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Dynamic interfacial properties of human tear-lipid films and their interactions with model-tear proteins in vitro

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ABSTRACT

This review summarizes the current state of knowledge regarding interfacial properties of very complex biological colloids, specifically, human meibum and tear lipids, and their interactions with proteins similar to the proteins found in aqueous part of human tears. Tear lipids spread as thin films over the surface of tear-film aqueous and play crucial roles in tear-film stability and overall ocular-surface health. The vast majority of papers published to date report interfacial properties of meibum-lipid monolayers spread on various aqueous sub-phases, often containing model proteins, in Langmuir trough. However, it is well established that natural human ocular tear lipids exist as *multilayered* films with a thickness between 30 and 100 nm, that is very much disparate from 1 to 2 nm thick meibum monolayers. We employed sessile-bubble tensiometry to study the dynamic interfacial and rheological properties of reconstituted multilayered human tear-lipid films. Small amounts (0.5–1 µg) of human tear lipids were deposited on an air-bubble surface to produce tear-lipid films in thickness range 30–100 nm corresponding to ocular lipid films. Thus, we were able to overcome major Langmuir-trough method limitations because ocular tear lipids can be safely harvested only in minute, sub-milligram quantities, insufficient for Langmuir through studies. Sessile-bubble method is demonstrated to be a versatile tool for assessing conventional synthetic surfactants adsorption/desorption dynamics at an air-aqueous solution interface. (Svitova T., Weatherbee M., Radke C.J. Dynamics of surfactant sorption at the air/water interface: continuous-flow tensiometry. *J. Colloid Interf. Sci.* 2003;261:1170–179). The augmented flow-sessile-bubble setup, with step-strain relaxation module for dynamic interfacial rheological properties and high-precision syringe pump to generate larger and slow interfacial area expansions-contractions, was developed and employed in our studies. We established that this method is uniquely suitable for examination of multilayered lipid-film interfacial properties. Recently it was compellingly proven that chemical composition of human tear lipids extracted from whole tears is substantially different from that of meibum lipids. To be exact, healthy human tear lipids contain 8–16% of polar lipids, similar to lung lipids, and they are mostly double-tailed phospholipids, with C16 and longer alkyl chains. Rationally, one would assume that the results obtained for meibum lipids, devoid of surface-active components such as phospholipids, and, above all, in a form of monolayers, are not pertinent or useful for elucidating behavior and stability of an averaged 60-nm thick ocular tear-lipid films in vivo. The advantage of sessile-bubble technique, specifically, using a small amount of lipids required to attain multilayered films, unlocks the prospect of evaluating and comparing the interfacial properties of human tear lipids collected from a single individual, typically 100–150 µg. This is in sharp contrast with several milligrams of lipids that would be required to build equally thick films for Langmuir-trough experiments. The results of our studies provided in-depth understanding of the mechanisms responsible for properties and stability of human tear-lipid films in vivo. Here we summarize recent publications and our latest findings regarding human tear-lipid interfacial properties, their chemical composition, and their interaction with model proteins mimicking the proteins found in human tear-aqueous phase.

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Contents

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1. Introduction

1.1. Tear film structure and functions

Most of the reactions and interactions in biological systems occur at the interfaces, hence it is difficult to overestimate the impact of interfacial phenomena investigations on the advancement of knowledge and the development of new technologies in biology and medical applications [1,2].

Human tears are seemingly a simple, clear, slightly salty fluid; however, they are in fact extraordinarily multifarious biological colloid systems. Tears are essentially similar in the multitude and complexity of their components and functions to blood serum and lymph. The structure and functions of these films are not fully explored and understood because of huge variability and complexity of their properties [3–12].

A stable tear film, retaining uniform thickness over the entire corneal surface between eye-blinks, is critically important for acute vision and ocular-surface health [3,7]. In a healthy eye, human tear-film composition is precisely balanced to maintain optimal functions [2–10]. The tear-film thickness typically ranges from 3 to 10 μm, depending on individual variations among the subjects and method of measurements [3, 11]. The aqueous part comprises approximately 90% of its total thickness.

A schematic representation of tear film [3] is shown in Fig. 1.

There is a diffuse mucin-enriched layer adjacent to the Glycocalix, and next to it a tear-aqueous layer. The outermost oily layer is tear-lipid film. According to Dilly, [3], the aqueous layer for humans is about 7-μm deep. Normally, the lacrimal glands produce most of the tear-aqueous phase. The important functions of tear fluid are to provide a lubricating layer between the moving surfaces of the eye and eyelids, to remove foreign materials or particles, and to nurture the corneal and conjunctival epithelia.

Tear aqueous contains 0.9–1.0 wt.% of inorganic salts, akin to lymph and blood plasma, and up to 7 mg/ml of the numerous proteins, including a vast variety of enzymes, immunoglobulins, glycoproteins, etc. The total number of protein-species identified in human tears has reached 1543 and is still growing [13–15]. Lysozyme (2–4 mg/ml), lactoferrin (1.2–3 mg/ml) and lipocalin (1–2 mg/ml) are found among the most abundant proteins [14].

When an eye is deliberately kept open for a prolonged period without blinking, which naturally occurs instinctively once every 5–7 s, the tear film eventually breaks. The most popular method for clinical evaluation of human tear stability involves instillation of few microliters of sodium-fluorescein solution into an eye and subsequent monitoring of the tear-film breakup as viewed by a biomicroscope with dye-specific filters. Alternatively, a non-invasive method requires a projection of evenly-spaced placido-rings onto the ocular surface. The representative images of a stable tear film with instilled fluorescein (a) and a tear film with placido rings projected onto its surface (b) are shown in Fig. 2. The

placido-ring based topographers work by assessing the reflection of a concentric set of alternating white and black rings, printed on placido-disk, from the convex surface of the anterior cornea. Human tear film is considered stable if a breakup time is ≥10 s.

The breakup time is defined as the time it takes after a blink to observe the first appearance of a dark spot after instilling fluorescein dye in the eye or the first appearance of distortions or breaks on the reflected placido rings.

1.2. Functions of tear-lipid layer

Intricate mixtures of oily substances, known as tear lipids, comprise the outermost layer of human and other mammalian's tear films. These films contain a considerable variety of components, such as non-polar and polar lipids, secreted mostly by meibomian and probably, in part, by lacrimal glands [6,10,12,16–19]. The meibomian glands (or tarsal
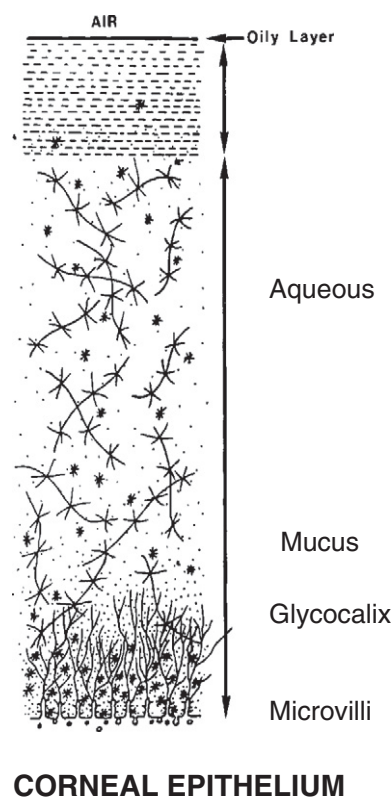

Fig. 1. Diagram of the structure of the tear film. The film is 30–40 μm thick. The immunoglobulins are represented by stars, and the glycoproteins (mucins) by the long lines with crosses [3] @ Springer 1994.
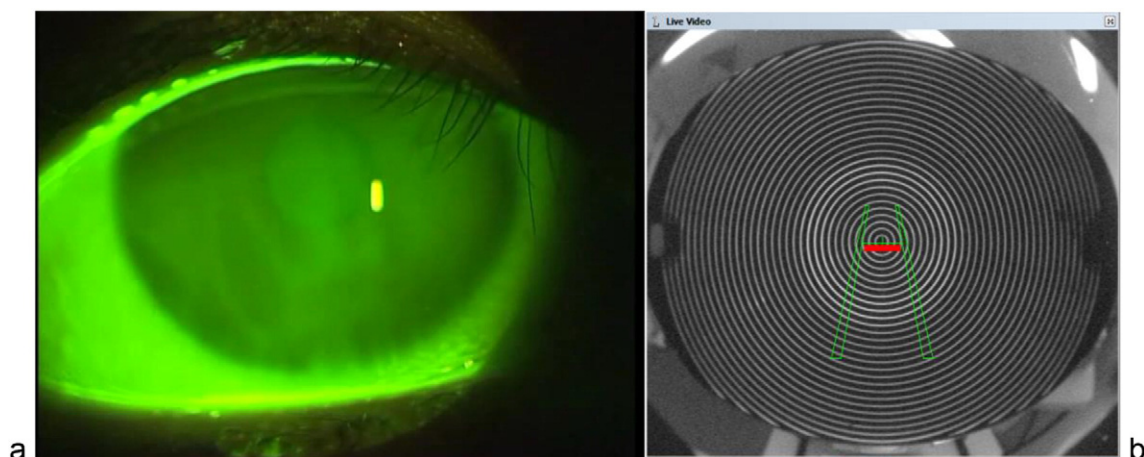
Fig. 2. Images of a stable tear films with fluorescein dye (a) and placido rings (b).

glands) are a special kind of sebaceous gland in the eyelids, responsible for the production of meibum.

The tear-lipid film plays vital roles in maintaining ocular surface homeostasis by facilitating re-spreading of whole tear film over the cornea after a blink, and by retarding tear-aqueous evaporation [3,10, 12,16–22]. Bron et al. [22] summarized the major functions attributed to tear-lipid layer as follows:

1. To spread over the aqueous sub-phase, lower free energy, and impart stability to the tear film
2. To thicken the aqueous sub-phase by Marangoni effect
3. To retard evaporation
4. To provide a smooth optical surface for the cornea
5. To provide a barrier against foreign particles
6. To provide some anti-microbial activity
7. To seal the lid margins during prolonged closure.

In a recent review [23], the Marangoni effect, mentioned above as one of the possible tear-lipids functions, was criticized and practically dismissed as an important factor based on the premise that an aqueous-air interface has to be exposed and the tear-lipid film has to be broken in order for the Marangony flow to take place. The results of our studies of tear-lipids dynamic interfacial properties suggest [24–26] that Marangony flows do take place at the interface between air and lipid film due to surface-pressure gradients arising when lipid films are irregularly stretched or compressed during blink. The surface-pressure gradients between thick and thin lipid-film areas cause the Marangony flows in tear-lipid films and consequently, the viscous drag between lipid film and tear aqueous, which in turn creates flows in tear-aqueous fluid. Thus, the tear-aqueous layer tends to thicken when the stretched areas of lipid films begin to condense and tear aqueous moves to fill in the thinner regions of tear-lipid film. A true-to-life example of such an event has been captured by King-Smith et al. [27] and is illustrated by Fig. 3. The images on the left represent fluorescein-stained tear-aqueous film while the images on the right are of tear-lipid film made visible by using spectral interferometry. These images demonstrate that there is a movement in the lipid layer causing gaps of the thinner areas to shrink. The lipid-film shrinking causes noticeable thickening of underlying the tear-aqueous layer seen as the area of substantially increased fluorescence intensity.

2. Lipid sampling and composition

2.1. Methods of tear fluid, meibum and tear-lipid collection

As emphasized in the late 1980s, there is "no typical meibum composition" and it varies greatly among human subjects [8]. The same is likely true for human tear lipids. The major differences between meibum and tear-lipid compositions and properties are linked to collecting samples from different sources using different methodologies [28,29]. To obtain meibum-lipid samples, meibomian gland secretion is expressed manually by applying gentle pressure on a cleaned lower eyelid, and then collecting the exuded meibum lipids using glass capillary or spatula. Tear aqueous and tear lipids are collected in less invasive or forcible ways by imbibing several microliters of whole tear fluids either into glass capillaries or sterile strips of filter papers, so-called Schirmer strips. Then the lipids from collected strips are extracted by mixtures of organic solvents [29,30]. Tear lipids can also be extracted from worn soft contact lenses [30]. Lam et al. [29] have shown that Schirmer-strip collection provides significantly (~10 times) higher yield of human tear lipids than samples collected using glass capillaries.

Recently, it was shown that the chemical composition of human meibum lipids is substantially different from the composition of human tear lipids, although the amount of data on human tear-lipid composition is not plentiful [31–37].

