Characterization of Monoolein Langmuir Monolayers Spread at the Salt Solution/air Interface

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ABSTRACT:

The Langmuir monolayer is commonly described at interfaces for an insoluble homogenous single molecular layer. Langmuir monolayers have demonstrated various issues regarding soft matters and complex fluids by forming ideal uniform two-dimensional structures over the air-water interface. This monolayer has advantages for evaluating physicochemical properties at interfaces and, for the insoluble molecules, can be applied simultaneously to the different interaction occurrences at interfaces. For this experiment, monoolein lipid was used as a spreading solvent to create a Langmuir monolayer, and five different types of salt sub-phases were applied for the physicochemical properties’ interaction studies. On the air-water interface, the surface properties of monoolein lipids were investigated for interfacial phase behaviors, using the Wilhelmy plate pressure sensor technique compression isotherm (π-A). Data and analysis were also contributed to the correspondent, precise verification of physical state behavior with the surface pressure measurements on the interfaces through the compressibility modulus and the elasticity modulus parameters on the surface. In the experiments, the interfacial activity of the monoolein lipids was found to be stable on the aqueous sub-phase, while the area per molecule over the interface did not have much impact as a sub-phase with a change in salts. The repeatability and reproducibility of tests were affirmed by the difference in the Langmuir monolayer’s particular phase transition orientation behavior and the stability of colloidal lipid dispersion. However, Langmuir monolayer formation contributes to several special groups being restructured and is found to be a more remarkable natural process for their attractive organic dynamic structural properties over the interface, but the interfacial molecular dynamics have proven to be difficult to calculate.

Keywords: Air-water interface; Compression isotherm; compressibility modulus; elastic modulus; Monoolein; Salt sub-phase

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Graphical Abstract:

Highlights:

- The Langmuir monolayer approach to measuring the surface pressure-area of molecules’ (π-A) interaction phase behavior curves at the interface between air and water.
- The Wilhelmy plate surface pressure sensor is applied to the monoolein monolayer with added salts solution in the sub-phases.
- Phase behavioral changes are confirmed with the determination of lipid compressibility and elasticity modulus Langmuir monolayer.
- The desired orientation and stability of colloidal dispersions distinguishes various salt sub-phase interactions.
1. Introduction

In the past, from 1920 to 1932, the Langmuir monolayer technique had gained popularity. The popularity in terms of uniform homogeneous monolayer and two dimensional phase behavior. A similar trend has been observed in 2020. The discoverer of this technique, Irving Langmuir, was awarded the Nobel Prize for this great achievement in chemistry in 1932 [1]. Many researchers subsequently studied the various applications of this technique, such as in science and technology, primarily in the form of protein-lipid macromolecules at air-water interfaces [2], protein-protein nanocomposites [3], molecular thin films [4], polymers [5], colloids [6], nanomaterials [7], and more recently, monoparticular layers as vehicles for bioactive macromolecules membrane [8]. Recently, a study on a peculiar disease with the surface chemistry of Alzheimer’s disease was successfully carried out with the Langmuir monolayer approach [9]. Furthermore, Langmuir monolayer techniques are most commonly used in interaction-based application studies [7]. Therefore, nowadays, it has become possible to produce monolayer colloidal films that will broadly describe particle size and shape, which may be used for enhancing interfacial properties for the coating of layers by using the advantages of a uniformly distributed monolayer [2, 10]. Because of this and other reasons, such as its ability to control accurate measurements of the monolayer at interfaces and its extension to the deposition of substrates for coating purposes and its molecular dimensions scalability, the Langmuir monolayer method has become an attractive source [11, 12]. Although it is difficult to perform real-time characterization of Langmuir monolayer experimentation and uniform deposition of molecules on the liquid interface, surface properties at the interface were observed for two non-identical side groups that are not soluble and contain the head or tail groups that contribute advantages to the air-water interfaces [2, 4, 9].

Depending on the structure of the head group that interacts with the aqueous phase, natural lipids can carry a negative net charge or be neutral [13]. Lipids are widely used for the bio-membrane process, and they are largely heterogeneous in terms of chain length, chain saturation, and head group structure [14]. They are commonly used in the food industry, as well as for low-fat and instant food mix surfactants in traditional foods [15–17]. Langmuir monolayer formation for lipid is an easy and simple technique, which is used for lipid drug delivery purposes. Since, the lipid molecules are the second-most essential type of macromolecules after the protein bio-macromolecules, due to their surface activity and lower molecular weight. In molecular species, lipid macromolecules are highly surface-active
amphiphilic materials and are purely hydrophobic. Many researchers have studied the monolayer approach of Langmuir lipids using a compression isotherm [4, 18]. The lipid monolayer macromolecule assists in the understanding of the folding-unfolding behavior of interacting molecules along with the structural analysis of the formed monolayers [19]. Recently, elastic energy was measured with the topographical changes of lipid macromolecules using the Langmuir monolayer approach [20–23]. Similarly, several other theoretical methods were developed and used for various experiments to better understand the driving force of the folding-unfolding phase transition behavior. In addition, the heterogeneity of the molecules plays a significant role in determining the lipids film 3D structure and stability [24, 25].

