

Temperature-Induced Transitions in the Structure and Interfacial Rheology of Human Meibum

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Supplementary Information

This supplementary information is intended to demonstrate the effect of solvent on isocycles and interfacial rheology of meibum. Figure 1 shows surface pressure versus trough area for meibum spread neat compared to meibum deposited from chloroform and hexane solutions at 24°C. Each curve represents a meibum sample collected from a single individual. The curves from all three solvent conditions show increasing slope with compression, but further interpretation of the molecular behavior is difficult. The two isotherms of lipids spread from chloroform and hexane are quite similar, suggesting that the form of organic solvent does not strongly effect lipid organization. At equivalent trough areas the hexane curve is shifted to higher pressures, presumably because the sample dissolved in hexane contained more material.

On the other hand, the isotherm of the sample spread neat had a greater slope compared to the samples spread by chloroform and hexane. The liftoff (the area at which surface pressure became non-zero) for the neat sample lies at a low trough area compared to the two solvated samples, suggesting that the mass of material in the neat sample was lower than the solvated samples. But, the neat sample achieved higher surface pressures with equivalent compression. While the difference in isocycles of neat compared to solvated samples could be due solvent-induced organization or the presence of proteins, we suspect that the enhanced surface pressure of the neat sample is primarily due to heat cycling. The trough was heated to 35°C to spread the neat sample, while samples spread from hexane or chloroform were kept at 24°C at all times. Mudgil and Millar have reported that temperature cycles have a strong influence on surface pressure versus area isotherms of human meibum. Meibum spread from chloroform was capable of achieving higher surface pressures after a heat-cool cycle compared to meibum maintained at room temperature [1].

The interfacial shear rheology of meibum is dependent on surface pressure. Figure 2 shows results from a meibum sample spread without solvent. Upon deposition the interfacial viscous modulus was fairly low, around 0.08 mN/m, and the surface elastic modulus was not measurable. But with minimal compression to 3 mN/m the film became predominantly elastic. With further compression the surface moduli, or film strength, continued to increase up to 10 mN/m, which is quite high for an interfacial film. For this particular sample, compression of the monolayer from 1-15 mN/m resulted in an increase in surface moduli of three orders of magnitude. These results are similar to those reported of human meibum spread

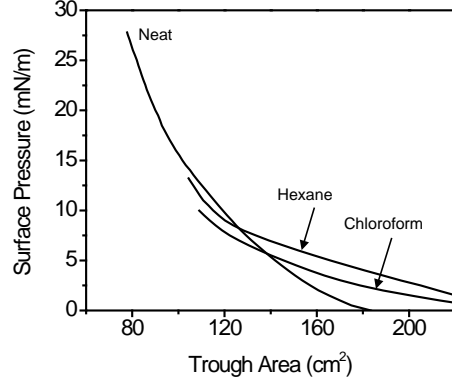


Figure 1: Surface pressure versus area isotherms for meibomian lipids spread from different solvent conditions.

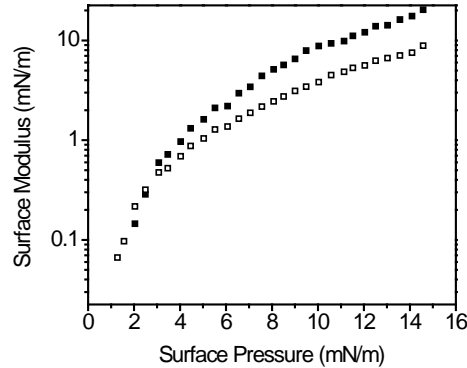


Figure 2: Interfacial shear rheology as a function of surface pressure for meibum spread neat. The closed symbols represent the surface elastic modulus, G' , while the open symbols represent the surface viscous modulus, G'' .

from chloroform [2].