As one can see from the diagrams (Fig. 4), there are striking similarities between meibum and tear lipids regarding non-polar parts of lipids. However, human tear lipids contain $12 \pm 7\%$ of highly polar and amphiphilic lipids, such as phospholipids and sphingolipids, and $4.4 \pm 0.6\%$ of hydroxyl fatty acids [32].

In a recent paper [29] it was reported that HPLC/MS-based approaches were very successful in the comprehensive, qualitative, and comparative characterization of human whole tears and meibum lipidomes from individual subjects. The analysis revealed that human whole tears actually contain more than 600 individual lipid species from 17 distinct lipid classes. The authors also established, for the first time, that there is approximately 0.1–0.15 mol% of novel lipid amphiphile, cholesteryl sulfate, in the human tears and meibum. The authors suggested that this lipid might have considerable impact on the lipid-film properties. They also convincingly demonstrated that tear-fluid collection using Schirmer strips provided tear lipids containing 6–8 mol% of phospholipids [29]. Note that the majority of this study cohort was Chinese. That might be the reason why there is such a significant difference in total phospholipids content detected in this study in comparison with the previously shown data on Fig. 4. However, both studies have presented cogent proof that human tear lipids are significantly enriched by phospholipids and other polar components in comparison with corresponding (patient-matched) meibum [32].

The major representatives of lipids identified in human tears have been reported in [33–37] along with the quantitative data obtained for Asian subjects in [29], are summarized below in Table 1.

Here some examples of polar lipid chemical structure are depicted:

As one can see, most of polar lipids are natural, double-tailed, highly surface-active, molecules. The amount of lyso-phospholipids, single-tailed products of initially double-tailed phospholipid enzymatic
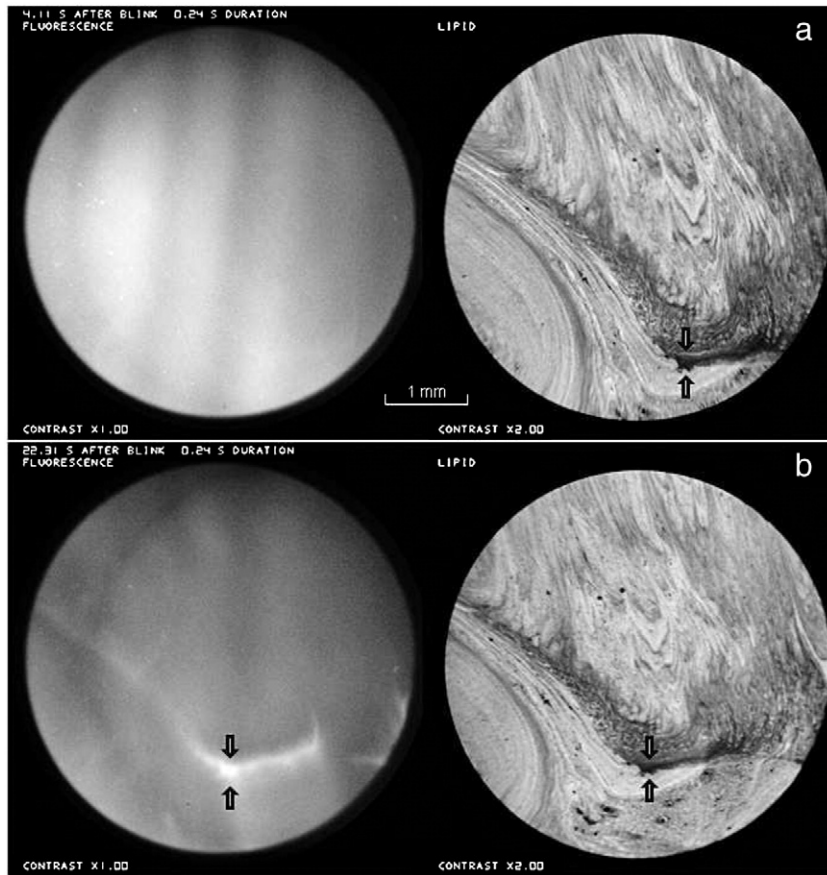
Fig. 3. Contraction of the thinner lipid between the arrows, in the 18.2 s between (A) and (B), causes thickening in the aqueous layer, with a corresponding bright streak between the arrows, in the fluorescence image in (B). Healthy tear film, 25-year-old white male [26] ©The American Academy of Optometry 2013.

hydrolysis, in tear aqueous was less than or comparable with the amounts of their original double-tailed counterparts. When double-tailed surfactant molecules are compared with single-tailed molecules, for the same equilibrium area per head group a_e, the double-tailed molecule has a packing parameter $v_o/a_e l_o$ twice as large as that of the single-tailed. Thus, double-tailed surfactants self-assemble into bilayers and vesicles while the corresponding single-tailed surfactant aggregates into spherical or globular micelles. [38]. It is also well known that double-tailed surfactants are more surface active and reduce surface tension to lower values, 22–24 mN/m, in comparison with 30–35 mN/m typical for single-tailed hydrocarbon surfactants.

More detailed information regarding classification, content, and importance of polar components in meibum and human tear lipids is available in recently published reviews [36,37]. From a surface and interfacial chemistry point of view, it is obvious that the interfacial behavior of meibum-lipid monolayers should be very different from that of 60–100 nm thick tear-lipid films enriched with polar components, both under in vivo ocular and in vitro experimental conditions. Our pilot study has shown that the dynamic interfacial behavior of tear-lipid films was indeed distinctly different from the behavior of films formed using meibum lipids collected from the same individuals [39]. These observations suggest that the results of meibum monolayer studies [40–44] can provide only circumscribed insights for elucidating mechanisms responsible for tear-film stabilization.
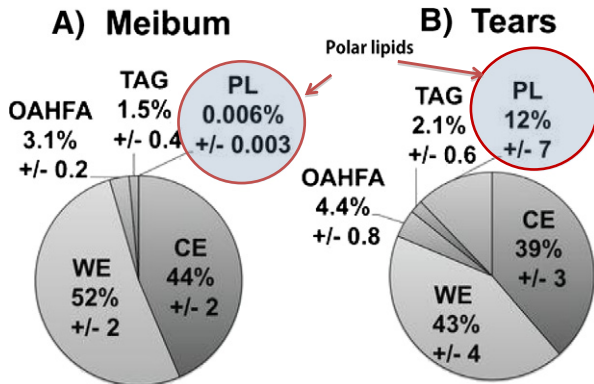

Fig. 4. A comparison of patient matched meibum and tear lipidomes [31] ©The American Academy of Optometry 2013.

Table 1
The major representative classes of lipids found in human tears.

Neutral lipids		Molar % of total [28]
Cholesteryl esters	ChE	44.82
Wax esters	WE	35.2
Tricylglycerides	TAG	2.8
Diacylglycerides	DAG	0.3
Free cholesterol	Ch	5.9
Sphingolipids		
Sphingomyelins	SM	1.5
Ceramides	Cer	0.26
Phospholipids		
Phosphotidylcholines	PC	2.96
Phosphotidylethanolamines	PE	2
Phosphotidylserine	PS	0.57
Phosphatidic acid	PA	0.14
Phosphotidylinositol	PI	0.76
O-acyl-ω-hydroxy-fatty acid	OAHFA	2.5
Cholesteryl sulfate	CS	0.14

SM

Structure of predominant species

Cer

Structure of predominant species

PC

Structure of predominant species

PE

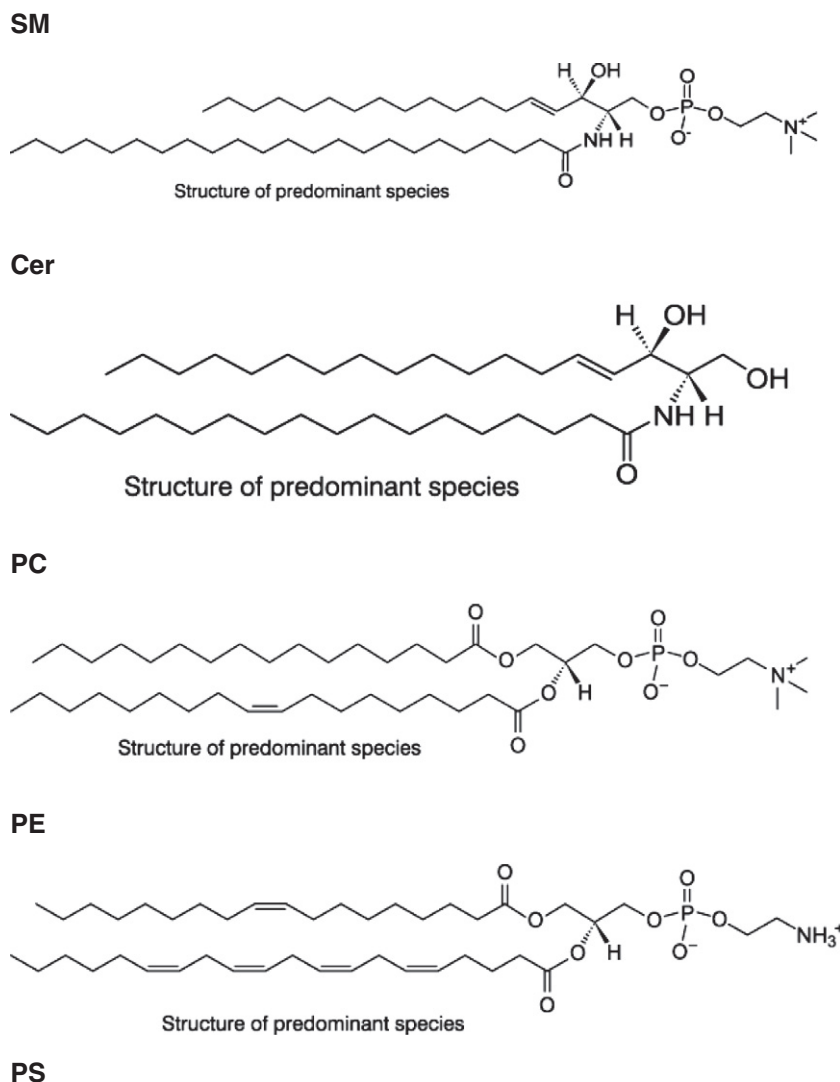

Structure of predominant species

PS

Fig. 5. Chemical structures of the representative molecules belonging to different classes of lipids found in human tears.

3. Tear lipids and protein interfacial properties

Tear-film instability and ruptures can cause ocular surface irritation or inflammation and dry eye symptoms. Therefore, understanding the mechanisms responsible for tear-film instability is of public health significance, as dry eye is one of the leading ocular complaints that clinicians encounter [45].

Tear-lipid layer interfacial properties, specifically interfacial elasticity and viscosity, are key factors affecting tear-film stability [22–26]. The interactions between tear-lipid films and tear-aqueous constituents (e.g., natural tear proteins, ingredients of topical ophthalmic medications and/or lens-care solutions) are also important factors and may influence overall tear-film stability [40–44]. Systematic data regarding physico-chemical and interfacial properties of human tear-lipid film in the range of natural ocular thicknesses are limited [24–26] since most of the studies were focused on interfacial properties of human or animal meibum lipids spread on the aqueous phase in a form of monolayers.

3.1. Lipid sample preparation and quantification

At the beginning of our studies, we used human tear lipids extracted from lotrafilcon A contact lenses (Focus® Night & Day™, CIBA Vision Corp., Duluth, GA) worn continuously (during day and sleep at night, without removal and cleaning) for 1 month [24,25]. Focus® Night &

Day™ (FND) lenses were chosen because they are FDA-approved for continuous overnight wear. The FND lenses were inserted onto subjects' eyes and then collected by clinicians wearing powder-free examination gloves. The clinical examinations were conducted before and after lens removal to ensure that none of the subjects had shown any adverse consequences from continuous lens wear. The collected lenses were then rinsed in deionized distilled water, and excess water was removed with filter paper. Each lens was placed in a separate glass vial containing 4 ml of toluene + iso-propanol (5:1, v:v) solvent, sonicated for 15 min at medium power level. Then the lens was removed from the vial, allowing the solvent to evaporate under a vacuum at ambient temperature.

For our later studies of human tear-lipid interfacial properties [26, 39], human tears were collected using standard Schirmer strips — sterile 5×35 mm strips of filter paper routinely used in clinical practice for tear production evaluations. Four to six Schirmer strips were collected from each subject, then stored at −80 °C. The lipids were extracted from strips using 4 ml of chloroform:methanol (2:1 v/v) mixture in glass vials submerged in an ultrasound bath and sonicated for 15 min at a medium level.