A commonly known chemical name of monoolein lipid is 9-Octadecenoic acid (Z)-monoester with 1,2,3-propanetriol. It is also known as 1-Monoolein (1-(cis-9-Octadecenoyl)-rac-glycerol) [26]. In terms of physicochemical properties, it is a viscous pale yellow liquid with a characteristic odor. In terms of molecular structure, as shown below, it is composed of a hydrocarbon chain that is attached by an ester bond to a glycerol backbone as shown in Figure 1. Macroscopically speaking, the moiety of glycerol provides polar characteristics to this part of the molecule, which leads to formation of a hydrophobic head. This implies that the formation of hydrogen bonds with water by the hydrocarbon chain is known as the hydrophilic tail, which has a double bond position and is highly hydrophobic[1, 26]. In water and cold alcohol, monoolein is insoluble, but it is regularly soluble in oil, petroleum ether, chloroform, and even hot alcohol. As it is a non-toxic, biodegradable, and biocompatible material, monoolein lipids can have very desirable properties such as food emulsion [27].

![Figure 1. Chemical structure of monoolein lipid](image)

Interest in evaluating the lipid Langmuir monolayer technique on the air-water interface has increased to understand the effect of a change in sub-phase containing liquids on the interaction of lipid macromolecules. The surface properties of monoolein lipid macromolecules were investigated in this paper by using the Wilhelmy plate surface pressure sensor on an air-water interface. For the phase behavioral orientation of the lipid molecules and their interaction with various ionic salt sub-phase molecules on an interface, a compression isotherm ($\pi$-$A$) was
observed. The shift in the phase transmission of the lipid macromolecules was expressed by surface pressure-area measurements per molecule ($\pi$-A) for aqueous salt sub-phases. We took advantage of the sub-phase surface and interface head groups in this experiment to examine the interaction at the interface. Monoolein lipid was used to spread from 0.01 M to 0.1 M across various forms of salt sub-phases and to observe their phase transition in molar concentration. NaCl, KCl, KH$_2$PO$_4$, (NH$_4$)$_2$SO$_4$, and (NH$_4$)$_2$S$_2$O$_8$ were the sub-phase salts used for lipid interactions. Extension to compression isotherm leads to establishing parameters of compressibility and elasticity modulus with the use of $\pi$-A isotherms’ mean molecular area to find out the added salt physical state behavior and identify lipid interaction at the interface.

2. Materials and Methods

2.1. Materials

Monoolein lipid was purchased from TCI Chemicals Pvt. Ltd., Chennai, India. The molecular weight of monoolein lipid, which has an empirical formula of $C_{21}H_{40}O_4$, was 356.55 g/mol and the boiling point was 238–240°C with a density of 0.94 g cm$^{-3}$ [26]. Hexane and chloroform were used as the spreading spectroscopic grade lipid solvent, purchased from Finar Chemicals Ltd., Ahmedabad, India. Acetone and ethanol were purchased from Rankem Laboratory Reagent, Gurugram, India. The numerous laboratory reagents purchased were NaCl, KCl, KH$_2$PO$_4$, (NH$_4$)$_2$SO$_4$, and (NH$_4$)$_2$S$_2$O$_8$. At room temperature, i.e., at 20 ± 0.3°C, the surface tension of the pure aqueous solution was 72.4 ± 0.3 mN/m. Deionized ultrapure water (18.2 MΩ cm$^{-1}$, 0.22 μm filtration, Millipore, ELIX, Bangalore, India) was used for all experiments. Within three weeks of sample preparation, all isotherms were measured to minimize the effects of solvent evaporation and lipid oxidation.

2.2. Methods

2.2.1. Surface pressure measurements

The surface pressure measurement was conducted on a fully automatic and customized Langmuir Blodgett trough (APEX Instruments Co. Pvt. Ltd., India). The total trough area was 26 cm × 15 cm. The surface pressure v/s area per molecule isotherms was obtained for different sub-phases by applying a Wilhelmy plate pressure sensor [6, 28]. The Teflon trough was thoroughly cleaned with acetone twice and then rinsed with Milli-Q water before each
experimental run. The purity of the sub-phase was verified by ensuring that there was no increase in the surface pressure during barrier compression until the solution was distributed. The sub-phase in the trough was either Milli-Q water or dissolved salt solutions of NaCl, KCl, KH$_2$PO$_4$, (NH$_4$)$_2$SO$_4$, and (NH$_4$)$_2$S$_2$O$_8$. Each experiment was performed at room temperature (20 ± 0.3°C) in Indian winter. The effective surface area of the trough was controlled from both sides by a moving pair of Derlin barriers; an illustrative schematic of Langmuir trough can be seen in Figure 2. Monoolein lipid was spread on the interface and was given enough time to evaporate organic volatile solvent for the optimal level of adsorption of macromolecules on the sub-phase.

