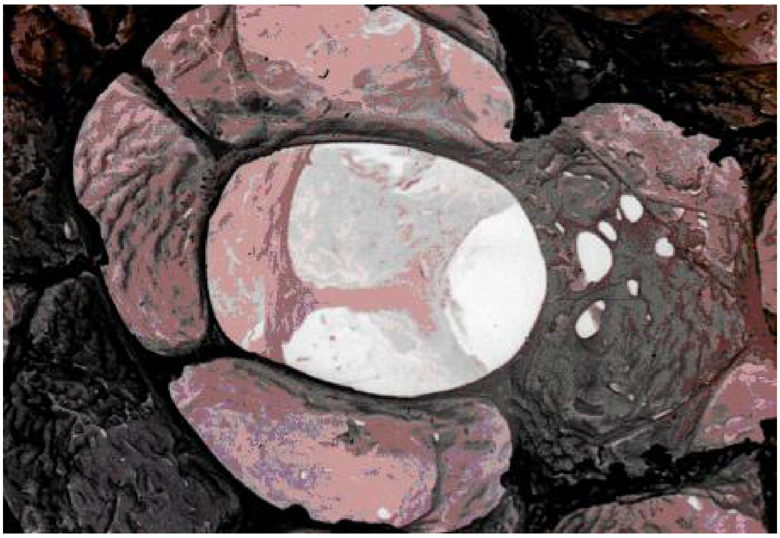
Effects of Nicotine on a Primary Lipid of Lung Surfactant: Testing the Stability and Efficiency Using Subphases of Different Ion Strengths and pH



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11/2006

Suomen Akatemia
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Abstract

The thin surfactant film lining the alveolar air–liquid interface, where gas exchange in the lung takes place, plays an important role in proper lung function. The stability and efficiency of this surface-active material is responsible for lowering surface tension to near zero values on expiration, and preventing lung collapse, maintaining effective re-spreading during expansion, and minimizing the work required for breathing.

The aim of this research was to investigate how nicotine affects the stability and efficiency of a pure phospholipid monolayer (used as a model membrane for lung surfactant) when added to aqueous solutions of different ion strengths and pH as well as to study nicotine-phospholipid interactions. The technique applied for this purpose was the Langmuir–Wilhelmy method, where the surface pressure of a monolayer at an air–aqueous interface, such as in the lung, is recorded as the monolayer area is changed by mechanical barriers. From the specific phase behaviour of the surfactant, observed in surface pressure-area isotherms, it was found that nicotine generally destabilizes the surfactant in water, calcium chloride ($CaCl_2$) and sodium chloride (NaCl) subphases, promoting the likelihood of getting respiratory diseases such as edema and emphysema. At pH 10, nicotine is negatively charged and the surfactant is generally a little more stable than at other studied pH. $CaCl_2$ and NaCl at pH 4 and 10, respectively, stabilize the surfactant in the presence of nicotine, presenting possibilities for therapeutic treatment of lung surfactants exposed to nicotine.

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- **Abstract**

1. Introduction

A thin monolayer, mainly composed of lipids,[1] lines the alveolar walls of our lungs. This monolayer, called lung surfactant, takes about 25% of the entire surface area of the human body, and about fifty times the surface area of skin[2]. Thus, the monolayer is extremely susceptible to even the tiniest particles in air, and to the structural alterations caused by them.

The lung surfactant has the function to reduce surface tension at the air-to-liquid interface in the alveoli. Without the surfactant the surface tension would rise during expiration (when the alveoli become smaller) due to water molecules coming closer together in the water film lining the alveoli. The property that enables the surfactant to reduce the surface tension during expiration is its amphiphilic[3], or spreading behaviour, at gas-to-liquid interfaces. The self-assembling favours expansion of the air-water interface, and therefore lowers the surface tension. This keeps the alveoli spherical to allow gaseous exchange to take place, and prevents them from collapse.

A symptom of the respiratory disease, emphysema, is a hard work of breathing as a result of alveolar wall rupture: a difficulty of re-expanding alveoli during inhalation. (Figure 1 b) shows alveolar wall rupture as a result of the absence of lung surfactant). It has already been investigated that respiratory diseases are associated with exposure to toxic contaminants in air pollution and smoking. This is partly due to the direct cause: structural alterations of the surfactant that cause the surfactant to lose its stability and efficiency. It is also partly due to indirect causes such as triggering the release of chemicals that interfere with the surfactant.

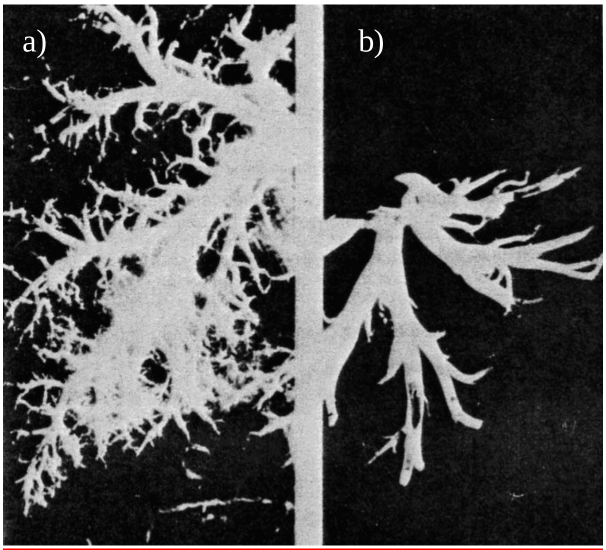

Fig.1.[4] The vitality of lung surfactant in the lungs: extreme cases showing a) presence of normal surfactant and b) absence of surfactant, as in Respiratory Distress Syndrome (RDS).

In this work, the effect of nicotine on the stability and efficiency of a primary lipid of the lung surfactant is investigated. This primary lipid is a phospholipid, the component of cell membranes. Thus, the change in the properties of also a cell membrane as caused by changes in its structure is

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[1] A pie chart showing lung surfactant composition is shown in Appendix (1).

[2] http://www.vendian.org/envelope/dir2/lungsout.html

[3] The amphiphilic property of phospholipids is due to their hydrophobic (non-polar) fatty acid chains and hydrophilic (polar) headgroups that ten to form self-assembling layers at interfaces.

[4] Molly M. Bloomfield; p. 489.

observed. The toxic compound, nicotine, $C_{10}H_{14}N_2$, is chosen from the 2000-4000 chemicals contained in tobacco, a substance in smoke. This is because nicotine is a highly addictive compound, causing dependency, and it is of additional health concern to investigate whether it does structural damage on the lung surfactant.

It is of further interest to see how the effect of nicotine on the surfactant properties varies in different conditions, such as pH and ion strength, in order to draw conclusions on the general trends in regard to the condition. Suggestions can be made on respiratory diseases and on the possibilities for therapeutic treatment.

The method for the investigation is based on the idea that the normal functioning of the surfactant changes when contaminants interact with it. This is the basis of the Langmuir-Wilhelmy method.

2. Theoretical background

2.1. An outline of the Langmuir-Wilhelmy method

The effect of contaminants on the surfactant may be positive or negative depending on the change in its functioning. This change is 'visualized' in graphs, or isotherms[5], where surface pressure (the change in surface tension) is plotted against the molecular area of a DPPC monolayer (a simplified lipid monolayer model of the lung surfactant). The structure of DPPC is shown in Figure 2. As the molecular area of the lipid monolayer at an air-aqueous interface is decreased by mechanical barriers, the surface tension is decreased more, so the surface pressure rises, seen in the isotherms. The method thus provides a model for the functioning of lung surfactant. (This mechanism is explained in the next section, section 2.2)

To know whether the interaction of the contaminant with the surfactant is positive or negative, and speculate on interactions, there must be a reference isotherm and parameters from which qualitative comparisons between other isotherms can be made. The reference isotherm, which illustrates the functioning of a healthy lung surfactant, has a certain shape, or phase behaviour (which is interpreted in section 2.3).

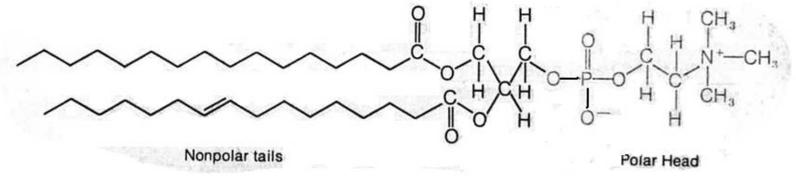

Fig.2.[6] Dipalmitoyl phosphatidylcholine (DPPC), the primary lipid of lung surfactant used in this investigation.

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[5] A line of constant temperature in a graph

[6] Molly M. Bloomfield; p. 489.

2.2. Equipment and measurement

The reduction of surface tension at the air–water interface by the surfactant can be defined by the surface pressure, $\gamma_0 - \gamma$,[7] where γ_0 is the surface tension of the clean surface and γ is the surface tension with surfactant. The surface pressure can be varied as a function of the area per molecule of the surfactant, i.e. a surface pressure-area (π–A) isotherm can be produced. The isotherm is based on Rayleigh's principle: πA = constant.[8]

The method works by having mechanical barriers compress and expand a monolayer at an air-water interface, where the aqueous subphase is held in a Langmuir trough (see Figure 3). As the area changes, the surface pressure is measured by a platinum Wilhelmy plate (see Figure 4). The data is recorded and π–A isotherms yielded via a computer processor.

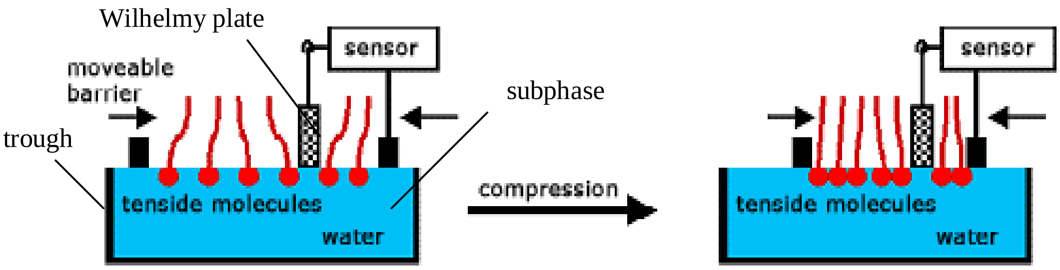

Fig.3.[9] Compression of a lipid monolayer at an air–water interface.

