Soft Matter

Accepted Manuscript

ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/softmatter

"Polysaccharide films at an air/liquid and a liquid/silicon interface: effect of the polysaccharide and liquid type on their physical properties"

Yasunori Taira[1] and Cathy E. McNamee[2,*]

[1] Department of Medical Engineering and Cardiology, Tohoku University, Japan.

[2] Faculty of Textile Science and Technology, Shinshu University, Tokida 3-15-1, Ueda, Nagano 386-8567 Japan.

* Corresponding author: email: mcnamee@shinshu-u.ac.jp

Tel.: +81-(0)268-21-5585

Keywords: chitin; chitosan; Atomic Force Microscopy; Langmuir monolayer; friction; forces; water; buffer.

Abstract

We investigated the effect of the polysaccharide type, the subphase on which the Langmuir monolayers were prepared, and the liquid in which the properties of the transferred monolayers were measured on the physical properties of the polysaccharide films at an air/aqueous interface and at a silicon substrate, and the forces and friction of the polysaccharide transferred films when measured in solution against a silica probe. Chitosan was modified with a silane coupling agent to make chitosan derived compounds with a low and a medium molecular weight. Chitin and the chitosan-derived compounds were used to make Langmuir monolayers at air/water and air/pH 9 buffer interfaces. The monolayers were transferred to silicon substrates via a Langmuir-Blodgett deposition, and the chitosan-derived compounds subsequently chemically reacted to the silicon substrates. Atomic Force Microscope force and friction measurements were made in water and in the pH 9 buffer, where the water and the pH 9 buffer acted as a good and a bad solvent to the polysaccharides, respectively.

The polysaccharide type affected the friction of the polysaccharide film, where the physically adsorbed chitin gave the lowest friction. The friction of L-Chitosan was higher than that of M-Chitosan in water, suggesting that the molecular weight of the polymer affects its lubricating ability. The forces and friction of the polysaccharide films changed when the subphase on which the Langmuir monolayers were formed was changed or when the liquid in which the properties of the films adsorbed at the silicon substrate were being measured was changed. The friction increased significantly when the liquid was changed from water to the pH 9 buffer. This increase was explained by

the reduced charge of the chitin and chitosan-dervied materials due to the pH increase, the screening of the charges by the salts in the buffer, and the possible hardening of the monolayer caused by the adsorption of salts from the buffer.

1. Introduction

Chitin is a natural polysaccharide that is synthesized by numerous living organisms,[1] such as arthropods.[2] Chitosan is the N-deacetylated derivative of chitin, see Fig. 1 for the structures of chitin and chitosan. Chitosan is the most important derivative of chitin as it contains more than 50% free amine in its structure,[3] which makes it soluble in aqueous acidic media due to the protonation of the primary amine function on the D-glucosamine repeat unit.[1] Additionally, chitosan is important, as it is the only pseudonatural cationic polymer.[1] Chitin and chitosan show biocompatibility, biodegradability, and non-toxity,[4] and are therefore used in pharmaceutical and biomedical applications,[1] such as artificial skin,[5] materials to help bone rebuild,[6] and drug delivery system carriers.[7,8] The successful applications of chitin and chitosan require the ability to create well-defined films that display the required properties in the working environment. This ability requires an in-depth understanding on the physical properties of the films created using chitin or chitosan and the way to control these properties in different environments. The polysaccharide type, its conformation and packing in the film, and the surrounding liquids in the working environment contribute to the forces and friction of the system, which affect the properties of the polysaccharide films.

Films of chitin or chitosan at a substrate can be made by their adsorption from the bulk solvent to a substrate,[9] or the transfer of monolayers of chitin or chitosan that are formed at an air/liquid interface to a substrate via a Langmuir-Blodgett or Langmuir-Schaefer deposition.[10] Substrates adsorbed with monolayers of polysaccharide from

the bulk have been reported to give low friction in aqueous solutions.[9] The structure and properties of the adsorbed polysaccharide film have also been reported to contribute to the friction of that film.[11,12,13,14] One advantage of using a Langmuir monolayer over films that are adsorbed from the bulk is that the molecular properties of the film, *e.g.*, packing density and conformation of the polymer chains, can be controlled. Additionally, the film thickness can be controlled by varying the number of depositions. The conformation and intra- and inter-molecular interactions of polymer chains in the film and the film thickness will affect the forces resulting from the polysaccharide film and therefore the lubrication properties of the film. Numerous studies exist on films of chitin or chitosan adsorbed to a substrate from the bulk.[13,15,16,17] However, less research exists about the physical properties of films created via Langmuir monolayers of chitin or chitosan and the forces and friction of their transferred films.

Chitin and chitosan do not dissolve in spreading solvents, such as ethanol or chloroform.[18] Chitin additionally does not dissolve in water.[18] However, chitin can dissolve in organic acids, such as formic acid, strong polar protic solvents, such as trichloroacetic acid, and in methanol that is saturated with $CaCl_2 \cdot 2H_2O$.[18] Thus, chitin can be spread at an air/water interface to form a Langmuir monolayer, if a $CaCl_2 \cdot 2H_2O$ saturated methanol is used as the spreading solution. Chitosan is soluble in acidic aqueous solutions. The hydrophilicity of chitosan therefore makes chitosan unable to form insoluble monolayers at the air/water interface. Langmuir monolayers of chitosan have, however, been successfully made at an air/water interface by modifying the structure of chitosan to include hydrophobic groups.[19,20]

Chitin and chitosan films are commonly designed in the laboratory and their physical properties tested in air or water.[9] Water acts as a good solvent for polysaccharide films, as seen by the fact that a polysaccharide film acts as a good lubricator under high load in water.[21] However, polysaccharide films are often used in physiological liquids, which contain salts and pH different than that of pure water. Changing the salt concentration and pH of the solution may affect the physical properties and performance of the polysaccharide films, as the solution may change from being a good solvent to a bad solvent.

The environment is reported to affect the structure of the chains in a polymer.[22] A good solvent causes the chains in a polymer to expand, in order to maximize the number of polymer-solvent contacts. Changing the solvent to a bad solvent causes the polymers to collapse to form a compact conformation, as the polymer-polymer self-interactions are preferred over the polymer-solvent interactions.[23] The solvent quality can be changed via the temperature, solvent molecules, and the chemical composition of the polymer.[22] Changing the liquid from a good to a poor solvent is expected to change the physical properties of the polymers, and therefore the forces and friction arising from the polysaccharide film. It is therefore important to determine how the liquid used in the measuring environment, *i.e.*, working environment, affects the physical properties and performance of the polysaccharide films.

We made Langmuir monolayers of three different polysaccharides at an air/aqueous interface. The chitin monolayer ("Chitin") was made by dissolving chitin in methanol

that was saturated with $CaCl_2 \cdot 2H_2O$ and then by spreading it at an air/aqueous interface. The monolayer was then physically attached to a silicon wafer via a Langmuir-Blodgett deposition. The chitosan monolayer was made by firstly modifying chitosan to be more hydrophobic by chemically attaching a silane coupling agent to chitosan.[24] The chitosan-derived compounds with a lower and a higher molecular weight were "L-Chitosan" or "M-Chitosan", respectively, see Fig. 7B. This modification method had the advantages of enabling Langmuir monolayers to be made at the air/aqueous interface and also of allowing the chitosan-derived compound to chemically attach to a silicon wafer after the deposition by using a method based on that described by Ohno and others.[25] The effect of the polysaccharide type on the physical properties of the films was studied by comparing the physical properties of the Chitin, L-Chitosan and M-Chitosan films. The contribution of the subphase used in the preparation of the polysaccharide Langmuir monolayers on the final properties of the polysaccharide adsorbed substrates was determined by using water and a pH 9 buffer. As the pKa of chitin and chitosan is 6.1[26] and between 6 and 7[27,28], respectively, water was expected to act as a good solvent and the pH 9 buffer to act as a bad solvent. The physical properties of the Langmuir monolayers at the air/aqueous interface were determined via surface pressure (Π)-area (A) isotherms. The physical properties of the monolayers that were transferred to a silicon wafer were investigated via Atomic Force Microscopy. The effect of the working environment, *i.e.*, measuring liquid, on the physical properties of the transferred films was determined by measuring the force and friction of the transferred films in water and in the pH 9 buffer against a silica probe.

2. Materials and Methods

2.1. Materials

Chitin (Wako 1st Grade, Wako, Japan, Fig. 1A), low molecular weight chitosan (Molecular weight: 50-190 kDa, Aldrich, USA), medium molecular weight chitosan (Molecular weight: 190-310 kDa, Aldrich, USA), acetic acid (4% v/v, Alpha Aesar, UK), ethanol (EtOH, 99.5% purity, Wako, Japan), methanol (MeOH, JIS Special Grade, Wako), chloroform (JIS Special Grade, Wako, Japan), acetone (high purity, Wako, Japan), calcium chloride dehydrate (~98% purity, Wako, Japan), glycidoxypropyltrimethoxysilane (purity ≥98%, Aldrich, USA), sodium hydrohyde (JIS Special Grade, Wako, Japan), sodium hydrogen carbonate (JIS Special Grade, Wako, Japan), and sodium chloride (99.5% purity, Wako, Japan) were used in this study. The water was distilled and de-ionised using a water purification system (Direct-Q3 UV, Millipore, USA). The pH 9 buffer consisted of 0.1 M $NaHCO_3$, NaOH and 0.9 wt % NaCl.