![Illustrative schematic of Langmuir trough with movable barriers](image)

**Figure 2. Schematic of the Langmuir monolayer trough diagram with movable barriers**

### 2.3. Molecular stability of monolayer phases

The Langmuir monolayer approach analysis for the investigation of surface properties of lipid macromolecules was conducted on an air-water interface. Langmuir monolayer formation involves molecules spreading across the surface of the sub-phase, using a volatile organic solvent. As the organic solvent evaporates within 15 minutes of waiting time, molecules at the interfaces drive the system to an equilibrium state. The stock solution was prepared by 10 percent chloroform: hexane (v/v) and macromolecules spreading carried using the traditional Trurnit method [29, 30]. A gas-tight Hamilton micro-syringe was used to spread the molecules. During preliminary trials, the optimal volumes and spreading concentrations were standardized. The two symmetrically moving barriers were operated at a speed of 1 mm/min. In a standard monolayer experiment, it is appropriate to spread a few micrograms of the
solution compounds on a sub-phase. The resulting compact monolayer film is stable for a long period if the surface pressure is not too high.

The monolayer will provide information on two-dimensional surface behaviors and immediate access to the disordered and organized ordered layer of confirmations of physical state transmission under a compression isotherm. The phase transition sequence, under compression, follows the order of a gas-like, liquid-like, and solid-like monolayer formation on the air-water interface from right to left. Furthermore, during the Langmuir isotherm process, the compact macromolecule ensures the hydrophobic heads and tails of present molecules. Repeating the same tests confirmed reproducibility. The schematic representation for monolayer formation on the air-water interface, as shown in Figure 3.

![Figure 3. Schematic diagram of monolayer formation on the air-water interface](image)

The spreading will continue until the surface pressure has reached an equilibrium value until the solid-like monolayer colliding surface pressure occurs. The colliding surface pressure is defined as when the formed Langmuir monolayer breaks and indicates a sudden reduction in surface pressure value. The spreading pressure of the equilibrium is produced spontaneously when the solid bulk is put in contact with the surface of the water. Again, the vapor pressure of the solid bulk will disburse compression. In its vapor phase, an equilibrium pressure occurs for the solid bulk. If this vapor pressure reaches, when the vapor is over-saturated, deposit on the solid surface will occur. In certain experiments, however, relatively stable surface pressure can be calculated to higher values than the spreading pressure of the equilibrium. When water soluble amphiphiles are injected under an insoluble monolayer onto the sub-phase surface, various types of interactions are possible. “Penetration” is one of the interactions that can take place; it involves adsorption on the monolayer interface of the soluble amphiphilic molecules.
The penetration of monolayers provides a useful model for many biological processes and is important for many industrial and food processing operations [31].

### 3. Results and Discussion

For this paper, the surface properties of monoolein lipid macromolecules and the single molecule’s Langmuir monolayer were developed using the Wilhelmy plate pressure sensor on the air-water interface for the interaction of different salt sub-phases. The Langmuir monolayer confirms the positioning of different transition phases, such as gas-like, liquid-like, and solid-like behavior, on a compression isotherm. It is recognized by the Langmuir monolayer that the force that causes the spreading of lipid on the surface of a water sub-phase is due to the attraction or repulsion of interacting lipid molecules and the surface activity of monoolein lipid. Using the equilibrium surface pressure results observed for thermodynamics change. The compressibility and surface elasticity calculations were performed with the mean molecular area of the isotherm. Therefore, an active group of lipid molecules carried a spreading in the uniform for a single layer molecule on the sub-phase.

#### 3.1. Effect of sub-phase on monoolein lipid Langmuir monolayer

The compression isotherm [surface pressure (\(\pi\)) - area per molecule (A)] (\(\pi\)-A) study allows us to determine the surface pressure increase as a function of decreasing surface area with compression, illustrating the monolayer packing on the interface shown in Figure 4(a). The liftoff of compression isotherm observe for the area per molecule from 1.2-0.88 nm\(^2\). The pure monoolein lipid isotherm was observed to undergo a sharp transition at a molecular area of \(\sim 0.88-0.17\) nm\(^2\); this was consistent with previous studies [1, 26, 32]. The compactness of the molecules on barrier compression observed and the phenomenon of liftoff surface pressure observed for monoolein lipid monolayer have been shown in Figure 4(a). Due to the compactness of the molecules on barrier compression and the phenomenon of liftoff surface pressure observed for monoolein monolayer has been shown in Figure 4(a). On the experimental side, the values of salt-free monoolein studies for the area per lipid for monoolein monolayers were recorded for various temperature ranges [32–34]. The observed value of the area per lipid is, therefore, in good agreement with the available experimental data at room temperature [33].

In the gaseous phase, there is the initial compression of lipid molecules, which implies that molecules don’t interact with each other. The lipid molecules come closer at extended
compression, and an increase in surface pressure was observed, as shown in Figure 4(a). The monolayer is still in the gaseous phase at this point; thus, it can be assumed that there was no interaction. This surface pressure increases until there is an inflection, which is denoted as the liquid-expanded lipid monolayer phase. After more compression with the use of the interfaces, attached barriers move progressively to a liquid-condensed phase and then to a solid phase in which the third phase of transition is observed. This means that because of the presence of hydrogen bonds, the liquid condensation phase induces the van der Waals interaction and the intermolecular interaction phase is established. The maximum surface pressure inflection was observed for the pure water sub-phase at 45.5 mN/m for the monoolein lipid monolayer, as shown in Figure 4(a). Following the determination of maximum surface pressure, further compression of the lipid monolayer breaks the isotherm horizontally, which implies the collapse of monolayer surface pressure.

