Surfactant Properties of Human Meibomian Lipids

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PURPOSE. Human meibomian lipids are the major part of the lipid layer of the tear film. Their surfactant properties enable their spread across the aqueous layer and help maintain a stable tear film. The purpose of this study was to investigate surfactant properties of human meibomian lipids in vitro and to determine effects of different physical conditions such as temperature and increased osmolarity, such as occur in dry eye, on these properties.

METHODS. Human meibomian lipids were spread on an artificial tear solution in a Langmuir trough. The lipid films were compressed and expanded to record the surface pressure-area (II-A) isocycles. The isocycles were recorded under different physical conditions such as high pressure, increasing concentration and size of divalent cations, increasing osmolarity, and varying temperature.

RESULTS. II-A isocycles of meibomian lipids showed that they form liquid films that are compressible and multilayered. The isocycles were unaffected by increasing concentration or size of divalent cations and increasing osmolarity in the subphase. Temperature had a marked effect on the lipids. Increase in temperature caused lipid films to become fluid, an expected feature, but decrease in temperature unexpectedly caused expansion of lipids and an increase in pressure suggesting enhanced surfactant properties.

CONCLUSIONS. Human meibomian lipids form highly compressible, non-collapsible, multilayered liquid films. These lipids have surfactants that allow them to spread across an aqueous subphase. Their surfactant properties are unaffected by increasing divalent cations or hyperosmolarity but are sensitive to temperature. Cooling of meibomian lipids enhances their surfactant properties. (Invest Ophtalmol Vis Sci. 2011;52:1661–1670) DOI:10.1167/iovs.10-5445

Interfacial tension is an important physical property of tears because it enables the tears to spread and form a stable film across the ocular surface. It has been reported that a low surface tension is related to higher tear film stability and higher tear breakup time, which are important for preventing dry eye. The nature and behavior of surface active molecules (surfactants) are keys to interfacial tension. At the ocular surface, it is believed that membrane-bound mucins are the surfactants and at the lipid-aqueous interface at the outer surface of the tear film, there is evidence that a number of different types of surfactant molecules might be involved.

Techniques for studying surfactant properties of the tear film and its components were introduced by Holly in the 1960s and important was the measurement of surface tension. Surface tension of whole tears at the air interface has been found to be between 42 to 46 mN/m. This surface tension is a result of complex interactions of various tear components, particularly from the aqueous layer. The likely importance of individual components of tears to the surface tension has also been examined in several studies. Ocular mucins have been found not to be surface active by themselves, but indirectly contribute to lowering the surface tension by condensing other surface active components such as lipids. Major tear proteins lysozyme, lactoferrin, and lipocalin are surface active and contribute to the low surface tension of normal tears. Lipids in whole tears also contribute to lowering surface tension because removal of lipids from whole tears was shown to increase surface tension to ~54 mN/m and this was restored to normal values when the extracted lipids were added back.

To evaluate the possible role that meibomian lipids may contribute to the tear film as surfactants, it is necessary to examine their physical properties under controlled conditions. Earlier studies of the physical properties of human meibomian lipids have provided baseline data, but there still are many gaps remaining. Studies on various lipid films in the physical chemistry literature indicate that the parameters such as pH, temperature, and ionic composition of the subphase, can affect surface pressure measurements. Few studies of meibomian lipids have taken all these parameters into account. Holly used either saline solutions or water at 37°C and Kaercher et al. used water at pH 5.5 at room temperature or 18°C. Measuring surface activity profiles of lipids above and below their melting temperature alters the orientation and interactions of lipids at air-liquid interfaces and hence strongly influences the results. For meibomian lipids, being a complex mixture of lipids, their melting range is such that they are solid at 20°C and liquid at 35°C. Therefore, measurements at room temperature would be expected to differ from those from experiments carried out at physiological temperature. Indeed, this has been shown to be the case.

It has been shown in many studies that the osmolarity (ionic composition) of tears varies and in most cases there has been an association of dry eye with increased osmolarity. This increase in osmolarity could potentially affect the spread of lipids across the tear film because it has been shown, for example, that increasing divalent cations (increased with increased osmolarity) leads to the formation of complexes with fatty acids and phospholipids and this alters their surfactant properties. In tears, the levels of divalent cations are likely to increase substantially in dry eye due to an overall increase in osmolarity of the subphase.