We also collected meibum lipids and whole tear samples [39] using microcapillary tubes. Whole tear-fluid samples were collected by placing a glass microcapillary tube in the pool of tear fluid near the lower lateral corner of the eye (i.e., tear meniscus). The total amount of tear fluid
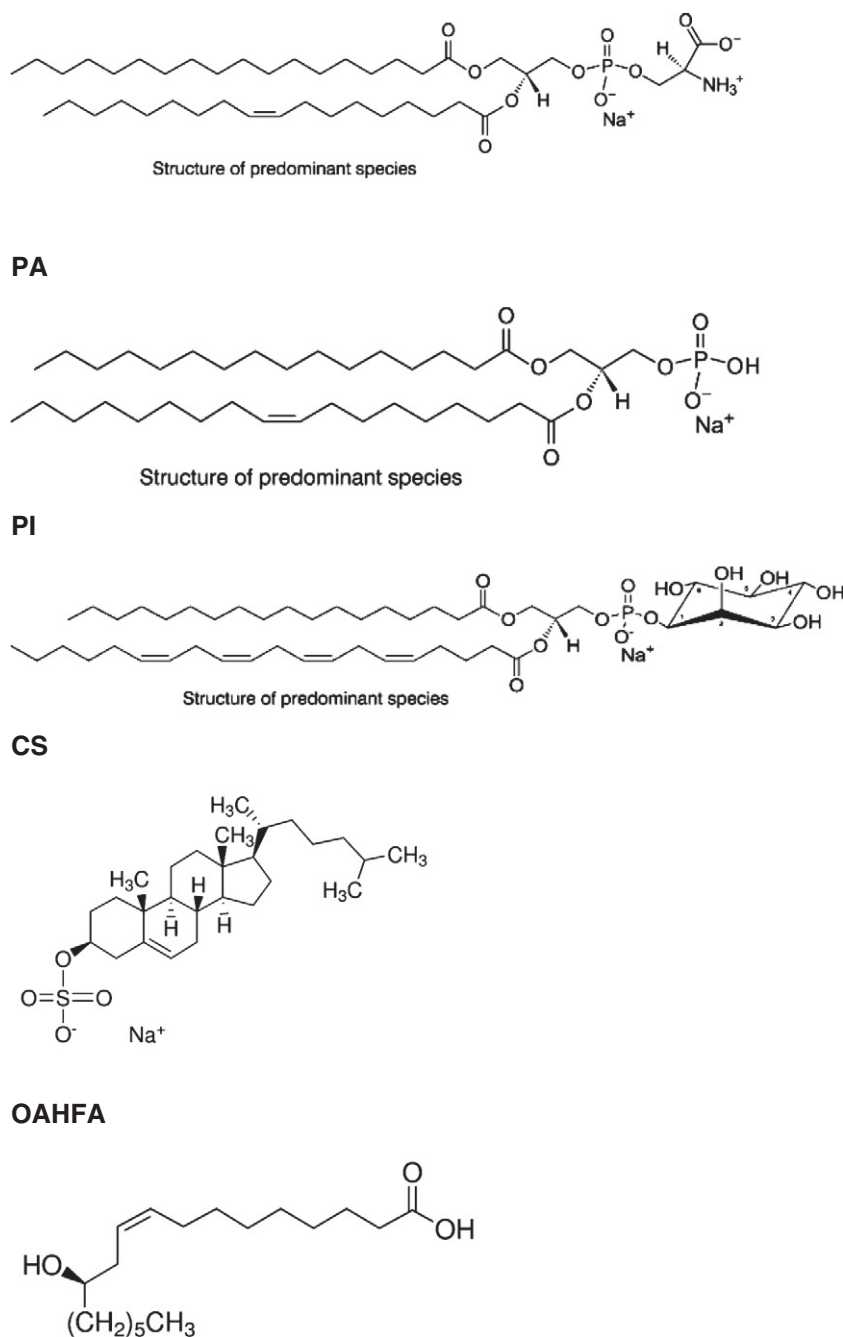
Structure of predominant species

PA

Structure of predominant species

PI

Structure of predominant species

CS

OAHFA

Fig. 5 (continued).

collected from two eyes was 15–20 μl per subject. Meibum lipids were expressed by gentle pressure applied with the index finger to the lower eyelids. The microcapillary tubes were placed directly in the small pool of freshly expressed meibum over the gland orifice and meibum lipids were drawn into the tubes. The collected samples were kept at −80 °C. Meibum lipids were extracted from glass tubes immersed in a mixture of 3 ml of chloroform + methanol solvent by sonicating for 5 min at the medium power level. Then the tubes were removed and solvents were evaporated under a vacuum at ambient temperature.

All lipid extracts, regardless of collection methods, were dried under a vacuum and stored in a freezer at −20 °C. The samples were re-dissolved in 100 μl of toluene + iso-propanol solvent prior to their de-position on the air–water interface for tensiometric and interfacial rhe-ological measurements.

An SE-400 Ellipsometer (Sentec Instruments GmbH, Germany) was used to measure the thickness of lipid films deposited on a silicon wafer with a 2-nm layer of native SiO_2, and these measurements were then used to calculate the total amount and concentration of lipids in reconstituted lipid-extract solutions. A 1-μl drop of reconstituted lipid extract was deposited and evenly spread over 3 different circular spots with area of 45 mm^2 outlined on a SiO_2 wafer. After complete evaporation of solvent, the thickness of the deposited lipid film was measured across the entire circular area. A total number of ellipsometer measurements were 75–100 points and the data were averaged for each of three spots and then between them. The variations in thickness for each film did not exceed 15%. The average thickness of the dry remnants left by pure solvent served as a baseline. Average thicknesses of wafer-deposited lipid films varied from 10 to 30 nm. Based on the results of ellipsometric measurements, we calculated the volume of lipid-extract

solution necessary to build an interfacial layer around an air bubble of a known surface area with a thickness comparable to ocular tear-lipid thicknesses.

Distilled and deionized water from a MilliQ® filter system (Millipore Co., Bedford, WA) was used for solution preparations. The aqueous phase in all experiments was a buffered model tear electrolytes (MTE) solution composed of 5 g/l NaCl (Sigma, USA) with 4 g/l of sea salts (Sigma, USA) added to provide other ions found in human tears (i.e., K$^+$, Ca^{2+} and Mg^{2+}, phosphate and bicarbonate). [46] The pH value of MTE solution was adjusted to 7.3 by additions of small amounts (0.5–0.8 ml) of 250 mM KH$_2$PO$_4$, so that the aqueous phase in our experiments simulated the salt content and pH of human tears. Proteins (Sigma, USA) and lens-care solutions listed in Table 2 were used as received.

3.2. Tensiometry

A sessile-captive bubble configuration (a small air bubble pinned to the underside of a straight hydrophobic capillary vertically immersed in an aqueous phase) was used to create an air–water interface and to examine interfacial properties of reconstituted ex vivo tear lipids. Similar techniques have been employed in studies of surface activity and protein–lipid interactions in pulmonary-surfactant systems [47].

A detailed description of the sessile-bubble with flow apparatus and technique has been published elsewhere [24,25,48,49]. The major advantage of this method over the widely used Langmuir-trough technique is very small interfacial areas (10–15 mm^2) of air-bubbles, requiring only approximately 1 µg of lipids to cover the entire bubble-surface with a 100 nm-thick multi-layered film.

A Ramé-Hart tensiometer (Ramé-Hart Instrument Co., Netcong, NJ, USA) with DropImage Advanced software, v.2.2, and an automated dispensing system was used for real-time surface-tension data acquisition. Fig. 5 displays the experimental setup. Tear-lipid extract containing 0.6–1.0 µg of lipids was deposited onto the surface of the air bubble from underneath, using an L-shaped needle and a 5 µl-high precision syringe (Hamilton Co., NV, USA). This amount of tear lipids was enough to form the film with initial thickness of 60–100 nm. The aqueous phase in the optical cell was continuously stirred to provide a uniform distribution of lipids at the air-bubble aqueous interface and to accelerate dissolution of solvents into the aqueous phase. The aqueous phase was then displaced with 250 ml of MTE solution at the flow rate of 2–4 ml/min to remove traces of organic solvents. After the solvents were washed out, the bubble coated with the lipid film was left to equilibrate for 17–24 h with stirring. Interfacial tension was monitored during each of these steps and the bubble surface area was kept constant during equilibration processes. Interfacial rheological properties were then measured. The schematics of sessile bubble setup are depicted on Fig. 6.

Surface-pressure-vs-film-thickness iso-cycles were recorded for film thicknesses ranging from 5 to ~120 nm and surface pressures

ranging from 2 to 50 ± 2 mN/m to evaluate the compressibility, reversibility, and extent of compression-expansion hysteresis. Analogous to the bulk compressibility, the compressibility of monolayers is defined as.

$$C = -1/A(dA/d\Pi) \qquad (1)$$

where A is the area per molecule. Thus the compressibility properties of Langmuir monolayers can be determined in a simple way from the slope of the Π–A isotherms [50]. In our case of thick multilayered films, we characterized compressibility C as the slope of Π-h, surface pressure vs. film thickness isotherms.

Our studies focused on tear-lipid multilayers in a wide range of film thicknesses (i.e., 5 to ~120 nm) and surface pressures (i.e., 2 to 50 ± 2 mN/m) as well as quantitative analysis of the Π-h isotherms. In contrast to our experimental conditions, previous studies of interfacial properties of meibum-lipid monolayers [40–44] with Langmuir trough reported surface pressure as a function of the film area at surface pressures not exceeding 35 mN/m, film thickness at 2 nm at most, and un-quantified lipid-film dynamic interfacial properties.

The examples of surface-pressure-vs-film-thickness iso-cycles obtained for one of human tear-lipid samples, measured using three different bulk-flow rates, thus, corresponding to different surface deformation rates, are presented in Fig. 7.

As one can see from this plot, there is a reasonable agreement between the curves representing dynamic surface pressure as a function of lipid-film thickness at surface-deformation rates between 0.1 mm^2/s and 0.2 mm^2/s. The bulk-flow rate of 8 mm^3/min, corresponding to 0.1 mm^2/s surface-deformation rate, was typically used in our experiments unless specified otherwise.

3.3. Interfacial rheology

A dilation step-strain technique was used to study the interfacial dilatational visco-elastic properties of lipid layers. The air bubble, previously coated with lipids and equilibrated for 17–24 h, was expanded or contracted very fast within 0.2 s, so that its change in surface area (ΔA) was 5–7% of the initial surface area (A_0). The process of interfacial tension relaxation after surface perturbation was monitored and recorded by the DropImage software for 30 min. This time span was typically sufficient for the interfacial tension to reach a nearly constant value. The decay over time of the transient elasticity ($E(t)$) was then determined as:

$$E(t) = A_0 \Delta\gamma(t)/\Delta A \qquad (2)$$

where A_0 is the initial bubble surface area (mm^2) and $\Delta\gamma(t)$ (mN/m) is the change in surface tension induced by the change in the surface area [49,51–55]. Lipid layers studied in this project were ~50 times thicker than a monolayer and 5–8 times thicker than the reconstituted lipid layers we studied in our earlier publication [24]. To quantify their

Table 2
Lens care solutions and their ingredients.

