ANNEX: Study of phospholipid-cholesterol mixtures for mimic cell membranes.

Final Master Project

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ANNEX 1: Langmuir-Technique

The Langmuir-Blodgett method is a fabrication technique of ordered monomolecular films on a liquid surface where this monolayer could be transferred, at a constant surface pressure, by the vertical or horizontal method onto a solid substrate, leading to mono or multi-layered films depending on the passages of the substrate through air-water interface. [28] This technique provides many possibilities for preparing ordered monolayers with densely packed structure and controlled thickness.

In order to form a Langmuir monolayer is necessary to work with an insoluble compound in water and soluble in a volatile solvent such as chloroform. Typical compounds have two major parts, a hydrophilic and a hydrophobic part, which usually consist of an aliphatic chain, for example, amphiphilic molecules. These compounds suitably dissolved in an appropriate solvent can be spread on the surface of the water and decreasing the area of available to the molecules with movable barriers; the molecules are organized into a film whose thickness corresponds to a molecule and these films are known as "Langmuir monolayers".

Monolayer formation is carried out inside a Langmuir through consisting of several parts: a vessel filled of water; one or two movable barriers moving on the surface of the water; a surface pressure sensor, generally a Wilhelmy balance and finally a mobile support where the substrate is placed to make the transference. Figure A1 illustrates the components of a Langmuir through. The trough is located inside a semi-white room, where the use of gloves and shims is mandatory. The investigator has the responsibility to do a periodically cleaning; it is important because any particle in suspension can cause distortion in the monolayer. In addition to carry out the experiment, the trough must be closed to avoid any interference and the trough is on a platform that avoids or minimizes the vibrations of the building.

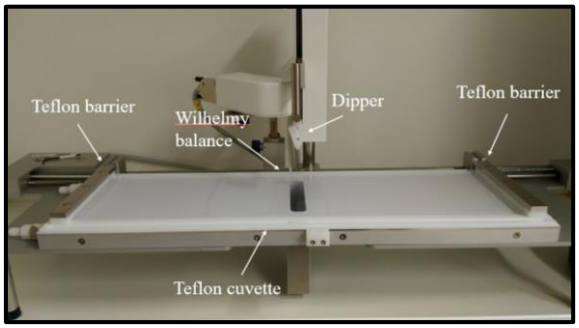


Figure A1: Representation of Langmuir instrument.

The surface pressure is defined as the difference between the surface pressure at clean subphase (without molecules) (γ_0) and surface pressure once the molecules are dispersed (γ).

$$\pi = \gamma_0 - \gamma$$

Langmuir technique allows identifying phase transitions in the monolayer. An example of π-A isotherms is represented in Figure 3 where all phases and phase transitions known are depicted although not all systems exhibit all of them. These phases and phase transitions include: G (gas phase), G-LE (coexistence of gas-liquid expanded phase) LE (liquid expanded phase); LE-LC (coexistence of liquid expanded and liquid-condensed phases; LC (liquid condensed phase; S (solid) phase and collapse.

At the beginning of the compression process, no surface pressured is detected due to large distance between molecules, gas phase. Upon the compression process, a surface pressure increase occurs and it is indicative of changes in the phase of the monolayer. In the liquid expanded phase molecules interact each with each other but only weakly. If the monolayer compression continues, the slope of the monolayer becomes sharp, indicating a new phase change, this is liquid condensed phase where molecules orient themselves in a preferential direction and inter-molecular interactions occur. Finally, an abrupt change in the slope is indicative of a new phase called solid phase. This phase is compact and rigid and the molecules interact strongly. If the monolayer is further compressed the collapse of the film into multi-layered (ordered or disordered films) occurs.

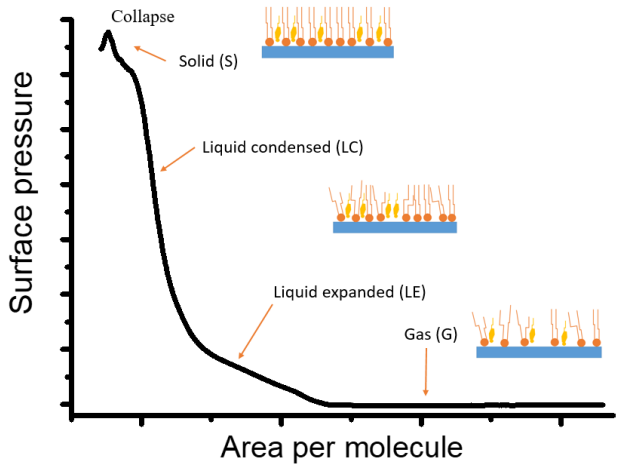


Figure: Example π-A isotherm showing the different phases and phase transitions of a Langmuir monolayer.