Another important consideration in surface tension measurements is the nature of the techniques that are used. Various methodologies that have been used to study surfactant properties of tear components include: contact angle measurement, capillary tube method, pendant drop method, and Wilhelmy plate method. Contact angle and capillary tube methods are
static, so only one condition at a time can be measured e.g., one concentration of lipids at the surface. This makes the measurements tedious. Like the capillary tube method, the pendant drop method has the advantage of using small sample volumes but applying lipids accurately to the surface is difficult. Evaporation can also be a problem with the pendant drop and contact angle measurements, particularly for experiments that are done over longer times or at higher temperatures, because it causes surface area changes during experiments as the volume of the subphase is reduced. Although the Wilhelmy plate method needs large volumes of subphase and therefore is not suitable for whole tears, it lends itself to modeling the surface activity of tears at the air-liquid interface. It is simple and used with a Langmuir trough with movable barriers allows dynamic measurements as the concentration of the lipids is increased or decreased by closing or opening the barriers. Evaporation does not change the surface area of the Langmuir trough.

Several previous studies investigating the surface activity of meibomian lipids have used either meibomian lipids from animals or mixtures of commercial lipids to emulate human meibomian lipids. However, because of the complex nature of meibomian lipids, human meibomian lipids have been shown to behave differently from these models. Therefore, human meibomian lipids are preferred over various substitutes for understanding their surfactant behavior.

In this study, we have explored the surfactant behavior of human meibomian lipids using a Langmuir trough and Wilhelmy plate method, with a focus on determining how the physical conditions such as osmolarity and temperature affect their surfactant properties.

**Materials and Methods**

HPIc grade chloroform (Sigma Chemical Co., Australia) was used as a solvent for lipid solutions. The water used in all experiments was purified by ion exchange and had a resistance of 18.2 MΩ (Milli-Q; Millipore, Billerica, MA). The commercially available lipids used in some experiments were cholesterol, cholesteryl palmitate, stearic acid, and dipalmitoyl phosphatidylcholine (all from Sigma Chemical Co., Millipore, Billerica, MA). The commercially available lipids used in some experiments were cholesterol, cholesteryl palmitate, stearic acid, and dipalmitoyl phosphatidylcholine (all from Sigma Chemical Co., Castle Hills, NSW, Australia).

**Collection of Human Meibomian Lipids**

Human meibomian lipids were collected from healthy volunteers (23-, 54-, and 52-year-old males) with no apparent ocular pathology nor self-reported ocular conditions such dry eye symptoms. The samples were pooled to provide a consistent sample for all the experimental protocols and to allow for multiple repeats of experiments. To ensure that there was no difference between the pooled sample and the individual samples, some selected experiments were carried out on individual samples and the results were the same as the pooled sample. The collection protocol was approved by the University of Western Sydney’s Biosafety and Human Research Ethics Committee and was in accordance with the Declaration of Helsinki for Medical Research.

**Measurement of the Surfactant Properties of Human Meibomian Lipids**

Meibomian lipids (20 μL of 1 mg/mL) dissolved in chloroform were applied drop-wise from a microsyringe (Hamilton Co, Bonaduz, Switzerland) onto an air-artificial tear (AT) buffer interface between the barriers of a double-barrier Langmuir trough (NIMA 102M, Nima Technology Ltd., Coventry, UK). The AT buffer was chosen to emulate the salt composition and pH of tears. The lipid film was compressed and expanded by moving the barriers such that the surface area between the barriers varied from a maximum of 80 cm² to a minimum of 15 cm². The surface pressure (π) was continuously monitored using a Wilhelmy plate (Whatman, Chertsey, UK) and a plot of Π-A isocycle was obtained. All the experiments were repeated at least three times to ensure reproducibility of the data. For these reproducible data, the variations were <1 cm² for the area and <1 mN/m for the surface pressure. The relative humidity in the laboratory ranged between 35 to 55%.

Another trough, a ribbon trough (302 M; Nima Technology Ltd.), was used to provide a larger compression ratio of the film. This allowed testing for collapse of films at high surface pressures because the surface area could be reduced without risk of breaching the sides of the trough. The maximum surface area was 200 cm² and minimum 20 cm². Dynamic Π-A isocycles were obtained and if the film collapsed during the first compression, subsequent expansion and compression cycles provided information on whether the collapse was reversible or irreversible. These experiments were performed with human meibomian lipids and commercially available lipids.