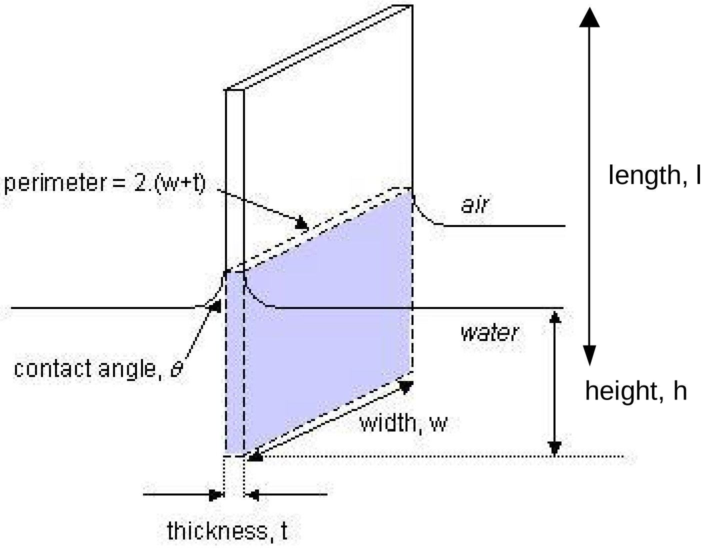

Fig.4.[10] Schematic representation of a Wilhelmy plate and its physical properties.

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[7] Banerjee R; p. 422
[8] Banerjee R; p. 422
[9] http://www.itc.tu-bs.de/Abteilungen/Makro/makro.html
[10] http://www.nima.co.uk/Literature/Manuals/NimaCh3-L-B.pdf

The forces acting on the plate consist of gravity and surface tension downward, and buoyancy due to displaced water upward. For a rectangular plate of dimensions l, w and t, of material density ρ_p, immersed to a depth h in a liquid of density ρ_l, the net downward force is given by the following equations[11]:

$$F = \rho_p glwt - \rho_l gtwh + 2(t + w)\gamma \cos\theta \tag{1}$$

Force = weight – upthrust + surface tension (2)

where γ is the liquid surface tension, θ is the contact angle of the liquid to the plate and g is the gravitational constant.

The weight and upthrust terms are eliminated since the pressure reading is zeroed before making any measurements and the plate is always kept at a constant level by the balance. Since the plate is completely wetted by the liquid, $\cos\theta = 1$. Therefore[12],

$$\gamma_0 - \gamma = \Pi = \frac{\Delta F}{2(t + w)} \tag{3}$$

2.3. *π–A* isotherm parameters for a lung surfactant

Comparisons between the isotherms are made in terms of the extent they meet the set parameters. Since the monolayer serves as a model of the lung surfactant in this investigation, efficiency parameters are set in addition to the stability parameters that are only set if the monolayer is assumed to be a cell membrane. The efficiency and stability determining properties of a healthy, or unaffected, lung surfactant are shown in Table 1, and produce an isotherm of certain phase behaviour (see Figure 5). In the investigation, the conditions that produced the reference isotherm were a water subphase in pH 7.4 in a temperature of 25°C.

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[11] http://www.nima.co.uk/Literature/Manuals/NimaCh3-L-B.pdf

[12] http://www.nima.co.uk/Literature/Manuals/NimaCh3-L-B.pdf

Table 1. Stability and efficiency parameters

Property: Stability and Efficiency	See Fig. 5.
Achievement of near-zero surface tension (i.e. high surface pressure) on compression to prevent collapse during expiration and maintain spherical alveoli. This minimizes the work of breathing.	1.
[13]The limiting area per molecule, A_0[14]. For an efficient surfactant, the target pressure should occur close to A_0 (e.g. not too low so that less surface area reduction is required for high surface pressure, thus leaving a large area in the lungs for gas exchange.) For a compressed, stable surfactant, A_0 can be very low. For a stably expanded surfactant, A_0 can be high.	2.
Compressibility (generally low surface pressure) in the gaseous and expanded phases.	3.
A steep condensed phase curve.	4.
A long phase transition (constant pressure) plateau.	5.
A low minimum pressure.	6.
Clear phase transitions.	-
High re-spreading of the surfactant during expansion, or small hysteresis.	-

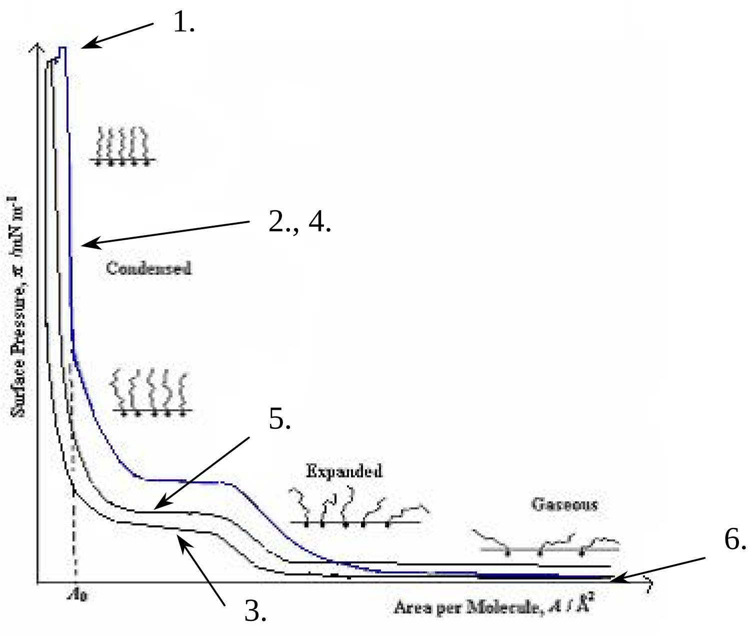

Fig.5.[15] Surface pressure–area isotherms showing the properties of a phospholipid monolayer phase behaviour.

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[13] P J Quinn, M Kates, J F Tocanne, M Tomoaia-Cotişel; pp. 377-381.

[14] A_0 is obtained by extrapolating the condensed phase curve of the isotherm for water at pH 7.4 to zero surface pressure.

[15] Isotherms modified from: http://www.dur.ac.uk/sharon.cooper/lectures/colloids/interfacesweb2.html

2.4. Hypothetical chemical interactions

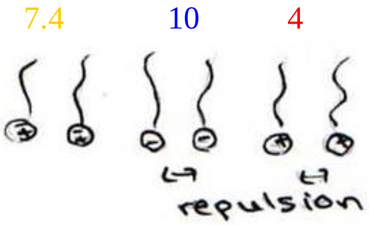
The interactions of calcium (Ca^{2+}), sodium (Na^{+}) and aluminium (Al^{3+}) ions with the monolayer are studied. Speculating the electrostatic and ionic interactions of these monovalent, divalent and trivalent metal ions are of importance in order to establish reasons for the shapes of the isotherms and draw more supportive conclusions on the contributions of nicotine on respiratory diseases. The interactions give information on the electrostatic properties of a cell membrane[16].

The possible interactions between the subphase contents and the phospholipid headgroups of the monolayer can be categorised into three types: binding, adsorption and penetration. These can be speculated by analyzing the properties in the measured isotherms, using the stability and efficiency parameters outlined in section 2.3.
Binding is a surface interaction that does not extensively affect stability and efficiency. The molecules or ions are not adsorbed into the layer: they do not promote attraction, into a more crystalline structure, or repulsion, into a more expanded state.
Adsorption[17] of an ion in the headgroup region causes attraction of adjacent headgroups due to ionic bonds or electrostatic interactions. This is likely to stabilize the monolayer, but cause inefficiency if the monolayer is too compressible. With a single positive charge, sodium ions can adsorb onto the monolayer surface – the interaction is electrostatic. With a double positive charge, calcium ions can crosslink adjacent lipid headgroups by forming ionic bonds. Aluminium ions adsorbed on the monolayer can result in charge reversal,[18] in which positively charged ions are drawn and adsorbed to an already positively charged surface, resulting in stability.

Penetration involves molecules moving deeper (than in adsorption) between polar headgroups of the monolayer. Stable and unstable penetration cause instability: the former causing tight and lowly compressible monolayers, and the latter causing rigid, expanded films with unclear phase transitions and perhaps large hysteresis due to lipid loss.

The following diagrams show theoretically possible subphase-monolayer interactions[19] in different subphases[20].

Water without nicotine



Legend
pH 7.4: 7.4
pH 10: 10
pH 4: 4

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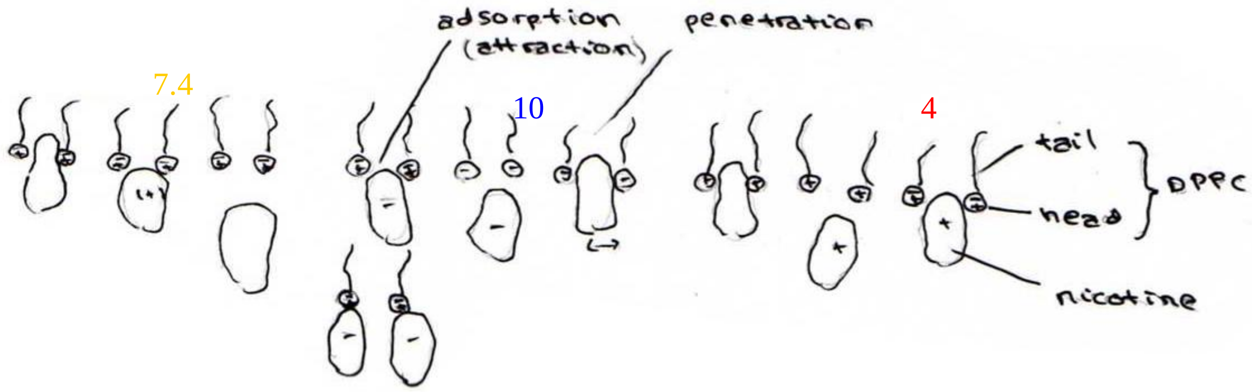
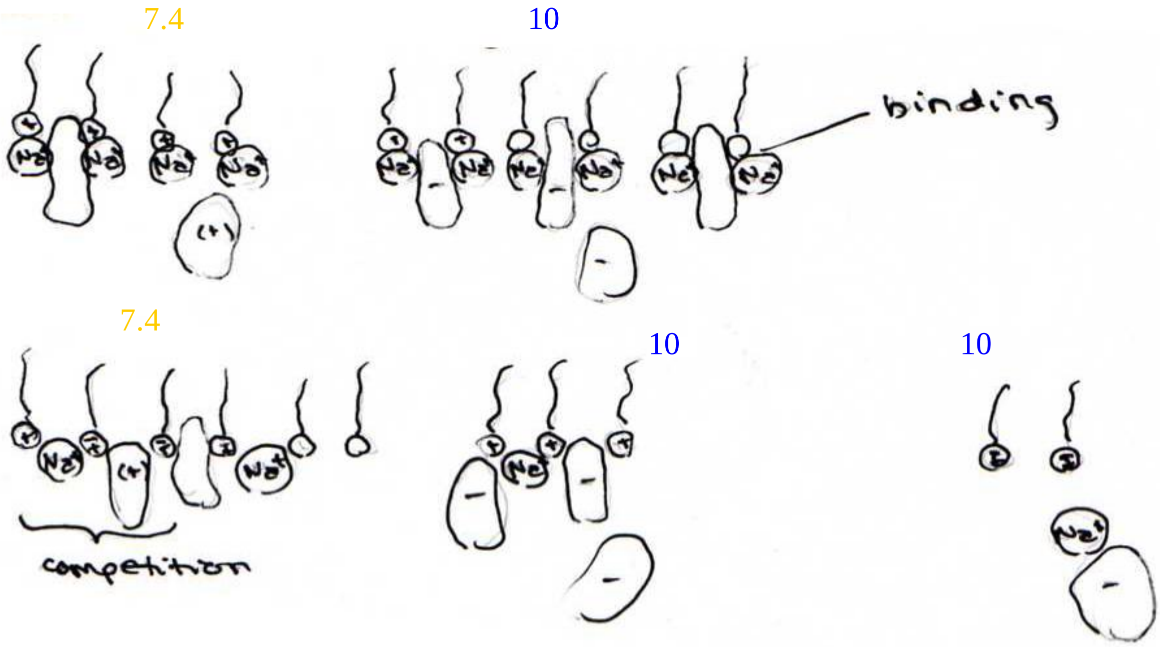
[16] For the biologically existent calcium and sodium ions, the properties are important for a number of processes including neural transmission, membrane fusion, cell excitation and the adsorption of charged species to the membrane.