The manufacturing process of LB films can be divided into three stages: dispersion, compression and transference, although it is necessary in the first place to carry out a series of steps to ensure that the trough is cleaned. The cleaning process consist of a first rinse with acetone, followed by two ethanol and chloroform rinse, waiting 30 minutes between each of them. Finally three Milli-Q water rinse. Once cleaning process is ended, spreading process starts (Figure A2a). This stage consists in a slowly deposition drop by drop, of the compound solution on the water surface. The drop should fall as close to the surface as possible, keeping the surface pressure close to 0 $mN \cdot m^{-1}$. Both the concentration of the solution and the volume to disperse must be known to know the number of molecules deposited in the air-water interface. Once the deposition process is finished, 15 minutes must be waiting until solvent evaporation. When the molecules are deposited on the aqueous surface the hydrophilic region interacts with the water molecules; while the hydrophobic region is arranged in a conformation as far away as possible from the water, thus decreasing the total energy of the system. Therefore, the molecules located at the air-water interface are strongly oriented with the tendency to form a single layer of molecular thickness.

After spreading process, the compression of the barriers begins (Figure A2 b), during this process Upon the Langmuir compression, the surface pressure is measured together with area per molecule and the surface pressure vs. the area per molecule (π-A) isotherms are recorded, isotherm π - A.

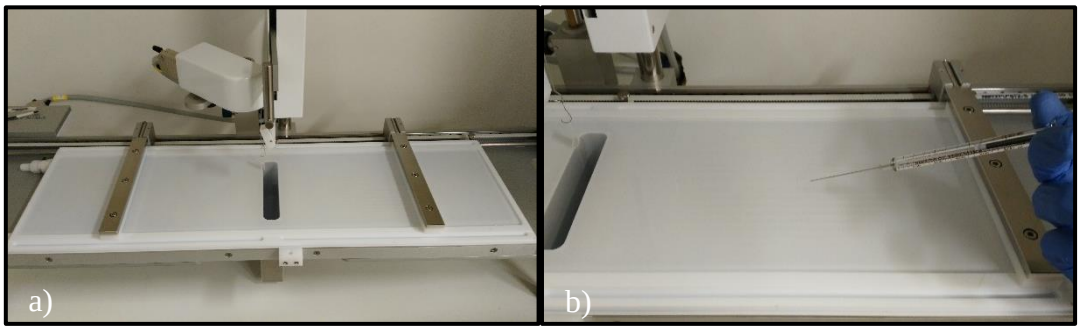


Figure A2: Representation of two first stages: a) dispersion and b) compression

The Langmuir-Blodgett technique allows the immobilization of the monolayer on solid substrates by the transference of the Langmuir films onto solid supports. Following this methodology, a supported mono or bilayer can be obtained and subsequently these films can be characterized using a variety of surface techniques including Atomic Force Microscopy (AFM). [29] In addition to the LB methodology, the Langmuir-Schaefer (LS) [30] technique can also be employed to immobilize the floating Langmuir film onto a solid

support [31]. While for phospholipid films the LB technique provides a first good quality monolayer, the second monolayer often results in the detachment of the first monolayer or uncontrolled reorganization of the film resulting in not well-organized bilayers. On the contrary, LS technique involves the transfer of the second monolayer by horizontal deposition resulting in high quality bilayers. This methodology is very useful in the formation of asymmetric bilayers, however, a special equipment is required and a rigorous study of the conditions for an optimal transfer.

ANNEX 2: Mole fraction experiments for ternary Langmuir monolayers

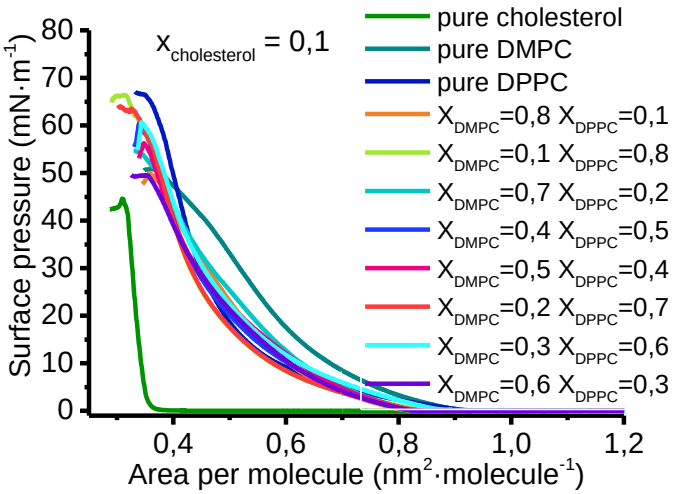
In the follow table the composition of ternary Langmuir monolayers appears.