The purpose of using different salt sub-phase in monolayer formation was to observe the effect of interaction of added salt on monoolein lipids on the air-water interface. Also, along with the lipid monolayer effects on mono valiant, it also implies that lipid monolayer affects divalent changes. And to identify the effect of lipid monolayer on mono valiant changes in the distribution of solvent at interfaces. Lipids also have a weak interaction with monovalent salts and a strong one with divalent salts [35]. Effects from the observations shown in Figure 4(b-f), for the compression isotherm, indicate the lipid macromolecule spread on various ionic salt sub-phase compression isotherms on the air-water interface. It forms a thin layer on the interface after lipid spread due to its inbuilt hydrophobic nature, which makes it stay on interfaces. The compression isotherm occurs from the initial liftoff of molecules starting from 0.88 nm², as shown in Figure 4, for different condensed individual salt sub-phases inflection. Furthermore, compression of two-dimensional homogenous lipid molecules on the interface changes the order of the gas-like phase to liquid expansion arrangements, such as physical state, since this transition does not occur at constant surface pressure. For all the salt sub-phases, the compression isotherms’ liquid transition surface pressure is observed at 5–15 mN/m. Finally, to increase the salt sub-phase concentration from 0.01 to 0.1 M, the collapse phase/solid transition surface pressure for monoolein lipids was required to be approximately 35 to 40 mN/m. Finally, to increase the salt sub-phase concentration from 0.01 to 0.1 M, the collapse phase/solid transition surface pressure for monoolein lipids was measured at approximately 35 to 40 mN/m. It determined the maximum surface pressure by which the highest number of molecules are adsorbed on the surface.
In the monolayer, the interactions between components can be investigated through the excess area from the added salt physical state behavior between components in the sub-phase. These results suggest that higher surface pressures are dominated by all salt solution. This is represented in Figure 4(a): the hypothetical \( \pi \)-A isotherms for compression monolayer calculated for lipid molecules on pure water sub-phase are present on the air-water interface. Other salts’ compression isotherm plots are shown in Figure 4(b-f). Similarly, they were observed for any changes in the physical state after compression with symmetrically moving barriers. The observed physical state was a gas-like, liquid-like, and solid-like monolayer with surface pressure value of 5, 30, and 35 mN/m on the air-water interfaces, respectively. Moreover, the area per molecule of 0.9 to 0.7 nm\(^2\) for gas-like, 0.7 to 0.4 nm\(^2\) for liquid-like, and 0.4 to 0.17 nm\(^2\) for solid-like monolayer was measured at interfaces with various salt sub-phases for monoolein Langmuir monolayer formation. On the surface, as well as on the interface, the main parameters affecting the analysis were distinguished: pH scale, ionic strength, charge density, concentration, surface activity, structure, added salt physical state, film formation kinetics at fluid interfaces, biopolymer penetration on the insoluble sub-phase interfaces, etc. [36].
3.2. Determination of elasticity modulus and surface compressibility modulus

Figure 5(a-j) demonstrates the measured compressibility and elastic modulus for the previously obtained Langmuir isotherm data in various experiments performed with a change in salt.
concentrations, for the formation of lipid Langmuir monolayer. The compressibility and elastic modulus were computed using Eq. 1 and Eq. 2. The mean molecular area obtained utilizing compression isotherm is respective. Many researchers conduct the elasticity modulus recognition based on Eq. 1 for the selection of parameters based on the physical state of the lipid monolayer, in their extension analysis toward the Langmuir isotherm [37–39]. The information provided by the π-A isotherm, the elasticity modulus, was determined to be evaluated in more detail as follows.

\[ K_s^{-1} = -\frac{1}{A} \frac{\partial A}{\partial \pi} \]

(1),

where \( A \) denotes the mean molecular area and \( \pi \) - the surface pressure at room temperature \( T \) (approx 20°C). From the experimental observation, it can be seen that the elasticity modulus is \( K_s < 10 \) mN/m for the liquid expanded (LE) state, 10-25 mN/m for the liquid condensed (LC) state, and >25 mN/m for the solid (S) state. These experimental findings for the elasticity modulus are consistent with the results previously published [21]. Also, their LE state did not start from zero surface pressure; it decreased later, as observed in Figure 5. their LE state did not start from zero surface pressure and then decrease, as observed in Figure 5. With the shift in slope values, the physical state transitions behavior can be discovered from the \( K_s - \pi \) curve study. Furthermore, \( K_s \), according to the Davies and Rideal description, offers information on the monolayer phases and phase transitions physical state [40]. Similarly, the compressibility modulus \( C_s \) was derived using the following equation from the π-A compression isotherms:

\[ C_s^{-1} = -A \frac{\partial \pi}{\partial A} \]

(2)