[17] http://www.answers.com/topic/adsorption
[18] Israelachvili, Jacob; pp. 213-214, 237
[19] For explanations, see Appendix (2).
[20] DPPC lipids are zwitterionic: they contain an acidic and basic group.

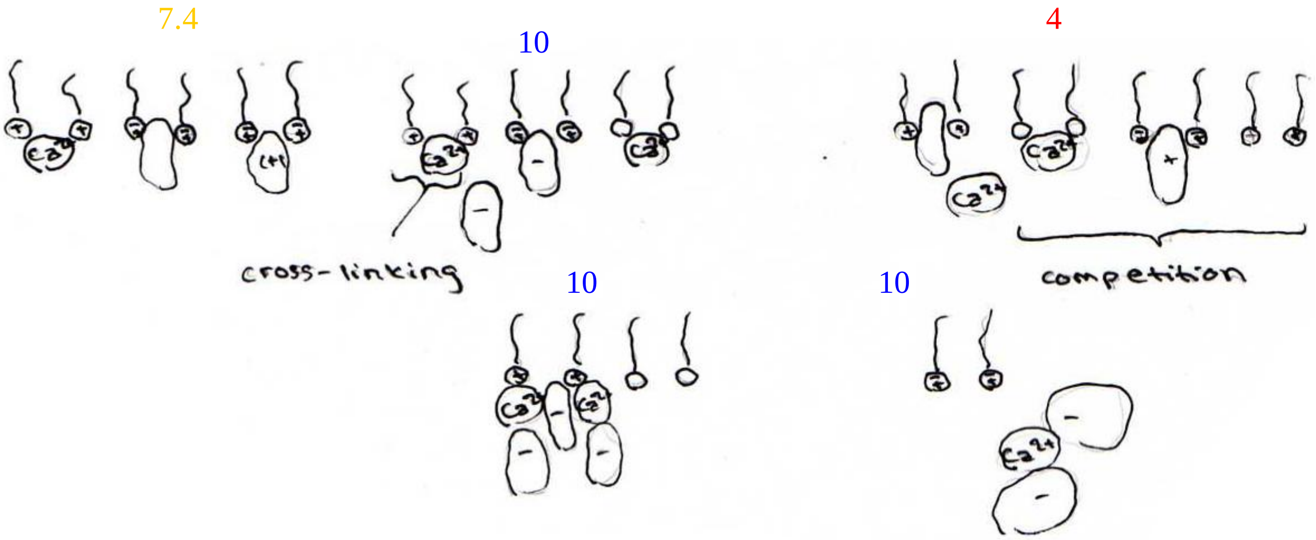
Water with nicotine



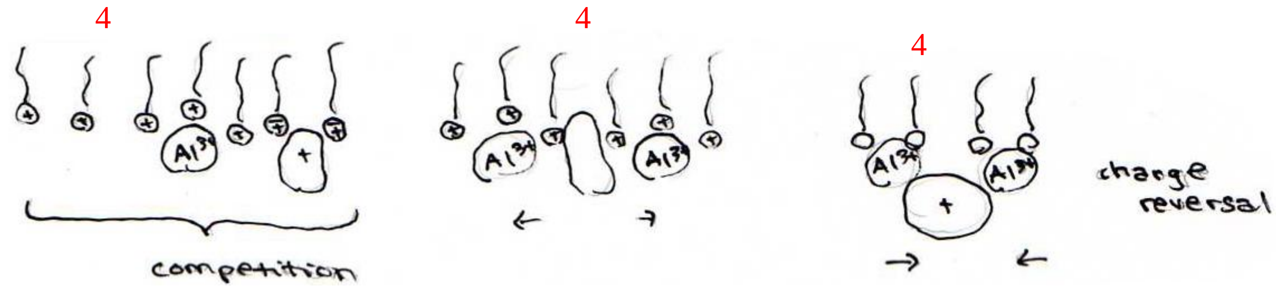
NaCl



$CaCl_2$



$AlCl_3$



It can be hypothesized that the interaction of nicotine at pH 7.4 is harmful, causing the destabilization of the surfactant monolayer. However, whether nicotine may cause destabilization or stabilization at other pH solutions depends on the action of hydrogen ions. If hydrogen ions are bonded to or are removed from the lipid headgroups it is likely that the repulsion, which eases possible penetration of nicotine, would cause destabilization. If, on the other hand, nicotine becomes charged instead of hydrogen ions taking part in lipid interaction, the resulting adsorption of nicotine on the lipid headgroups would cause stabilization. The resulting stability of the monolayer also depends on the possible interactions with different ions: binding or adsorption, and the competition between ions and hydrogen ions (and nicotine) in interacting with the lipid monolayer.

Possible respiratory outcomes include emphysema and edema. Emphysema is characterized by loss of the normal elasticity of the lung that helps to hold airways open. Progressive inelasticity leads to the collapse of the airways on expiration, making it impossible to fully exhale air.[21] Previous research shows that the alveolar wall rupture can be due to a loss of surface tension lowering function of surfactant and an increase in pressure gradient across the alveolar wall[22] (at large limiting areas per molecule of the surfactant). It is also thought that tobacco may release chemicals in the lungs that trigger the disease: this would be an indirect cause. The properties of a lung surfactant that link to edema (inflammation) include a decrease in the maximum surface pressure (or collapse pressure[23]) and an increase in hysteresis, possibly due to loss of surfactant lipids.[24]

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[21] http://www.healthscout.com/ency/68/149/main.html

[22] G Devendra, RG Spragg.

[23] The collapse pressure is the maximum to which a monolayer can be compressed without a detectable expulsion of molecules from it.

[24] Bringezu F, Pinkerton KE, Zasadzinski JA; pp. 2900-2907.

3. Experimental procedure

3.1. Set-up and preparation

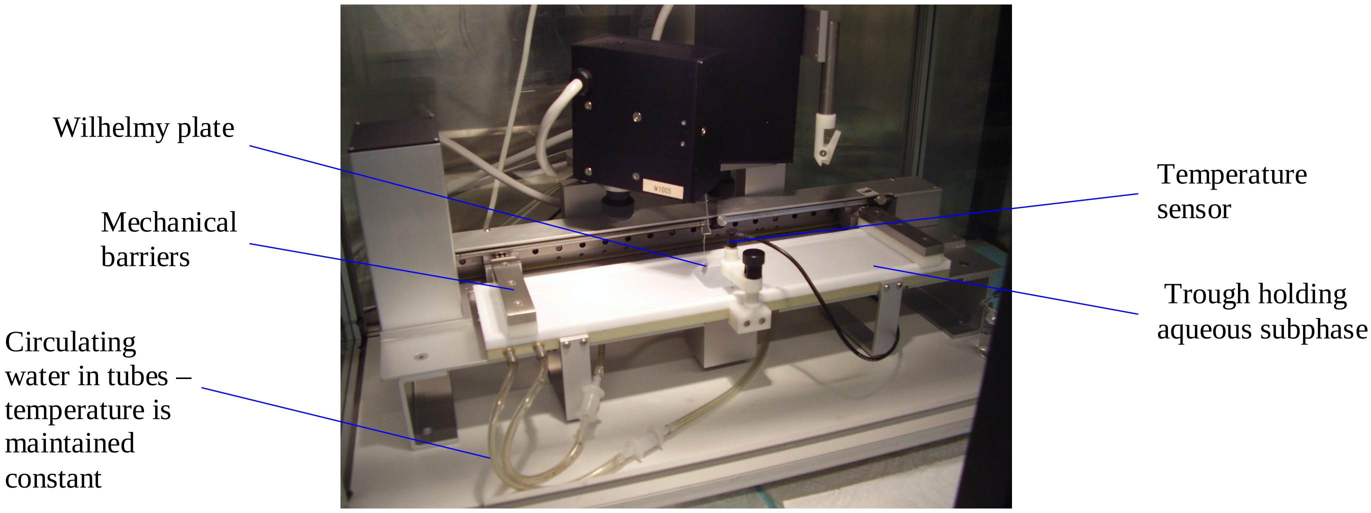

Fig.6. The actual equipment of the Langmuir–Wilhelmy[25] balance used in this work.

The experiment was carried out in a laboratory at the Physical Chemistry and Electrochemistry Department of Helsinki University of Technology.

Table 2. Preparation of the subphases (marked with X)

150µl			30mM		
Nicotine	**pH**	**Water**	**NaCl**	**$CaCl_2$**	**$AlCl_3$**
Not present	7.4	X	X	X	
Present	7.4	X	X	X	
Present	10	X	X	X	
Present	4	X	[26]	X	X

The aqueous subphase composition, pH and concentration were varied while the monolayer composition was kept constant[27].