**Effect of Subphase**

Π-A isocycles of human meibomian lipids were obtained on different types of subphases: water (MilliQ water), phosphate buffer saline (PBS; 10 mM phosphate buffer, 0.9% NaCl, pH 7.4), and an artificial tear (AT) buffer that emulated the salt concentration of human tears (NaCl 6.6 g/L; KCl 1.7 g/L; NaHCO₃ 1.4 g/L; CaCl₂ 2H₂O 0.15 g/L; NaH₂PO₄ 2H₂O 0.1 g/L; MOPS 4.18 g/L; and pH 7.4) derived from the artificial tear solution of Mirejovsky et al.

**Effect of Concentration and Size of Divalent Cations**

Π-A isocycles of human meibomian lipids were obtained on AT subphases with three different types of divalent cations: Mg²⁺, Ca²⁺, and Ba²⁺ (these have different ionic sizes: Mg²⁺ ion, radius 0.72 Å; Ca²⁺ ion, radius 1 Å; and Ba²⁺ ion, radius 1.35 Å). Their salts with Cl⁻ such as MgCl₂ (Sigma Chemicals Co.), CaCl₂ 2H₂O (Ajax Chemicals, Burnt, NSW, Australia), and BaCl₂ 2H₂O (Univar, Ingleburn, NSW, Australia) were added in the subphase. The final concentrations of divalent cations used were 0.2–1 mM.

**Effect of Osmolarity**

The effect of osmolarity of the subphase on the Π-A isocycles of human meibomian lipids was explored by varying the concentration of the AT buffers: normal AT and 1.5, 2, and 3 times the salt concentration (1.5 × AT, 2 × AT, and 3 × AT, respectively). Osmolarity of these buffers was measured using a vapor pressure osmometer (Vapro 5520; Wescor, Inc., Logan, UT).

**Effect of Temperature**

Temperature of the subphase in the Langmuir trough was regulated by a circulatory thermostat (Ecoline RE106; Lauda, Lauda-Königshofen, Germany), which pumped coolant through the water jacket of the trough. The temperature of the subphase was measured using a temperature probe (MadgeTech, Inc., Warner, NH) immersed into the subphase outside the barriers and the temperature was maintained within ±1°C of those stated for each experiment.

The effects of heating and cooling cycles were also studied. For the heating cycle, meibomian lipids were first spread on AT buffer at 20°C and the Π-A isocycles were recorded. The temperature of the subphase
was then gradually increased and Π-A isocycles were recorded at 30°C, 40°C, and 50°C. The selected temperatures reflected the values in and above the melting range of meibomian lipids with the upper limit as 50°C because warm compress of eyelids generally raises the temperature to 40°C. This was followed by a cooling cycle, in which the temperature was gradually decreased and Π-A isocycles were recorded at 40°C, 30°C, and 20°C.

In some experiments, to avoid the use of solvent, meibomian lipids collected on a spatula were directly applied onto the surface of the subphase at 37°C and then cooled to 20°C. For this temperature transition, in some experiments isocycles were recorded at both temperatures, and in others change in pressure was monitored while keeping the area constant. In another set of experiments, subphase was heated to 41°C and then cooled down to 35°C; isocycles were recorded at both the temperatures.

RESULTS

Surfactant Properties of Human Meibomian Lipid Films

During the compression phase of a Π-A isocycle of human meibomian lipids at 37°C, there was a slow but continuous increase in pressure reaching ~12 mN/m (Fig. 1). At 20°C, it showed a continuous increase in pressure but with some small inflections and reached a higher pressure value of ~20 mN/m (Fig. 1). The expansion curves followed mostly the compression curves, except that at 20°C, there was a pronounced hysteresis at higher pressures. Overall, the two isocycles imply that human meibomian lipids form largely liquid films, show features of liquid condensed phase at lower temperatures, and show expansion on heating at higher surface areas, but take up less area at small surface areas. The estimated surface area per molecule at the highest pressure, calculated using an average molar mass of 720 Å ² and assuming all components lie on the surface, was ~10 Å ². This surface area is too small for a lipid molecule, therefore, it is likely that the film comprises multiple layers, and the lower surface pressure for the higher temperature at 15 cm² is indicative of more layers in the film.