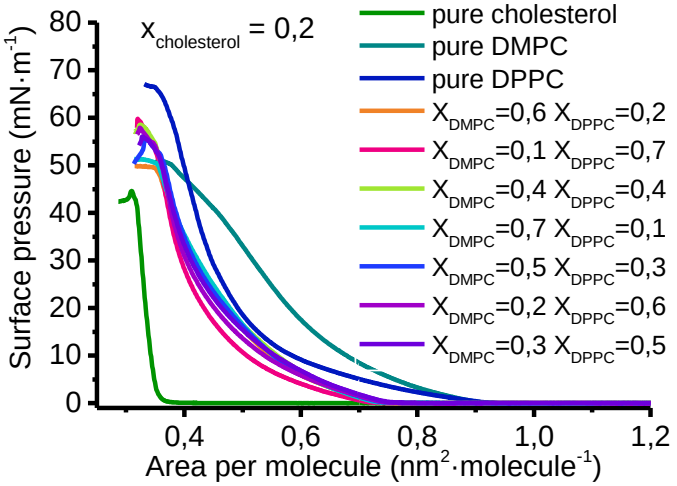
Table 1: Composition from ternary Langmuir monolayers

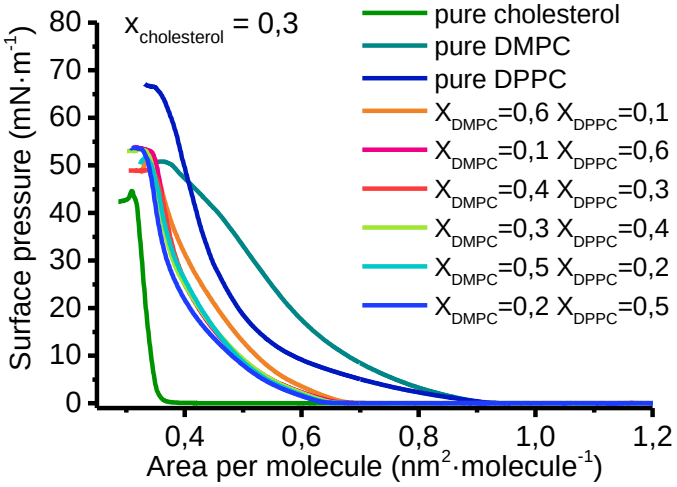
Nº	X cholesterol	X DMPC	X DPPC
1	0.9	0.05	0.05
2	0.8	0.1	0.1
3	0.7	0.1	0.2
4	0.7	0.2	0.1
5	0.6	0.1	0.3
6	0.6	0.2	0.2
7	0.6	0.3	0.1
8	0.5	0.1	0.4
9	0.5	0.2	0.3
10	0.5	0.3	0.1
11	0.5	0.4	0.1
12	0.4	0.1	0.5
13	0.4	0.2	0.4
14	0.4	0.3	0.3
15	0.4	0.4	0.2
16	0.4	0.5	0.1
17	0.3	0.1	0.6
18	0.3	0.2	0.5
19	0.3	0.3	0.4
20	0.3	0.4	0.3
21	0.3	0.5	0.2
22	0.3	0.6	0.1
23	0.2	0.1	0.7
24	0.2	0.2	0.6
25	0.2	0.3	0.5
26	0.2	0.4	0.4
27	0.2	0.5	0.3
28	0.2	0.6	0.2
29	0.2	0.7	0.1
30	0.1	0.1	0.8
31	0.1	0.2	0.7
32	0.1	0.3	0.6
33	0.1	0.4	0.5
34	0.1	0.5	0.4
35	0.1	0.6	0.3
36	0.1	0.7	0.2
37	0.1	0.8	0.1
38	0.05	0.05	0.9
39	0.05	0.9	0.05

