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AFM Study of a Lipid Multilayer with Cholesterol

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***Abstract*— Atomic force microscopy and Uv-Vis Spectra is used to study the effect of cholesterol in distribution and absorption of 1,2-Distearoyl-sn-glycero-3-phosphocholine lipid multilayer. The lipid/cholesterol is deposited on to a glass surface by spreading of suspension and spin coating. The results show high distribution of lipid multilayer increase with increasing of cholesterol. The absorption spectra of the lipid multilayer don't change with increase of cholesterol concentration in multilayer.**

***Keywords* — AFM, Cholesterol, Lipid, UV-Vis**

I. INTRODUCTION

Basic photonic ingredients can be generated from a large diversity of materials by top-down lithography. Examples include simple gratings and two- or three-dimensional photonic materials. The main challenge is to find new materials for generation of complex device in the simple way with efficient way, [1]. Phospholipids are fundamental structural of biological membranes that they are self-organize to form liposomes. Lipids include a category of naturally molecules that consist of waxes, fats, phospholipids, sterols. Lipids have a lot of application in nanotechnology and in food industries. Membrane lipids are compounds which form the double layered surface of all cells. The main major of membrane lipids are phospholipids, glycolipids, and cholesterol. Biological membranes include of membrane lipids and proteins. The lipids are formed as a bilayer, providing a wall between the outside and inside of a cell. The lipids have a hydrophilic head and hydrophobic tail so they are similar to the surfactants and they are self organisme that they have behavior as microemulsion,[2-5]. Since in biomembranes many different lipid and protein type are present, membranes are heterogeneous mixtures in which lateral dissociation could occur, leading to the formation of domains. A lot of study was done by Atomic force microscopy on lipids membranes. More study was focused effect of cholesterol on lipid membrane. A study on mixture of ternary lipid mixtures with 1:1:1 sphingomyelin/dioleoylphosphatidylcholine/cholesterol monolayer leads to increase of ordered on bilayers,[6]. A studied with NMR experiment done on the effects of cholesterol on the structure and mesoscopic dynamics of the DPPC bilayer as a function of cholesterol concentration. The main effects observed are a significant ordering of the DPPC chains a reduced fraction of gauche bonds, a reduced surface area per lipid, less undulations—corresponding to an increased bending modulus for the membrane, smaller area fluctuations, and a reduced lateral diffusion of DPPC-lipids as well as cholesterols, [7].A studied was done on the effect of cholesterol on phospholipid bilayers was investigated as a function of cholesterol concentration by X-Ray scattering. The measured of phosphate-to-phosphate distances shows increases of phosphate-to-phosphate distances nonlinearly with the cholesterol concentration, [8].

In this work, we study the effect of cholesterol on structure and optical property of 1,2-Distearoyl-sn-glycero-3-phosphocholine (DSPC) lipid multilayer. AFM and Uv-Vis spectra are used to study the lipid multilayer in the absent and present of cholesterol. AFM can image biological systems with a good resolution, and therefore it is an excellent tool to study domains. The DSPC is a phospholipid with two chains and a polar head group, fig.1,[9,10].

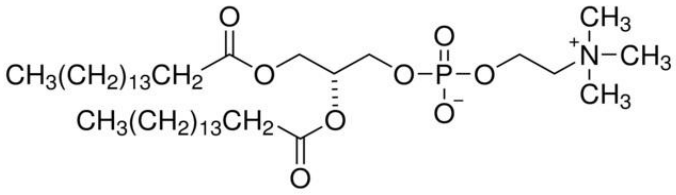

Fig.1. The chemical structure of 1,2-Distearoyl-sn-glycero-3-phosphocholine (DSPC).

Cholesterol is a lipid with a structure completely different from that of phospholipids. It is made from four hydrocarbon rings. A non-polar hydrocarbon chain is at one end of the steroid while a polar hydroxyl group is attached to the other end, fig.2,[11].

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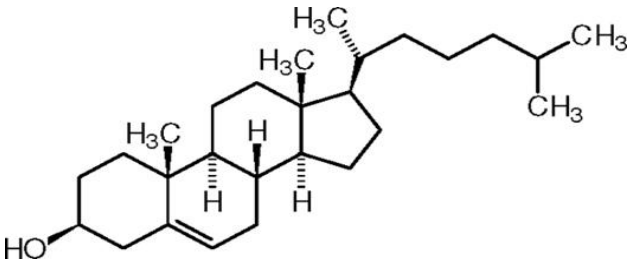


Fig.2. Chemical structure of cholesterol, the main chain built of four hydrocarbon rings.