Effect of High Pressure

Human meibomian lipid films were found to be highly compressible and hence did not collapse at high surface pressures (Fig. 2). This was seen not only at 37°C but also at 20°C, when the film was expected to be more rigid. In this experiment, 10 times the amount of meibomian lipids were spread on the ribbon trough and it was compressed to its minimum surface area. This meant that the starting pressure was >0 mN/m and the maximum surface pressures were much higher. However, the films did not collapse as indicated by the overlay of successive cycles. During first cycle, some reorganization of components happens, which is typical for these types of experiments and as a result the initial pressure for the subsequent cycle(s) was lower than the first cycle, but still not 0 mN/m. The estimated area per molecule at highest pressure was ~1 Å ², indicating multiple layer formation. This feature contrasted with films of individual lipids that are reported components of meibomian lipids. Cholesterol films collapsed during compression but the collapse was reversible on expansion (Fig. 3). Films of phosphatidylcholine (DPPC as a pure commercially available representative of phospholipids), cholesteryl palmitate, and stearic acid collapsed irreversibly (Fig. 3).

Effect of Subphase

Human meibomian lipids (30 μL of 1 mg/mL) showed higher maximum surface pressures on buffered subphases (PBS and AT) compared with water (Fig. 4) at 20°C, indicating that the orientation of the lipids at the surface was affected by the subphase. There was little difference between having

![Figure 1. Π-A isocycles of human meibomian lipids on AT buffer at 20°C and 37°C. A slow and continuous increase in pressure indicated features of a liquid film.](http://jovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/jovs/932971/)

![Figure 2. Π-A isocycles of human meibomian lipids on a ribbon trough. The film did not collapse at high pressures and very small molecular areas indicated multilayer formation that was reversible.](http://jovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/jovs/932971/)
PBS or AT as the subphase, and the nature of the subphase had little effect on the overall shape of the II-A isocycles. Experiments conducted at 37°C (data not shown) gave similar results.

**Effect of Divalent Cations**

In the standard AT buffer, Ca\(^{2+}\) was the divalent cation. Neither increasing the concentration, nor changing the divalent cation to Ba\(^{2+}\), which is larger in size than Ca\(^{2+}\), or to Mg\(^{2+}\), which is smaller, affected the II-A isocycles of meibomian lipids (Fig. 5). Only the lowest and highest tested concentrations of these ions are shown.

**Effect of Osmolarity**

Osmolarity of different AT solutions both calculated and measured using osmometer ranged between 305 (normal) and a very hyperosmolar 920 mOsm/Kg. The values for each of the buffers were: AT: 305 mOsm/kg; 1.5 \times AT: 478 mOsm/kg; 2 \times AT: 920 mOsm/kg.
AT: 633 mOsm/kg; 3 × AT: 920 mOsm/kg, and there was only ~2 mOsm/kg difference between the calculated and measured osmolarity. Increasing osmolarity had little effect on the II-A isocycles of human meibomian lipids at 20°C, the isocycles were mostly overlapping, but at 37°C increasing osmolarity resulted in slight increases in the overall pressures (Fig. 6).

**Effect of Temperature**

As films of human meibomian lipids were heated, there was an increase in surface pressure at maximum area, little change in maximum surface pressure at the smallest area, and the II-A isocycles became less complex (Fig. 7). This is consistent with the molecules at higher temperature taking up more space on the surface (increase of pressure at maximum surface area with temperature) and more readily forming multiple layers at minimum surface area. The cooling cycle, after heating, gave completely unexpected results (Fig. 7). As the film was cooled, there was an unexpected increase in surface pressure at smaller areas or higher compressions (approximately area <50 cm² or pressure >10 mN/m), indicating molecules taking more space on cooling. At bigger areas or low compressions (approximately area >50 cm² or pressure <10 mN/m), the film seemed to occupy less area on cooling, which one would normally expect (Fig. 7, inset: for simplicity only the compression parts of isocycles are shown). The II-A isocycles of the heating and cooling cycle did not overlap with each other: the isocycles at equivalent temperatures were very different after the film had been heated (Fig. 8). Once the lipid film had been heated, the meibomian lipids occupied more surface area at the same temperature than before heating, greater hysteresis, and higher maximum surface pressures. This indicates that the input of external thermal energy into the film had caused a dramatic reorganization of the molecules, which meant that they were more resistant to collapse (higher maximum surface pressure) and cohesion also seemed to be increased (increased hysteresis).