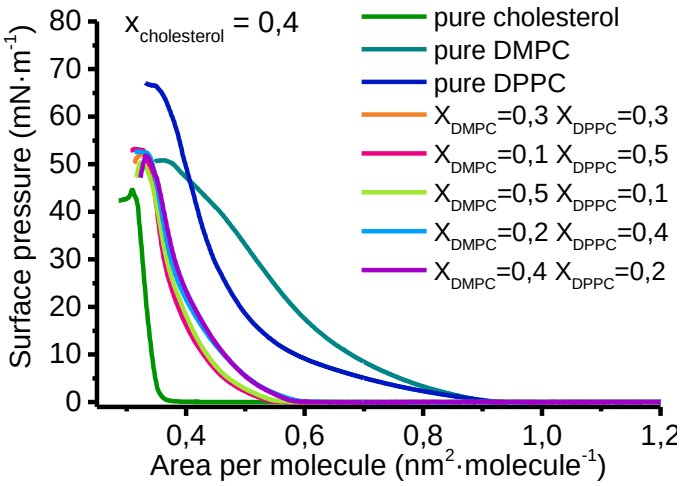
ANNEX 3: Isotherms obtained for ternary system monolayer

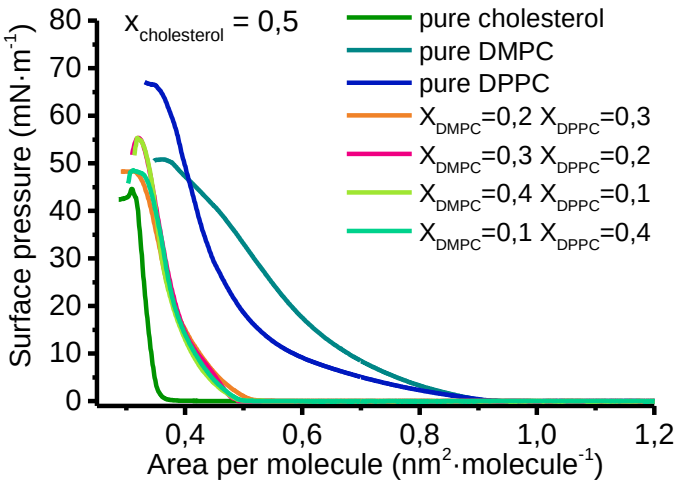
Surface pressure vs area per molecule isotherms register in the ternary systems at different cholesterol mole fraction.

