, the predominance of hydrophobic interaction in surface pressure is getting bigger. The nature of the phospholipids in the membrane helps keep it fluid and semi-permeable, so that some molecules like oxygen, carbon dioxide and small hydrocarbons can move through it and enter the cell, while other molecules that might be harmful or unneeded by the cell are kept out. The composition of a membrane can affect its fluidity. The membrane phospholipids incorporate fatty acids of varying length and saturation. Lipids with shorter chains are less stiff and less viscous because they are more susceptible to changes in kinetic energy due to their smaller molecular size and they have less surface area to undergo stabilizing van der Waals interactions with neighboring hydrophobic chains. Cells function best at normal physiological temperature, which is 36.6°C in warm-blooded cell like humans. If body temperature increases, for example during a high fever, the cell membrane can become more fluid. This happens when the fatty acid tails of the phospholipids become less rigid and allow more movement of proteins and other molecules in and through the membrane. This can change the permeability of the cell, possibly allowing some potentially harmful molecules to enter. Both integral and peripheral proteins in the membrane can also be

damaged by high temperatures and, if extremely high, heat might cause these proteins to break down, or denature.

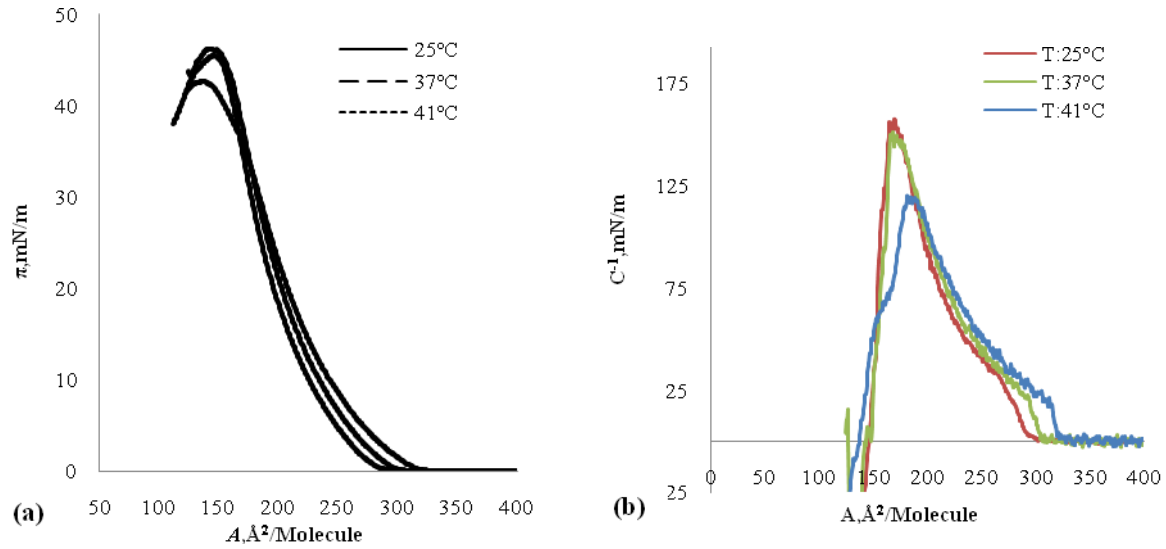

Figure 2: The (π-A) isotherms of DOPC in purified water subphase at 25, 37 and 41C° (a), comparison with the compression modulus $C_S^{-1}$and(π-A) isotherms of DOPC monolayers at 25, 37 and 41C° (b).

The (π-A) isotherms of DOPC monolayer in *L*- and *D*- AscA as a subphase in concentration 10^{-3}, 10^{-2} and 10^{-1} Mat 25°C.

Table 1, explain the changes in area A° of (π-A) isotherms, pressure collapse and the compression modulus $C_S^{-1}$of monolayer formation in 25°C. From results observed the significant increase in areaA° of (π-A) isotherms of monolayer formation for both*L*- and *D*-AscA as a subphase in10^{-2} ,10^{-1}M,and significant increase of collapse formation in 10^{-2}M for *D*-AscA as a subphase while significant increase in10^{-3}M for *L*-AscA, and significant decrease in collapse formation in10^{-2},10^{-1}M for *L*-AscA as a subphase ,these difference's due to, the synergistic effect of DOPC structure as mention above and the natural , synthetic varieties of *L*- and *D*-AscA, figure 3.