The salts were weighed on an analytical balance and diluted in pure water with a buffer solution to reach physiological standards. Solutions were prepared by mixing the weighed salts in volumetric flasks of 250 ml[28] without nicotine[29]. The solutions were homogenised by inverting the flasks to prevent insoluble particles interfering with the monolayer.

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[25] The Langmuir equipment was produced by KSV Instruments Ltd, a Finnish company specialized in the development and manufacture of high performance instruments for Langmuir-Blodgett Film and surface chemistry research.

[26] A subphase of NaCl at pH 4 was not prepared since only a limited amount of nicotine was available for security reasons.

[27] The DPPC properties are shown in Appendix (3)

[28] approximately the volume of the trough

[29] Some solutions were mixed with nicotine for preliminary isotherm measurements.

3.2. Assumptions

1. Nicotine is added to the aqueous subphase due to a lack of uniformity of nicotine exposure in the air subphase. Thus, it is assumed that the subphase-monolayer interactions are similar to those occurring when nicotine is in the air subphase as when entering the lungs, although nicotine interacts with the phospholipid polar headgroups at the aqueous subphase and with the non-polar tails at the air subphase.
2. The nicotine content in cigarettes varies with brand, ranging between 0.1-1.8mg[30] per cigarette. The maximum nicotine amount per monolayer used in this investigation was 150μl, or approximately 0.2 mg, a smaller amount.[31]
3. It is assumed that the only component of lung surfactant is DPPC in order to achieve more accurate conclusions on the subphase-monolayer interactions and simplify the model membrane.
4. Temperature is maintained at 25°C, although the physiological temperature is 37°C. This is due to the shorter waiting time to reach a lower temperature, and due to clearer phase transitions and easier analysis of the isotherms at lower temperatures.
5. It is assumed that nicotine exposure occurs during expiration, when area per molecule and surface tension of the surfactant are the lowest. I.e., nicotine is injected in the aqueous subphase when the barriers have compressed up to a target pressure. This is done in order to have a larger concentration of nicotine able to interact with the monolayer and cause a clearer difference between the isotherm for pure water at pH 7.4.
6. During normal respiration, lung surface area is reduced about 54%[32]. Experimentally, the area per molecule of DPPC should decrease from about 1.22 nm^2/molecule to 0.57 nm^2/molecule. However, according to this experiment, since the collapse pressure of the DPPC at a subphase of pure water at pH 7.4 occurs at about 0.4 nm^2/molecule[33] it is assumed that this is the minimum surface area of a normal alveoli during expiration.

3.3. pH measurement

Different pH solutions were used in order to charge the DPPC and/ or charge nicotine. pH was measured using a pH meter and adjusted by the addition of small amounts of either sodium hydroxide for more basic solutions (pH 10, and 7.4) or hydrochloric acid for more acidic solutions (pH 4).

To achieve the physiological pH of 7.4, the pH of pure water had to be increased significantly. This is because the theoretical pH of 7.0 decreases to about 5.6 due to absorption of carbon dioxide from air[34]:

$$CO_2 + H_2O \rightarrow H_2CO_3 \quad (4)$$

$AlCl_3$ solution could only be acidified to pH 4 due to its formation of aluminium hydroxide, $Al(OH)_3$, as the solution reaches pH 7.4 and 10[35].

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[30] http://www.erowid.org/plants/tobacco/tobacco_nic.shtml
[31] For security reasons.
[32] Banerjee R; p. 421.
[33] indicating the relative strength of the experimental surfactant
[34] http://www.ilpi.com/msds/ref/carbondioxide.html
[35] http://www.chemguide.co.uk/inorganic/period3/chlorides.html

$AlCl_3(s) + 6H_2O\ (l) \rightarrow [Al(H_2O)_6]^{3+}(aq) + 3Cl^-(aq)$ (5)

The equilibrium in the following reactions shifts to the right[36].

$[Al(H_2O)_6]^{3+}(aq) + H_2O(l) \rightarrow [Al(H_2O)_5OH]^{2+}(aq) + H_3O^+(aq)$ (6)

A pH of 10 was used in order to negatively charge nicotine ($pK_a \approx 8.5$)[37]. The following equilibrium shifts to the right:

$C_{10}H_{14}N_2 + H_2O \rightleftharpoons C_{10}H_{13}N_2^- + H_3O^+$ (8)

3.4. The Langmuir-Wilhelmy method

To prevent contamination, the Langmuir trough and barriers[38] were cleaned before and after each experiment with ethanol and pure water using a soft brush. The Wilhelmy plate and the temperature sensor were rinsed with pure water and kept in ethanol after each experiment.

The experiment type, hysteresis or kinetics run, was selected after the subphase solution was poured from its flask to the clean trough, and the temperature control targeted to 25°C. DPPC was taken out of the freezer and left to equilibrate at room temperature. The aqueous surface of the subphase was carefully aspirated in order to reduce the surface pressure to near-zero values and minimize the effect of surface-active impurities. Then, 23 µl of the lipid solution was spread evenly onto the subphase drop by drop with a micrometer syringe (Hamilton) to form an insoluble monolayer at the air–aqueous interface[39]. The monolayer was allowed to equilibrate for 10–15 min before starting the first compression to ensure that the lipid solvent, chloroform, had evaporated.

If kinetics run was selected, the barriers were signalled to compress with a rate of 5 mm/min up to a target pressure of +55.0 mN/m. At this pressure, nicotine was injected using a micrometer syringe evenly beneath both barriers under the compressed monolayer. Twenty minutes was waited to allow the nicotine to interact with the lipid monolayer (see Figure 7). After this, the hysteresis run was selected, with 4 sweeps of compression and expansion cycles (each one taking about 20 min) and the isotherms obtained by the computer.

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[36] The small, highly charged aluminium ion of the hexaaqua complex polarizes the water molecules that are attached to the aluminium ion through coordinate bonds. This makes the hydrogen atoms δ^+ and susceptible to attack from solvent water, which acts as a base. The complex ion is deprotonated, causing the solution to be acidic from the formation of oxonium ions H_3O^+.

[37] http://www.inchem.org/documents/pims/chemical/nicotine.html

[38] The Langmuir trough and barriers are made of Teflon or polytetrafluoroethylene to prevent leakage of liquids over the edges

[39] Ambient lighting was switched off to prevent heating and the glass and container doors, which prevent airborne contamination, closed slowly to prevent vibration of the monolayer.

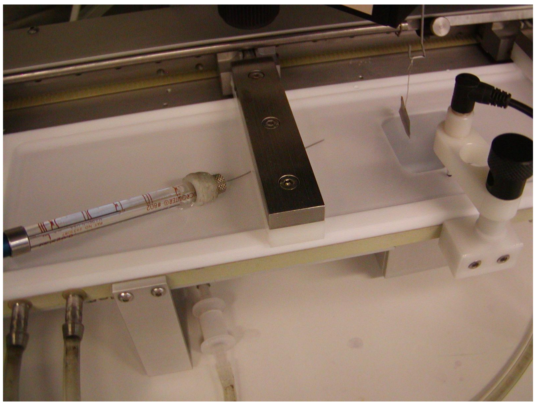

Fig.7. Injection of nicotine using a micrometer syringe beneath both barriers under the compressed monolayer.

4. Results and Discussion

4.1. Results[40]

Hysteresis of the isotherms was compared instead of comparing their collapse pressures, since re-spreading is an important efficiency determining property of lung surfactants. When not mentioned, the temperature is 25 °C.
π – surface pressure (the reduction in surface tension relative to that of pure water)
mma – mean molecular area
inj. - injection

4.2. Analysis of Results

4.2.1. Preliminary isotherms

Preliminary experiments were performed before combining nicotine with different salt solutions. The effect of temperature and different nicotine and ion concentrations (using different methods of nicotine exposure) on the phase behaviour of the monolayer surfactant were analysed from the isotherms.[41]

4.2.2. Experimental isotherms

By comparing the shape of the isotherms the following observations can be made in terms of subphase-monolayer interactions.

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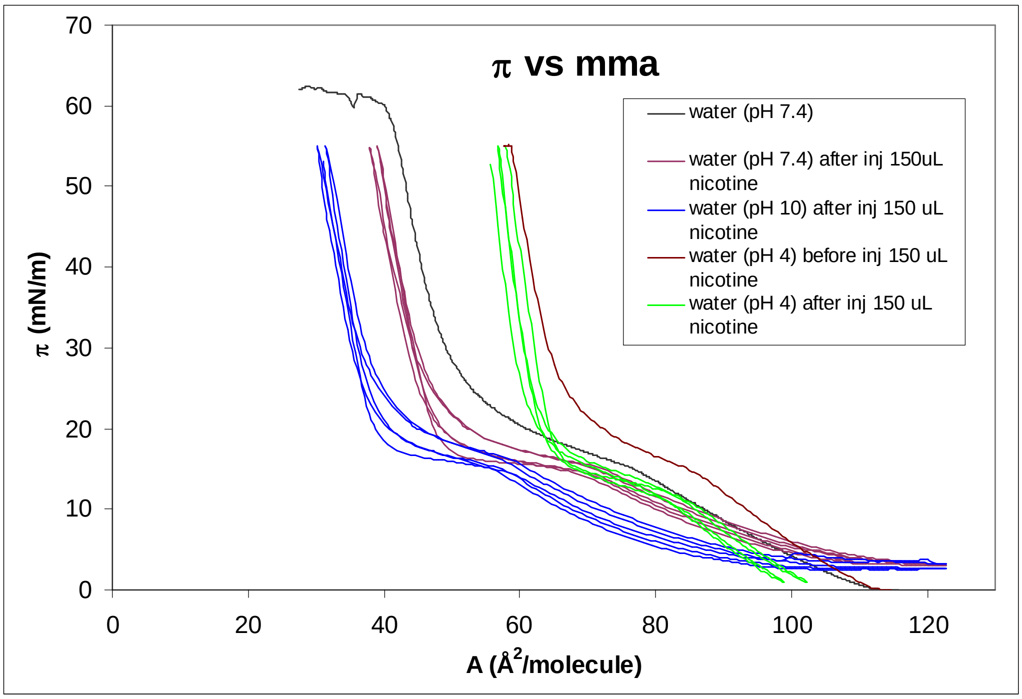
[40] See Appendix (4).
[41] See Appendix (5).

4.2.2.1. Water subphase

Graph 1: water



In comparison to the black isotherm, the pink isotherm suggests the possibility that nicotine has penetrated the monolayer. A loss of some lipids might explain the observed low surface pressure. Another possibility could be that slightly positively charged nicotine has adsorbed between lipid headgroups, stabilizing the monolayer.