However, lipid surfactants have attracted a lot of attention on the air-water interface to create a Langmuir monolayer, to monitor the interface of the molecular structure. Additionally, combinations of various sub-phase solutions are employed to understand both the fundamental knowledge of lipid interaction and the monolayer formation influence, as well as the role of net loading [38, 40]. A derived compressibility equation of the modulus is defined using the mean molecular area of the monolayer on the air-water interface to deduce the physical state and orientation of the formed monolayer Langmuir. Concerning the monoolein lipid, the compression of the lipid spreading isotherm (π-A), which occurs at the lowest level of the \( C_s^{-1} \) surface pressure isotherms, which in turn occurs at the lowest level of the \( C_s^{-1} \) surface pressure isotherm, was observed on the plateau of transitional changes in gas-like, fluid-like,
and solid-like behaviors. The obtained $C_s^{-1}$ values were below 50 mN/m, indicating a liquid-expanded phase, and a change in the phase-state of the lipid on the air-water interface was not due to any change of the $\pi$-A isotherms [41]. Even at low surface pressures, the compressibility module is not directly linked with its true rheological and shear properties [42].
Figure 5. Compression isotherm effect with the value for the elasticity and compressibility modulus of lipid monolayers by added salt solution sub-phase on the air-water interface, (a-b) elasticity and compressibility of NaCl salt, (c-d) elasticity and compressibility of KCl salt, (e-f) elasticity and compressibility of KH$_2$PO$_4$ salt, (g-h) elasticity and compressibility of ammonium sulphate salt, (i-j) elasticity and compressibility of ammonium persulphate salt.

The range of the value of $C_s^{-1}$ depends on the specific system being characterized. The compression isotherm ($\pi$-$A$) from a lipid monolayer describes various salt sub-phases in a liquid-condensed state ($\pi > 25$ mN/m); there is a drop in $C_s^{-1}$ values in NaCl and KCl, but in the case of KH$_2$PO$_4$, (NH$_4$)$_2$S$_2$O$_8$, and (NH$_4$)$_2$SO$_4$, no substantial change in $C_s^{-1}$ values was observed. The introduction of NaCl or KCl into the monolayer phospholipids in the liquid-condensed state indicates the development of an additional liquid-extended surface layer [43]. The golden rule says that the less the compression module value is, the more the monolayer is compressible [44]. Furthermore, the overall compressibility values can help to improve lipid monolayer stability. Adding salt to the sub-phase of lipid monolayers causes a reduction in the values of $C_s^{-1}$, implying that the salt and the monolayer components may be partly miscible.

The well-formed physical state displays the sequential order of gas, liquid-expanded to liquid-condensed, and in the end, solid phase transition. But sometimes, the distribution of the homogeneous layer, penetration time, and size of the molecule is deformed in such a way that they are no longer circular [45]. These superficial structural characteristics might be the cause of deformity of our monoolein lipid, which shows only one transition phase, the acceptable version of a phase transition asymmetric peak, as shown in Figure 5. However, the effect is different for sub-phase conditions used on interfacial interactions of a monoolein lipid monolayer. More data on the dynamic and elastic properties and the application of $C_s^{-1}$ to membrane lipids and their mixtures can be found in several studies [4, 46–48]. The presence
of interacting forces is almost the same in the compression and elasticity equations as surface pressure $\alpha$-A isotherm measurements. The slit complement to the interactions between van der Waals, the hydrocarbon chain of the lipid, and hydrogen bond links on the interface form the network with the lipid polar head group. This enhances the attraction or repulsion of the uniform distributive single-molecule lipid layer, particularly the affinity to change the sub-phase of salt in the bulk aqueous process [34, 49]. It may also be associated with the bulk aqueous phase with monolayer molecular loosening by collapse and dissolution, as seen for the same lipid propagation in the aqueous solution [50].

With salt surface groups and lipid heads being loaded, electrostatic interactions seem highly likely to be the driving force, but interactions with the lipid tail might justify all this. This could allow the salt to penetrate the lipid layer deeper and therefore lead to significantly higher changes in surface pressure as compared to other molar concentrated sub-phases in 0.1 M salt forms. Figure 5(a-f) shows that higher concentrations of 0.1 M salt have resulted in monoolein interaction, while monoolein monolayer binding did not affect this. Interestingly, the anionic salt sub-phase demonstrated substantially greater interaction with both monolayers at high concentrations than the cationic salt sub-phase. As Na ions are almost not bound, the high concentration of sodium does not directly affect the density of the surface, but a high level of Na$^+$ ion will indirectly affect the density of surface charges, thus their impact on the surface concentration of lipids, in particular by an increase in Na$^+$ ion concentration [13]. This study can, however, be useful in developing finished products and determining the physicochemical properties of a stable system. The main goal is to connect different possible sub-phase interaction behaviors to understand the fundamental research behind the interactive lipid interface affected by different molecules on the air-water interface.
4. Conclusion

Using the Langmuir monolayer technique on air-water interfaces, we observed the surface properties of various salt sub-phases and their change in concentration to facilitate the spread of monolein lipid macromolecules on interfaces. This characterizes the effect of sub-phase ions on the monolein lipid monolayer’s phase behavior, conformation, and head group structure. The inflection observed for monolein lipid surface pressures was 45.5 mN/m for pure water sub-phase on the air-water interface. Collapse pressure for lipid was about 40, 39, 40, 37, and 42 mN/m, for the change in salt sub-phases types. The concentrated salt sub-phases of NaCl, KCl, KH₂PO₄, (NH₄)₂SO₄, and (NH₄)₂S₂O₈ have shown feasible data analyses on the air-water interface. During compression of the lipid monolayer, the π-A isotherms reveal high accuracy in transition phases and transforms the lateral disordered molecules to ordered molecules at the interfaces for all the solutions. The modulus of compression and elasticity are also used to gain knowledge of the miscibility of salt-lipid molecules. $C_s^{-1}$ offers insights on the lateral packaging elasticity of the monolayer system and responds better during lateral interactions and to slight changes in the monolayer structure. Preliminary studies of lipid monolayer interactions are important for understanding the organized relationship of function to various areas, such as ion transportation, photosynthesis, cell recognition, and numerous disease conditions. The Langmuir lipid monolayer must be established and monitored in various food systems to ensure improved structural stability for its physiochemical properties, macrostructure, and the resulting rheological surface characteristics.