To confirm that these data were due to the meibomian lipids and not somehow due to the solvent or to the very high temperature (50°C), 0.06 mg of meibomian lipids were immediately, after collection, spread from the collection spatula onto the AT buffer in the Langmuir trough maintained at 37°C. At this temperature, the meibomian lipids spread across the subphase without the need of a solvent. A II-A isocycle of the film was taken at 37°C before cooling to 20°C, when another II-A isocycle was taken (Fig. 9). This experiment was repeated on three other occasions with similar, but not exactly the same, amount of lipids. The results were similar to those obtained on cooling of meibomian lipid films using a solvent. In another experiment, the surface area in the Langmuir trough was kept constant. Meibomian lipids were spread from a spatula onto the AT buffer at 37°C and the pressure was allowed to stabilize (3 mN/m). The pressure was monitored as the subphase was cooled to 20°C (Π-time profile). The result was a pressure increase to 8 mN/m at 20°C (Fig. 9). Overall, these results indicate that cooling meibomian lipid films from 37°C or above caused a relative increase in surface pressure, hence such cooling apparently enhances the surfactant properties of meibomian lipid films.
To further explore the possible significance of cooling of meibomian lipids after warm compresses that are generally recommended for MGD patients, Π-A isocycles of the meibomian lipid film were taken at 41°C (warm compress temperature) and after cooling to 35°C (ocular temperature). An increase in surface pressure was observed on cooling of lipids from 41 to 35°C (Fig. 10). The results were similar for lipids spread with or without solvent.

**DISCUSSION**

Besides confirming what is intuitively obvious—the physical conditions of the experiments for measuring surface pressure affect the results—these experiments emphasize which of the physical conditions need particular attention when dealing with meibomian lipids. In addition, they highlight some special properties of meibomian lipids that are not typical characteristics of simple lipid films: in particular, the unexpected effects of temperature where the molecules increase their average molecular area on cooling.

A characteristic feature of human meibomian lipids, as shown by their Π-A isocycles, is that they form liquid films. They remain liquid even when compressed to high pressures. Another feature of human meibomian lipids films is that they are highly compressible and characteristically non-collapsible. During the natural phenomenon of blinking, it is assumed that the lipid layer of the tear film is subjected to enormous surface pressures. For maintaining integrity of the tear film, it should be able to reversibly compress and expand during multiple blinks, and be able to withstand high surface pressures without collapsing. Our data, particularly on the ribbon trough with high compression ratios, are consistent with these ideas. The lack of collapse could be due to reversible folding or reversible formation of multiple layers. The formation of multiple layers is supported by the impossibly low area per molecule observed at high pressures. Petrov et al. have shown that during compression, the hydrophobic part of the meibomian lipids are transferred over the top of hydrophilic lipids forming multilayer. At low pressures, the available surface area is occupied mainly by polar lipids and partially by non-polar lipids. From our data, it is likely that transfer of molecules into multiple layers is a continuous process during compression, because no plateau or sudden changes in the Π-A isocycles were observed. Our results show that the compressible nature and multilayer formation are characteristic features of the human meibomian lipids. The fact that none of the individual lipids tested possessed this property might indicate that combination of different lipids and their interactions with each other contribute toward making the lipid layer non-collapsible that is well adapted to blinking. The corollary of this is that changes to the lipid composition could lead to collapsible meibomian lipid films, although this phenomenon has never been observed.

**FIGURE 6.** Π-A isocycles of human meibomian lipids at 20°C and 37°C on various AT buffers with increasing osmolarity. Increasing osmolarity had little effect on the isocycles.

**FIGURE 7.** Π-A isocycles of human meibomian lipids while heating from 20°C to 50°C (left) and cooling from 50°C to 20°C (right). Cooling of lipids resulted in increased surface pressure and enhanced surfactant properties. Inset shows the compression parts of the isocycles of the cooling cycle.
been reported in clinical studies which could indicate that the lipid film is very tolerant to changes in component composition.