2.2. Preparation of the Chitin, L-Chitosan and M-Chitosan spreading solutions

The chitin spreading solution was made by adding 30 g $CaCl_2 \cdot 2H_2O$ to 40 mL MeOH, after which it was stirred for at least 3 h. The solution was then filtered, and 1.2 mg Chitin was added to 10 mL of the prepared MeOH solution that was saturated with $CaCl_2.2H_2O$.

The chitosan-derived spreading solutions were made in the following way. Low or medium molecular weight chitosan was reacted with a silane coupling agent based on scheme 20 that was given by Kurita and others, see Figure 1B.[24] Briefly, chitosan was

dissolved in a 1wt% acetic acid solution to give a concentration of 8 g/L. 1 mL of glycidoxypropyltrimethoxysilane was added to 60 mL of this solution, after which 80 mL of water was added. The pH of the solution was adjusted to be above 10 by the addition of NaOH. The reaction was allowed to continue for at least 3 h, after which a gel was formed. The solution was then centrifuged and the upper liquid layer removed and 20 mL EtOH added. The resulting solution was then mixed using a sonificator, after which it was centrifuged. The EtOH layer was subsequently removed and 20 mL of new EtOH was added and mixed with the gel. The mixing and sonification process was repeated three times. The resulting solutions were the chitosan-silane coupling agent in EtOH solutions. The low molecular weight chitosan and the medium molecular weight chitosan solutions are referred to as "L-Chitosan" and "M-Chitosan" further throughout this work.

2.3. Preparation of Langmuir monolayers

The monolayers were prepared using a Langmuir-Blodgett Trough (Large microscopy Langmuir trough, Nima Technology Ltd, Coventry, UK) made from poly(tetrafluoroethylene) (PTFE) and equipped with two PTFE barriers that compressed around the centre of the trough (maximum surface area of trough = 290 cm^2). The surface pressure was measured using a Wilhelmy plate of wet filter paper (No.2 240mm, Toyo, Japan) suspended from a strain gauge (Nima PS4 surface pressure sensor, Nima Technology Ltd, Coventry, UK).[29]

The ∏-A isotherms were acquired by firstly cleaning the Langmuir trough with chloroform and then with EtOH. Water was subsequently added to the trough and the

water surface cleaned by compressing the barriers to maximum and suctioning the water surface between the two barriers clean, after which the barriers were fully expanded. The water subphase was maintained at a temperature of 25°C. The monolayers were prepared by spreading 500 μL of the spreading solutions with a 250 μL syringe (725RN, Hamilton, Switzerland). The complete evaporation of the spreading solvent was ensured by waiting 10 min. The monolayers were then compressed or compressed and expanded (isotherm cycle) with a speed of 1.852 cm^2s^{-1}. The surface pressure and area were simultaneously recorded to give the ∏-A isotherms. The ∏-A isotherm of each monolayer was measured a minimum of three times to ensure reproducibility of the results. The same solutions and volumes of Chitin, L-Chitosan and M-Chitosan were spread at the air/aqueous interface when the subphase was water or the pH 9 buffer.

2.4. Attachment of the Langmuir monolayers to silicon wafers

The Chitin, L-Chitosan and M-Chitosan monolayers were attached to silicon wafers via a Langmuir-Blodgett deposition. Briefly, the Langmuir trough was cleaned in the manner described above, after which water was added to the trough. The naturally oxidized silicon wafer (Silicon Quest INT., USA) was then cleaned by washing with acetone, EtOH, and water, after which it was dried. The wafer was subsequently cleaned in a plasma cleaner (Harrick, USA) on high for 1 min. Next, the surface of the water in the Langmuir trough was suctioned cleaned. The silicon substrate was then attached to the mini-dipper (DC-20 small, Nima Technology Ltd, UK), and immersed into the subphase so that only the top few mm of the substrate remained in the air. The Chitin, L-Chitosan or M-Chitosan monolayer was then spread, and a waiting time of 10 min allowed for the spreading solvent to evaporate. The monolayer was subsequently

compressed to the desired surface pressure using the surface pressure control mechanism. Once the surface pressure stabilized, the wafer was moved out of the subphase using the slowest speed available on the dipper (1 mms^{-1}).

The Chitin, L-Chitosan and M-Chitosan monolayers were physically attached to the silicon wafer upon the Langmuir-Blodgett deposition. L-Chitosan and M-Chitosan were subsequently chemically bonded to the silicon substrate by reacting their silane coupling groups with the silicon substrate by using a method based on that described by Ohno and others.[25] Briefly, the substrates covered with L-Chitosan or M-Chitosan were washed with ethanol, and then with water. The substrates were subsequently dried. Optical images of the substrates before and after the washing procedure showed no change, indicating little or no removal of L-Chitosan or M-Chitosan with the procedure.

2.5. Atomic Force Microscopy Imaging

The silicon substrates were imaged with an Atomic Force Microscope (AFM, Digital Instruments NanoScope III Multimode, Santa Barbara, USA) in contact mode by using a liquid fluid cell and a Si_3N_4 cantilever (OMCL-TR800PB-1, Olympus, Japan) with reflective gold coating. The V-shaped cantilevers were 200 μm long and 166 μm wide, and had a nominal spring constant of 0.16 Nm^{-1}, and square pyramidal tips with an end-tip radius of curvature of 30 nm. Briefly, the physically attached Chitin, chemically attached L-Chitosan, and chemically attached M-Chitosan silicon substrates were firstly washed with water and then dried. The substrates were subsequently attached to the piezo using double sided tape (NW-15SF, Nichiban, Japan). The cantilever was then set in the liquid cell, and the liquid cell attached to the AFM. Next, the cantilever was

brought into close contact to the silicon substrate, after which water was injected into the liquid cell. The substrates were then imaged with a scan rate of 2 Hz (4 μm/s) and a scan size of 1×1 μm^2. The number of lines and the resolution of data points per line were both 512. All the images are unmodified (*i.e.*, non-filtered), except for a 1st order flattening along the scan lines. The images shown for each substrate type represent the images made at a minimum of three different positions. The images were reproducible upon multiple scans, and did not change if they were scanned from top to bottom or from bottom to top. Imaging over a wider scale of 5×5 μm^2 gave smaller but similar topographical features as those obtained in the 1×1μm^2 scale image, indicating the absence of tip artifacts.

2.6. Atomic Force Microscopy colloid probe preparation

The colloid probes for the force and friction measurements were prepared by evaporating the water from a small volume of a diameter=6.84 μm silica particle dispersion (Bangs Laboratory, USA), and then by attaching a single particle to a cantilever by using an XYZ micromanipulator and an epoxy resin (rapid araldite, Showa polymer company, Japan). Rectangular cantilevers (NSC12/ no Al, nominal spring constant=14 N/m, length= 90±5 μm, width=35±3 μm, thickness=2.0±0.3 μm, Micromasch, USA) were used for the force and friction measurements.

2.7. Atomic Force Microscopy force curve acquisition

The surface forces were measured between the bare SiO_2 colloid probe and the polysaccharide covered substrates in water or the pH 9 buffer solution as a function of

separation *relative to hard wall contact* by using the AFM and a liquid fluid cell. Briefly, the colloid probe was brought into contact with the substrate by using a piezo. During this time, a split photodiode measured the change in the deflection of the cantilever (Δx) as a function of the piezo displacement. Next, the detector signal-versus-piezo position curves were converted to force-versus-distance curves by firstly using the slope of the linear compliance region, then by subtracting the cantilever deflection (Δx) from the piezo position, and finally by using Hooke`s law, $F = k\Delta x$. The zero force position was defined at large cantilever-substrate separations, where no surface forces were acting on the cantilever. The force and the nominal spring constant of the cantilever are given by F and k, respectively. The forces were measured with speeds of 593, 1993 and 27901 nm/s, corresponding to frequencies of 0.3, 1 and 14 Hz, respectively. As the forces did not change with the measuring speed, only the forces measured at 1993 nm/s are shown.

2.8. Atomic Force Microscopy friction data acquisition

The friction (lateral force) was measured between the silica colloid probe and the polysaccharide covered substrates in water or the pH 9 buffer solution by using the AFM (Digital Instruments NanoScope III Multimode, Santa Barbara, USA) in the "friction mode" and a liquid fluid cell. Briefly, the colloid probe was pressed against the substrate in solution at a known load, while the substrate was moved horizontally underneath the cantilever at a speed of 4.0 μm/s (scan frequency = 0.3 Hz). The lateral frictional force (F_F) was calculated using

$$F_F = \frac{V_L S_L K_L}{2H} \quad (1).$$

Here, V_L is the difference in the lateral force detector signal in one complete scan and H is the distance from the bottom of the sphere to the mid-point of the cantilever. S_L is the lateral detector sensitivity, whose value was determined from the method of Meurk and others[30] to be 3.1×10^{-4} rad/V. K_L is the lateral spring constant and was calculated using

$$K_L = \frac{2K_N L^2}{3(1+\nu)} \quad (2).$$

Here, K_N is the normal spring constant, L is the length of the cantilever, and ν is the Poisson`s ratio, where we used a typical value[31] of 0.27. The error in the friction values was less than 25 nm.