The blue isotherm is highly compressible in the liquid expanded phase and the condensed phase curve is relatively steep, which suggests that negatively charged nicotine has adsorbed between lipid headgroups, pulling them together.[42]

The green isotherm is at the most right side of the graph, which can be explained by the expansion of the monolayer due to high repulsion between positively charged lipid headgroups as a result of hydrogen ions binding to phosphate groups. The green isotherm does not show a remarkable difference between the orange isotherm, suggesting that nicotine does not have a significant effect.[43] Furthermore, the fact that the blue isotherm is to the left side of the graph (thus generally more compressed) in contrast to the green isotherm shows that the $N(CH_3)_3$ groups of the lipid headgroups are more likely to keep hydrogen ions and that phosphate groups are more likely to gain hydrogen ions.

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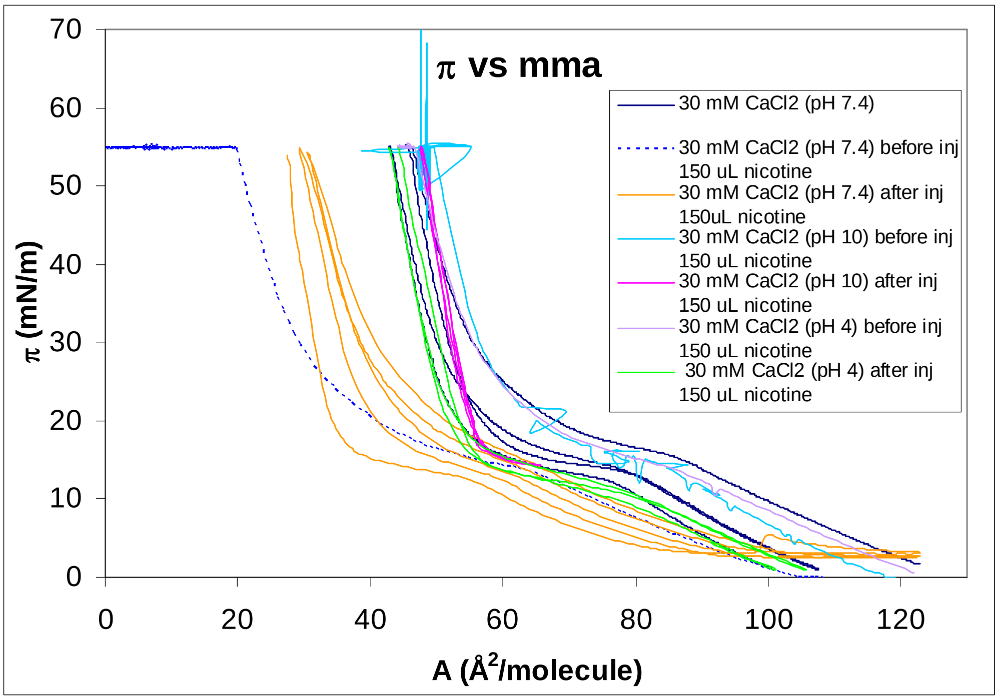
[42] Other possibilities, such as negatively charged lipid headgroups repelling each other, would cause expansion of the monolayer, resulting in a less stable monolayer than at pH 7.4, which is not the case here.

[43] Also, clear penetration is not observed since the minimum pressure is relatively low.

4.2.2.2. $CaCl_2$ subphase

Graph 2: $CaCl_2$



The large hysteresis of the dark blue isotherm suggests the presence of impurities in the aqueous subphase. As the compression cycles progress the isotherm moves leftwards, making it hard to determine A_0. The high rigidity and compressed state of the monolayer on the first expansion suggests the cross-linking action of calcium ions adsorbed between lipid headgroups. As cycles progress, more and more nicotine molecules penetrate unstably between lipid headgroups, as can be seen from the unclear phase transitions and absence of a phase transition plateau at constant pressure. Perhaps even loss of lipids has occurred.[44]

The pink isotherm shows high penetration as evident from the withstanding high minimum pressure and a very steep and early condensed phase curve. This may be due to negatively charged or neutral nicotine penetration between lipid headgroups, to which calcium ions have bonded with attached negatively charged nicotine. The calcium binding not only leaves space for nicotine penetration, but also makes nicotine more attached between the lipid headgroups, accounting for the very small hysteresis of the isotherm. The monolayer is relatively more penetrated than for sodium ions[45] (which may also be due to large calcium-nicotine complex formation). Cross-linking of calcium over adsorbed nicotine increases the stability.

It is possible that the monolayer exposed to nicotine is more expanded in $CaCl_2$ at pH 4 than at pH 7.4 due to hydrogen ions binding to phosphate groups and ‘winning the competition’ between cross-linking calcium ions. This results in the repulsion between the lipid headgroups. The monolayer exposed to nicotine at pH 4 is slightly more compressed than the monolayer at pH 10 due to penetration having a greater destabilizing affect than repulsion.

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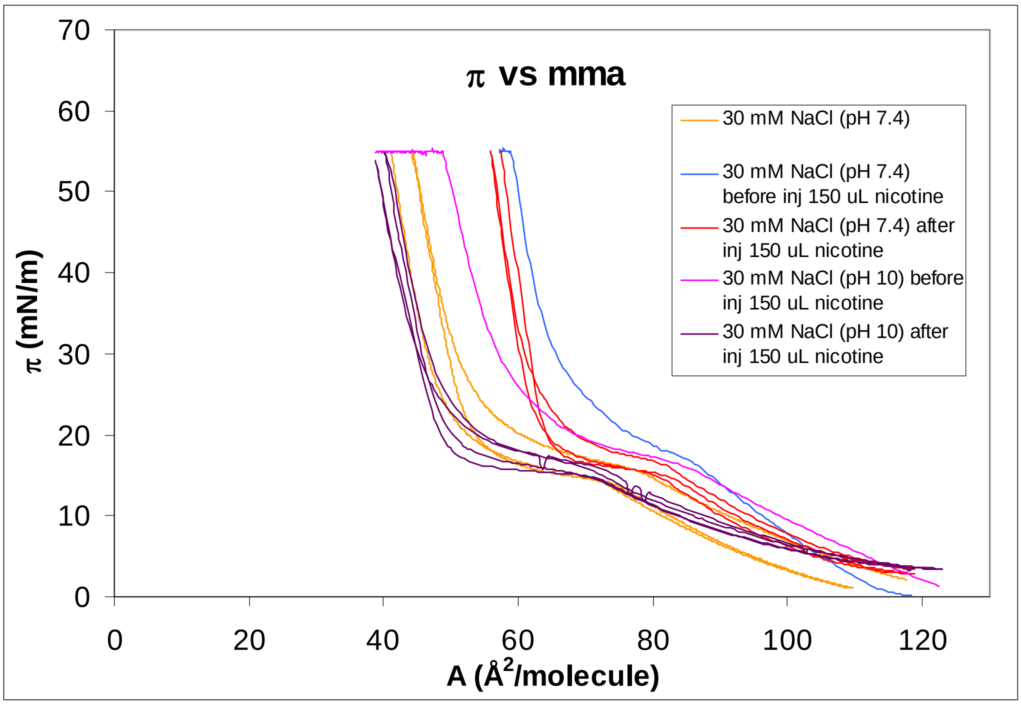
[44] Bringezu F, Pinkerton KE, Zasadzinski JA; p. 2901.

[45] See Appendix (4), graph 5.

4.2.2.3. NaCl subphase

Graph 3: NaCl



The orange isotherm has a less steep condensed phase curve and lower pressure than the red isotherm. This suggests that sodium ions have bonded onto negatively charged phosphate groups and not adsorbed in between them, leaving space for the penetration of nicotine. The resulting long lipid headgroups keep the penetrated nicotine molecules more stuck between the headgroups, preventing them from escaping.

The purple[46] isotherm is similar to both the pink isotherm and the isotherm for water at pH 7.4 with nicotine (see Graph 1). This suggests that nicotine has not affected the monolayer properties. Sodium ions and nicotine or one of them is likely to have adsorbed into the monolayer, causing the shift leftwards relative to the blue and orange isotherms.

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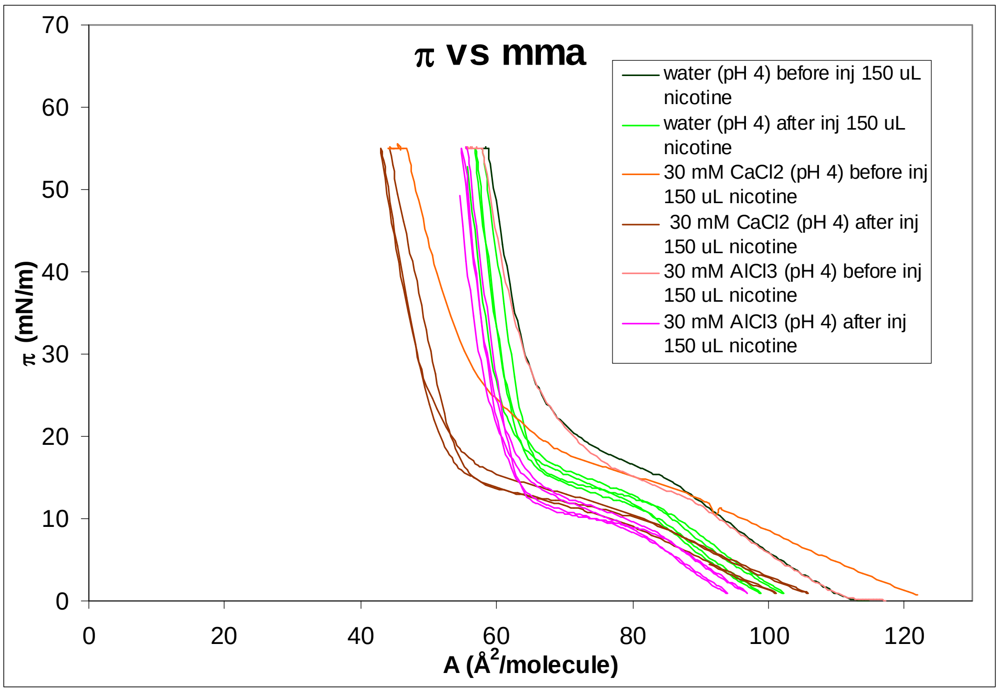
[46] Penetration of some sodium-nicotine complexes into the monolayer may account for some of the impurities, which are negligible to the shape of the isotherm.