Table 1: The(π-A) isotherms of DOPC monolayers A°, π_{COLL} ,C_S^{-1}in *L*- and *D*-AscA as a subphase concentration 10^{-3} , 10^{-2} and 10^{-1} Mat 25°C.

Temperature:25°C	*Subphase*	*C*, M	*A*,Å²	π_{coll},*mN/m*	C_S^{-1}, *mN/m*
	D-AscA	**10^{-3}**	225	46	171
		10^{-2}	242	49	172
		10^{-1}	250	46	162
	L-AscA	**10^{-3}**	240	48	193
		10^{-2}	250	45	166
		10^{-1}	295	44	117

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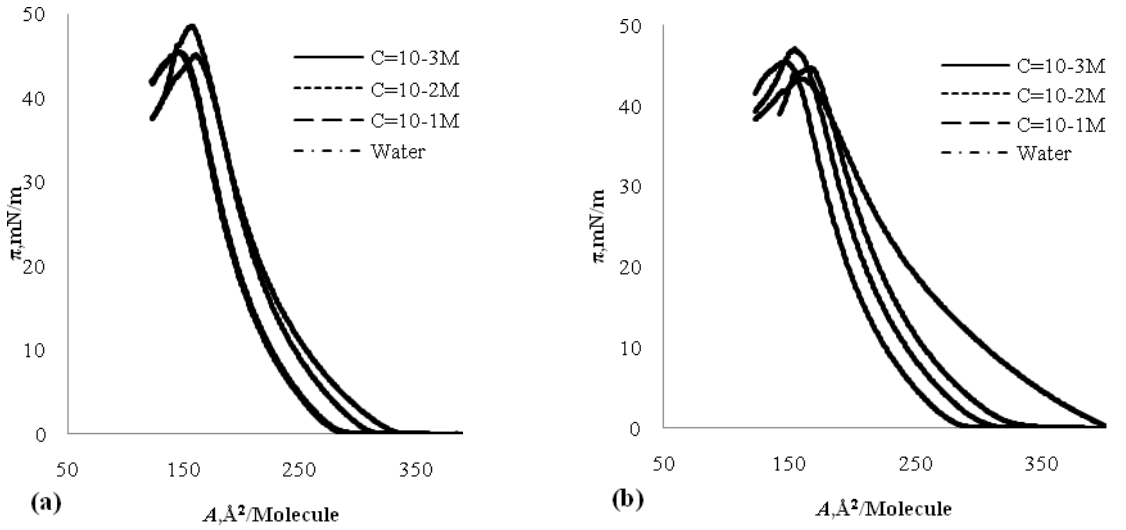

Figure 3: The(π-A) isotherms of DOPCmonolayerin *D*- AscA (a) and *L*-AscA (b) as a subphase concentration 10^{-3} , 10^{-2} and 10^{-1} Mat 25°C.

The (π-A) isotherms of DOPC monolayers in *L*- and *D*-AscA as a subphase at different temperatures 37 and 41°C

From table 2, there is similar significant effect of temperature and concentration on area A° of (π-A) isotherms of DOPC monolayersformation in *L*- and *D*-AscA subphase. In temperatures 37 and 41°Cno significant effect of temperature 37°C on collapse formation and compression modulus C_S^{-1}on*L*- and *D*-AscA subphase in concentration 10^{-3} and 10^{-2}M,butin high *L*- and *D*-AscA concentration10^{-1}M have a significant effect, and observed the effect of natural *L*-AscA and synthetic isoform *D*-AscA. Between the natural and synthetic varieties, the reactions will be different with DOPC as a subphase under the synergistic effects of concentration and temperature, in natural *L*-AscA showed significant and more sensitivity to the reaction conditions effect like high concentration 10^{-1}M at high temperature 41°C in comparison with synthetic isoform *D*-AscA by ripped reaction with DOPC, these changes are clear in the area of compressionisotherms,G, L andLC phase and collapses formation. While synthetic isoform *D*-AscA influence the monolayer formation by increasing the LC phase rigidity and make it more stable by increasing collapses formation at different conditions, figure 4, 5. This effect of high temperature 41°Con monolayer make it relatively flexible due to, the weak interaction first between the DOPC chains, second between hydrogen bonding of head group and the active site (hydroxyl group) of *L*- and *D*-AscA subphase because they become more soluble especially the natural isoform *L*-AscA, and the surface pressure through full range is reduced these fact cold be seen clearly by compression modulus C_S^{-1}, figure 6.