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Consent for publication: This article does not contain any studies from any individual person/researcher. Please state “Not applicable” in this section.

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References

1. Carrera Sánchez, C., Cejudo Fernández, M., Rosario Rodríguez Niño, M., & M. Rodríguez Patino, J. (2006). Thermodynamic and Dynamic Characteristics of Monoglyceride Monolayers Penetrated by β-Casein. *Langmuir, 22*(9), 4215–4224. https://doi.org/10.1021/la053506+

2. Gallier, S., Gragson, D., Jiménez-Flores, R., & Everett, D. W. (2012). β-Casein–phospholipid monolayers as model systems to understand lipid–protein interactions in the milk fat globule membrane. *International Dairy Journal, 22*(1), 58–65. https://doi.org/https://doi.org/10.1016/j.idairyj.2011.08.007

3. Ali, S. S., Tang, X., Alavi, S., & Faubion, J. (2011). Structure and Physical Properties of Starch/Poly Vinyl Alcohol/Sodium Montmorillonite Nanocomposite Films. *Journal of Agricultural and Food Chemistry, 59*(23), 12384–12395. https://doi.org/10.1021/jf201119v

4. Elderdfi, M., & Sikorski, A. F. (2018). Langmuir-monolayer methodologies for characterizing protein-lipid interactions. *Chemistry and physics of lipids, 212*, 61–72. https://doi.org/10.1016/j.chemphyslip.2018.01.008

5. H. Schmidt, R., L. Illingworth, B., C. Deng, J., & A. Cornell, J. (2002). Multiple regression and response surface analysis of the effects of calcium chloride and cysteine on heat-induced whey protein gelation. *Journal of Agricultural and Food Chemistry, 27*(3), 529–532. https://doi.org/10.1021/jf60223a026

6. Dhopte, B., & Lad, V. N. (2020). Alteration of surface pressure of macromolecular monolayer at the air-water interface and electrochemical impedance characteristics. *Colloids and Surfaces B: Biointerfaces, 187*, 110638. https://doi.org/10.1016/J.COLSURFB.2019.110638

7. Park, J. young, & Advincula, R. (2011). Nanostructuring polymers, colloids, and
nanomaterials at the air–water interface through Langmuir and Langmuir–Blodgett techniques. *Soft Matter*, 7, 9829–9843. https://doi.org/10.1039/C1SM05750B

8. Livney, Y. D. (2010). Milk proteins as vehicles for bioactives. *Current Opinion in Colloid & Interface Science*, 15(1–2), 73–83. https://doi.org/10.1016/j.cocis.2009.11.002

9. Thakur, G., Micic, M., & Leblanc, R. M. (2009). Surface chemistry of Alzheimer’s disease: a Langmuir monolayer approach. *Colloids and surfaces. B, Biointerfaces*, 74(2), 436–456. https://doi.org/10.1016/j.colsurfb.2009.07.043

10. Rodríguez Patino, J. M., & Fernández, M. C. (2004). Structural and Topographical Characteristics of Adsorbed WPI and Monoglyceride Mixed Monolayers at the Air–Water Interface. *Langmuir*, 20(11), 4515–4522. https://doi.org/10.1021/la036190j

11. Rojewska, M., Skrzypiec, M., & Prochaska, K. (2017). Surface properties and morphology of mixed POSS-DPPC monolayers at the air/water interface. *Colloids and surfaces. B, Biointerfaces*, 150, 334–343. https://doi.org/10.1016/j.colsurfb.2016.10.047

12. Chumakov, A. S., Ermakov, A. V, Gorbachev, I. A., Kossovich, E. L., Kletsov, A. A., & Glukhovskoy, E. G. (2016). The influence of redistribution ions in subphase at the properties Langmuir monolayer: physical and theoretical experiments. In *Proc.SPIE* (Vol. 9917). Retrieved from https://doi.org/10.1117/12.2228787

13. Kensbock, R., Ahrens, H., & Helm, C. A. (2019). Interactions of Monovalent and Divalent Cations with Cardiolipin Monolayers. *Langmuir*, 35(10), 3624–3633. https://doi.org/10.1021/acs.langmuir.8b03637

14. O’Mahony, J. A., & Fox, P. F. (2014). Milk: An Overview. *Milk Proteins*, 19–73. https://doi.org/10.1016/B978-0-12-405171-3.00002-7

15. Dickinson, E., & McClements, D. J. (1995). *Advances in food colloids*. Springer Science & Business Media.

16. Krog, N. J., & Sparso, F. V. (2004). *Food emulsifiers and their chemical and physical properties. Food emulsions* (Vol. 12). Marcel Dekker Inc., New York, USA.