3. Results and Discussion

3.1. Influence of the polysaccharide type and monolayer subphase on the physical properties of the polysaccharide film

3.1.1. Properties of the polysaccharide monolayers at an air/water or an air/pH 9 buffer interface

Fig. 2 shows the ∏-A isotherms of the Chitin, L-Chitosan, and M-Chitosan monolayers at the air/water interface. A change in the slope of the ∏-A isotherm indicates a phase transition and signifies a change in the compressibility of the monolayer.[32] The ∏-A isotherm of the Chitin monolayer showed three distinct phases, while that of the L-Chitosan and M-Chitosan monolayers showed only one phase. The structure of the polymers in a polymer monolayer can change from pancake (region I), mushroom (region II), and to brush (region III) upon compression[33] Fig. 3 schematically depicts the structures of these three regions.

The pancake region occurs at large areas and shows a slow increase in the surface pressure with an area decrease. It was observed for Chitin, L-Chitosan and M-Chitosan for $\prod \leq 7$mN/m, $\prod \leq 10$ mN/m, and $\prod \leq 30$ mN/m, respectively. The hydrophobic backbone of the glycoamine repeat group in Chitin, L-Chitosan and M-Chitosan, and the alkyl tail of L-Chitosan and M-Chitosan are thought to be anchored to the air/water interface, while the hydrophilic groups are in contact with the water subphase. The Chitin, L-Chitosan and M-Chitosan chains are expected to show a flat almost two-dimensional conformation without long loops and tails protruding into the bulk phase at large areas. Increasing the monolayer compression within the pancake region from large areas (Area=250 cm^2) to smaller areas, caused the surface pressure to increase. This increase is explained by the increased steric repulsion, caused by the formation of loops and tails in a Chitin, L-Chitosan or M-Chitosan chain and/or due the formation of entanglements within the monolayers.

The other two distinct regions at higher compression areas observed for Chitin are explained as mushrooms and brushes. The phase transitions were interpreted by the change in the $\prod$-A slopes in the isotherm. However, a direct observation of the monolayers at the different regions by optical or scattering techniques is required to confirm these assignments and the fact that these changes are truly thermodynamic phase transitions.[3,4]

The mushroom region for Chitin occurred at intermediate surface pressures of 7-10 mN/m. It is recognized by the pseudo-plateau in the surface pressure-area isotherm, see

the arrow in Fig. 2. The pseudo-plateau is associated with the dissolution of the Chitin chains into the water subphase. The phase change from pancake to mushroom occurs as the denser packing of Chitin causes the hydrophilic portions to start to sink. Above a critical density, Chitin forms a larger number of loops in its chains, which are squeezed upon compression. The gradual surface pressure increase at lower areas is explained by the interactions between the neighboring Chitin chains.

The brush region occurs at the high surface pressure region of > 10 mN/m for Chitin. Here, the surface pressure increases rapidly as the area is decreased. This increase is explained by the interactions between the neighboring Chitin chains, which cause brushes to form and the onset of entanglements.

The L-Chitosan and M-Chitosan monolayers did not show the mushroom phase transitions that were seen in the Chitin monolayer, as the monolayers were compressed from large to small areas. The fact that a phase transition was not apparent for the L-Chitosan and M-Chitosan monolayers suggests that the L-Chitosan and M-Chitosan polymer chains did not sink upon compression. The silane alkyl chain that was attached to the glycoamine repeat group is thought to have given the L-Chitosan and M-Chitosan molecules enough hydrophobicity to have prevented all or part of the molecules from dissolving into the water subphase. Thus, the mushroom phase was not observed. However, the fact that the surface pressure increased from zero to a maximum value of 16 and 31 mN/m for the L-Chitosan and M-Chitosan monolayer, respectively, as the monolayer was compressed shows that the L-Chitosan and M-Chitosan polymer chains

could be compressed. The polysaccharide chains were squeezed upon compression, causing the steric repulsion to increase.

Langmuir monolayers of chitosan-derived materials have been reported by other groups. An N,N-dilauryl chitosan pentamer, which was synthesized from chitosan, enabled stable Langmuir monolayers to be formed at an air/water interface.[20] The Π-A isotherms showed a steep increase in surface pressure, similar to the one observed for our M-Chitosan monolayer, and was explained by a condensed monolayer. In the case of the amphiphilic chitosan deriviative of octanoylchitosan cinnamate,[19] the Π-A isotherm also showed a steep increase in surface pressure. However, the collapse pressure was lower than that reported for the N,N-dilauryl chitosan pentamer; the collapse pressure was approx. 24 mN/m and 52 mN/m in the former and latter cases. This difference was explained by the larger surface area occupied by the chitosan-derived amphiphile and the rigid and bulky cinnamate groups, which prevented the polymer backbones from being compressed tightly. This resulted in a less densely packed monolayer for octanoylchitosan cinnamate. Our chitosan-derived amphiphile of M-Chitosan did not collapse for surface pressure values less than 30 mN/m. This suggests that M-Chitosan packed better than octanoylchitosan cinnamate.

The effect of the subphase type on the Chitin, L-Chitosan, and M-Chitosan monolayers can be seen from the Π-A isotherms of Chitin, L-Chitosan, and M-Chitosan at an air/pH 9 buffer, see Fig. 4. The same polymer solutions and spreading volumes were used as for the pure water subphase case, allowing the Π-A isotherms of Fig. 2 (water

subphase) and Fig. 4 (pH 9 buffer subphase) to be directly compared. Using a pH 9 buffer as the subphase instead of water caused the shape of the isotherms to change, and the maximum surface pressure to increase. The Chitin and M-Chitosan monolayers showed a pancake (region I) and a brush phase (region III). The transition was estimated to occur at approx. 7 and 37 mN/m, respectively, by inspecting the relevant ∏-A isotherms, see the arrows in Fig. 4. The pseudo-plateau that was seen for the Chitin monolayer at an air/water interface was not apparent when the subphase was changed to the pH 9 buffer. The L-Chitosan monolayer showed only the pancake phase (region I). However, the slope of the ∏-A line was much steeper in the buffer case compared to that seen in the water case. The change in the shapes of the isotherms for the Chitin, L-Chitosan and M-Chitosan monolayers when the subphase was changed from water to the pH 9 buffer indicates that the physical properties of the monolayers depended on the subphase type.

Further information on the properties of the monolayers was obtained by compressing and expanding the Langmuir monolayers of Chitin, L-Chitosan and M-Chitosan, when the subphase was water or the pH 9 buffer.

The compression and expansion isotherms the Langmuir monolayers of Chitin, L-Chitosan and M-Chitosan at an air/water interface are shown in Figs. 5A, 5B, and 5C, respectively. Upon the first compression-expansion of the monolayers, the Chitin, L-Chitosan and M-Chitosan ∏-A isotherms showed hysteresis, where the monolayers shifted to smaller areas upon expansion when compared to the areas obtained in the compression isotherm at the same surface pressure. The greatest hysteresis was seen in

the pancake region. In the case of the Chitin monolayer, subsequent compressions and expansions did not cause hysteresis. The Chitin isotherms also did not shift upon the second and third compression-expansion cycle. The isotherms of L-Chitosan and M-Chitosan, however, showed hysteresis upon subsequent compressions and expansions, and continued to shift to smaller areas as the number of compressions-expansion cycles increased. The degree of hysteresis increased in the order of Chitin < L-Chitosan<M-Chitosan.

A shift in the compression-expansion cycles to smaller areas indicates a loss of material from the air/water interface. Hysteresis between the compression and expansion isotherms shows irreversible structural changes in the monolayers. In the case of the Chitin monolayer, the chains in the monolayer did not regain their original pancake structure upon expansion after being compressed the first time. This indicates a more densely packed structure after compression, possibly due to the presence of entanglements that could have been induced in the structure when it was compressed to the brush phase. The second and third compression-expansion cycles did not show hysteresis. The isotherm also did not display a measurable shift to the left upon expansion and compression, indicating no significant loss of material from the surface. In the case of the L-Chitosan and M-Chitosan monolayers, the isotherms continued to shift to smaller areas upon subsequent compression-expansion cycles. This result suggests entanglements or fusion between the chains in the L-Chitosan and M-Chitosan monolayers or dissolution of part of the chains into the subphase upon compression. However, as a phase change was not observed for the L-Chitosan and M-Chitosan monolayers, the inter- and intra-chain molecular associations are thought to dominate.

The hysteresis in the L-Chitosan and M-Chitosan monolayers indicates that the polymer chains in the L-Chitosan and M-Chitosan monolayers could not recover their original structures upon expansion after their compression. The inter- and intra-chain interactions and entanglements are thought to contribute to this stiffness.