| Brand name	Surface-active components	Equilibrium surface tension, mN/m (1:10 diluted)
Clear Care,(CC) CIBA Vision	Pluronic 17R4 (polyoxiethylene–polyoxypropylene block copolymer) (3% H$_2$O$_2$ for disinfection)	43.6 ± 0.15
Biotrue (BT), Bausch& Lomb	Poloxamine (polyoxyethylene-polyoxypropylene block copolymer), sulfobetaine (zwitterionic surfactant), hyaluronane, and edetate disodium. Preservatives: Polyaminopropyl biquanide, 0.00013%, Polyquaternium-1 −0.001%	38.3 ± 0.17
Revitalens (RL), Abbott	Tetronic 904 (polyoxyethylene-polyoxypropylene block copolymer with amino-groups), edetate disodium. Preservatives: alexidine dihydrochloride −0.00016%; Polyquaternium-1 −0.003%	36.5 ± 0.18
Pure Moist (PM), Alcon	Tetronic 1304 (polyoxyethylene-polyoxypropylene block copolymer with amino-groups), HydraGlyde (EOBO-4™ polyoxyethylene–polyoxybutylene block copolymer) Preservatives: POLYQUAD -0.001%; ALDOX (myristamidopropyl dimethylamine) 0.0006%	32.8 ± 0.23
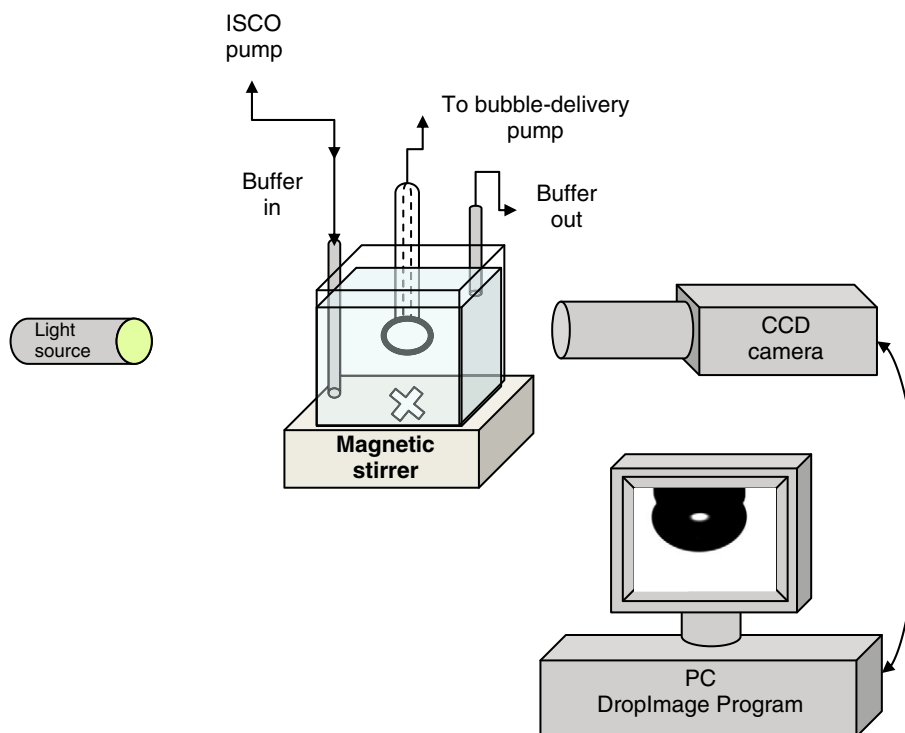
Fig. 6. Schematics of sessile bubble tensiometer with bulk phase exchange [24–26] ©The American Academy of Optometry 2010.

response to dilatational perturbations, these thick layers required an approach different from the technique used previously — single-exponential decay fits. Here, we adopted a combined Maxwell visco-elastic and diffusion-relaxation model used earlier [25,26,48,49,51–54] to describe dilatational behavior of mixed polymers and surfactants thick layers.

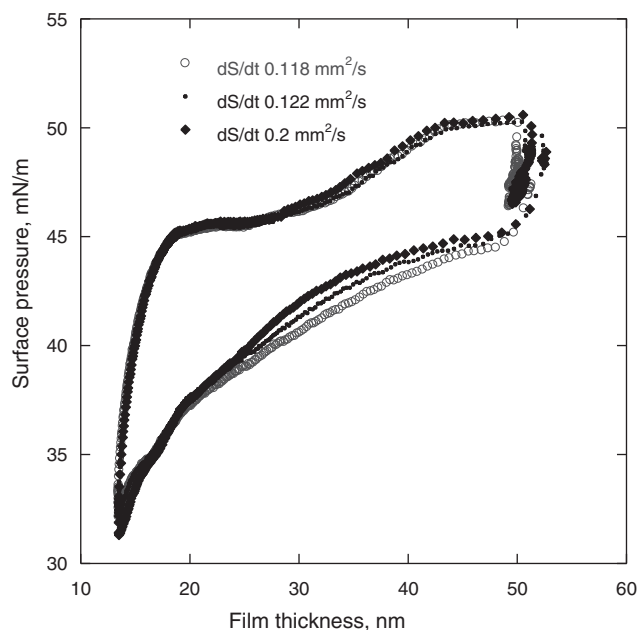$$E(t) = E_\infty + A_M \exp(-t/\tau_M) + A_D \exp(2t/\tau_D) erfc \sqrt{2t/\tau_D} \qquad (3)$$

Fig. 7. Dynamic surface pressure as a function of lipid-film thickness at three different surface-deformation rates.

where the first two terms on the right account for visco-elastic contribution in the relaxation of an interfacial layer and the last term reflects diffusion of active species from a polar lipid surface interface into the bulk layer during compression and diffusion in the other direction when the film is expanded. τ_M and τ_D represent characteristic times for visco-elastic relaxation and diffusive exchange, respectively. E_∞ is elastic modulus of the interface at time $t \rightarrow \infty$, and A_M and A_D are constants characterizing the relative contributions of visco-elastic and diffusion mechanisms into transient elastic modulus $E(t)$, respectively.

4. Comparison of tear lipids, meibum, and whole human tear interfacial properties

4.1. Equilibrium surface tension of whole-tear-aqueous and reconstituted multilayered tear-lipid films

As a first step in our studies of human tear lipids, we assessed surface tension of the reconstituted lipid films as a function of lipid-layer thickness. Fig. 8 shows that the surface tension of lipid films reduced from approximately 32–35 to 22.5 mN/m as lipid-layer thickness increased from 2 to 20 nm. Surface tension did not significantly change with further thickening of the interfacial lipid layer. The minimum surface tension, 22.5 ± 0.8 mN/m, is consistent with the values reported for lung surfactants and condensed phospholipid monolayers [47,53,56].

Cerrano in 1910 [57] was the first to measure stimulated calves' tears' surface tension in vitro and that was close to surface tension of pure water at 72.3 mN/m. Miller in 1969 measured human tears' surface tension in vivo and reported a value of 46.2 mN/m [58]. In the late 1980s [8], surface tension of pooled human tears was measured by employing Ferguson and Kennedy method (a version of maximum pressure technique) and was found to be between 42 and 46 mN/m for healthy asymptomatic subjects, but rose to ~56 mN/m after lipid extraction. These tension values were significantly higher than what was measured with reconstituted lipid films reported in Fig. 8 even at the lowest film thickness of 2 nm. There are several possible reasons for this disparity, one being that the method used in these studies did not allow for
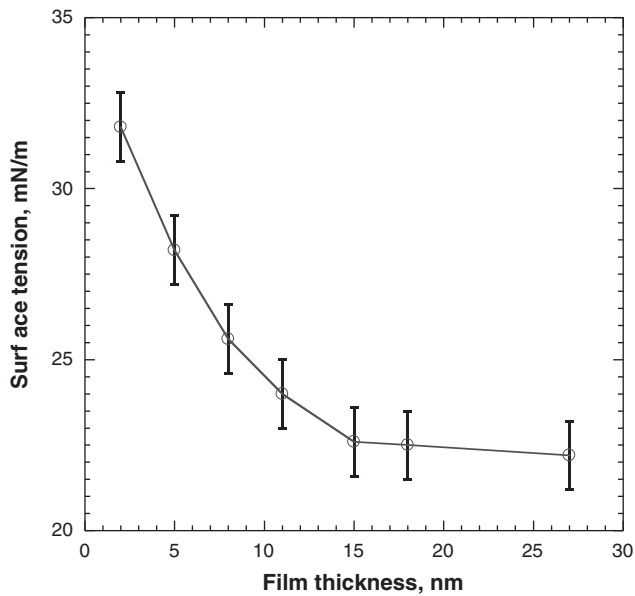
Fig. 8. The surface tension of the reconstituted ex vivo lipid films as a function of the film thickness [24] ©The American Academy of Optometry 2010.

long-term measurements and aging of the interface since the liquid sample isolated in a glass capillary would eventually evaporate. Applying the sessile-bubble method, we measured the surface tension of model proteins (e.g., lysozyme, Bovine Submacillary Mucin (BSM), and Human Serum Albumin (HAS) [25]) and found that surface tension of the protein solutions strongly depended on the age of the interface and would reduce significantly after several hours of aging [59]. Fig. 9 shows typical examples of surface tension *vs.* time dependence for 2 mg/ml lysozyme, 0.03 mg/ml BSM and 0.1 mg/ml HSA solutions (these concentrations are close to the concentration of analogous proteins in the tear-aqueous phase) in the presence of model tear electrolytes measured over 20 h of equilibration at 35 °C.

Surface tension of these model proteins continued to decrease for at least 4–6 h and reached 48–50 mN/m for lysozyme, 48 mN/m for BSM,
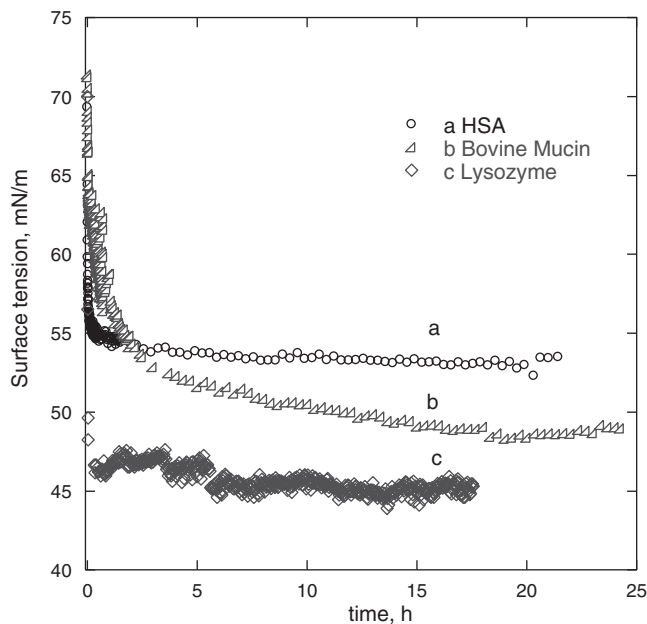
and 52 mN/m for HAS at 22 °C. These values of surface tension were 4–6 mN/m lower than what would be found at the physiological temperature of 35 °C. It is likely that the previously reported values of 42–46 mN/m [5,6,42] as surface tension of whole tears do not reflect the actual value, as they are very close to the tension of protein solutions alone, without any lipids. It is indeed the case, as one can see from Fig. 10, where the surface tension is presented as a function of interface age for two samples of whole tear fluids.

Surface tension of two whole-tear samples was measured using the sessile drop technique. The drop was formed at the top of hydrophobic capillary, in a sealed glass cell containing MTE solution with RH 90% to minimize tear-drop evaporation during measurements, and the volume of these drops was kept constant. It is clearly seen that surface tension dynamics were very different for these two samples — one equilibrated within 4–6 h similar to the lysozyme solution shown on Fig. 9, compared with the other human whole-tear samples with its tension continuing to decrease even after an aging period of 22 h. Fig. 11 summarizes our results for static surface tension of whole-tear samples collected from 8 individuals after 24 h of aging.

There were significant variations in static surface-tension values for these samples. For one sample, the tension dropped down to 22 mN/m, which was typical for condensed phospholipid monolayers and tear-lipids thick films, while for the other samples it remained high, up to 48 mN/m. The average value was 38.7 ± 8.2 mN/m, which was lower than previously reported values [8,58] The reason for these variations is most likely an uncontrolled and unknown amount of surface-active tear lipids in a capillary tube during tear-fluid collection. Taking these rationales into account, all of our studies were conducted with a known amount of lipids deposited at the interface, and with lipid films aged for 16–24 h.