Table 2: **The(π-A) isotherms of DOPC (C_8) monolayers A°, π_{COLL} and C_S^{-1}in *L*- and *D*-AscA as a subphase concentration 10^{-3} , 10^{-2} and 10^{-1} M at 37°C.**

*Temperature:*37°C	*Subphase*	*C*, M	*A*,Å²	π_{coll},*mN/m*	C_S^{-1}, *mN/m*
	D-AscA	10^{-3}	209	45	176
		10^{-2}	225	45	192
		10^{-1}	212	47	154
	L-AscA	10^{-3}	221	44	155
		10^{-2}	230	44	151
		10^{-1}	270	41	122

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Table 3: The (π-A) isotherms of DOPC (C_8) monolayers A°, π_{COLL} and C_S^{-1} in *L*- and *D*-AscA as a subphase concentration 10^{-3} , 10^{-2} and 10^{-1} M at 41°C.

Temperature:41°C	*Subphase*	*C*, M	*A*,Å²	π_{coll},*mN/m*	C_S^{-1}, *mN/m*
	D-AscA	10^{-3}	270	44	152
		10^{-2}	271	45	165
		10^{-1}	285	44	109
	L-AscA	10^{-3}	272	42	140
		10^{-2}	280	44	149
		10^{-1}	300	40	118

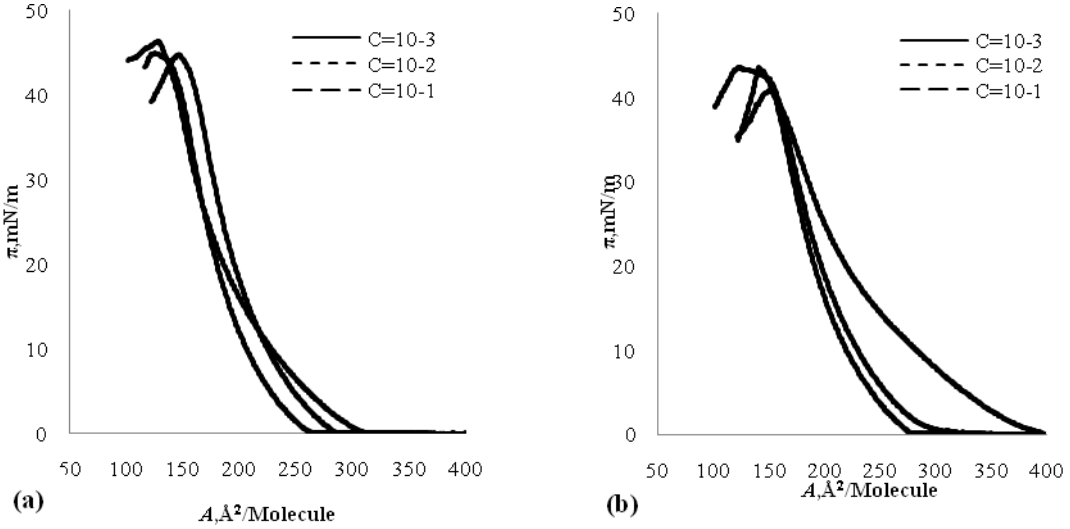

Figure 4: The(π-A) isotherms of DOPC monolayerin *D*- AscA (a) and *L*-AscA (b) as a subphase concentration 10^{-3} , 10^{-2} and 10^{-1} M at 37°C