The ∏-A isotherm compression and expansion cycles of the Chitin, L-Chitosan, and M-Chitosan monolayers at an air/pH 9 buffer interface are shown in Figs. 6A, 6B, and 6C, respectively. Each monolayer showed hysteresis between the compression and expansion isotherms. The isotherms shifted to smaller areas upon subsequent compression-expansion cycles. The hysteresis was larger when the subphase was the pH 9 buffer than when it was water. Previously, in the case of a water subphase, the Chitin monolayer did not shift to smaller areas upon a continued compression and expansion of the monolayer. This result is in contrast with that seen in the pH 9 buffer case. Thus, the presence of NaCl and NaOH in the subphase changed the conformations of the chains in the polysaccharide monolayers and the visco-elastic properties of the polysaccharide monolayers.

Changing the subphase from water to the pH 9 buffer caused the pH to increase and the ionic concentration to increase from 1.6×10^{-3} mM to 262.33 mM. The pKa of chitin and chitosan is 6.1[26] and between 6 and 7[27,28], respectively. Thus, the amino group in chitin and chitosan is protonated in water with a pH of 5.8. Increasing the pH of the solution to 9 by changing the solution from pure water to a pH 9 buffer reduces the charge of chitin and chitosan. This reduction in charge causes less repulsion between the chitosan chains, resulting in less extended structures. At pH > pKa, chitosan has been

reported to be insoluble in water due to the depronated amines.[35,36] The addition of salt to the subphase of a monolayer has also been shown to cause charged monolayers at air/water interfaces to become harder.[37] The addition of salt also reduces the intra- and inter-chain repulsion in the polymer films, due to screening of the charged repeat units. Thus, the change in the subphase from water to the pH 9 buffer would cause the conformation and interaction of the chains in the Chitin, L-Chitosan and M-Chitosan monolayers to change. The physical properties of the transferred monolayer films are therefore also expected to have changed as a result of the subphase liquid type.

3.1.2. Properties of the polysaccharide monolayers transferred to a solid substrate

The above monolayers were prepared with the aim of use as films to be used in medical and biological applications. Thus, the monolayers showing high density coverage, lubrication and durable properties were desired. The properties of the monolayers that were compressed to the maximum stable surface pressure and transferred to a hard substrate were therefore investigated.

The surface pressures at which the Chitin, L-Chitosan, and M-Chitosan at the air/water interface were transferred were 16.0, 10.6 mN/m, and 33.3 mN/m, respectively. The Chitin, L-Chitosan, and M-Chitosan monolayers at the air/pH 9 buffer interface were compressed to their maximum stable surface pressures of 22.0, 25.0, and 41.0 mN/m, respectively. The monolayers were transferred to silicon wafers via a Langmuir-Blodgett deposition. The L-Chitosan, and M-Chitosan surfaces were washed with ethanol, enabling L-Chitosan and M-Chitosan to chemically attach to the silicon surface via a silanization reaction.

The $1\times1\mu m^2$ AFM height images of the Chitin, L-Chitosan, and M-Chitosan films made at an air/water interface and transferred on silicon wafers are shown in Figs. 7A, 7B, and 7C, respectively. The height sections of the lines shown in the Chitin, L-Chitosan, and M-Chitosan images are shown in Figs. 7D, 7E, and 7F, respectively. The properties of polymer brushes are reported to change when measured in air and in water.[38] As our polymer films were envisioned to be used in a liquid environment, we measured the images in water. The polymers showed circular regions with a diameter of approx. 100-200 nm, explained by the aggregation or compression of the polymer chains. The circular shapes were explained as polymer brushes for Chitin or pancake conformations for L-Chitosan and M-Chitosan, as the surface pressures at which the Chitin, L-Chitosan, and M-Chitosan were transferred were designated as polymer brushes or pancakes from the Π-A isotherms. The surface showed a height difference of less than 1 nm, indicating a flat surface. Such a surface can be achieved if the polysaccharide chains adsorb horizontally to the substrate. The small peaks seen in the image for M-Chitosan suggest that there were small attractive interactions between the cantilever tip and M-Chitosan.

The Chitin, L-Chitosan, and M-Chitosan films all gave films with a similar morphology, in spite of the fact that the Chitin film was formed by brushes while the L-Chitosan and M-Chitosan films were formed from compressed pancakes. A film made from chains with a pancake structure is expected to give larger aggregates with a lower packing density than that observed for the films made from chains with a brush structure. However, the above images showed that the packing of Chitin brushes is comparable or

less than that observed of compressed pancakes formed from L-Chitosan or M-Chitosan. This result suggests that the chains in Chitin and those in L-Chitosan and M-Chitosan interact with each other and with the silicon and water differently. Thus, if only the morphology of the films control the physical properties of the Chitin, L-Chitosan and M-Chitosan films, then the compressed pancakes of L-Chitosan or M-Chitosan Chitin brushes are expected to give similar results.

The $1\times1\mu m^2$ AFM height images of the Chitin, L-Chitosan, and M-Chitosan films made at an air/pH 9 buffer interface and transferred onto silicon were imaged in water and are shown in Figs. 8A, 8B, and 8C, respectively. The height sections of the lines shown in the Chitin, L-Chitosan, and M-Chitosan images are shown in Figs. 8D, 8E, and 8F, respectively. Chitin gave the flattest film with a surface variation of less than 5 nm. The diameter of the circular regions was approx. 100 nm, a size comparable with those obtained when the subphase was water. L-Chitosan and M-Chitosan, however, gave very rough surfaces, with surface height differences of approx. 80 nm. These values are more than 80 times larger than those obtained when the subphase was water. The diameter of the circular regions formed by L-Chitosan and M-Chitosan were up to 600 nm, values approx. three times larger than those obtained for the water subphase case.

L-Chitosan and M-Chitosan showed larger surface height deviations in the AFM images when it was prepared at an air/pH 9 buffer interface (Fig. 8) than when it was prepared at an air/water interface (Fig. 7). This difference can be explained by the fact that the chains were elongated in the air/water interface case, causing little surface height deviations, *i.e.*, a flat or nearly flat surface. The chains were, however, compacted in the

air/pH 9 water interfacial case, causing many compact globules to exist at the silicon surface. This resulted in a rough surface. The fact that that L-Chitosan and M-Chitosan showed a higher roughness than Chitin can be explained in terms of the differences in the deacetylation of chitosan and chitin. Chitin and chitosan are reported to be 14.28 and 80% deacetylated, respectively.[42] Thus, chitosan is more charged than chitin at low pH. At pH 6.0, chitin and chitosan are 7.14% and over 40% charged, respectively, while chitin and chitosan are charged only a maximum of a few percent at pH 9.[26] The change in the charge for chitin with pH is small. Therefore only a small change will be seen in the physical properties of the Chitin film with a pH change. L-Chitosan and M-Chitosan, however, exhibit a large charge change, when the pH is increased from pH 5.8 (pure water) to pH 9 (buffer). As a result, the structure is expected to change significantly as a result of the subphase change.

The causes for the differences in the AFM images obtained when the monolayers were made on a water subphase and on a pH 9 buffer subphase were investigated by measuring the forces in water between a silica particle (probe) and the Chitin, L-Chitosan, and M-Chitosan covered silicon substrates that were prepared at air/water interfaces and at air/pH 9 buffer interfaces.

The forces between a silica particle (probe) and the Chitin, L-Chitosan, and M-Chitosan covered silicon substrates that were prepared at air/water and measured in water are shown in Fig. 9. Repulsive forces were observed in the approach force curves of the L-Chitosan and M-Chitosan films. The repulsive forces started at approx. 15-20 nm for the L-Chitosan and M-Chitosan films. The inset of Fig. 9 shows that an attractive force was

observed in the approach force curve of the Chitin film for separation distances between 20 and 5 nm. A repulsion was then observed for separation distances of less than approx. 5 nm. The zero separation distance indicates the position where the silica was in contact with a hard surface, *i.e.*, the position where the film could not be compressed any further. The AFM images showed a height difference between the pancakes or brushes of approx. 1 nm. The fact that the repulsion forces were larger than 1 nm indicates that these films were thicker than 1 nm or that these films showed strong repulsive force against silica.

Silica is negatively charged at pH 5.8, the pH of the water used in these studies, as the isoelectric point of silica is between 2 and 4.[39] The silicon wafers to which the Chitin, L-Chitosan and M-Chitosan films were transferred were covered by the Chitin, L-Chitosan and M-Chitosan films, as seen by the AFM images. Thus, the charge of the substrate is explained by the charge of the Chitin, L-Chitosan and M-Chitosan films. The amino group in chitin and chitosan is protonated in acidic to neutral solutions, as the pKa of chitin and chitosan is 6.1[40] and between 6 and 7[27,41], respectively. Thus, Chitin, L-Chitosan and M-Chitosan films were positively charged at pH 5.8, the pH of our water. As silica is negatively charged at pH 5.8, the pH of the water used in these studies, the silica probe used in the force studies was negatively charged.