4.2.2.4. $AlCl_3$ subphase

Graph 6: pH 4



The pink isotherm shows some degree of repulsion or penetration relative to the brown isotherm, and adsorption relative to the green isotherm. Aluminium ions each adsorbed between three lipid headgroups can cause dislodgment of middle lipids due to unstable penetration. If aluminium ions have adsorbed between just two lipid headgroups this can cause charge reversal.[47]

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[47] See section 2.4.

4.2.3. Summary of analysis

Table 3. Comparison of the stability and efficiency of the lipid monolayer when nicotine is added to the aqueous subphases of different ion strengths and pH

Graph	Isotherm (subphase + inj. of 150 ul nicotine)	Steep condensed phase curve	Clear phase transition plateau at constant pressure	Target pressure occurs near 40Å^2/molecule[48]	Hysteresis	Stability ranking according to A_0	Stability and efficiency ranking*
4 (pH 7.4)	water	~	yes	yes	small	1/2	1
	Ca^{2+}	no	no	~	large	1/2	3
	Na^{+}	yes	~	no	small	3	2
5 (pH 10)	water	~	~	no	small	1	2/3
	Ca^{2+}	yes	(yes)[49]	no	very small	3	3/2
	Na^{+}	~	yes	yes	small	2	1
6 (pH 4)	water	yes	~	no	small	3	2/3
	Ca^{2+}	~	~	yes	small	1	1
	Al^{3+}	yes	~	no	small	2	2/3

* Stability and efficiency ranking in terms of parameters in the isotherms:
1 – most stable, 3 – least stable. ~ between yes and no.

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[48] The assumed minimum area at expiration.

[49] A high minimum surface pressure.

Table 4. Comparison of the interactions and resulting respiratory outcomes of the lipid monolayer when nicotine is added to aqueous subphases of different ion strengths and pH

Graph	Isotherm (subphase + inj. of 150 ul nicotine)	Stability and efficiency ranking,	Likely interaction(s) of nicotine	Relative respiratory outcome(s)
4 (pH 7.4)	water	1	Stable, although loss of lipids due to penetration (or slightly stabilizing)	□ / ○
	Ca^{2+}	3	Loss of lipids due to penetration (or slightly stabilizing)	○
	Na^{+}	2	Stable, expanded penetration	●
5 (pH 10)	water	2/3	Stable, compressed due to adsorption	■
	Ca^{2+}	3/2	Stable penetration	●
	Na^{+}	1	Adsorption of sodium ions and nicotine binding, or stable penetration	□
6 (pH 4)	water	2/3	Nicotine does not interact (repulsion between lipid headgroups due to H^{+} ions)	●
	Ca^{2+}	1	Nicotine does not interact (or loss of lipids due to unstable penetration)	□
	Al^{3+}	2/3	Stable and compressed due to adsorption (charge reversal), or loss of lipids due to unstable penetration	--

□ – stable;
■ – high compressibility, inefficiency;
○ – inflammation, edema;
● – alveolar wall rupture, emphysema

5. Conclusions and Evaluation

Trends can be established for the effect of pH and nicotine from the stability and efficiency ranking of the monolayer of each aqueous subphase studied. The ranking corresponds to the magnitude of stability difference between isotherms at pH 7.4 without nicotine. There is no visible trend for ion strength; the change in order of the stability and efficiency of the surfactant for different ions as the pH changes (see Table 4) confirms the existence of many possibilities for ion interactions, which are seen to depend on pH and nicotine state. Thus, there is trend for pH, instead.

In pH 10, the $N(CH_3)_3$ groups of lipid headgroups are more likely to keep than release hydrogen ions, giving the possibility for other subphase contents, like nicotine, to become negatively charged. In pH 4 the phosphate groups are more likely to gain hydrogen ions from solution, leaving other subphase contents remaining unaffected by the low pH. From the isotherms, it was observed that nicotine is either positively charged or neutral. Thus, pH 10 has a stronger effect in determining interactions.

When comparing isotherms of water, $CaCl_2$ and NaCl at pH 7.4 and 10 it can be observed that the negatively charged nicotine at pH 10 tends to cause subphase-monolayer interactions that are relatively more stable than at pH 7.4. Isotherms for water and $CaCl_2$ are compared at pH 7.4, 10 and

4, the latter in which the competitive contribution of H^+ ions to the stability of the monolayer is compared with nicotine activity at the other pH solutions. Generally, monolayers at pH 10 were still more stable. The main difference between isotherms at pH 7.4 and 4 is that for $CaCl_2$ at pH 7.4 the unstable penetration of nicotine possibly caused loss of lipids, while for water at pH 4, the high repulsion between positively charged lipid headgroups (due to H^+ ion binding) caused stable expansion. The resulting instability and respiratory result for both subphases, though, is quantitatively similar.

The hypothesis is confirmed and additional information is gained from the investigation. Nicotine seems to have an overall negative effect on the stability and efficiency of the surfactant, causing different destabilizing interactions, which may contribute to respiratory diseases, such as edema and emphysema. However, calcium chloride with nicotine at pH 4 and sodium chloride with nicotine at pH 10 seem to keep the surfactant very stable and efficient, suggesting possible treatments for nicotine-exposed lungs. Speculative conclusions can be established for the surface interactions of $AlCl_3$ on the monolayer exposed to nicotine. More isotherms could be obtained for the effects of $AlCl_3$ at different pHs in order to make more comparisons.

These respiratory diseases, however, are not only the result of structural damage caused to the lung surfactant. As well as direct causes, there can be indirect causes, such as nicotine affecting some part of the alveoli that release destructive chemicals. Also, the role of nicotine in tobacco in contributing to these disease-triggering interactions remains unresolved.

Errors are not that evident as there are many possible interactions when observing an isotherm. The obtained isotherms match possible hypothetical interactions and so are sufficiently reliable in confirming the general effects of nicotine and pH. Some errors could have been due to airborne contamination, impurities in subphase solutions, inhomogenous solutions and chloroform in monolayers. For example, the isotherms of $CaCl_2$ with nicotine at pH 7.4 and 10 are likely to have been affected by impurities. This is shown by the exceptionally large hysteresis at pH 7.4 and large minimum surface pressure at pH 10, observed from the quick shifts in surface pressure already before nicotine is injected.

Chloroform has most likely affected the shape of the isotherms in Graph 6. This is due to lipid loss being unlikely, unless the hysteresis of the isotherm is large, and that positively charged nicotine was found not to adsorb in the monolayer at pH 4. The isotherms before injection of nicotine have a generally higher surface pressure and are not similar to the ones after injection. This is most likely a result of chloroform penetration in the monolayer, due to not waiting long enough (20 minutes) for it to evaporate before starting compression.

For further investigations, the monolayers could be visualized using fluorescence microscopy to show the variation in morphology that accompanies the changes in the isotherms. The effect of a different toxicant or component of tobacco on the properties of the surfactant could be investigated to compare with the effect of nicotine, using the Langmuir-Wilhelmy method. Also, another method, such as infrared reflection-absorption spectroscopy could be used to gain more details on the positions of the subphase specimen on the monolayer surface. Moreover, quantitative data in addition to the present qualitative data could be obtained using electrochemistry, to obtain more reliable and definitive conclusions.

References

Banerjee R. Surface Chemistry of the lung surfactant system: Techniques for in vitro evaluation. Current Science 2002; pp. 420-423.

G Devendra, RG Spragg. Lung Surfactant in Subacute Pulmonary Diseases. BioMed Central Ltd 2002.

Bringezu F, Pinkerton KE, Zasadzinski JA. Environmental Tobacco Smoke Effects on the Primary Lipids of Lung Surfactant. American Chemical Society 2003; pp. 2900-2907

Schurch S, Lee M, Gehr P. Pulmonary surfactant: Surface properties and function of alveolar and airway surfactant. Pure and Applied Chemistry 1992; pp. 1745-1750

Scott JE. The Pulmonary Surfactant: Impact of Tobacco Smoke and Related Compounds on Surfactant and Lung Development. Tobacco Induced Diseases 2004; pp. 3-25

Kaganer CM, Möhwald H, Dutta P. Structure and phase transitions in Langmuir monolayers. Reviews of Modern Physics 1999; pp. 779-786

Grandell D. Phospholipids in Monolayers and Bilayers. 1996

Gennis, Robert B. Biomembranes: Molecular Structure and Function.

Mälkiä A. Biomembrane mimetics. 2000

Petty, Michael C. Langmuir-Blodgett films: An Introduction. UK: Cambridge University Press, 1996.

Roberts, Gareth. Langmuir-Blodgett Films. New York: Plenum Press, 1990

Israelachvili, Jacob. Intermolecular and surface forces. California: Academic Press, 2003 (10th edition); pp. 213-214, 237

Wennerström, Håkan and Evans, D. Fennell. The Colloidal Domain: Where Physics, Chemistry, Biology and Technology Meet. Canada: Wiley-VCH, 1999; pp. 79-84

Manahan, Stanley E. Toxicological Chemistry: A guide to toxic substances in chemistry. Michigan: Lewis Publishers, inc., 1989; pp. 10, 51-55, 94-95, 228

P J Quinn, M Kates, J F Tocanne, M Tomoaia-Cotişel. Surface characteristics of phosphatidylglycerol phosphate from the extreme halophile Halobacterium cutirubrum compared with those of its deoxyanalogue, at the air/water interface. Biochem J. 1989; pp. 377-381

Molly M. Bloomfield: Chemistry and the living organism. John Wiley & Sons, Inc, 1984; p. 489.

http://www.ksvltd.com

http://www.dur.ac.uk/sharon.cooper/lectures/colloids/interfacesweb2.html

http://www.nima.co.uk/Literature/Manuals/NimaCh3-L-B.pdf http://www.answers.com/topic/adsorption

http://www.itc.tu-bs.de/Abteilungen/Makro/makro.html

http://www.vendian.org/envelope/dir2/lungsout.html

http://www.healthscout.com/ency/68/149/main.html

http://www.ilpi.com/msds/ref/carbondioxide.html

http://www.chemguide.co.uk/inorganic/period3/chlorides.html

http://www.inchem.org/documents/pims/chemical/nicotine.html

http://www.erowid.org/plants/tobacco/tobacco_nic.shtml

Front cover picture modified from:
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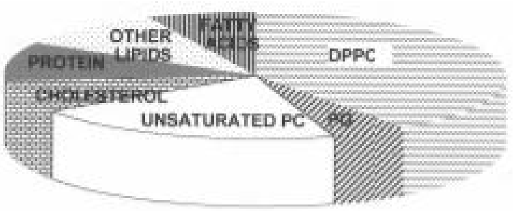
Appendices

Appendix (1)



Pie chart of the composition of lung surfactant[1]

Appendix (2) Possible subphase-monolayer interactions

Water without nicotine

pH 7.4: The positively and negatively charged zwitterionic phospholipids balance each other.

pH 10: A hydrogen ion from the positively charged $N(CH_3)_3$ group may form intramolecular H bonds with the negatively charged phosphate group, increasing the headgroup volume and leading to a more expanded monolayers than at pH 7.4.

pH 4: A hydrogen ion binds to the negatively charged phosphate group leading to a positively charged lipid headgroup that can repel each other and promote more expanded monolayers as at pH 10.