The aging of lipid films was found to be an essential step in experimental procedures because dynamic properties continued to change within 12–16 h of equilibration. The measurements of the monolayer equilibration times for saturated diacyl phospholipids at the water–air interface conducted with the Wilhelmy plate suggest that for phospholipids with longer acyl chains (longer than 12 carbons), monolayer equilibration required more than 1 day [59]. This is the most probable reason for the discrepancies found among literature values reported as "equilibrium surface tension" of phospholipids, especially for monolayers

Fig. 9. Lysozyme,2 mg/ml, BSM,0.03 mg/ml, and HAS, 0. 1 mg/ml, solution surface tension histories for 20 h [42].
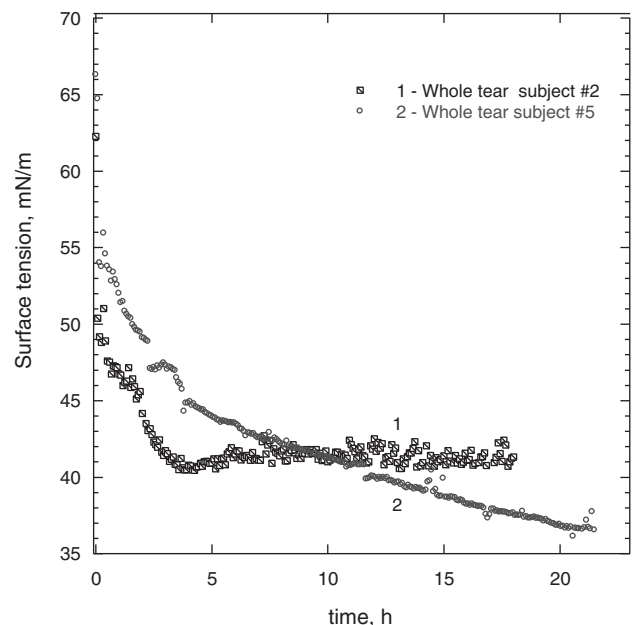

Fig. 10. Surface tension history for two samples of human whole tear fluid [39].

Fig. 11. Summary of static surface tension values for samples of human whole tear fluids.

oil–water interfaces and the non-polar part comprises the major bulk of the deposited film. The other purpose of aging in our experiments was to allow lipid films to equilibrate with proteins or other ingredients introduced into the aqueous phase.

4.2. Interfacial tension dynamics

Our first experimental studies of human tear lipids were conducted using tear lipids extracted from worn contact lenses. How well these films composed of contact-lens-deposited lipids represented actual human tear-lipid films and whether these films were similar to the films formed by meibum lipids remained unclear. An ad hoc video-microscopic experiment provided images of thick films formed using meibum lipids and tear lipids extracted from worn lenses. The ~100 nm lipid films were spread on the surface of the MTE solution in a mini-trough with a surface area of 45 cm^2 at the ambient temperature of 22 °C. Fig. 13 shows images of thick films formed by (a) cow, (b) human meibum (samples courtesy of Dr. C. Cerretani, UC Berkeley, Department of Chemical and Biochemical Engineering), and (c) pooled lipid samples extracted from 10 lenses worn by human subjects.

These images demonstrate substantial differences in appearance and uniformity in the films constituted from meibum lipids and human tear lipids. Images of meibum films displayed intricately shaped areas with rainbow-like coloration and some dark spots, all together indicating that meibum films were of uneven thickness and unstable. Reconstituted tear-lipid film was much more uniform in color and thus in thickness. It is remarkable that reconstituted tear-lipid films were stable for over 6 h and exhibited neither dark spots, which would indicate film thinning and rupture, nor a combination of thicker islands formed by condensed liquid with some de-wetted areas, whereas meibum films formed island-like structures and eventually breakup. The differences in stability of these thick films may imply that contact-lens-extracted human tear lipids may better represent healthy in vivo tear-lipid films than meibum films, which eventually showed instabilities and breakup. These observed distinctions between meibum and ex vivo tear lipids may be related to a substantially higher level of amphiphilic lipids such as phospholipids, sphingolipids, and OAHFA found in tear lipids compared with meibum lipids [31,32,60]. For comparison with reconstituted lipid films, Fig. 13 also shows the images of human tear-lipid films in vivo, captured using LipiView® (TearScience®) used for interferometric evaluation of human tear-lipid film thicknesses in vivo. Lipid films observed in vivo do not look absolutely uniform; however, this image is much more similar to the picture of reconstituted human tear lipids than meibum lipids.

Saville et al. [31] have pointed out that phospholipids deposited on worn contact lenses show the molecular profile similar to that of tear lipids; however, the relative abundance of polar lipids might be higher in lens extracts. Fig. 14 demonstrates a comparison of expansion-contraction iso-cycles for two lipid samples from the same individual, one extracted from Schirmer strips and the other extracted from worn lenses.

As seen from these plots, the shapes of iso-cycles were different to some extent, but both samples reached the same maximum dynamic surface pressure 50 mN/m at approximately the same film thickness of 25–30 nm. This observation suggests that both samples should behave similarly when subjected to small step-strain deformations in a course of interfacial rheology experiments, provided that the films are of similar thickness above the transition point.

An example of meibum monolayer expansion–contraction curves obtained using Langmuir trough and pendant-drop techniques are shows below (Fig. 15).

As one can see, for meibum monolayers at low surface pressures, there is a reasonable agreement between the iso-cycles obtained using these two different techniques. Note that "equilibration" time for these measurements did not exceed 2 h because of complications related to aqueous-phase evaporation. Thus, it is difficult to compare these

formed at a temperature below gel-to-liquid crystalline phase transition temperature [59].

As seen from the examples of expansion–contraction cycles acquired for the same human tear-lipid sample after 4 and 24 h of interface aging (Fig. 12), dynamic interfacial pressure responses changed with interface aging as lipid-film continuously goes through restructuring. Specifically, the hysteresis between expansion and contraction branches became smaller and the entire cycle shifted toward the lower range of film thicknesses, suggesting widening of lipid-film stability region.

We stipulate that in the process of aging, the films that were initially deposited as randomly distributed mixtures of polar and non-polar components undergo re-organization and finally, during constant stirring of aqueous phase for 16–24 h at 35 °C, the proper film-structures emerged. In aged films, the polar lipids are adsorbed at the oil–air and
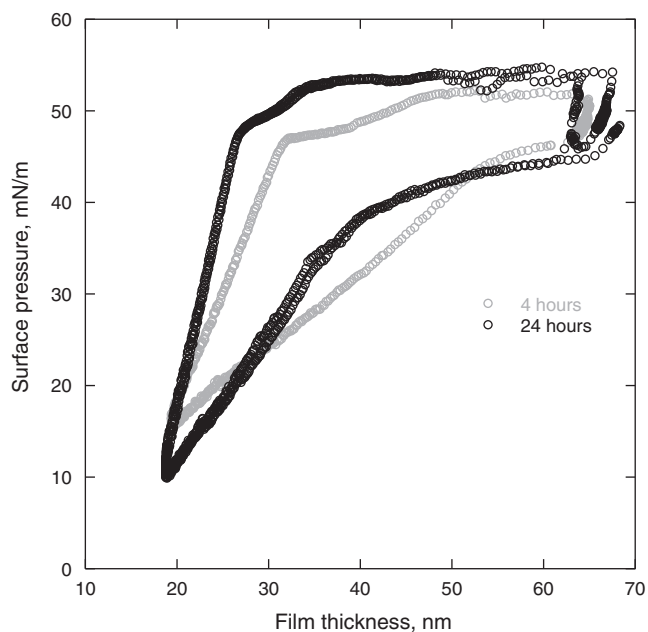

Fig. 12. The expansion-contraction iso-cycles for the same human tear lipid sample after 4 and 24 h of interface aging.

a) Cow meibum film   b) Human meibum film   c) Human *ex-vivo* tear lipids films
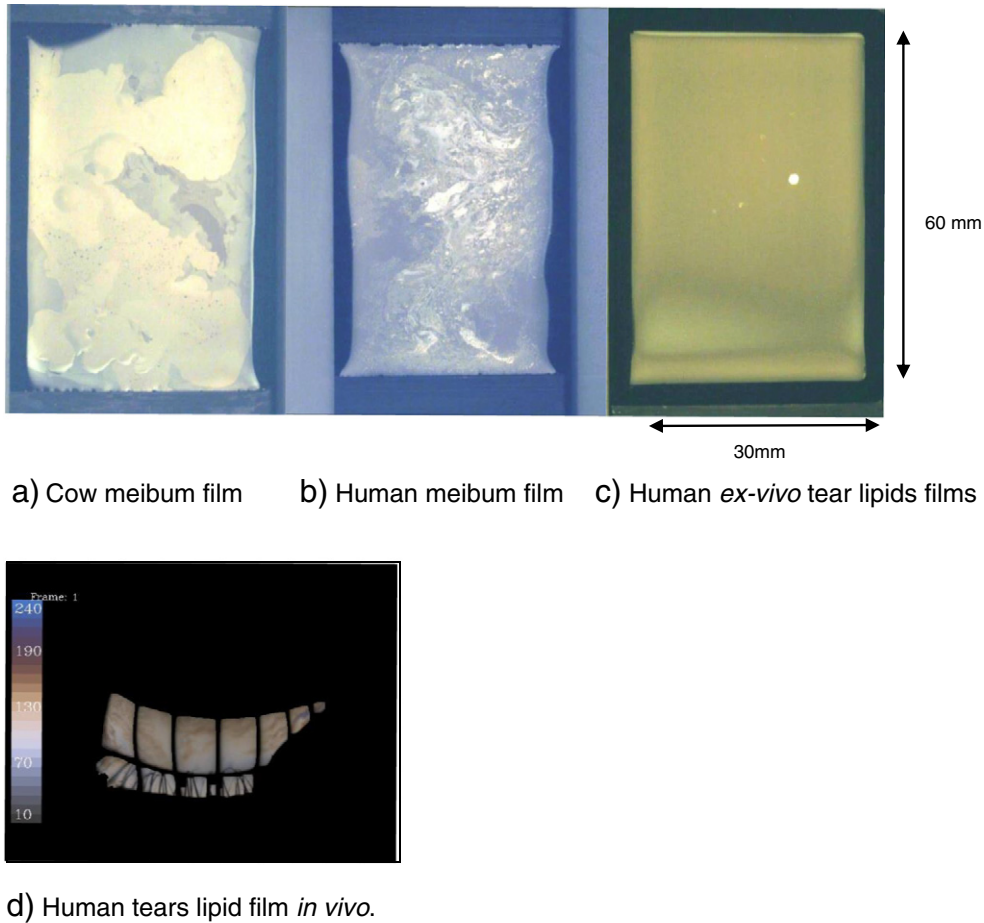
d) Human tears lipid film *in vivo.*

Fig. 13. Images of cow meibum, human meibum and FND-extracted lipid films and Lipiview photo of human tear lipid film in vivo [25]. ©The American Academy of Optometry 2013.
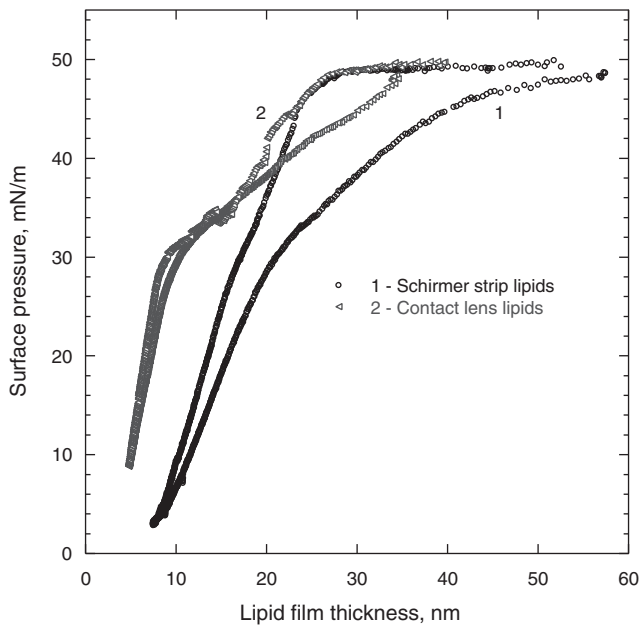
data with the expansion-contraction isotherms we obtained for thick lipid films, aged for 24 h at high surface pressure of ~48–50 mN/m, employing sessile bubble methodology.

The surface pressure vs film thickness isotherms for two lipid samples, meibum lipids(a) and tear lipids (b) collected from the same individual, are depicted in Fig. 16.