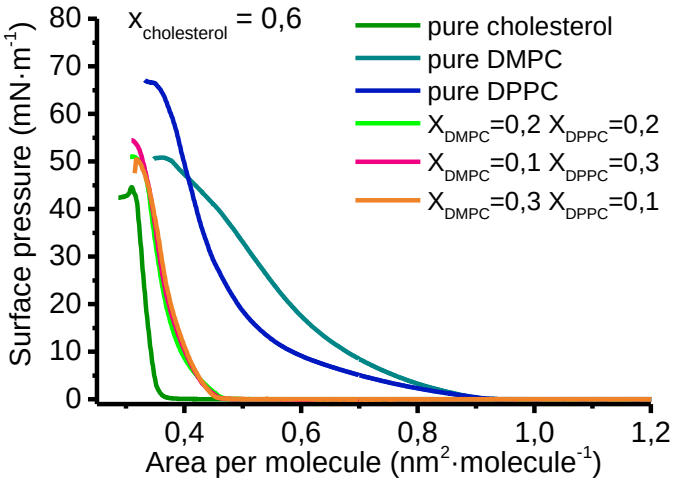


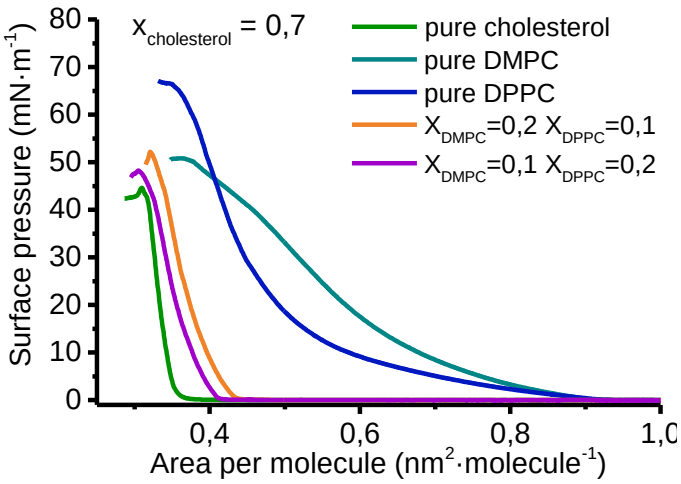


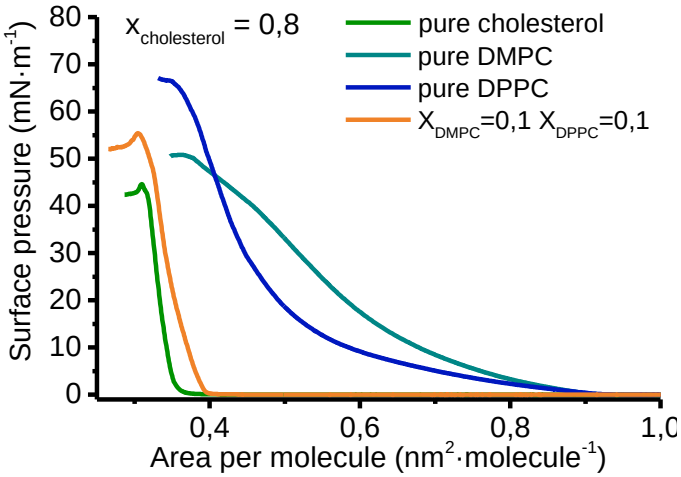


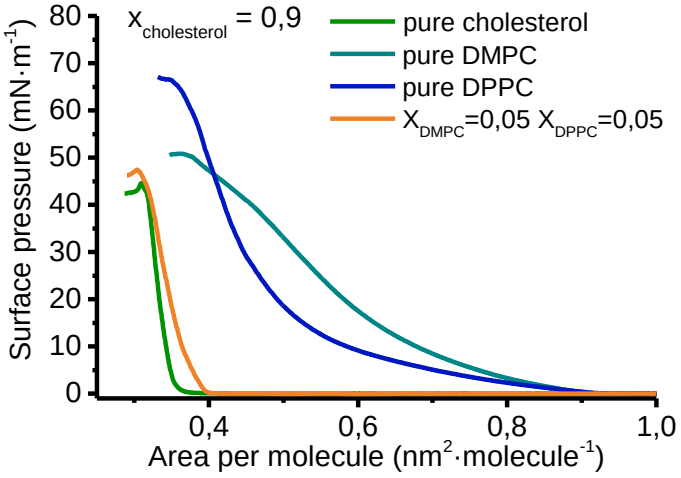

x cholesterol = 0,4
pure cholesterol
pure DMPC
pure DPPC
X DMPC=0,3 X DPPC=0,3
X DMPC=0,1 X DPPC=0,5
X DMPC=0,5 X DPPC=0,1
X DMPC=0,2 X DPPC=0,4
X DMPC=0,4 X DPPC=0,2
Surface pressure (mN·m-1)
Area per molecule (nm2·molecule-1)
0
10
20
30
40
50
60
70
80
0,4
0,6
0,8
1,0
1,2


x cholesterol = 0,5
pure cholesterol
pure DMPC
pure DPPC
X DMPC=0,2 X DPPC=0,3
X DMPC=0,3 X DPPC=0,2
X DMPC=0,4 X DPPC=0,1
X DMPC=0,1 X DPPC=0,4
Surface pressure (mN·m-1)
Area per molecule (nm2·molecule-1)
0
10
20
30
40
50
60
70
80
0,4
0,6
0,8
1,0
1,2


x cholesterol = 0,6
pure cholesterol
pure DMPC
pure DPPC
X DMPC=0,2 X DPPC=0,2
X DMPC=0,1 X DPPC=0,3
X DMPC=0,3 X DPPC=0,1
Surface pressure (mN·m-1)
Area per molecule (nm2·molecule-1)
0
10
20
30
40
50
60
70
80
0,4
0,6
0,8
1,0
1,2


x cholesterol = 0,7
pure cholesterol
pure DMPC
pure DPPC
X DMPC=0,2 X DPPC=0,1
X DMPC=0,1 X DPPC=0,2
Surface pressure (mN·m-1)
Area per molecule (nm2·molecule-1)


x cholesterol = 0,8
pure cholesterol
pure DMPC
pure DPPC
X DMPC=0,1 X DPPC=0,1
Surface pressure (mN·m-1)
Area per molecule (nm2·molecule-1)


x cholesterol = 0,9
pure cholesterol
pure DMPC
pure DPPC
X DMPC=0,05 X DPPC=0,05
Surface pressure (mN·m-1)
Area per molecule (nm2·molecule-1)