The anti-evaporative mechanism of wax esters – Implications for the function of tear fluid

Riku O. Paananen, Antti H. Rantamäki and Juha M. Holopainen*

Helsinki Eye Lab, Department of Ophthalmology, University of Helsinki, Helsinki, Finland

Funding: The Finnish Eye Foundation and the Sigrid Juselius Foundation supported this study.

*Corresponding author: Juha Holopainen, Helsinki Eye Lab, Department of Ophthalmology, University of Helsinki, P.O. Box 220, 00029 HUS, Finland; E-mail: juha.holopainen@hus.fi; Tel.: +358-9-471 77197; Fax +358-9-471 75100

Supporting information

BAM calibration

According to the Fresnel equations, the reflectance for p-polarized light at an interface of two materials with refractive indices n_1 and n_2 is

$$R_p = \left| \frac{n_1 \sqrt{1 - \left(\frac{n_1}{n_2} \sin \theta_i\right)^2} - n_2 \cos \theta_i}{n_1 \sqrt{1 - \left(\frac{n_1}{n_2} \sin \theta_i\right)^2} + n_2 \cos \theta_i} \right|^2 \quad (\text{A1})$$

where θ_i is the angle of incidence. The refractive index of the aqueous subphase can be varied by adding different concentrations of NaCl.¹ Reflected intensity per unit time was measured from the surface of water containing 0, 9, 20, 30, 50, 60 and 90 g/L NaCl. Intensity per unit time was used instead of average intensity in order to obtain intensity measurements from a wider range of reflectance values without working near the sensitivity limit of the detector or having overexposed pixels in the images. Reflectance at different concentrations of NaCl was calculated using eq A1 to create the calibration curve shown in Figure S1. A power function was fitted to the data and used to obtain reflectance from intensity-per-unit-time data in further measurements.

Figure S1. Calibration curve relating the intensity per unit time (I , mean $\pm$ SE) measured from BAM images to the reflectance (R) of the film.

1-Octadecanol, 1-Docosanol, 1-Hexacosanol and 1-Triacontanol were added to the air-water interface of a Langmuir trough in 5 mM chloroform solution and chloroform was allowed to evaporate for 10 minutes. Trough temperature was maintained within 36 ± 1 °C during the experiments. Films were compressed to a mean molecular area of $18 \text{ \AA}^2$ at a speed of $1.2 (\text{ \AA}^2/\text{molecule})/\text{min}$. Average image intensity per unit time was measured from all of the films and intensity values were converted into reflectance using the fit shown in Fig. S1. A theoretical thickness for the linear alcohols in all-trans conformation was calculated according to Tanford.² A linear fit (Fig. S2) was used to obtain values of $11.6 \pm 0.4 \times 10^3 \text{ \AA}$ (value $\pm$ SE) for A and $1.3 \pm 0.2 \times 10^{-6}$ for R_{bg} (eq 2).

Figure S2. Fit between theoretical film thickness (d) and measured reflectance (R , mean $\pm$ SE) of linear alcohol monolayers with chain lengths of 18, 22, 26 and 30 carbons at a mean molecular area of $18 \text{ \AA}^2$.

Humidity calibration

In non-vacuum conditions, evaporation rate J from an open water surface can be expressed by³

$$J = \frac{C_s^{\text{sat}}(T_s) - C_\infty(T_\infty)}{R_k + R_e} \quad (\text{A2})$$

where C_s^{sat} is the water vapor concentration in equilibrium with the liquid phase and C_∞ is the water vapor concentration far from the surface. T_s is the temperature of the water surface and T_∞ is the temperature far from the surface. R_k is the evaporation resistance for the kinetic escape of the water molecules from the

liquid water into the thin vapor layer adjacent to the surface and R_e is the evaporation resistance for mass transfer by diffusion and convection from the vapor layer to the surrounding atmosphere.