Electrostatic forces between the probe and the chitin or chitosan modified substrates would be attractive, as the probe and substrate were negatively and positively charged, respectively. The attraction that was observed between the Chitin film and the silica probe may be due to this electrostatic attraction. Alternatively, as a polysaccharide chain

in Chitin contains multiple positive sites and as Chitin was not chemically attached to the silicon wafer, one chain may have bound to both the silicon wafer and the silica probe. This would result in an attraction, due to bridging forces. The repulsion observed for the Chitin, L-Chitosan and M-Chitosan films cannot be explained by an electrostatic force, as the Chitin, L-Chitosan and M-Chitosan films were positively charged and the silica probe was negatively charged. The repulsion observed in the force curves, however, can be explained as a steric force that results from the compression of the Chitin, L-Chitosan or M-Chitosan chains by the silica particle. As the repulsion distance was 5 and 15-20 nm for the Chitin and L-Chitosan or M-Chitosan films and as the silica probe was bare, the compression region of the Chitin film is thought to be at least 5 nm thick, and that of the L-Chitosan or M-Chitosan films to be at least 15 nm thick.

L-Chitosan and M-Chitosan were also positively charged and contained multiple positively charged sites which could also have adsorbed onto to the negatively charged silica particle. In spite of this, no clear attractions were observed in the approach force curves of the L-Chitosan or M-Chitosan films. Bridging and/or electrostatic attractive forces were seen in the Chitin case to commence at 20 nm. The steric forces were seen in the L-Chitosan or M-Chitosan films to commence around 15-20 nm. Thus, it is possible that the attractive forces were being superimposed onto the steric force. If the repulsion in the steric force was stronger than the attraction, then the repulsion would dominate.

The retract force curves for Chitin, L-Chitosan and M-Chitosan show a single adhesion. This adhesion indicates that the polysaccharides interacted with the silica surface. As

the Si-$(OCH_3)_3$ group on L-Chitosan and M-Chitosan chemically reacted with the silanol groups on silicon, it is the glycosamine repeat unit on the L-Chitosan and M-Chitosan chains that contacted water and which could interact with silica. Chitin physically adsorbed to the silicon substrate. Thus, its chains could interact with both the silicon wafer and the silica probe. The Chitin, L-Chitosan and M-Chitosan chains possessed positively charged groups at pH 5.8, the pH of our water, and our silica probe was negatively charged at pH 5.8. Thus, the adhesion observed in the retraction force curve can be explained by an electrostatic attraction between the Chitin, L-Chitosan or M-Chitosan transferred films and the silica probe in water.

The strength of the adhesion increased in the order of Chitin>M-Chitosan> L-Chitosan. The stronger adhesion seen for M-Chitosan when compared to L-Chitosan is explained by the fact that the molecular weight of M-Chitosan is greater than that of L-Chitosan. This results in M-Chitosan having more positively charged sites on its chains that could adhere to the negatively charged sites on the silica probe.

Chitin and chitosan are reported to be 14.28 and 80% deacetylated, respectively.[42] At pH 5.8, chitin and chitosan are 7.14% and over 40% charged.[26] Thus, L-Chitosan and M-Chitosan are more charged than Chitin at pH 5.8. As a result, an electrostatic attraction should have caused L-Chitosan and M-Chitosan to show a stronger attraction to the silica probe than Chitin. The fact that the adhesion between Chitin and the silica probe was greater than that of L-Chitosan or M-Chitosan and the silica probe indicates that this adhesion was not due to electrostatic forces alone. A bridging force between Chitin and the silica probe would also give an adhesion. The combination of the

electrostatic attraction and the attractive bridging force is thought to give the strong adhesive force that was measured for Chitin. As Chitin was not chemically adsorbed to the substrate, the Chitin chains had more freedom to change their conformation and adsorb onto the silica probe. The L-Chitosan and M-Chitosan films were chemically attached to the silicon wafer. Thus, their chains did not have as much freedom to change their conformation. As a result, the L-Chitosan and M-Chitosan are thought to have adsorbed less to the silica probe than Chitin.

The forces between a silica particle (probe) and the Chitin, L-Chitosan, and M-Chitosan covered silicon substrates that were prepared at air/pH 9 buffer interface and measured in water are shown in Fig. 10. Repulsive forces were observed in the approach and retract force curves for all the films, when the monolayers were prepared on a pH 9 buffer subphase and the forces measured in water. The repulsive forces extended to approx. 20-25 nm for the Chitin, L-Chitosan and M-Chitosan films. Comparison of Figs. 9 and 10 shows that the attraction between the Chitin film and the silica probe in the approach force curve disappeared, when the monolayer subphase was changed from water to the pH 9 buffer. The retract force curves measured in water for the pH 9 subphase systems also showed no adhesion. The absence of an attraction in the approach force curve and the absence of an adhesion in the retract force curve shows that the films did not adhere to the negatively charged silica particle. Although the measuring liquid was the same, *i.e.*, water, the force of the polysaccharide films depended on which subphase the polysaccharide monolayers were prepared.

The samples whose forces were measured in water were made by firstly preparing the monolayers at an air/aqueous interface and then by transferring them to a silicon substrate. The subphase directly affected the conformation of the chains in the polysaccharide monolayers in the preparation process, as the polysaccharide was at the air/aqueous interface and therefore was in direct contact with the subphase. A pH 9 buffer reduced the charge of the Chitin, L-Chitosan and M-Chitosan monolayers, due to the screening by the buffer ions and the lowered protonation of the amino groups in the chitin and chitosan monolayers. As a result, the chains are expected to collapse and form globules. The result will be a less flat monolayer made at the air/pH 9 buffer interface than that which was made at the air/water interface. The properties of the monolayers that were transferred to a silicon substrate were investigated in water. The measuring liquid (water) has a pH of 5.8, which is less than the pKa of chitin or chitosan, and also has a low ionic concentration. Thus, the chitin and chitosan chains should be protonated and the charges non-screened. The monolayers made at an air/water interface, however, showed different forces than those made at an air/pH 9 buffer interface. This difference shows that the polymer chains did not change to give a stretched conformation that is typical of polyelectrolytes in good solvents (water), when the monolayers were made at an air/pH 9 buffer interface and the properties of its transferred film measured in a good solvent.

The repulsive forces observed for the Chitin, L-Chitosan and M-Chitosan films prepared at an air/pH 9 buffer interface and measured in water were assigned to a steric repulsion. This is because the films that were made on the buffer subphase were non-charged, and as any part of the polysaccharide chain that was charged due to the

measuring liquid being water, *i.e.*, pH<pKa, would be positively charged while the silica probe would be negatively charged. The repulsive forces extended to 20-25 nm, meaning that the thickness of the compressible part of the Chitin, L-Chitosan and M-Chitosan films were at least 20 nm. This value is higher than that measured for the films produced on a water subphase and can be explained by the large globules that were seen in the AFM image (Fig. 8). The polysaccharide film made at an air/water interface gave a flatter surface, explained by the more stretched chains that could attach more horizontally to the substrate. The globules are thought to act as hairy layers, which give rise to a steric force when compressed.

The effect of the polymer type and monolayer subphase on the lubricating ability and durability of the adsorbed Chitin, L-Chitosan, and M-Chitosan films were investigated by measuring the friction in water between a silica probe and the films prepared at an air/water interface or an air/pH 9 buffer interface , when the probe was brought in contact with the polymer films.

The friction of the adsorbed Chitin, L-Chitosan, and M-Chitosan films that were prepared at an air/water interface and measured in water are shown in Fig. 11. The friction was measured as a function of the load applied to the probe, when the load was varied from low to high values (loading) and then from high to low values (unloading). In general, the friction increased in the order of Chitin~M-Chitosan< L-Chitosan. The friction of Chitin and L-Chitosan was comparable at low loads, but the friction of Chitin was lower than L-Chitosan at high loads.

The difference in the magnitude of the charges of the chains in Chitin, L-Chitosan, and M-Chitosan may also affect the lubricating abilities of the polymer films. At pH 5.8, L-Chitosan and M-Chitosan were more positively charged than Chitin at pH 5.8. As a result, L-Chitosan and M-Chitosan should show a stronger electrostatic attraction to the negatively charged silica probe than Chitin. The magnitude of this attraction should then increase as the load is increased. A stronger adhesion gives a higher friction,[43] which reduces the lubricating ability of the surfaces. The facts that the friction of Chitin and L-Chitosan was comparable at low loads and that the friction of Chitin was lower than L-Chitosan at high loads shows that the charge of the film affected the lubricating abilities of the film.

M-Chitosan has a higher molecular weight than L-Chitosan. Therefore, M-Chitosan should have a greater number of positively charged sites than L-Chitosan and should adsorb to the silica probe stronger than L-Chitosan. The friction data, however, shows that M-Chitosan gave a lower friction than L-Chitosan. Thus, the lubricating ability of M-Chitosan must be due to a different cause.