17. Rosario Rodríguez Niño, M., J. Wilde, P., C. Clark, D., & M. Rodríguez Patino, J.
(1996). Surface Rheological Properties of Monostearin and Monoolein Films Spread on the Air–Aqueous Phase Interface. *Industrial & Engineering Chemistry Research, 35*(12), 4449–4456. https://doi.org/10.1021/ie960333y

18. de la Fuente Feria, J., & M. Rodriguez Patino, J. (2002). Destabilization of Monoglyceride Monolayers at the Air-Aqueous Subphase Interface. 1. Kinetics. *Langmuir, 10*(7), 2317–2324. https://doi.org/10.1021/la00019a049

19. Hostetler, M. J., Green, S. J., Stokes, J. J., & Murray, R. W. (1996). Monolayers in three dimensions: synthesis and electrochemistry of ω-functionalized alkanethiolate-stabilized gold cluster compounds. *Journal of American Chemical Society, 118*(17), 4212–4213.

20. Lee, D., Redfern, O., & Orengo, C. (2007). Predicting protein function from sequence and structure. *Molecular cell biology, 8*(december), 995–1005. https://doi.org/10.1038/nrm2281

21. Giri, R. P., Mukhopadhyay, M. K., Mitra, M., Chakrabarti, A., Sanyal, M. K., Ghosh, S. K., … Sinha, S. K. (2017). Differential adsorption of a membrane skeletal protein, spectrin, in phospholipid membranes. *EPL (Europhysics Letters), 118*(5), 58002. https://doi.org/10.1209/0295-5075/118/58002

22. P. Giri, R., Chakrabarti, A., & K. Mukhopadhyay, M. (2017). Cholesterol-Induced Structural Changes in Saturated Phospholipid Model Membranes Revealed through X-ray Scattering Technique. *The Journal of Physical Chemistry B, 121*(16), 4081–4090. https://doi.org/10.1021/acs.jpcb.6b12587

23. J, T.-B., & R, R. (2019). Effect of lysozyme subphase and insertion on several lipid films. *Advanced Material Science, 4*(1), 1–7. https://doi.org/10.15761/AMS.1000149

24. Zhang, L., Lu, Q., & Liu, M. (2003). Fabrication of Chiral Langmuir–Schaefer Films from Achiral TPPS and Amphiphiles through the Adsorption at the Air/Water Interface. *The Journal of Physical Chemistry B, 107*(11), 2565–2569. https://doi.org/10.1021/jp026621r

25. Piknova, B., Schram, V., & Hall, S. B. (2002). Pulmonary surfactant: phase behavior and function. *Current opinion in structural biology, 12*(4), 487–494. https://doi.org/10.1016/s0959-440x(02)00352-4
26. Kulkarni, C. V, Wachter, W., Iglesias-Salto, G., Engelskirchen, S., & Ahualli, S. (2011). Monoolein: a magic lipid? Physical Chemistry Chemical Physics, 13(8), 3004–3021. https://doi.org/10.1039/C0CP01539C

27. Ariza-Carmona, L., Martín-Romero, M. T., Giner-Casares, J. J., Pérez-Morales, M., & Camacho, L. (2013). Elastic Nanocomposite Structures Formed by Polyacetylene–Hemicyanine Mixed Films at the Air–Water Interface. The Journal of Physical Chemistry C, 117(42), 21838–21848. https://doi.org/10.1021/jp406397q

28. Taylor, D. M., & Bayes, G. F. (1999). The surface potential of Langmuir monolayers. Materials Science and Engineering C, C(8–9), 65–71.

29. Trurnit, H. J. (1960). A theory and method for the spreading of protein monolayers. Journal of Colloid Science, 15(1), 1–13. https://doi.org/https://doi.org/10.1016/0095-8522(60)90002-7

30. Phadke, R. S., & Agarwal, G. (1999). Methods of deposition and characterization of thin films of amphiphiles. Materials Science and Engineering C, C(8–9), 113–118.

31. Sánchez, C. C., Fernández, M. C., Rodríguez Niño, M. R., & Rodríguez Patino, J. M. (2006). Thermodynamic and Dynamic Characteristics of Monoglyceride Monolayers Penetrated by β-Casein. Langmuir, 22(9), 4215–4224. https://doi.org/10.1021/la053506+

32. Rodríguez Patino, J. M., Cejudo Fernandez, M., Carrera Sanchez, C., & Rodriguez Nino, M. R. (2007). Structural and shear characteristics of adsorbed sodium caseinate and monoglyceride mixed monolayers at the air-water interface. Journal of colloid and interface science, 313(1), 141–151. https://doi.org/10.1016/j.jcis.2007.04.025

33. Gurtovenko, A. A., & Vattulainen, I. (2008). Membrane Potential and Electrostatics of Phospholipid Bilayers with Asymmetric Transmembrane Distribution of Anionic Lipids. The Journal of Physical Chemistry B, 112(15), 4629–4634. https://doi.org/10.1021/jp8001993