Figure 3 shows the surface pressure dependence of the interfacial rheology of meibum collected from the same donor but spread from different solvent conditions. To simplify the plots, complex viscosity, η^* , is shown. The complex viscosity is calculated from the interfacial elastic and viscous moduli as follows:

$$\eta^* = \frac{(G'^2 + G''^2)^{1/2}}{\omega^2}, \quad (1)$$

where G' and G'' represent the interfacial elastic and viscous moduli, respectively, and ω is the frequency of the oscillatory deformation. The surface rheology of two films spread from

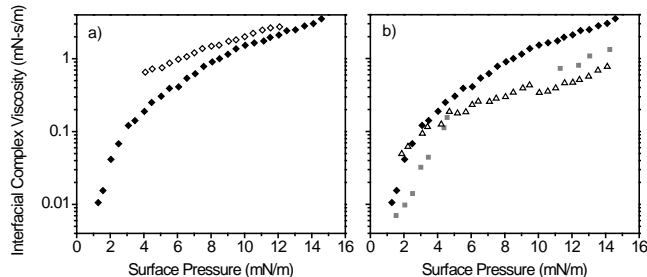


Figure 3: a) Interfacial complex viscosity as a function of surface pressure for two independently spread meibum films from neat storage conditions. The meibum for each curve was collected on separate occasions. b) The complex viscosity for meibum spread neat (diamonds), from chloroform (triangles) and from hexane (squares). All samples were collected from the same donor.

independently collected neat samples are shown in Figure 3a to explore reproducibility of meibum from a single donor and solvent condition. Figure 3b compares films spread from hexane, chloroform, and without solvent from a single donor. All four curves in Figure 3 show the same general trend: as surface pressure increased the complex viscosity increased. However, the complex viscosity at any particular surface pressure varied between trials. The two films spread from neat samples (a) did not achieve the same complex viscosity values, nor did samples spread from different solvents (b). There was a significant amount of variability in the interfacial moduli or complex viscosity for samples spread under the same condition, therefore it was difficult to discern any major trends between the samples spread under different conditions. Sources of variability could include amount of meibum initially spread and differences in the content of the meibum from day to day from a single individual.

Finally, the effect of solvent on the temperature dependence of interfacial rheology was explored. Figure 4 shows interfacial complex viscosity versus temperature curves for meibum spread neat, from chloroform, and from hexane, where all samples were collected from the same individual. For all three conditions, the complex viscosity is greater than 1 mN-s/m at temperatures between 20°C. As the films were heated, the interfacial complex viscosity values dropped about an order of magnitude between 26-28°C. By 34°C the values reached the lower sensitivity limit of the interfacial stress rheometer. However, the complex viscosity values of each curve are not equivalent, and there was no clear trend between solvent type. These findings are consistent with those shown in Figure 3: that interfacial rheology trends (by surface pressure or temperature) are consistent between different sample and solvent conditions, yet quantitative comparisons are difficult to make.

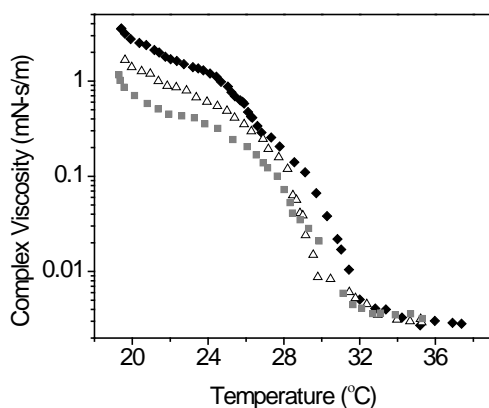


Figure 4: The interfacial complex viscosity as a function of temperature for meibum spread neat (diamonds), from chloroform (triangles) and from hexane (squares). The meibum for each curve was collected on a separate occasion from the same donor. The starting surface pressures were 14 mN/m for the neat and hexane samples and 13.75 mN/m for chloroform.

The results presented in this supplement demonstrate that although the solvation and spreading conditions of meibum should and probably do have some influence on the organization of interfacial meibum films, the techniques described here, isotherms and interfacial rheology, are not necessarily sensitive to such differences. We expect that such changes will be most obvious at the molecular level and may be elucidated by interfacial x-ray scattering techniques such as grazing incidence x-ray diffraction and x-ray reflectivity.

References

- [1] Millar, T. J., and P. Mudgil. 2011. Surfactant properties of human meibomian lipids. *Invest. Ophthalmol. Vis. Sci.* 10-5445.
- [2] Leiske, D. L., S. R. Raju, H. A. Ketelson, T. J. Millar, and G. G. Fuller. 2010. The interfacial viscoelastic properties and structures of human and animal meibomian lipids. *Exp. Eye Res.* 90:594-604.