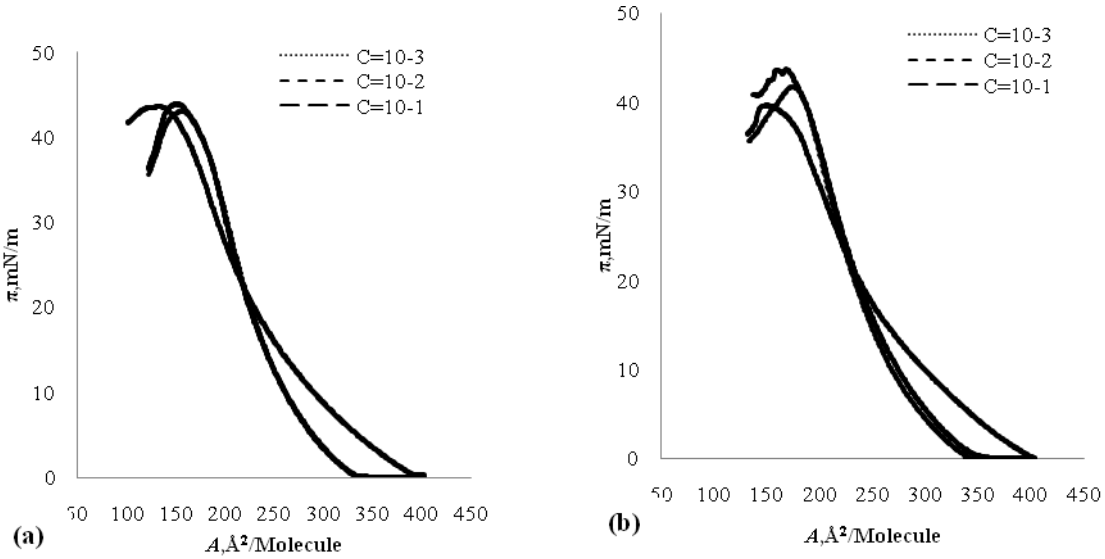

Figure 5: The(π-A) isotherms of DOPC monolayerin *D*-AscA (a) and *L*-AscA (b) as a subphase concentration 10^{-3} , 10^{-2} and 10^{-1} Mat 41°C

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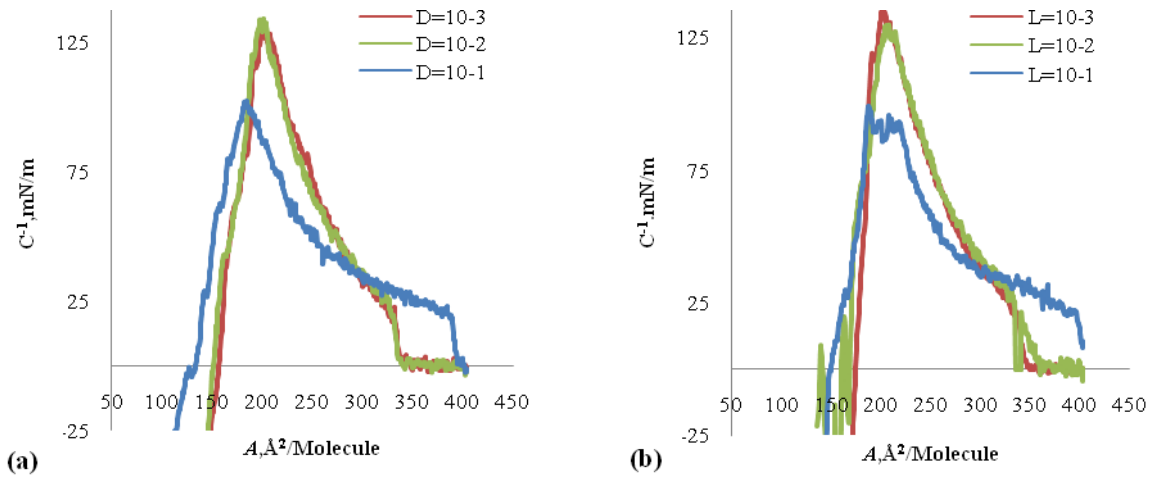

Figure 6: The compression modulusC_S^{-1} of DOPC monolayer in *D*-AscA (a) and *L*-AscA (b) as a subphase concentration 10^{-1} Mat temperature 41°C.

CONCLUSION

From results, we have significant differences in interactions between the AscA biomolecule isoforms and DOPC monolayer and this will be influence the packing and ordering of the films. The high concentrations of *L*- and *D*- AscA isoforms subphase have a significant effect on(π-A) isotherms of DOPC. There is a significant effect of temperature on the monolayer formation as a cell membrane model and this effect be more increased in high *L*- and *D*-AscA isoforms subphase concentration.

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