The differences between these two isotherms were remarkable: tear-lipid films reached the maximum surface pressure at a film thickness of ~25–30 nm, whereas for meibum lipids this happened at thicknesses above 60 nm, and the hysteresis between expansion and compression branches was much larger for meibum than was observed for tear-lipid films. Similar differences were observed for all pairs of

Fig. 14. The examples of expansion–contraction iso-cycles for two lipid samples from the same individual, (a) extracted from Schirmer strips and (b) extracted from worn FND lenses [25]. ©The American Academy of Optometry 2013.
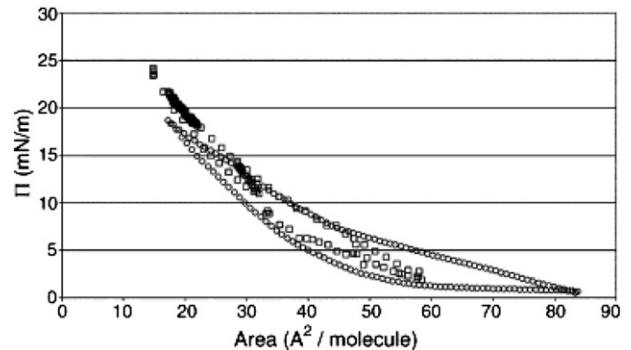

Fig. 15. Surface area–pressure plot of chloroform extract of bovine meibum over HBSS at 36 °C. Circles represent Whilelmy plate trough measurements, squares represent pendant drop measurements [40]. © Elsevier.
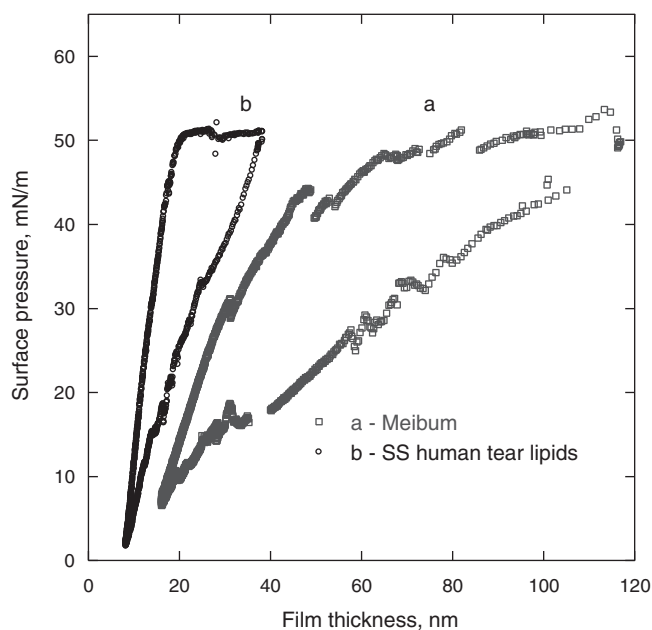
Fig. 16. Surface pressure vs film thickness isotherms for meibomian lipids (a) and tear lipids (b) collected from the same human subject [39].

Fig. 17. Surface pressure vs film thickness isotherms for tear lipid films formed using the samples collected from two different individuals, Asian and Caucasian [26]. © Elsevier.

meibum and tear-lipid samples collected from the same subject. As mentioned previously, tear lipids contain 12–16% of polar lipids, mostly phospholipids, whereas meibum lipids are practically devoid of phospholipids or have a very low concentration that is detectable by current technology. This lack of or a low amount of phospholipids is the most plausible explanation for the differences found in dynamic surface pressure between meibum-lipid and tear-lipid films at comparable film thicknesses.

In Fig. 17, we display representative examples of dynamic surface pressure as a function of film thickness for two tear-lipid samples, one from a subject of Asian ancestry, another from a subject of Caucasian decent. We studied 8 Asian and 7 Caucasian subjects, whose race-related differences in dynamic interfacial behavior of the tear-lipid films were found to be reproducible and statistically-significant [25].

As seen from this figure, the maximum surface pressure of 50 ± 2 mN/m was not subject-dependent; however, the differences in shape of iso-cycles for tear-lipid samples collected from individuals of different races were notable. At elevated pressures, Caucasian lipids exhibited greater slopes in both compression and expansion (lower) branches of these cycles and reached 50 mN/m maximum pressure at approximately 30 nm, whereas Asian lipid films had to be compressed to almost 55 nm-thick to attain the same high surface pressure. Nevertheless, inter-subject and inter-racial variations in tear-lipid film dynamic interfacial properties are significantly less pronounced than the differences found in the interfacial properties between meibum and tear lipids from the same subjects, as seen from Table 3.

Table 3 summarizes our findings regarding equilibrium and dynamic properties of meibum and tear-lipid samples from 10 individuals. One sample of each type of lipid was collected from each subject and the values of rheological parameters were averaged among 10 subjects. In Table 3, these numbers are compared with the rheological parameters of the lipids extracted from 10 worn FND lenses, also averaged. The results show that rheological parameters of lipids extracted from worn contact lenses were very close to those of the lipids extracted from whole tears absorbed by Schirmer strips. However, meibum samples exhibited significantly higher interfacial elasticity and viscosity than those of tear lipids. These findings suggest that the possible phospholipids deficit in meibum samples made meibum-lipid films more
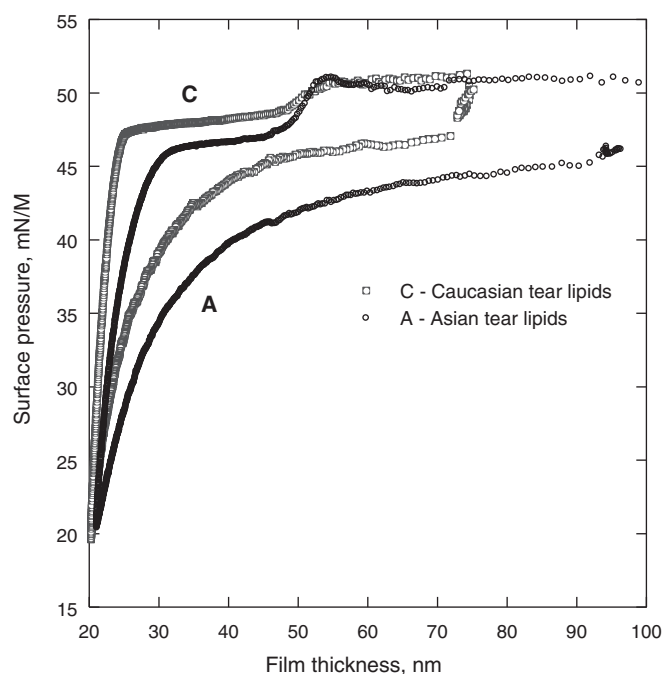
viscous, slower to relax, more irregular, and more unstable compared with tear-lipid films as shown in Fig. 13.

4.3. Effects of model proteins and lens-care solutions on tear-lipid interfacial properties

The human tear film is a very complex biological system and its composition must be delicately balanced in order to maintain its optimal functions [2–15]. The interactions between tear-lipid films and tear-aqueous constituents (e.g., natural tear proteins, or ingredients added in topical ophthalmic medications or lens-care solutions) are important and likely to influence overall tear-film stability [25,26,62]. Unfortunately, this intricate balance of essential components can be easily disrupted by many factors including contact lenses and lens-care solutions. For example, many lens-care solutions contain surface-active ingredients (i.e., surfactants) added to enhance their cleaning efficiency and lens-surface wettability (see Table 2). Some surfactants may destabilize the tear lipid layers by lowering the oil–water interfacial tension to nearly zero, which leads to emulsification of the lipid layer. All contact lenses significantly destabilize tear film as indicated by reduced pre-lens tear-film breakup time in comparison with pre-corneal breakup time. Contact lens wear is often discontinued because of gradual development of dry-eye symptoms. The concern regarding adverse effects of lens-care solutions on tear-film properties and ocular health has been raised in the literature [63–65]. Most market-available lens-care solutions contain significant amounts of surfactants added as either preservatives (e.g., Benzalkonium chloride, Polyaminopropyl biquanide, Polyquaternium) or cleansing and wettability-improving agents (e.g., polyoxyethylene-polyoxypropylene block co-polymers

Table 3
Interfacial rheological parameters of human meibum and tear-lipid extracts.

Lipids type	Equilibrium surface tension, mN/m	E_∞, mN/m	Relaxation time, s	Interfacial viscosity, N/m s
Meibum	26 ± 2.5	41.8 ± 23	302 ± 155	45 ± 2.2
SS lipids	22.4 ± 1.1	11.7 ± 3.5	124 ± 20	12 ± 0.277
FND Lipids	22.1 ± 0.9	12.4 ± 2.6	113 ± 28	10.3 ± 0.9

such as Tetronics™ or Pluronics™, Table 2). These substances adsorb and accumulate at the interface between tear-film lipids and aqueous layers [24,26]. Therefore, these surfactants introduced into an eye upon lens insertion can weaken already susceptible pre-lens tear film [63–65].

4.3.1. Effect of model proteins

Several studies [26–30] have explored monolayers of meibum lipids at relatively low surface pressures corresponding to a low surface coverage by polar lipids. Under these conditions, proteins were able to penetrate into lipid monolayer and reduce its surface pressure. Nagoyova et al. found that model tear proteins possessed significant surface activity. Using these and other results, the authors concluded that tear proteins were the major contributors to the surface pressure of the tear film [9,42].

However, as seen in Fig. 18, lysozyme injected into the aqueous phase surrounding a tear-lipid film did not significantly change the surface tension at the air-lipid film interface even after a long-time equilibration with continuous stirring. [62] The experimental evidence of tear-lipid interaction with lysozyme plus the formation of lipid-lysozyme complex was obtained using an interfacial rheology approach in combination with a washout option. We found significant differences in interfacial rheological parameters between human tear-lipid films alone and lipid-lysozyme mixed layers. These differences persisted even after a complete protein removal from the bulk phase, indicating that lysozyme was irreversibly bound to the lipid layer. At high surface pressures, the presence of lysozyme in the mixed interfacial layers was undetectable by surface tension measurements. However, it manifested itself by an approximately two-fold increase in mixed-film relaxation time when compared with that of a tear-lipid layer alone. The interfacial rheological properties of the reconstituted tear lipids in the thick mixed layers were much more sensitive indicators of their interactions with proteins than a static surface tension. Changes in interfacial rheological parameters after protein washout were most likely caused by the removal of loosely bound lysozyme molecules from the adsorbed interfacial layer, leading to increased interfacial viscosity.

Fig. 19 depicts π-S iso-cycles for proteins alone; here the surface pressure is plotted as a function of interfacial area.

The isotherms for these proteins differ significantly, and BSG mucin seemed to be the most surface active and its adsorbed layer could be compressed to as high a surface pressure as 47 mN/m, which was
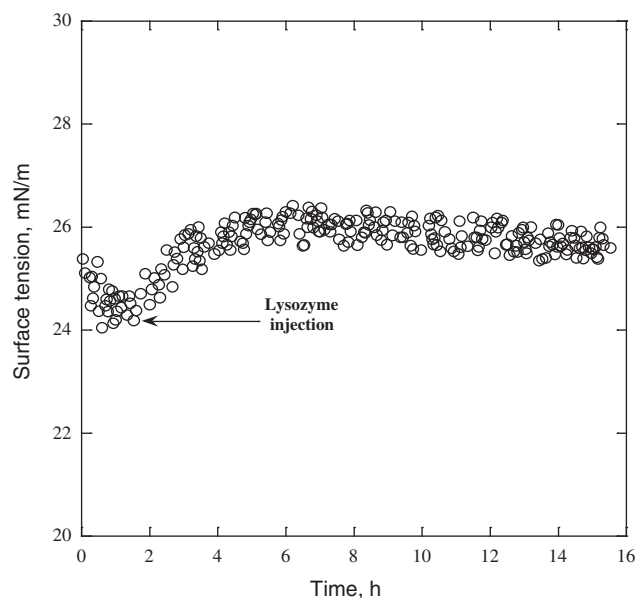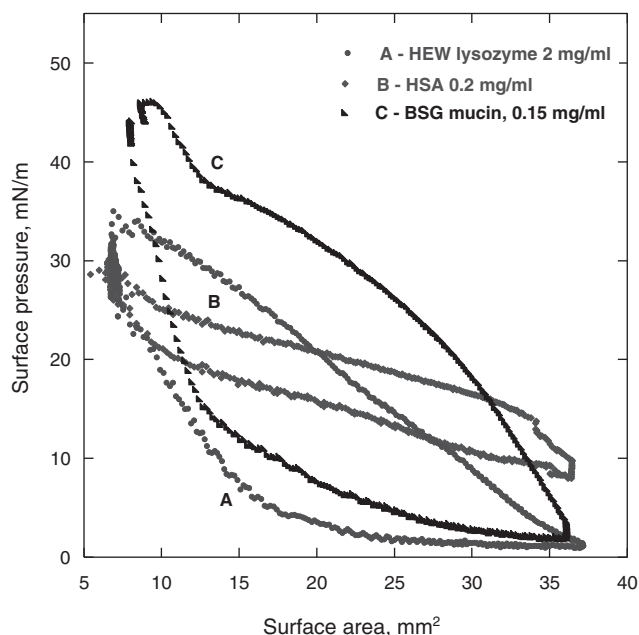

Fig. 19. The iso-cycles of proteins alone, BSM (a). HAS (b) and Lysozyme (c) [39].

comparable to the maximum surface pressure of phospholipids alone. The surface pressure was plotted as a function of interfacial area, not film thickness, because it was impossible to measure the amount of protein adsorbed thus the protein-layer thickness remained unknown.