The molecular weight of a polymer adsorbed to a substrate is also reported to affect the lubrication ability of the film. A polymer with a higher molecular weight gives better lubrication.[44] The conformation of a polymer at a substrate is also thought to affect its lubricating ability. The surface pressures at which the Chitin, L-Chitosan, and M-Chitosan were transferred were 16.0, 10.6 mN/m, and 33.3 mN/m, respectively. The AFM images shown in Fig. 7 showed that M-Chitosan had the smallest, most compact polymer globules, when the size of the polymer aggregates of Chitin, L-Chitosan, and

M-Chitosan were compared. The better lubrication ability of M-Chitosan compared to L-Chitosan is explained by the closer packing density of the chains in M-Chitosan compared to those in L-Chitosan. A polymer with a bottle-brush type architecture has been shown to give very low friction, when tested in water.[4,5] M-Chitosan is therefore thought to give the lowest friction due to its high molecular weight and compacted conformation.

Comparison of the loading and unloading friction curves of Chitin, L-Chitosan and M-Chitosan shows that the friction of all the polymer films decreased a little, after applying a high load to the films. The fact that only a small decrease was observed suggests only slight wear and degregation of the polymer films at high loads.

The friction of the Chitin, L-Chitosan and M-Chitosan transferred films prepared at an air/pH 9 buffer interface were measured against a silica particle in water, see Fig. 12. The friction was measured from low to high loads and then from high to low loads in order to determine the wearing of the films. The friction increased almost linearly as the load was increased. Additionally, little variation was observed between the friction values measured during loading and unloading. This result indicates little wearing of the surface. The friction values increased in the order of Chitin < M-Chitosan < L-Chitosan. Thus, the Chitin film gave the best lubrication. Comparing the friction curves obtained for the monolayers made on the buffer and those made on the water subphase, we see that the buffer subphase gave films with a significantly higher friction for the L-Chitosan and M-Chitosan films. Only slightly higher values were obtained for the Chitin case. The fact that the polysaccharide films showed higher friction for the buffer

case suggests that the Chitin, L-Chitosan and M-Chitosan chains were stiffer when the monolayers were made on the pH 9 buffer than when they were made on water. The addition of salt has been shown to cause charged monolayers at air/water interfaces to become harder.[37] Thus, this stiffening result may be due to the adsorption of ions to the chains. Alternatively, it may also be due to the formation of entanglements in the monolayer at the time the monolayer was formed at the air/pH 9 buffer interface.

The friction results also show that the buffer influences the L-Chitosan and M-Chitosan values much more than the Chitin values. The fact that the chains in the L-Chitosan and M-Chitosan films were chemically attached to the substrate and that the chains in the Chitin film were only physically attached may contribute to this difference. In the chemically attached case, the number of free parts of the chain is less than that in the physically adsorbed case. The chains may therefore change their conformation more in the physically adsorbed case, allowing a greater lubrication and therefore a lower friction. Additionally, as the charge of chitosan is higher than chitin, more ions can adsorb to chitosan than to chitin, resulting in the chitosan films being stiffer than the chitin films.

3.2. Influence of measuring liquid used in the force and friction measurements on the physical properties of the polysaccharide films

In order to determine how the measuring liquid affect the properties of the Chitin, L-Chitosan and M-Chitosan monolayers, the properties of the Chitin, L-Chitosan or M-Chitosan monolayers that were prepared on water or buffer subphases were investigated in the pH 9 buffer.

Figs. 13 and 14 show the force and friction curves measured in the pH 9 buffer and between a silica particle and the Chitin, L-Chitosan and M-Chitosan films, when they were prepared at an air/water interface. We observe that the order of magnitude of the repulsive force in the approach force curve increased in the order of Chitin< M-Chitosan < L-Chitosan. We also observe that the order of magnitude of the adhesive force in the retract force curve increased in the order of Chitin and L-Chitosan <M-Chitosan. The friction-load data show that the friction increased in the order of Chitin < M-Chitosan < L-Chitosan. Chitin showed a strong hysteresis in its friction-load curve, indicating wearing. The M-Chitosan film showed less hysteresis, indicating only some wearing. The L-Chitosan film showed little hysteresis, indicating little or negligible wearing. If we compare the forces and friction curves measured in water (Figs. 9 and 11) with those measured in pH 9 buffer (Figs. 13 and 14), we observe a reduced adhesion in the retract force curve and a higher friction, when the measuring liquid was changed from water to the pH 9 buffer. The extension of the repulsion observed in the approach force curves for the Chitin, L-Chitosan and M-Chitosan films were comparable, when the measuring solution was water or the pH 9 buffer. The magnitude of the hysteresis in the friction-load curve for the Chitin, L-Chitosan and M-Chitosan films increased when the liquid was changed from water to the pH 9 buffer.

Figs. 15 and 16 show the force and friction curves measured in the pH 9 buffer and between a silica particle and the Chitin, L-Chitosan and M-Chitosan films, when they were prepared at an air/pH 9 buffer interface. We observe that the order of magnitude of the repulsion measured in the force curves increased in the order of Chitin< M-Chitosan

~ L-Chitosan. The friction-load data show that the friction increased in the order of Chitin < M-Chitosan ~ L-Chitosan. Chitin showed a strong hysteresis in its friction-load curve, indicating wearing. The L-Chitosan monolayer showed less hysteresis, indicating only some wearing. The M-Chitosan monolayer showed little hysteresis, indicating little or negligible wearing. If we compare the forces and friction curves measured in water (Figs. 10 and 12) with those measured in pH 9 buffer (Figs. 15 and 16), we observe a change in the repulsive forces for the L-Chitosan and M-Chitosan monolayers. L-Chitosan showed a stronger and longer repulsive force and a higher friction than M-Chitosan, when water was used as the measuring liquid. The extension of the repulsion observed in the approach force curves for the Chitin, L-Chitosan and M-Chitosan films were comparable, when the measuring solution was water or the pH 9 buffer. The magnitude of the hysteresis in the friction-load curves for Chitin and L-Chitosan increased when the liquid was changed from water to the pH 9 buffer. The hysteresis in the friction-load curves for the M-Chitosan monolayers was not greatly affected by the measuring liquid.

The effect of the measuring solvent on the polysaccharide films can be seen by comparing the changes in the force and friction data when the measuring liquid was changed from water to the pH 9 buffer. In the case of the pH 9 buffer solution, there was in general less adhesion (stronger repulsion) and stronger friction than that which was observed when the measuring liquid was water. This result was observed, regardless of whether the monolayers were prepared at an air/water interface or an air/pH 9 buffer interface. The hysteresis seen in the friction-load curves of the monolayers also generally increased when the measuring solution was changed from water to the pH 9

buffer. The Chitin monolayer showed the strongest hysteresis and dependence on the measuring liquid.

The adhesion in the force curves was explained by an electrostatic attraction between the monolayers, which were positively charged when measured in a pH 5.8 solution (water), and the silica probe, which was negatively charged in the pH 5.8 solution. The silica probe became more negative at pH 9. Thus, the disappearance of the adhesion in the retract force curves and/or the increase in the magnitude of the repulsive forces when the measuring solution was changed from water to pH 9 buffer indicates a decrease in the positive charge of the monolayer. This decrease can be explained by the reduction in the dissociation of the amino groups in the chitin and chitosan monolayers, the screening by the ions, and/or by the adsorption of the ions to the polysaccharide films.

The extension of the repulsion observed in the approach force curves for the Chitin, L-Chitosan and M-Chitosan films were comparable when the measuring solution was water or the pH 9 buffer, regardless of whether the monolayers were prepared at an air/water interface or an air/pH 9 buffer interface. As this repulsion was explained by a steric force, this result suggests that the compressibility and/or conformation of the chains were not significantly affected by the measuring solution. Thus, if the ions from the measuring solution were adsorbing to the monolayers, they are thought to have adsorbed to the surface of the monolayers with only few ions penetrating inside the monolayers.

Comparing the friction versus load curves measured in water with those measured in a pH 9 buffer, we see a significant larger friction in the case of the pH 9 buffer. This result suggests a hardening of the surface. This hardening may result from a change in the conformation of the chains in the monolayers and/or due to the dehydration of the monolayers, caused by the adsorption of ions from the measuring liquid.[37]

The dependence of the magnitude of the hysteresis in the friction-load curve of the transferred monolayers on the type of measuring liquid indicates that its wearing depends on its environment. High hysteresis can be explained by the removal of ions from the surface due to a strong pushing. Low or negligible hysteresis indicates little loss of ions from the surface. Alternatively, stiffer surfaces show more damage than elastic surfaces, when a load is moved laterally across its surface. This would result in stiffer surfaces showing higher hysteresis than less-stiff surfaces. Non-lubricated surfaces have been reported to show a higher friction than lubricated surfaces.[46,47,48] Comparison of the wear of silicon in air, a non-lubricated surface, with the wear of silicon in water, a lubricated surface, showed that lubricated surfaces show less wear than non-lubricated surfaces.[49] This result indicates that high hysteresis is obtained with non-lubricating surfaces.