The ability of meibomian lipids to form multilayers for withstanding high pressure is akin to reversible folding in case of lung surfactants. Lung surfactants prevent alveolar collapse during breathing by reducing the surface tension at air-alveoli interface. They are able to sustain high pressure by forming multilayers under high compressive force of the breathing of lungs.41–43 The surfactant proteins present in the lung surfactants facilitate multilayer formation.44 Here, we have tested meibomian lipids alone, and although in situ it is likely that proteins are components of the lipid layer of the tear film,12–14,16 unlike lung surfactants, our data indicate that these are not needed to maintain a non-collapsible lipid layer.

Our data indicate that when carrying out such experiments, attention has to be given to the type of subphase used, its temperature, and pH.17 The effect of the subphase may explain the differences in results from previous studies on the surfactant properties of human meibomian lipids.18,19,45 For example, Kaercher et al.18 showed a maximum surface pressure of 12.4 mN/m at the highest compression on water (pH 5.5) at room temperature. This matched closely with the film pressure (12 mN/m) of meibomian lipids reported by Holly45 on water or physiological saline, although the temperature was 37°C. A slightly lower film pressure (10 mN/m) was reported by Holly3,4 earlier in 1973 on water or physiological saline at 37°C. These data and the profiles obtained in these studies were similar to ours, but there were some differences in the details that may be attributed to various pH, temperature, and amounts of lipids used. It would be useful to standardize these conditions across laboratories in the future so that there could be direct comparisons of the data.

It was somewhat surprising that the meibomian lipid films were resilient to large changes in osmolarity and to increases in divalent cations concentrations. The possibility that osmolarity might change the performance of the meibomian lipids is important physiologically because it has been reported that tears become hyperosmolar in dry-eye condition due to decreased tear production or increased evaporation.24,46,47 One would also expect that if the surfactant moiety of meibomian lipids were anions e.g., phospholipids or fatty acids, then a large increase in divalent cations would have a noticeable effect on the performance of the meibomian lipid films. It has been shown using polar lipid films that an increase in salt concentration generally results in increased surface pressure of polar lipids at air-liquid interface,48,49 and more specifically that complexation of metal ions with the acidic head groups of fatty acids, and divalent cations to phospholipids condenses these films.27,28,50,51 Presence of cations in the subphase induces decrease in surface tension of phospholipid monolayers.52 This was not the case for meibomian lipids which showed remarkable tolerance to salt concentrations. This observation is consistent with very low levels of free fatty acids or

**Figure 8.** Comparison of Π-A isocycles of human meibomian lipids from cooling and heating cycles at a particular temperature (40°C, 30°C, and 20°C). There was a general expansion, greater hysteresis, and higher maximum surface pressure after cooling.

**Figure 9.** Π-A isocycles (left) and Π-time profile (right) of human meibomian lipids spread directly on AT without any solvent at 37°C and then cooled to 20°C. Cooling enhanced the surfactant properties of meibomian lipids as indicated by higher surface pressure and area of the lipids.
phospholipids in meibomian lipids. The difference on the lipid films that was noticed between water subphase and buffered subphase might be accounted for in that a minimal salt concentration is required to provide enough ions to crosslink the lipids and above that there is no significant effect.

These results are also of potential use in the formulation of eye-drops because they tend to indicate that the concentration of divalent cations in the formulations is unlikely to affect the surfactant properties of meibomian lipids. Use of divalent cations is an important consideration in excipients used in eyedrop formulations. They are required for gel formation in case of gellen gum, gelrite, an ophthalmic preparation for dry eye. They can affect the conformation of different polymers used in eye-drops. Another application is their antibacterial potential as they are believed to inhibit certain Gram-negative bacteria.

Temperature is one of the foremost important factors that govern the surfactant properties of lipids. For single lipids, increase in temperature can increase the phase transition pressure or decrease in the collapse pressure. Melibomian lipids cannot be compared with single lipids in this context because they are mixture of many lipids, have melting range rather than a melting point, and form non-collapsible films. In 1969, using simple spreading technique, it was shown that temperature affected spreading of melibomian lipids; the melibomian lipids did not spread at 25°C on saline but warming the lipids to body temperature allowed their spread and formation of a multimolecular film. More recently, the infrared spectroscopy studies on melibomian lipids indicate that increase in temperature causes the hydrocarbon chain order to change from all trans (ordered, 25°C) to gauche (disordered, 42°C) conformation; in other words, a change from gel phase to liquid phase.