The changes in interfacial behavior caused by protein-lipids interactions became even more evident when we acquired surface pressure *vs.* lipids + proteins film thickness data. Fig. 20 a–c shows the iso-cycles for several human tear-lipid films alone (curves 1), 16–20 h after lysozyme, HAS, and BSG mucin injections and equilibration (curves 2), and washout of the proteins from bulk solution (curves 3). Note that these curves were plotted as a function of lipid-film thickness, since the total thickness after protein adsorption was unknown.

For human tear-lipid film alone, the maximum surface pressure π_{max} was obtained at h value of approximately 30 nm during film compression; in the presence of BSG mucin, the same value of π_{max} was obtained at h value less than 20 nm. These changes persisted after the protein was washed out. The entire isotherm shifted toward lower thicknesses indicating an expansion of the lipid-film stability region. Interaction of human lipid films with HSA reduced the π_{max} of iso-cycles by 3–5 mN/m and shifted the curve slightly to lower film thicknesses. It also reduced hysteresis between expansion and compression branches. These changes persisted after HSA was washed out; however, they were not as strong as the changes observed after interaction with BSG mucin.

Interaction of tear lipids with lysozyme decreased the π_{max} of tear lipids + lysozyme mixed films and shifted the region of maximum pressures to higher thicknesses. It also increased the hysteresis between expansion and contraction branches. Interaction of human tear lipids with this model protein, namely, hen-egg white lysozyme, made mixed films less stable than they were originally, unlike other proteins studied. However, it is quite unlikely that natural human-tear lysozyme would produce the same effect on tear-lipid films; therefore, it would not be sensible to draw conclusions based only on the results obtained from hen-egg white lysozyme [62].

Table 4 summarizes the parameters characterizing proteins + lipid films interfacial dynamic and rheological properties obtained in a course of the step-strain relaxation experiments.

As exhibited in Table 4, all proteins affected interfacial rheological properties of mixed tear-lipid–protein films, mostly by increasing relaxation time, whereas equilibrium surface tension and maximum surface pressure were changed only slightly, thus indicating that tear-lipid
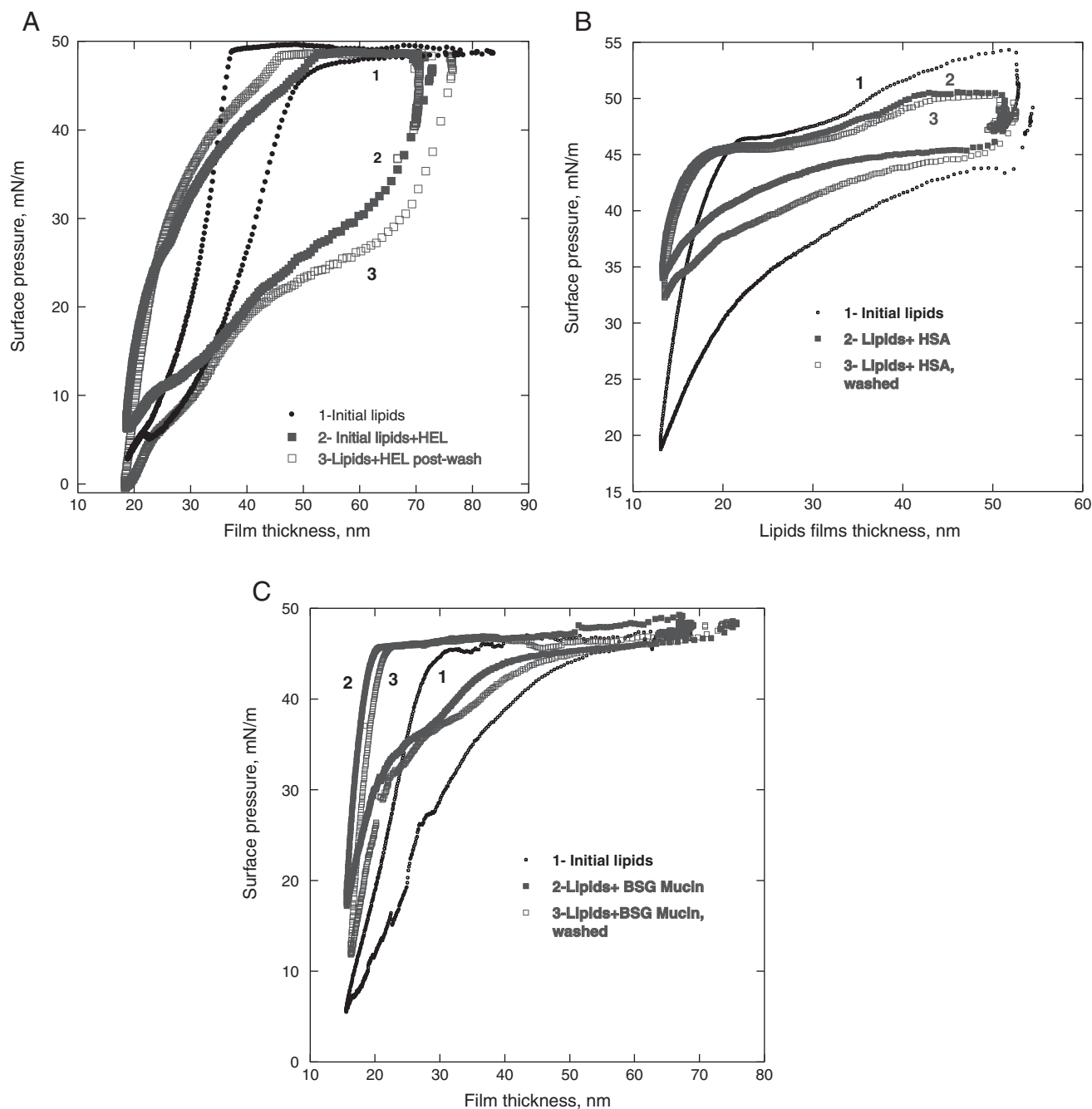

Fig. 18. Surface tension history of the lipid-lysozyme mixed film; stirring continued for 16 h [39].

Fig. 20. The iso-cycles for human tear lipid films alone aged for 24 h, 16–20 h after Lysozyme (a), HAS (b) or BSM (c) injections and equilibration followed by wash-out of the proteins from bulk solution [39].

Table 4
Interfacial and rheological parameters of human tear lipids (HTL) and mixed lipid–protein films.

System	γ_e, mN/m	E_∞, mN/m	τ, s	π_{max}, mN/m
HTL	22.2 ± 2.1	10.7–14.8	90–170	50.2 ± 2.5
HSA	40.4 ± 0.5	16.5	433 ± 42	30 ± 1.5
HEL	45.3 ± 1.5	18.5	458 ± 35	36.3 ± 2.1
BSM	36.7 ± 1.3	12.5	525 ± 40	47.3 ± 2.5
HTL + HSA	22.2 ± 2.7	15.5 ± 2.2	250 ± 40	48.5 ± 2.7
HTL + HEL	22.4 ± 2.1	14.5 ± 2.5	310 ± 34	46.3 ± 3.1
HTL + BSM	22.1 ± 2.5	11.6 ± 3.2	382 ± 40	50.4 ± 2.7

films, however altered, were not displaced or ruptured by model proteins adsorption and remained the major components defining the surface tension of these thick interfacial films.

4.3.2. Effect of lens-care solutions

As mentioned above, practically all lens-care solutions contain some surface-active additives introduced as disinfecting or wettability-improving agents. Also, conventional HEMA-based lenses might accumulate significant amounts of surface-active polymers, for instance, Tetronic, and then slowly release them during lens-wear. To expand the knowledge about human tear-lipid properties and their interaction with lens-care solutions, this part of our studies focused on evaluation of

dynamic interfacial and rheological behaviors of human tear lipids extracted from whole tears collected using Schirmer strips. We hypothesize that the dynamic and visco-elastic interfacial properties of human tear-lipid films may be influenced by the presence of surface-active ingredients of these lens-care solutions, as these ingredients are introduced into the eye during lens wear after lenses have been soaked/cleaned.

Surface pressure *vs.* film thickness (for lipids and lipids + lens-care solutions mixed films) and surface pressure *vs.* bubble surface area (for diluted lens-care solution alone) iso-cycles were recorded with the rate of area change 0.07 mm^2/s in both directions. Those iso-cycles were used for evaluation of lipid-film compressibility in a wide range of lipid-film thicknesses, according to Eq. (1).

The interfacial visco-elastic and dynamic properties of the tear-lipid films were first examined in MTE solution for films aged for 20–24 h without any lens-care solution, followed by exposure to one with lens-care solution for 2 h with 300 rpm stirring prior to interfacial properties re-evaluation. For the following lens-care solution test, a new lipid film was formed, aged, and its initial properties measured before and 2 h after lens-care solution injection.

Fig. 21a depicts two curves for lipids from two different subjects and their transient elasticity (calculated according to Eq. (2)) as a function of time after step-wise increase of tear-lipid film surface area with an initial thickness of 80–90 nm. These experimental data were fit with Eq. (3) to obtain numerical values for interfacial rheological parameters such as elastic modulus E_∞ and relaxation time τ_M. Fig. 21b illustrates transient elasticity for diluted lens-care solutions alone, without tear-lipid films deposited prior lens-care solutions injection.

The results of rheological experiments summarized in Table 5 along with data from Fig. 21a and b show significantly lower E_∞ and shorter τ_M for both lens-care solutions alone and for mixed human tear-lipid + lens-care solutions films, as compared to initial human tear-lipid films ($p < 0.05$, Student t-test).

Fig. 22a–d exhibits the surface pressure vs. surface area dependencies obtained for diluted and post-wash lens-care solutions alone.

One can see that all interfacial films formed by diluted lens-care solutions had noticeable hysteresis between compression and expansion branches, and that the overall shape of these iso-cycles was very different from the iso-cycles for human tear-lipid films or model tear proteins alone, as shown in Fig. 19.

Fig. 23a–d displays examples of the iso-cycles corresponding to initial human tear-lipid films in the presence of four different lens-care solutions, and 2 h after lens-care solutions were washed away (post-wash) by pumping MTE solution through the cell.

These figures demonstrate that exposure to all lens-care solutions altered the dynamic interfacial properties of human tear lipids, but to a different extent. For PureMoist and Revitalens lens care solutions, these changes remained evident even after complete removal of lens-care solutions from aqueous media in the cell.