High hysteresis can also occur for films that are only weakly attached to the substrate. Chitin was only physically adsorbed to the silicon substrate, while L-Chitosan and M-Chitosan were additionally chemically attached to the silicon substrate. In the pH 9 buffer, the chains were less charged, resulting in a weaker electrostatic attraction between the polysaccharide chains and the silicon wafer. This would cause a weaker

physical adsorption. The Chitin film would therefore be more easily removed from the silicon substrate in the pH 9 buffer than the L-Chitosan and M-Chitosan films because of its weaker adsorption to the silicon wafer. This would result in Chitin showing the greatest hysteresis out of the polysaccharide covered substrates in the case of the pH 9 buffer.

The environment is reported to affect the structure of the chains in a polymer film. [22] In the case of a polyelectrolyte in a good solvent, the chains extend due to the electrostatic repulsion between neighboring chains. A reduction of the electrostatic repulsion due to a reduced charge would cause the chains to collapse. Such a charge reduction can be achieved by decreasing the ionization of the polyelectyrolyte, e.g., increasing the pH above the pka, or by increasing the concentration of ions in the solvent, which acts to increase the screening of the polyelectrolyte charges. Changing the solvent from water to the pH 9 buffer acts to decrease the charge on Chitin, L-Chitosan and M-Chitosan, as the pKa of chitin and chitosan are 6.1 and between 6 and 7, respectively, and as the concentration of ions in the solution are increased from 1.6×10^{-3} mM to 262.33 mM. Thus, the chains in the Chitin, L-Chitosan and M-Chitosan monlayers are expected to be more expanded when the solution is water than when the solution is the pH 9 buffer. The fact that little difference was seen in the steric forces when the solution was changed from water to the pH 9 buffer indicates that the subphase on which the monolayer was prepared affected the conformation of the polysaccharide chains the most.

Other polymer systems have also shown differences in their physical properties when the measuring solution was changed. Rheological and small-angle X-ray scattering studies have shown that the poly(acrylamide-co-sodium acrylate-co-dihexylacrylamide) polyelectrolyte is extended in an aqueous solution due to charge repulsion. [50] The addition of salts to the solution caused intramolecular associations, due to the screening of the charges, and a decrease in the viscosity of the solution. Langmuir polymer monolayers have also been seen to display flow and possible reptation motion under good solvent conditions, but glassy behavior under poor solvent conditions. [51] The lubricating property of a polymer adsorbed to a substrate with a bottle-brush type architecture was also reported to change with the surrounding media. [45] Whereas a low friction was observed in water, a high friction was measured in a solution containing a high concentration of salt. This dependence of friction to the measuring liquid was explained in terms of the changed conformation of the polymer. Additionally, the low friction observed for a polymer in a good solvent has been explained in terms of the ability of water molecules, which act as good lubricants, to adsorb to the polymer. [38]

The friction of L-Chitosan was higher than that of M-Chitosan in water, a good solvent. This result suggests that the molecular weight of the polymer affects its lubricating ability. M-Chitosan had a larger number of charged sites and a longer chain-length. L-Chitosan had a shorter chain length than M-Chitosan. As a result, M-Chitosan had more charged sites than L-Chitosan in water. The lubrication between two charged surfaces increases with an increased charge. [52] This can be explained by the increased repulsion between the two surfaces, [12] and the increased lubricating ability of the polymers by the

solvent.[53] In the case of pH 9 buffer, L-Chitosan and M-Chitosan were less charged and the charges were screened. The buffer acted as a poor solvent, causing the chains in L-Chitosan and M-Chitosan to collapse and form globules. Thus, there was less difference in the adsorbed size and charge of the chains in the L-Chitosan and M-Chitosan monolayers. L-Chitosan and M-Chitosan therefore showed comparable friction in the pH 9 buffer measuring solution.

The friction of L-Chitosan and M-Chitosan was higher than that of Chitin, regardless of the measuring liquid. This cause is thought to be due to the ability the ions in the measuring liquid to adsorb to the polysaccharide chains in the case of the pH 9 buffer, and due to the electrostatic attraction between the polysaccharide films and the silica probe in the case of water. Chitin was less charged than L-Chitosan or M-Chitosan, resulting in less ions adsorbing onto Chitin than onto L-Chitosan or M-Chitosan and a weaker electrostatic attraction. As a result of the lower ion adsorption, the Chitin monolayer was less stiff than L-Chitosan or M-Chitosan. The Chitin monolayer was stiffer in the pH 9 buffer than in water, as the buffer contained more ions than water, which were also capable of adsorbing onto Chitin.

The difference in the structure between chitin and chitosan is also expected to affect the physical properties of chitin or chitosan adsorbed to the silicon substrate. If chitin were chemically adsorbed to silicon in such a way as L-Chitosan or M-Chitosan, then we would expect to see a difference in the force and lubrication properties between chitin and the chitosan-derived compounds due to a difference in their functional groups. The ether group in chitin may adhere to silica, due to its hydrogen bonding with the silanol

groups on silica. This would result in chitin showing a greater adhesion to silica than L-Chitosan or M-Chitosan. This is expected to cause chitin to show greater friction (less lubrication) than L-Chitosan or M-Chitosan.

4. Conclusions

Physically adsorbed polysaccharides (Chitin) appear to give better lubrication than chemically adsorbed polysaccharides (L-Chitosan and M-Chitosan) in good and bad solvents. However, the physically adsorbed polysaccharide films show more wearing than the chemically adsorbed ones, due to their weaker adhesion to the substrate. The lubrication is improved by the molecular weight of the polysaccharide, where polysaccharides with a high molecular weight appear to give better lubrication (lower friction) than polysaccharides with a low molecular weight. The change of the solvent from a good to a poor solvent decreases the charge of the polysaccharides and the strength of adsorption of a physically adsorbed polysaccharide to a substrate. It also decreases the lubrication ability of the polysaccharide films.

The physical properties of the Chitin, L-Chitosan, and M-Chitosan films could be changed via their subphase, when the monolayers were formed at an air/liquid interface, or the measuring liquid, when the properties of the monolayers were being measured. The environment as to where the films are made and the environment as to where the films are to be used should therefore be taken into account when designing chitin or chitosan based films. These results also indicate that the lubricating properties of a charged film can be changed from good lubrication to bad lubrication by changing the measuring solution from a good solvent to a bad solvent.

5. Acknowledgements

This research was partly supported by the Program for Dissemination of Tenure-Track System funded by the Ministry of Education and Science, Japan and the Promotion of Research by Young Scientists funded by Shinshu University, Japan.

6. References

[1] M. Rinaudo, *Prog. Polym. Sci.*, 2006, 31, 603-632.

[2] K.W. Buck, M.A. Obaidah, *Biochem. J.*, 1971, 125, 461-471.

[3] M. Prasitsilp, R. Jenwithisuk, K. Kongsuwan, N. Damrongchai, P. Watts, *J. Mater. Sci.-Mater. Med.,* 2000, 11, 773-778.

[4] M.N.V.R. Kumar, *React. Funct. Polym.*, 2000, 46, 1-27.

[5] R.A.A. Muzzarelli, *Carbohydr. Polym.*, 2009, 76, 167-182.

[6] Z. Li, H.R. Ramay, K.D. Hauch, D. Xiao, M. Zhang, *Biomaterials*, 2005, 26, 3919-3928.

[7] A. Miwa, A. Ishibe, M. Nakano, T. Yamahira, S. Itai, E. Jinno, H. Kawahara, *Pharm. Res.*, 1998, 15, 1844-1850.

[8] W. Liu, S.J. Sun, X. Zhang, K. De Yao, *J. Biomater. Sci-Polym. Ed.*, 2003, 14, 851-859.

[9] D. Gourdon, Q. Lin, E. Oroudjev, H. Hansma, Y. Golan, S. Arad, J. Israelachvili, *Langmuir*, 2008, 24, 1534-1540.

[10] A. Kalinouski de Brito, L. Caseli, C.S.F. Nordi, *Thin Solid Films*, 2013, 534, 312-

315.

[11] G. Dunér, E. Thormann, O. Ramström, A. Dėdinaitė, *J. Dispersion Sci. Technol.*, 2010, 31, 10, 1285-1287.

[12] T. Pettersson, A. Dėdinaitė, *J. Colloid Interface Sci.*, 2008, 324, 246-256.

[13] N. Kampf, U. Raviv, J. Klein, *Macromolecules*, 2004, 37, 1134-1142.

[14] K.J. Rosenberg, T. Goren, R. Crockett, N.D. Spenser, *ACS Appl. Mater. Interfaces*, 2011, 3, 3020-3025.

[15] P.M. Claesson, B.W. Ninham, *Langmuir*, 1992, 8, 1406-1412.

[16] D.W. Lee, C. Lim, J.N. Israelachvili, D.S. Hwang, *Langmuir*, 2013, 29, 14222-14229.

[17] A. Dėdinaitė, M. Ersnstsson, *J. Phys. Chem. B*, 2003, 107, 8181-8188.

[18] C.K.S. Pillai, W. Paul, C.P. Sharma, *Prog. Polym. Sci.*, 2009, 34, 614-678.