34. Rodríguez Patino, J. M., Rodríguez Niño, M. R., & Carrera Sánchez, C. (2003). Structure, Miscibility, and Rheological Characteristics of β-Casein–Monoglyceride Mixed Films at the Air–Water Interface. Journal of Agricultural and Food Chemistry, 51(1), 112–119. https://doi.org/10.1021/jf020197+
35. Honigmann, A., van den Bogaart, G., Iraheta, E., Risselada, H. J., Milovanovic, D., Mueller, V., ... Jahn, R. (2013). Phosphatidylinositol 4,5-bisphosphate clusters act as molecular beacons for vesicle recruitment. *Nature Structural & Molecular Biology, 20*, 679. Retrieved from https://doi.org/10.1038/nsmb.2570

36. Ettelaie, R., & Akinshina, A. (2014). Colloidal interactions induced by overlap of mixed protein þ polysaccharide interfacial layers. *Food hydrocolloids, 42*, 106–117. https://doi.org/10.1016/j.foodhyd.2014.01.020

37. Chitra, S., Bargavi, P., Balasubramaniam, M., Chandran, R. R., & Balakumar, S. (2020). Impact of copper on in-vitro biomineralization, drug release efficacy and antimicrobial properties of bioactive glasses. *Materials Science and Engineering: C, 109*, 110598. https://doi.org/https://doi.org/10.1016/j.msec.2019.110598

38. Torrent-Burgués, J., Cea, P., Giner, I., & Guaus, E. (2014). Characterization of Langmuir and Langmuir–Blodgett films of an octasubstituted zinc phthalocyanine. *Thin Solid Films, 556*, 485–494. https://doi.org/10.1016/J.TSF.2014.01.045

39. Jablonowska, E., Nazaruk, E., Matyszewska, D., Speziale, C., Mezzenga, R., Landau, E. M., & Bilewicz, R. (2016). Interactions of Lipidic Cubic Phase Nanoparticles with Lipid Membranes. *Langmuir, 32*(37), 9640–9648. https://doi.org/10.1021/acs.langmuir.6b01746

40. Davies, J. T. (2012). *Interfacial phenomena*. Elsevier.

41. Maget-Dana, R. (1999). The monolayer technique: a potent tool for studying the interfacial properties of antimicrobial and membrane-lytic peptides and their interactions with lipid membranes. *Biochimica et biophysica acta, 1462*(1–2), 109–140. https://doi.org/10.1016/s0005-2736(99)00203-5

42. Caruso, B., Ambroggio, E. E., Wilke, N., & Fidelio, G. D. (2016). The rheological properties of beta amyloid Langmuir monolayers: Comparative studies with melittin peptide. *Colloids and Surfaces B: Biointerfaces, 146*, 180–187. https://doi.org/10.1016/J.COLSURFB.2016.06.003

43. Sánchez-Martín, M. J., Haro, I., Alsina, M. A., Busquets, M. A., & Pujol, M. (2010). A Langmuir Monolayer Study of the Interaction of E1(145–162) Hepatitis G Virus Peptide with Phospholipid Membranes. *The Journal of Physical Chemistry B, 114*(1),
44. Lopez-Rodriguez, E., & Pérez-Gil, J. (2014). Structure-function relationships in pulmonary surfactant membranes: From biophysics to therapy. *Biochimica et Biophysica Acta (BBA) - Biomembranes, 1838*(6), 1568–1585. https://doi.org/https://doi.org/10.1016/j.bbamem.2014.01.028

45. Rodriguez Patino, J. M., Rodriguez Nino, M. R., Carrera Sanchez, C., & Cejudo Fernandez, M. (2001). The Effect of pH on Monoglyceride-Caseinate Mixed Monolayers at the Air-Water Interface. *Journal of colloid and interface science, 240*(1), 113–126. https://doi.org/10.1006/jcis.2001.7567

46. Brown, R. E., & Brockman, H. L. (2007). Using monomolecular films to characterize lipid lateral interactions. *Methods in molecular biology (Clifton, N.J.), 398*, 41–58. https://doi.org/10.1007/978-1-59745-513-8_5

47. Jurak, M., & Miñones, J. (2016). Interactions of lauryl gallate with phospholipid components of biological membranes. *Biochimica et Biophysica Acta (BBA) - Biomembranes, 1858*(8), 1821–1832. https://doi.org/10.1016/J.BBAMEM.2016.04.012

48. Zhai, X., Li, X.-M., Momsen, M. M., Brockman, H. L., & Brown, R. E. (2006). Lactosylceramide: Lateral Interactions with Cholesterol. *Biophysical Journal, 91*(7), 2490–2500. https://doi.org/10.1529/BIOPHYSJ.106.084921

49. de la Feria, J., & M. Rodriguez Patino, J. (2002). Destabilization of Monoglyceride Monolayers at the Air-Aqueous Subphase Interface. 2. The Role of Film Elasticity. *Langmuir, 11*(6), 2090–2097. https://doi.org/10.1021/la00006a041

50. Carrera Sánchez, C., de la Fuente Feria, J., & Rodríguez Patino, J. M. (1998). The importance of the monolayer structure in bidimensional miscibility in mixed films. 1. Kinetics. *Colloids and Surfaces A: Physicochemical and Engineering Aspects, 143*(2), 477–490. https://doi.org/https://doi.org/10.1016/S0927-7757(98)00378-1