Our data in the heating cycle and a similar recent study showed these effects agreeing well with these observations. Our results in the cooling cycle showed unexpected results and enabled us to identify another unique property of human melibomian lipids; that is, these lipids expand on cooling. To our knowledge, this is the first report of this unique property of human melibomian lipids. In similar experiments recently reported by Butovich et al., the expansion of melibomian lipids on cooling was not observed. The likely reason for this is the difference in the technique. Unlike us, they measured the changes in pressure at a constant area as the subphase was cooled. This constant area roughly corresponded to the area in our experiments where condensation of the film was observed. Here we report isocycles on cooling with pressure measurements for all trough areas including smaller areas. It was the smaller areas where the expansion on cooling effects was most apparent.

Expansion of meibomian lipids on cooling is contrary to the intuitive belief because one would expect lipids to condense at lower temperature. Although water shows a similar anomalous behavior of expansion on cooling due to crystallization, this would be unlikely for the melibomian lipid films. Formation of crystals on cooling by meibomian lipids would tend to make the film brittle and unstable, which is not the case because the films did not collapse at very high surface pressures. Another possible explanation is that proteins might be present in the melibomian lipid secretion and that it is these proteins that are responsible for this phenomenon. Although we cannot eliminate this as a possibility, it is extremely unlikely. Firstly, the same phenomenon was seen with meibomian lipids extracted with chloroform as those that were placed without solvent on the surface. One would expect that chloroform would have an effect on the possible proteins in meibomian lipid extracts and so give a different result from those applied directly to the subphase, this was not the case. Secondly, unfolding of proteins might explain the expansion seen on heating, but it does not explain expansion and increase in surface pressure seen on cooling. In unreported experiments, we have added a protein, lysozyme, to the subphase and heated the mixture. Heating causes expansion of the mixture at the surface, but isocycles taken on cooling do not show any further expansion/contraction. In particular, there is no further increase in surface pressures at small areas as were seen with meibomian lipids alone in heating/cooling experiments. Another possibility is that the observed changes occurred due to oxidation of the lipids. However, published evidence on the oxidation of oleic acid (the major unsaturated fatty acid in melibomian lipids) indicates that oxidation of the meibomian lipids could not occur under the conditions used and hence this could not account for the expansion of the film on cooling. Indeed, the products from oxidation of oleic acid films using ozone at air-water interface lead to a decrease in surface pressure, rather than an increased surface pressure as was observed here on cooling after heating.

Our results show that cooling enhances the surfactant properties of meibomian lipids, enabling it to reach higher surface pressures. An interesting possibility to consider is that this phenomenon might provide additional benefits to patients having meibomian gland dysfunction (MGD) associated dry-eye. These patients are recommended a warm compress to increase delivery of lipids to the ocular surface by making them more fluid. In addition, a warm compress may help by improving surfactant properties of the lipid layer. Warm compresses would raise the temperature of meibomian lipids to 40–42°C when the same lipids come on the ocular surface, they would be cooled down to 35°C (the ocular temperature), and this cooling will make them better performer on the eye in terms of decreased surface tension which would provide relief to the patients. This might help explain why warm compresses improve the tear break up time, and stability and uniformity of the lipid layer in MGD patients.

In summary, study of human meibomian lipid films at the air-tear buffer interface provides valuable information on the surfactant properties of meibomian lipids. These lipids form highly compressible, non-collapsible, multilayered, liquid films that are resilient to high pressure, high osmolarity, and divalent cations but are sensitive to changes in temperature. Expansion...
of meibomian lipids on cooling is an unexpected phenomenon and suggests enhanced surfactant behavior. Surfactant properties of meibomian lipids studied under different conditions in this article suggest that there should be some standard conditions for the future measurements by various researchers such as near physiological temperature, pH, and salt concentration, so that there is consistency when different data are compared. Particular attention should be given to the temperature of measurement, which can affect the data to a great extent.

References