[19] Y.S. Wu, K. Hisada, S. Maeda, T. Sasaki, K. Sakurai, *Carbohydr. Polym.*, 2007,68 ,766-772.

[20] M.C. Li, M.H. Xin, T. Miyashita, *Polym. Int.*, 2002, 51, 889-891.

[21] J.P. Gong, Y. Iwasaki, Y. Osada, *J. Phys. Chem. B*, 2000, 104, 3423-3428.

[22] A.V. Dobrynin, M. Rubinstein, *Prog. Polym. Sci.*, 2005, 30, 1049-1118.

[23] A.Y. Grosberg, A.R. Khokhlov, *Statistical physics of macromolecules*, AIP Press, New York, 1994.

[24] K. Kurita, *Prog. Polymer. Sci.*, 2001, 26, 1921-1971.

[25] K. Ohno, T. Morinaga, K. Koh, Y. Tsujii, T. Fukuda, *Macromolecules*, 2005, 38, 2137-2142.

[26] S.B. Murray, A.C. Neville, *Int. J. Biol. Macromol.*, 1998, 22, 137-144.

[27] T. López-León, E.L.S. Carvalho, B. Seijo, J.L. Ortega-Vinuesa, D. Bastos-González, *J. Colloid Interface Sci.*, 2005, 383, 344-351.

[28] D.R. Bhumkar, V.B. Pokrarkar, *AAPS PharmSciTech*, 2006, 7, E1-E6.

[29] G.T. Barnes, I.R. Gentle, *Interfacial Science An Introduction*; Oxford University Press, Oxford, 2005, p 86.

[30] A. Meurk, I. Larson, L. Bergström, *Mater. Res. Soc. Symp. Proc.*, 1998, 552, 427.

[31] P. Hess, *Appl. Surf. Sci.*, 1996, 106, 429-437.

[32] M. C. Petty, *Langmuir-Blodgett films An Introduction*; Cambridge University Press, Cambridge, 1996, p. 30.

[33] P.G. de Gennes, *Macromolecules*, 1980, 13, 1069-1075.

[34] V.M. Kaganar, H. Möhwald, P. Dutta, Rev. Mod. Phys., 1999, 71, 779-819.

[35] X. Luo, D.L. Berlin, J. Betz, G.E. Payne, W.E. Bentley, G.W. Rubloff, *Lab Chip*, 2010, 10, 59-65.

[36] J.-H. Chang, C.-L. Chen, A.V. Ellis, C.-H. Tung, *Int. J. Appl. Sci. Eng.*, 2012, 10, 293-306.

[37] C.E. McNamee, M. Kappl, H.-J. Butt, J. Ally, H. Shigenobu, Y. Iwafuji, K. Higashitani, K. Graf, *Soft Matter*, 2011, 7, 10182-10192.

[38] M. Kobayashi, H.Y amaguchi, Y. Terayama, Z. Wang, M. Kaido, A. Suzuki, A. Takahara, *Nihon Reoroji Gakkaishi*, 2008, 36, 107-112.

[39] M. Kosmulsi, *Colloid Surf. A-Physicochem. Eng. Asp.*, 2003, 222, 113-118.

[40] S.B. Murray, A.C. Neville, *Int. J. Biol. Macromol.*, 1998, 22, 137-144.

[41] D.R. Bhumkar, V.B. Pokrarkar, *AAPS PharmSciTech*, 2006, 7, E1-E6.

[42] R.A.A. Muzzarelli, *Chitin*, Pergamon Press, London, 1977.

[43] J. Ahmed, H. Guo, T. Yamamoto, T. Kurokawa, M. Takahata, T. Nakajima, J.P. Gong, *Macromolecules*, 2014, 47, 3101-3107.

[44] D.W. Lee, X. Banquy, S. Das, N. Cadirov, G. Jay, J. Israelachvili, *Acta Biomater.*, 2014, 10, 1817-1823.

[45] X. Banquy, J. Burdyńska, D.W. Lee, K. Matyjaszewski, J. Israelachvili, *J. Am. Chem. Soc.*, 2014, 136, 6199-6202.

[46] U. Raviv, J. Klein, *Science*, 2002, 297, 1540-1543.

[47] B. C. Donose, I. U. Vakarelski, K. Higashitani, *Langmuir*, 2005, *21*, 1834-1839.

[48] B.C. Donose, I.U. Vakarelski, E. Taran, H. Shinto, K. Higashitani, *Ind. Eng. Chem. Res.*, 2006, 45, 7035-7041.

[49] X.C. Chen, Y.W. Zhao, Y.G. Wang, H.L. Zhou, Z.F. Ni, W. An, *Appl. Surf. Sci.*, 2013, 282, 25-31.

[50] A.M.S. Maria, M.A. Viletti, R. Borsali, R.C. Balaban, *J. Braz. Chem. Soc.*, 2013, 24, 1871-1879.

[51] F. Monroy, L.R. Arriaga, D. Langevin, *Phys. Chem. Chem. Phys.*, 2012, 14, 14450-14459.

[52] G. Kagata, J.P. Gong, *Colloid Surf. B-Biointerfaces*, 2007, 56, 296-302.

[53] G. Kagata, J.P. Gong, Y. Osada, *Wear*, 2001, 1188-1192.

Figure captions

Figure 1. A. Structure of Chitin. B. Preparation of L-Chitosan or M-Chitosan by covalently binding chitisan to the silane coupling agent.

Figure 2. Surface pressure-area isotherms of the Chitin, L-Chitosan and M-Chitosan monolayers at an air/water interface. I, II, and III refer to the pancake, mushroom, and brush regions, respectively. The arrow shows the position of the pseudo-plateau.

Figure 3. Schematic diagram showing the pancake (region I, A), mushroom (region II, B) and brush (region III, C) structures in a polymer monolayer.

Figure 4. Surface pressure-area isotherms of the Chitin, L-Chitosan and M-Chitosan monolayers at an air/ pH 9 buffer interface. I and III refer to the pancake and brush regions, respectively. The arrow shows the position of the pseudo-plateau.

Figure 5. Surface pressure-area compression and expansion isotherms of the Chitin (A), L-Chitosan (B) and M-Chitosan (C) monolayers at an air/water interface.

Figure 6. Surface pressure-area compression and expansion isotherms of the Chitin (A), L-Chitosan (B) and M-Chitosan (C) monolayers at an air/ pH 9 buffer interface.

Figure 7. 1×1 μm^2 AFM height images of Chitin, L-Chitosan and M-Chitosan monolayers that were made at an air/water interface and transferred to a silicon wafer. The images were measured in water. A and D: Chitin; B and E: L-Chitosan; C and F:

M-Chitosan. A, B, and C show the height images, respectively. D, E, and F show the height sections of the lines shown in A, B, and C, respectively.

Figure 8. 1×1 μm^2 AFM height images of Chitin, L-Chitosan and M-Chitosan monolayers that were made at an air/pH 9 buffer interface and transferred to a silicon wafer. The images were measured in water. A and D, Chitin; B and E, L-Chitosan; C and F, M-Chitosan. A, B, and C show the height images, respectively. D, E, and F show the height sections of the lines shown in A, B, and C, respectively.

Figure 9. Forces measured in water between a silica probe and the polysaccharide monolayers that were prepared at an air/water interface. Open symbols, approach force curves; Solid symbols, retract force curves. The inset shows the approach force curves.

Figure 10. Forces measured in water between a silica probe and the polysaccharide monolayers that were prepared at an air/pH 9 buffer interface. Open symbols, Approach force curves; Solid symbols, Retract force curves.

Figure 11. Friction-load curves measured in water for the polysaccharide monolayers that were prepared at an air/water interface. Open symbols and solid lines, friction measured from low to high load; solid symbols and dashed lines, friction measured from high to low load.

Figure 12. Friction-load curves measured in water for the polysaccharide monolayers prepared at an air/pH 9 buffer interface. Open symbols and solid lines, friction measured from low to high load; solid symbols and dashed lines, friction measured from high to low load.

Figure 13. Forces measured in a pH 9 buffer between a silica probe and the polysaccharide monolayers that were prepared at an air/water interface. Open symbols, Approach force curves; Solid symbols, Retract force curves. The insert shows the approach force curves.

Figure 14. Friction-load curves measured in a pH 9 buffer between a silica probe and the polysaccharide monolayers that were prepared at an air/water interface. Open symbols, friction measured from low to high load; Solid symbols, friction measured from high to low load.

Figure 15. Forces measured in a pH 9 buffer between a silica probe and the polysaccharide monolayers that were prepared at an air/pH 9 buffer interface. Open symbols, Approach force curves; Solid symbols, Retract force curves.

Figure 16. Friction-load curves measured in a pH 9 buffer between a silica probe and the polysaccharide monolayers that were prepared at an air/pH 9 buffer interface. Open

symbols, friction measured from low to high load; Solid symbols, friction measured from high to low load.

Table of contents entry

Monolayers of three polysaccharides were made at an air/water or air/pH 9 buffer and the physical properties of the monolayers transferred to a silicon substrate investigated via Atomic Force Microscopy.