Water with nicotine

pH 7.4: The neutral nicotine molecule can penetrate between lipid heads of the monolayer, causing expansion and destabilization of the monolayer. Van der Waals forces between adjacent non-polar headgroups may become weaker if the nicotine is large enough and penetrative.

pH 10: If nicotine is negatively charged, it may adsorb between two zwitterionic lipid headgroups (containing positive charges) and cause stabilization. If nicotine remains neutral and the polar headgroups are negatively charged, the repulsion between the headgroups can promote nicotine penetration and cause expansion and destabilization of the monolayer. Furthermore, both nicotine and the polar headgroups may be negatively charged resulting in just repulsion between the lipid headgroups.

pH 4: The same phenomenon is observed as at pH 10, except with positive charges of nicotine and lipid headgroups.

NaCl

A sodium ion can bind to the negatively charged phosphate group of a polar headgroup, or electrostatically adsorb between two adjacent polar headgroups, making the monolayer more ordered or compressed and stable.

NaCl with nicotine

pH 7.4: Neutral nicotine can penetrate between lipid headgroups, to which sodium ions are bond to. Nicotine interference would be inhibited if sodium ions are adsorbed to the monolayer, preventing nicotine penetration.

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[1] Banerjee R.

pH 10: Negatively charged nicotine can either penetrate (more likely than when it is neutral) between lipid headgroups to which sodium ions are bond to, or bind to the positively charged monolayer surface where sodium ions are adsorbed. The stability of the monolayer would be unaltered if sodium ions bind to negatively charged nicotine in the subphase without interacting with the monolayer.

$CaCl_2$

A divalent calcium ion complexes with two phospholipids, i.e. Ca^{2+} adsorbs at the monolayer and cross-links two negatively charged phosphate groups. This gives to the monolayer a more crystalline and stable structure, as the headgroups are packed tighter. In pH 4, there is competition between hydrogen ion and calcium ion binding to the polar headgroups.

$CaCl_2$ with nicotine

pH 7.4: Calcium cross-links lipid headgroups and nicotine may penetrate the monolayer between two cross-linkages.

pH 10: Calcium cross-linking can be stronger than in pH 7.4 if the lipid headgroups looses positive charges, and the same phenomenon of nicotine would be observed as for water (see ‘water with nicotine’). Calcium can bind to the negatively charged nicotine and to a negatively charged phosphate group of the lipid headgroup, which is less stable than cross-linking, leaving space for nicotine penetration in the monolayer. Calcium ions can also bind to two negatively charged nicotine ions.

pH 4: Positively charged nicotine has little interaction with the monolayer due to repulsion of the positively charged polar headgroup where calcium ions and hydrogen ions have adsorbed.

$AlCl_3$

Trivalent aluminium ions cause high rigidity of the monolayer due to the cross-linking of three polar lipid heads.

$AlCl_3$ with nicotine

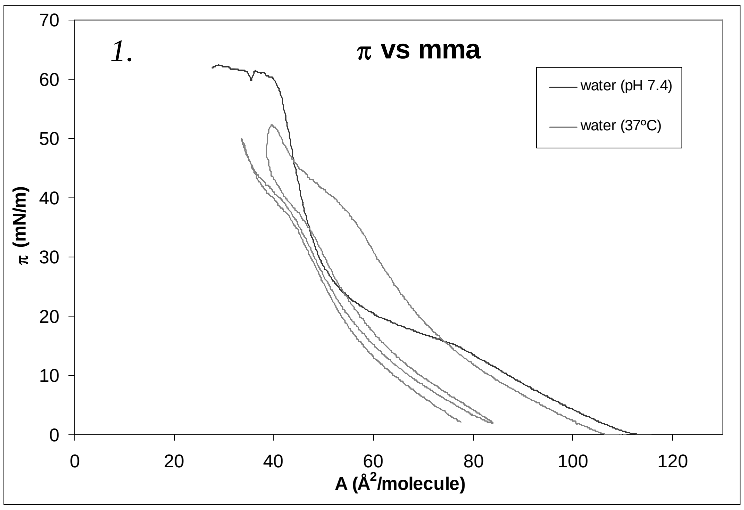
pH 4: Positively charged nicotine can be attracted to aluminium ions adsorbed in the monolayer due to charge reversal, wherein cations continue to adsorb onto a surface that is already positively charged. Competition between hydrogen ion adsorption can also be present.

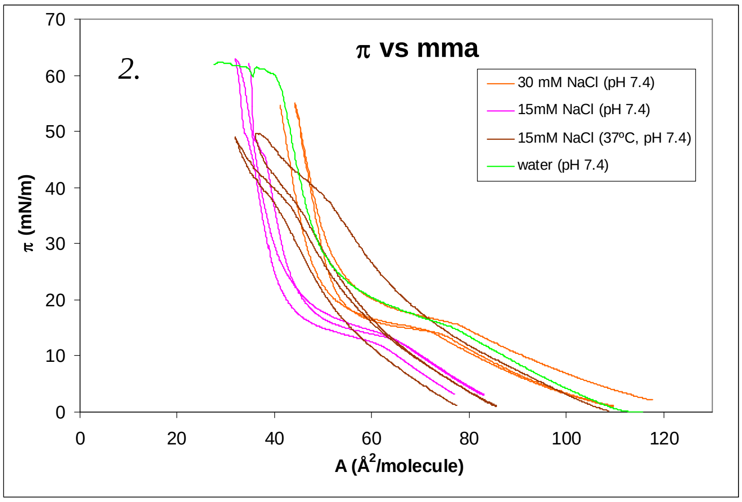
Appendix (3) Experimental Data

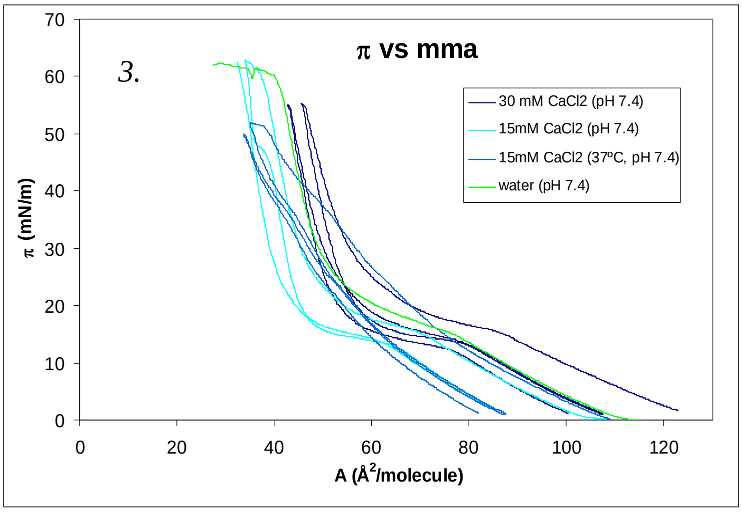
Dipalmitoyl phosphatidylcholine (DPPC) properties:
Concentration: 0.97 mg/ml
Molecular weight: 734 g/mol
Volume: ~23 µl
Solvent: chloroform