In the mixture of cholesterol with phospholipids, the polar hydroxyl group become close to the polar area of phospholipids and the hydroxyl group to interact with the head group of phospholipids. Moreover, the hydrocarbon tale become close to the hydrophobic tale of the phospholipids, fig.3.

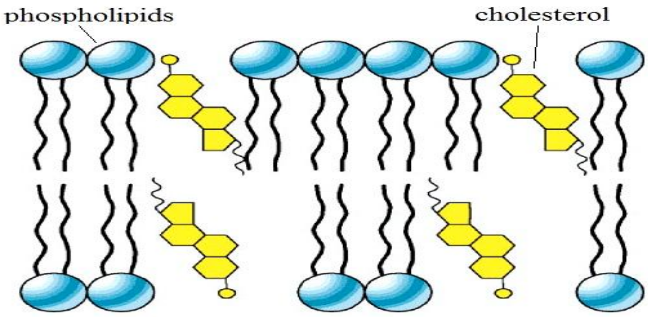


Fig.3. A schematic layer of mixture of cholesterol with phospholipids.

II. EXPERIMENTS

Material and preparations

1,2-Distearoyl-sn-glycero-3-phosphocholine (DSPC) and cholesterol (Chol) and chloroform was purchased from Sigma-Aldrich Company, Mainz Germany and methanol from Chemical Labs company, Iran. DSPC was dissolved in chloroform: methanol (1:1 v/v) solution with and without cholesterol in two different concentrations, the different composition of lipid suspensions is presented in the table 1. The solutions was deposited on to a glass surface by spreading of suspension and spin coating, and then dried overnight at the room temperature.

TABLE I
Composition of lipid suspensions at different concentration of lipid and cholesterol.

Sample Name	Composition(mg)
S1	DSPC/Chol. (5.5:10)
S2	DSPC/Chol. (5.5:7)
S3	DSPC (4)

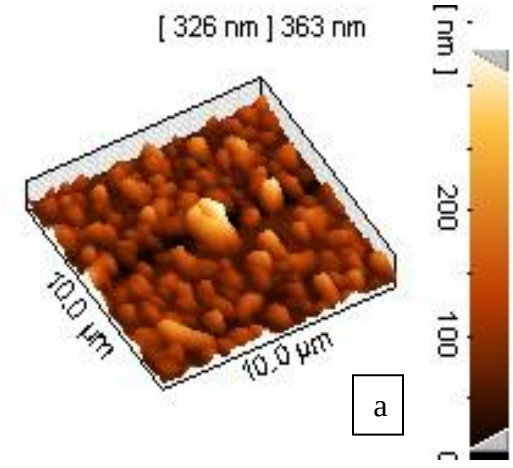
Methods

In this work, AFM is used to study the surface coated with different composition and lipid with and without cholesterol. The AFM observation is performed with a DME atomic force microscopy (Danish Micro Engineering A/S DK-2730 Denmark). The experiments were carried out at room temperature in non-contact mode. AFM images were obtained by measurement of the interaction forces between the tip and the sample surface [12].

UV-Vis absorbance spectra of the samples were recorded at room temperature by using PG instrument T80 UV-Vis spectrophotometer.

III. RESULTS AND DISCUSSIONS

The AFM measurements are carried out in non-contact mode on samples of the lipid solutions deposited on to a glass surface. As reported in several papers, both contact and tapping mode were able to capture soft samples, but the indentation and the vertical force deform the surface of soft or elastic materials [13]. In the fig.4 (a), shows 3D-veriws of DSPC/Chol with compositions (5.5:10) the X and Y scale is 10.0 µm and the Z scale is 363 nm. The composition of lipid with cholesterol (DSPC/Chol. (5.5:7)) is presented in the fig.4. (b) that the X and Y scale is 1.0 µm and the Z scale is 30.3 nm. Fig.5. (c) shows DSPC with composition (5.5) without cholesterol, the X and Y scale is 3.0 µm and the Z scale is 28.2 nm. The results show clearly that thickness of thin film decrease with decreasing of cholesterol. The height distribution of the DSPC/Chol.(5.5:10) and DSPC/Chol. (5.5:7) and DSPC without cholesterol are presented in the fig.5. This results shows with decrease of cholesterol the high distribution decrease from 90nm to lower that 10nm, fig.5(a) and (b). The fig.5(c), shows height distribution of lipid without cholesterol with combination of DSPC (5.5) is lower than 10nm that it can explain more change in the high distributions is observed in the higher combination of DSPC with cholesterol.