The resistance for kinetic escape from pure water surface is negligible,³ therefore evaporation rate from pure water surface is

$$J_w = \frac{C_s^{\text{sat}}(T_s) - C_\infty(T_\infty)}{R_e} = -\frac{C_\infty^{\text{sat}}(T_\infty)}{R_e} H_R + \frac{C_s^{\text{sat}}(T_s)}{R_e} \quad (\text{A3})$$

where H_R is relative humidity in the air above the film and C_∞^{sat} is the saturated water vapor concentration at T_∞ . Temperature far from the water surface remained constant at 22 ± 1 °C during the experiments independent of the measurement temperature. At this temperature, C_∞^{sat} is 19 ± 2 g/m³. Temperature variation also has very little effect on R_e .³ In order to estimate R_e and the effect of humidity on the evaporation rate, all the control measurements at 35 °C and 37 °C were pooled and a least squares method was used to simultaneously fit two linear functions to the data (Figure S3). Same slope was required for both fits but separate intercepts were allowed, since C_s^{sat} varies with measurement temperature.

Figure S3. Evaporation rate from the surface of pure PBS buffer at 35 °C and 37 °C. Error bars depict the average humidity variation during the experiment.

From the linear fit, a value of 1.9 ± 0.3 s/cm was determined for the mass transfer resistance R_e . According to the intercepts, water vapor concentration at the surface is 43 ± 6 g/m³ at 37 °C and 38 ± 5 g/m³ at 35 °C, corresponding to the saturated water vapor concentration at these temperatures.

Derivation of equation 7

In a monolayer film where solid and fluid phases coexist and mean molecular area of both phases is constant, the surface fraction covered by the solid phase can be expressed by

$$\Phi = \frac{a_2(a - a_1)}{a(a_2 - a_1)} \quad (\text{A4})$$

where a_1 and a_2 are the mean molecular areas of the fluid and solid phases and a is the total mean molecular area.

A monolayer residing at the air-water interface is considered to increase the resistance for the kinetic escape from water surface, R_k (see eq. A2). Assuming that the evaporation rates through a solid and a fluid monolayer of lipid are constant, the total evaporation resistance can be expressed as a function of condensed phase coverage as

$$R_k = \left[\Phi \frac{1}{R_{k,2}} + (1 - \Phi) \frac{1}{R_{k,1}} \right]^{-1} \quad (\text{A5})$$

where $R_{k,1}$ and $R_{k,2}$ are the evaporation resistances of the fluid and solid layer, respectively. When conditions between experiments are constant, evaporation reduction E can be defined as

$$E = 1 - \frac{J}{J_w} = 1 - \frac{R_e}{R_k + R_e} \quad (\text{A6})$$

By combining equations A5 and A6, E can be expressed as a function of surface coverage

$$E = \left[\Phi \frac{R_e}{R_{k,2}} + (1 - \Phi) \frac{R_e}{R_{k,1}} + 1 \right]^{-1} \quad (\text{A7})$$

Evaporation resistance of the fluid ($\Phi = 0$) and solid ($\Phi = 1$) film can be calculated from the beginning and plateau of the evaporation reduction curve (Fig. 3) by

$$R_{k,2} = \frac{R_e E_f}{1 - E_f} \text{ and } R_{k,1} = \frac{R_e E_0}{1 - E_0} \quad (\text{A8})$$

where E_f is the evaporation reduction caused by a solid monolayer completely covering the surface and E_0 is the evaporation reduction caused by a fluid monolayer completely covering the surface.

By combining equations A4 and A7, the total evaporation reduction can be expressed as a function of the total mean molecular area as

$$E = \left[\frac{a_2(a - a_1)}{a(a_2 - a_1)} \left(\frac{R_e}{R_{k,2}} - \frac{R_e}{R_{k,1}} \right) + \frac{R_e}{R_{k,1}} + 1 \right]^{-1} \quad (\text{A9})$$

(eq 7 in the article).

References

1. Grange, B. W.; Stevenson, W. H.; Viskanta, R. Refractive index of liquid solutions at low temperatures: an accurate measurement. *Appl. Opt.* **1976**, *15*, 858-859.
2. Tanford, C. *The Hydrophobic Effect: Formation of Micelles and Biological Membranes*, 2nd ed.; J. Wiley.: New York, 1980.
3. Cerretani, C. F.; Ho, N. H.; Radke, C. J. Water-evaporation reduction by duplex films: application to the human tear film. *Adv. Colloid Interface Sci.* **2013**, *197-198*, 33-57.