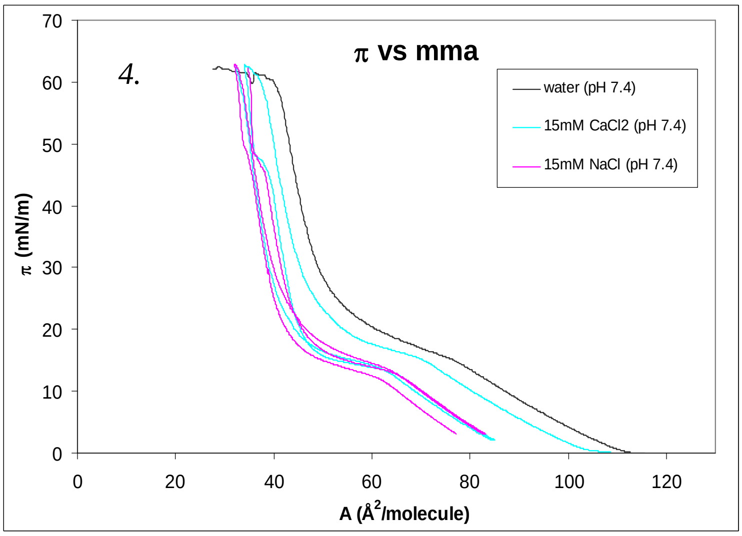
Appendix (4) Results

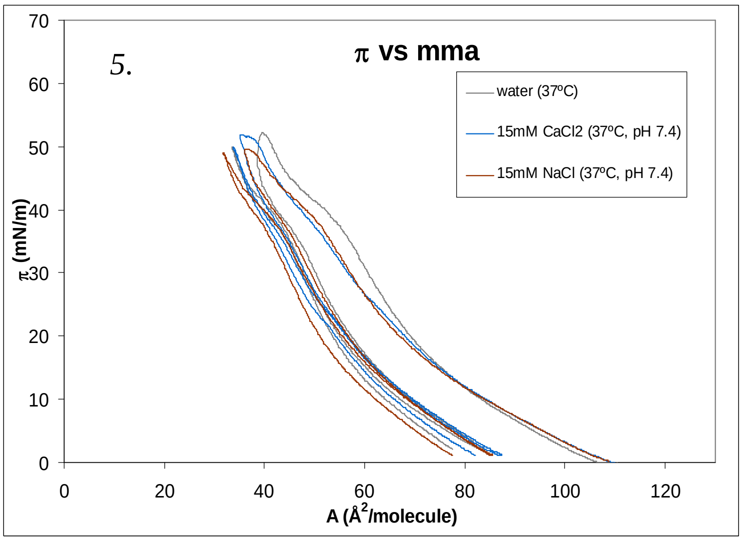
Preliminary Isotherms

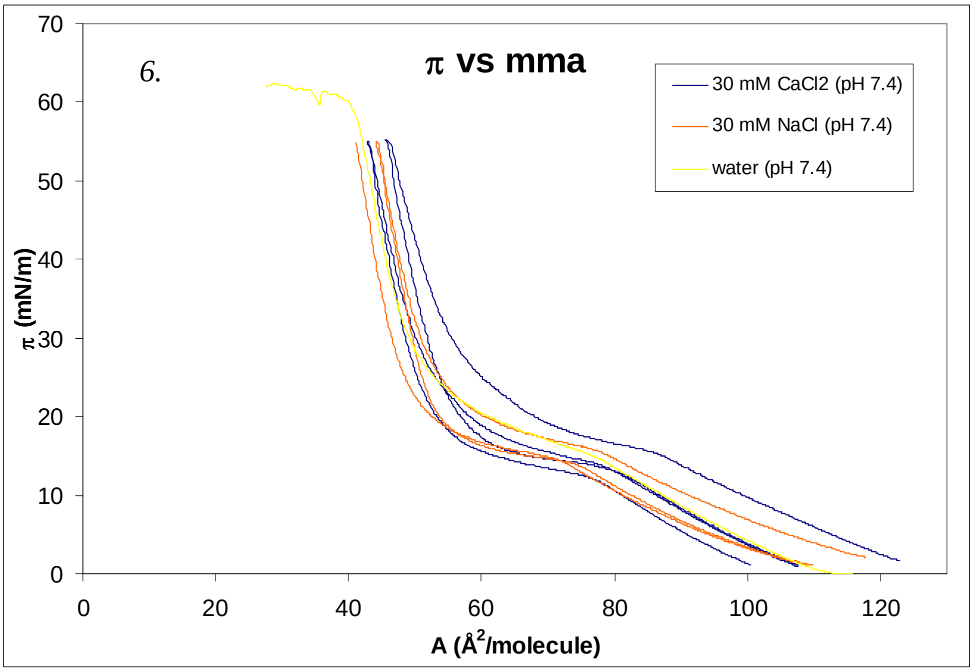


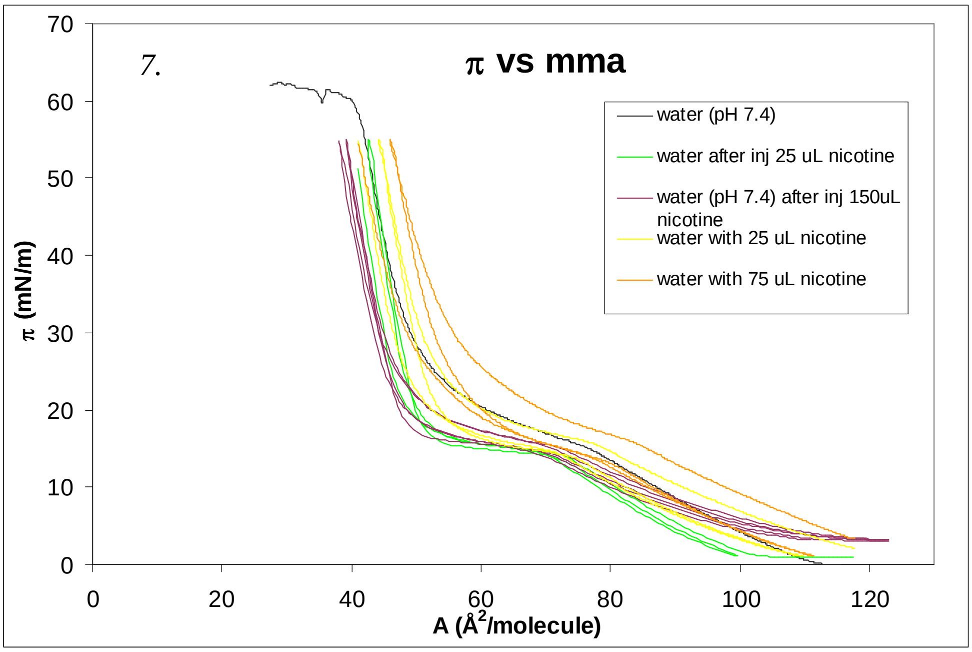



3.
π vs mma
30 mM CaCl2 (pH 7.4)
15mM CaCl2 (pH 7.4)
15mM CaCl2 (37ºC, pH 7.4)
water (pH 7.4)
π (mN/m)
0
10
20
30
40
50
60
70
0
20
40
60
80
100
120
A (Å^2/molecule)


4.
π vs mma
water (pH 7.4)
15mM CaCl2 (pH 7.4)
15mM NaCl (pH 7.4)
π (mN/m)
0
10
20
30
40
50
60
70
0
20
40
60
80
100
120
A (Å^2/molecule)

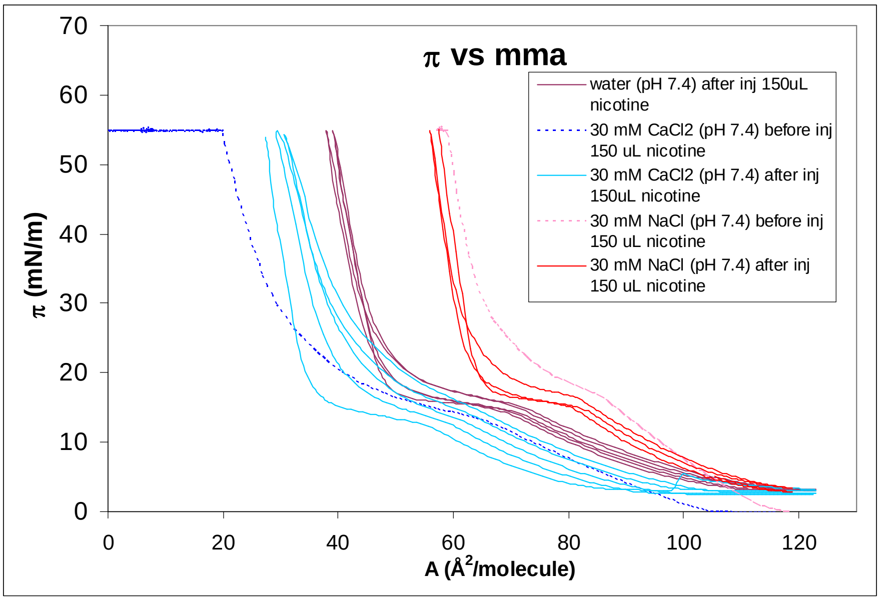

5.
π vs mma
water (37ºC)
15mM CaCl2 (37ºC, pH 7.4)
15mM NaCl (37ºC, pH 7.4)
π (mN/m)
0
10
20
30
40
50
60
70
0
20
40
60
80
100
120
A (Å^2/molecule)


6.
π vs mma
30 mM CaCl2 (pH 7.4)
30 mM NaCl (pH 7.4)
water (pH 7.4)
π (mN/m)
0
10
20
30
40
50
60
70
0
20
40
60
80
100
120
A (Å^2/molecule)

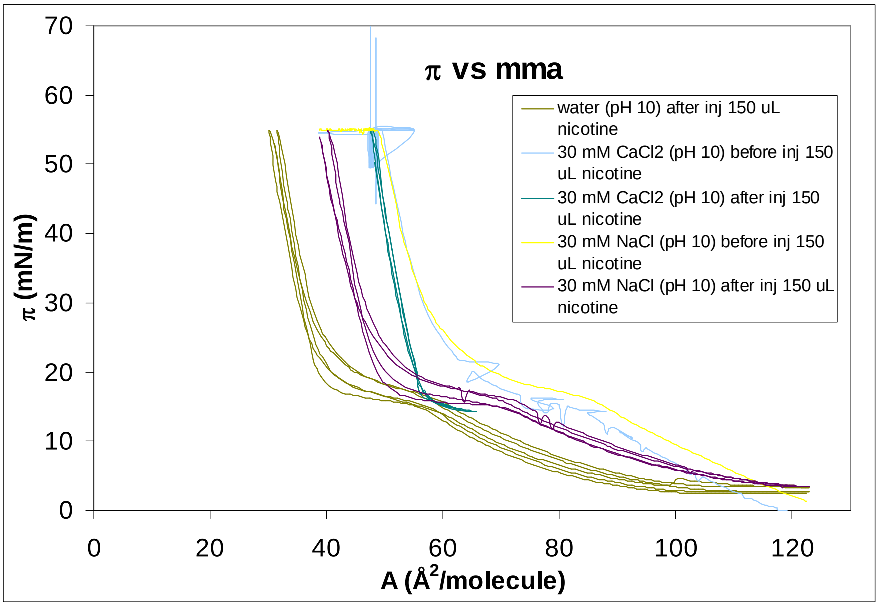

7.
π vs mma
water (pH 7.4)
water after inj 25 uL nicotine
water (pH 7.4) after inj 150uL nicotine
water with 25 uL nicotine
water with 75 uL nicotine
π (mN/m)
0
10
20
30
40
50
60
70
0
20
40
60
80
100
120
A (Å^2/molecule)

Experimental isotherms

Graph 4: pH 7.4



Graph 5: pH 10



Appendix (5) Analysis of preliminary isotherms

Graphs 1-5
Hysteresis is generally larger at 37˚C than at 25 °C. The monolayer is more expanded and disordered as can be seen from the unclear phase transitions and the absence of a phase transition plateau at constant pressure, which indicates the relative instability of monolayers at 37 °C.

Graphs 4 and 6
At 30 mM there is a clear difference between the effect of NaCl and $CaCl_2$ on the monolayer compared to at 15 mM. At 15 mM, there is smaller hysteresis for NaCl than for $CaCl_2$, although $CaCl_2$ should be more stabilizing, which suggests that chloroform was left in the DPPC on first compression. On the other hand, at 30 mM the phase transition plateau at constant pressure is slightly lower for $CaCl_2$, meaning that the monolayer is more compressed and stable than for NaCl.

Graph 7
Increasing nicotine concentration seems to have a negative effect on the stability of the monolayer. For isotherms where nicotine is present but not injected, the isotherm for the higher concentration (75 ul) has a generally higher surface pressure, indicating repulsion and penetration in the monolayer, and the phase transitions are less clear. Also for isotherms where nicotine is injected, the isotherm for the higher concentration (150 ul) shows less stability: penetration is evident from the higher minimum pressure and can be speculated from the less steep liquid condensed phase curve.

Graphs 1, 5 and 7
The monolayers for which pH was not controlled are generally less stable than for those at pH 7.4. This might be due to more repulsion between positively charged lipid headgroups as a result of hydrogen ions from the more acidic (about pH 5.6) subphase binding to the phosphate groups. This also gives more space for nicotine to penetrate, and thus contributing to the destabilizing effect.