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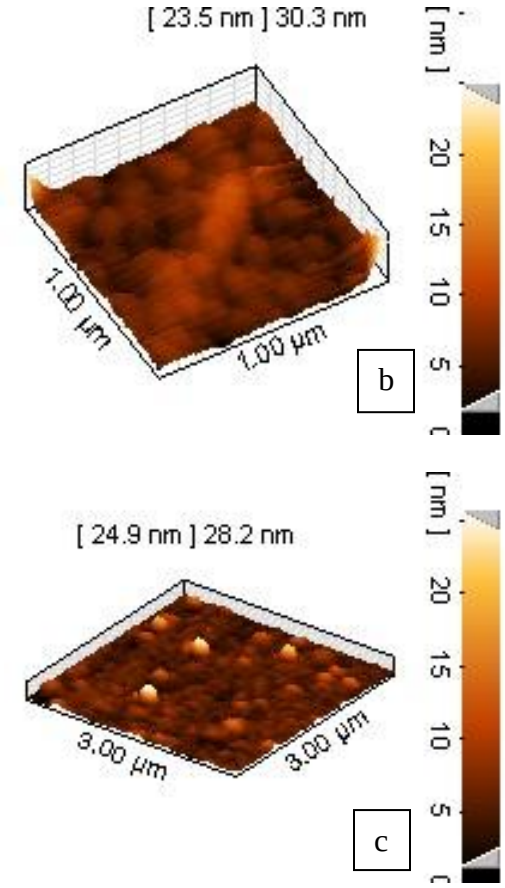
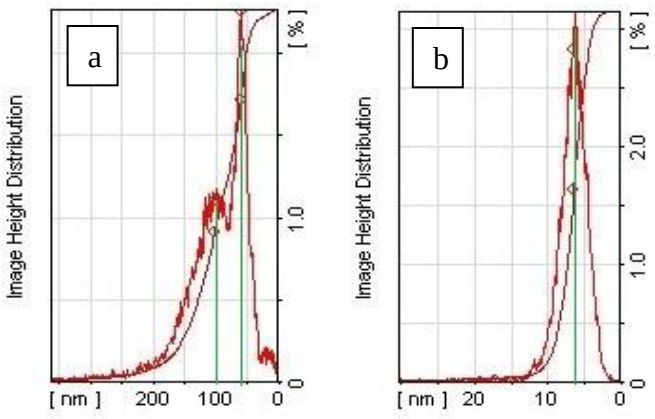


Fig.4. 3D- view of AFM topography of DSPC with and without cholesterol at different concentration of lipid/cholesterol (a)S1, (b)S2, (c)S3

The height different between the domains and the layers at DSPC with and without cholesterol are presented in the fig.6. The big different is observed around 110nm that it is lipid with high concentration of cholesterol, fig.6 (a) and in the lower concentration of the DSPC/Chol. (5.5:7) the different is around 12nm, fig.6 (b) and at absence of cholesterol the different is around 12nm, fig.6(c).

Analysis of the data indicates that the diameter of nanoparticles is much larger than the height for both in the presence and absence of cholesterol.

A previous study on DOPC/SpM (1:1) bilayers, in the absence of cholesterol, pure SpM domains appeared 1 nm above the level of the fluid monolayer and with increase of cholesterol this amount is increasing. This studied was suggested that cholesterol can induce bilayer coupling, [14].



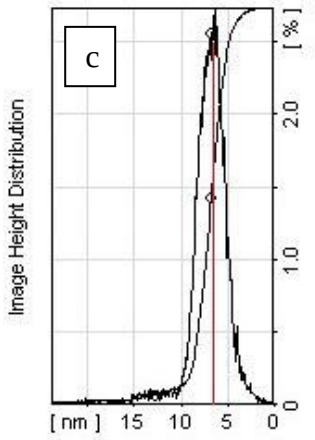
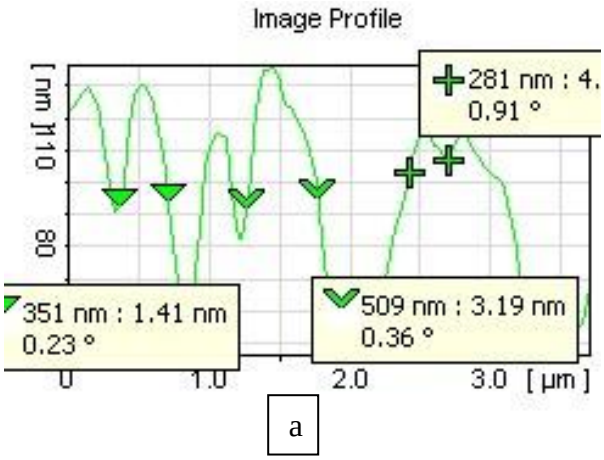
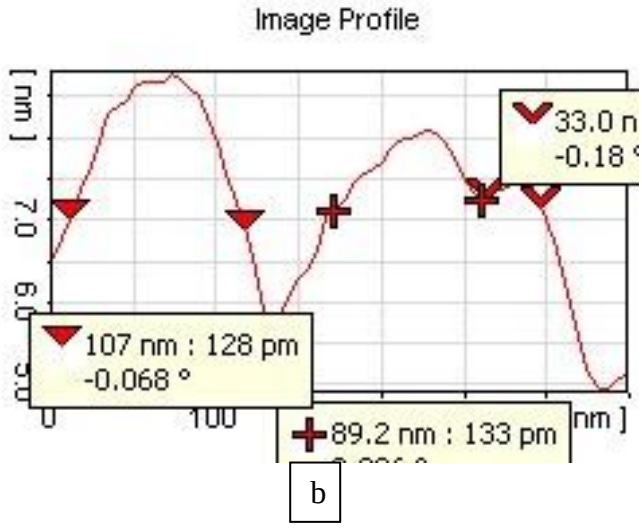


Fig. 5. Height distribution of DSPC with and without cholesterol at different combination of lipid and cholesterol (a) S1, (b)S2, (c)S3





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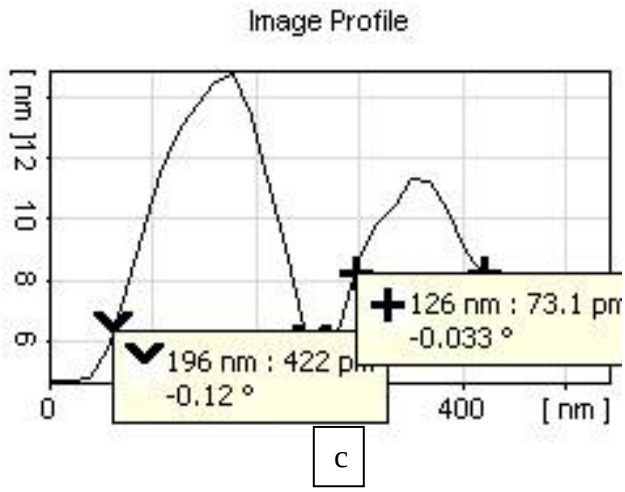
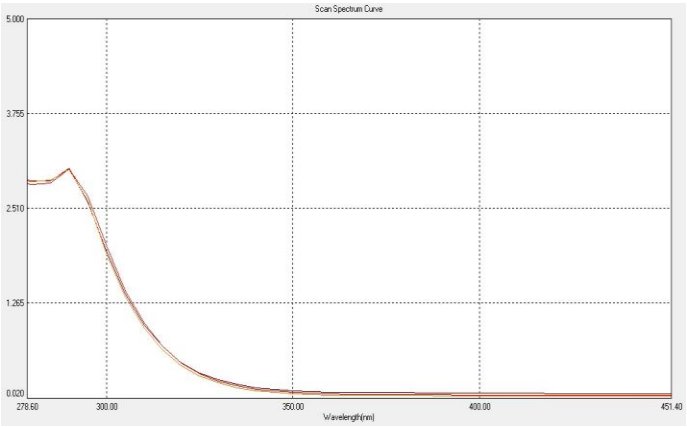


Fig.6. Image profile of sample with different combination of lipid/cholesterol, (a)S1, (b)S2, (c)S3

In this work, we studied Uv-Vis spectra of lipid lipid/cholesterol thin films. Our results shows, the spectra don't change with change the concentration of the cholesterol in the lipid structure, fig.7. It means, in the mixture of the DSPC/Cholesterol, the Cholesterol and also the thickness of multilayer don't have optical property of the lipid multilayers.

Fig. 7. Absorbance spectra for different combination of lipid/cholesterol miltilayer (a)S1, (b)S2, (c)S3



IV. CONCLUSIONS

In this work, we study studies the domain formation and high distribution of the DSPC with increase of cholesterol on a thin film by atomic force microscopy. The comparing high distribution and height different between the domains and the layers of DSPC/ Cholesterol (5.5:10) with DSPC/ Cholesterol (5.5:7) is showing that the both parameters depend to the Cholesterol consentration. In general, the Cholesterol can increase the stability of multilayer and also improve the formation of layers. The study of DSPC/ Cholesterol thin films with Uv-Vis shows the Cholesterol doesn't change the spectra data's.

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