We stipulate that the interfacial properties of multilayered lipid films formed using lipids extracted from whole tears imbibed by Schirmer strips, with an initial thickness close to the ocular lipid-film thickness (as in the present study) are better models for examining human tear-lipid behavior than meibum-lipid monolayers [40–44]. The results described above show that our sessile-bubble technique provides new essential information that is more representative and relevant to in vivo ocular lipid films than results obtained by studying monolayers. This is important because under ocular conditions tear lipids do not typically exist in the form of monolayers. The importance of lipid-film thickness and high surface pressures achieved in expansion-contraction iso-cycles of thick films become even more obvious when the results from human and animal meibum monolayers studies [61] are compared with our results from thick multilayered tear-lipid films. Previously reported [61] isotherms of human and animal meibum monolayers were qualitatively similar within a surface pressure interval of 0–30 mN/m. These iso-cycles exhibited little
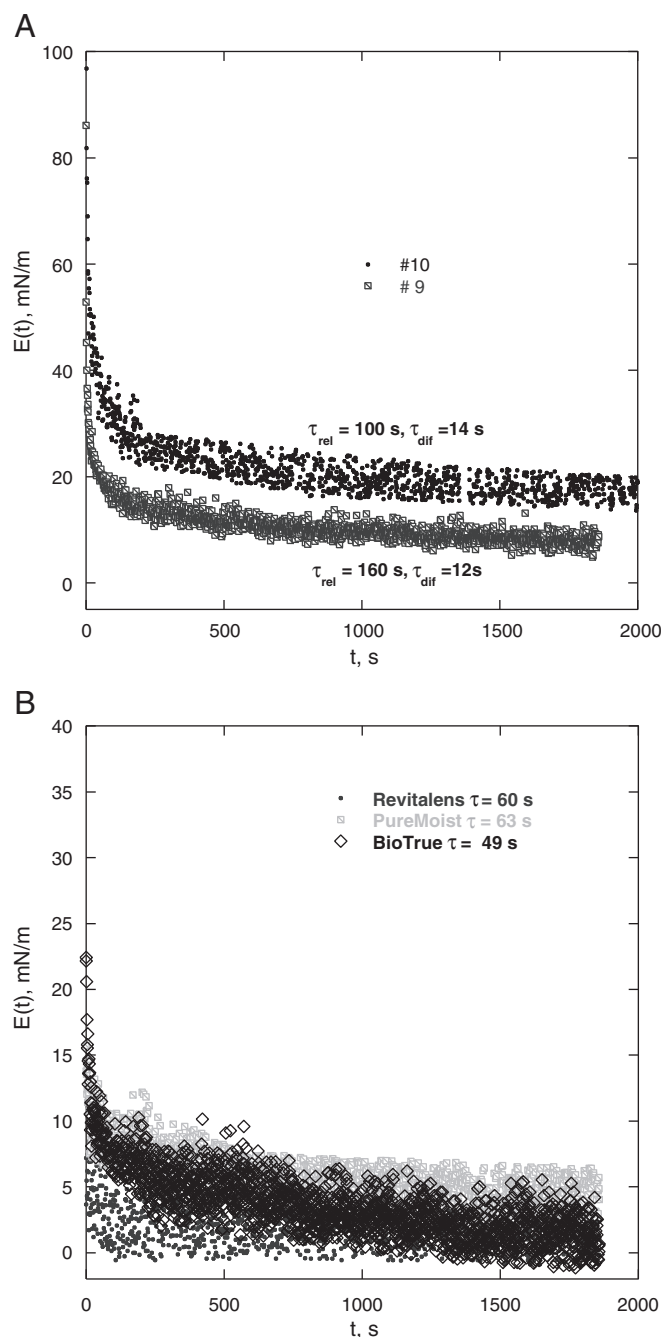

Fig. 21. Transient elasticity as a function of time for tear-lipid film (a) and for diluted lens-care solutions alone (b) [26]. © Elsevier.

hysteresis between expansion and compression cycles. The difference between these lipid samples becomes evident only at higher surface pressures corresponding to film thicknesses of ~20 nm and greater. It has been shown [64] that the addition of polar lipids such as Sphingomyelin and Dipalmitoylphosphatidyl Choline affected the shape of meibum-lipid monolayers iso-cycles and increased the maximum attainable surface pressure from 35 to ~50–55 mN/m, which is in excellent agreement with our results obtained from polar-lipids containing thick tear-lipid films. We assume that for thick tear-lipid films, the differences in iso-cycles at high surface pressures are related to variations in polar lipid content and composition of the samples collected from different subjects (Fig. 17).

It has been recently reported that the integrity of meibum-lipid monolayers could be compromised by penetration of surface-active

Table 5
Compressibility parameters for human tear lipids and mixed HTL + LCS films.

| LCS	π range, mN/m for thickness 60–90 nm	Compressibility, dπ/dh	π_{max}, mN/m	π_{min}, mN/m	Compressibility ratio Tear Lipid + LCS/TL
Tear-lipids alone	48–52	0.02–0.11	52	12	NA
ClearCare	48–50	0.126	52	20	6.3
ClearCare washed	47–50	0.13	52	11	6.5
Biotrue	46–50	0.24	50	28	12
Biotrue washed	43–50	0.18	50	33	9
Revitalens	46–52	0.8	52	33	40
Revitalens washed	46–50	0.24	50	28	12
PureMoist	48–54	3.2	52	28	32
PureMoist washed	46–50	0.14	50	29	28
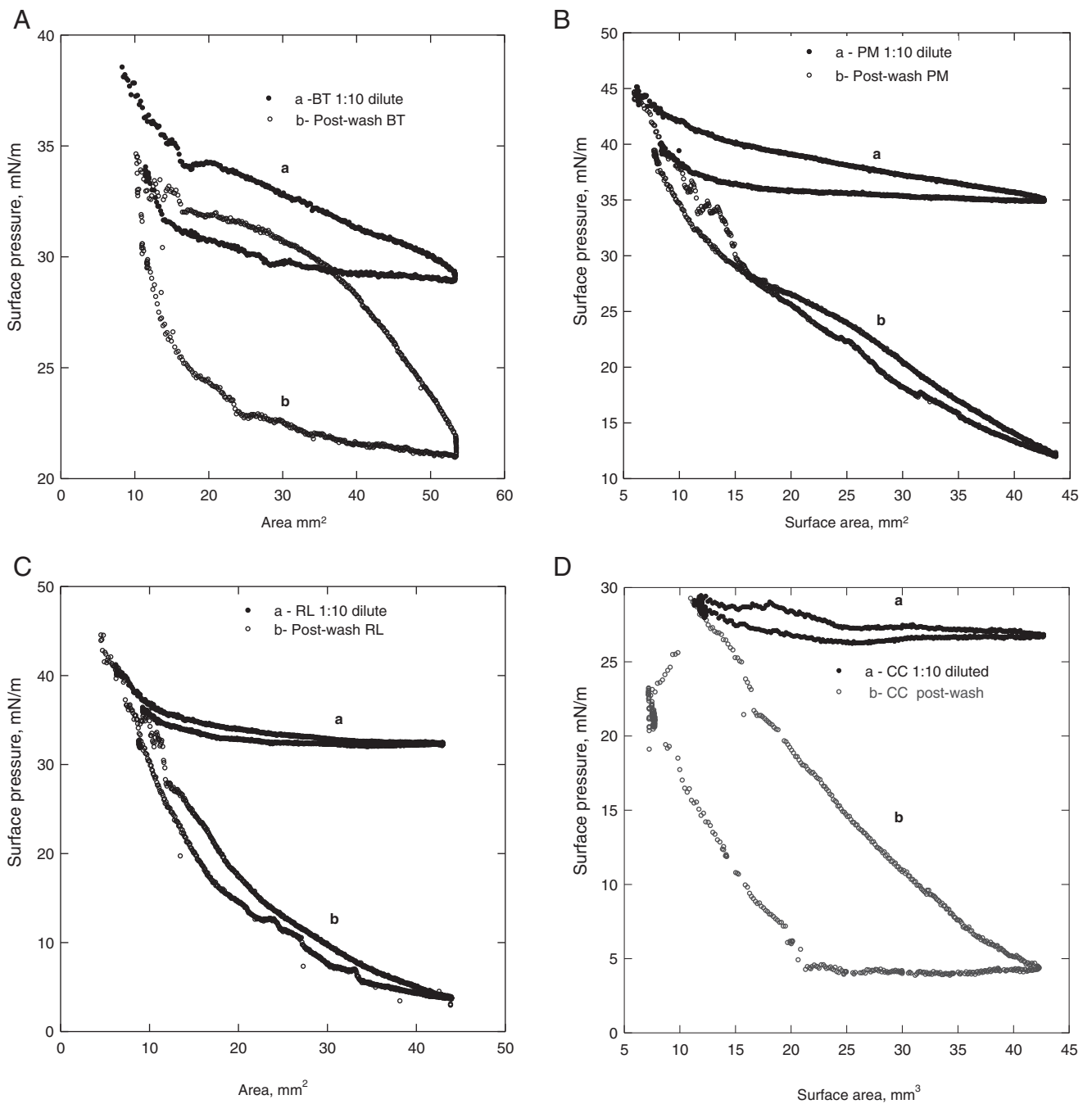
Fig. 22. The surface pressure vs surface area dependencies obtained for diluted and post-wash lens-care solutions alone [26]. © Elsevier.
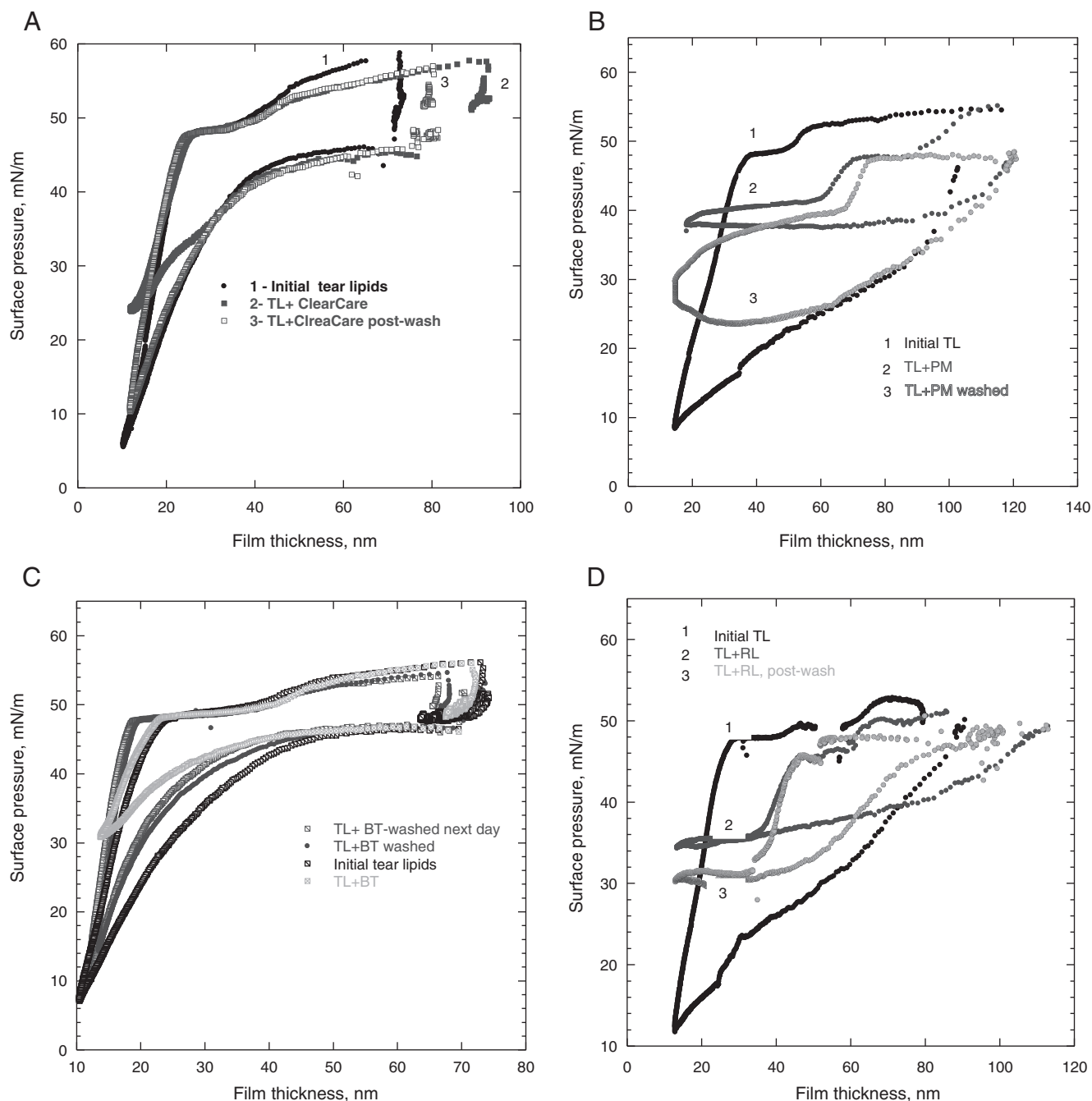
Fig. 23. The iso-sycles corresponding to initial human tear-lipid films and the same films in the presence of CC (a), PM (b), BT (c) and RL (d) lens care solutions, and 2 h after the lens-care solutions was washed away [26]. © Elsevier.

preservative (e.g., benzalkonium chloride, BAC) found in ophthalmic formulations (e.g., Travatan). [63] However, no substantial evidence of long-term effects from this particular preservative or Travatan formulation on meibum-lipid properties was reported. These authors did not examine effects of surface-active components typically present in lens-care solutions. The effect of lens-care solutions (e.g., OptiFree Express and OptiFree Replenish) on human tear-lipid interfacial properties was first described in our work. [24].

Our studies were focused on thick multilayered human tear lipids, as opposed to meibum-lipid monolayers [39–43], and on both short-term and long-term effects of diluted lens-care solutions, for example, reversibility of the changes caused by exposure to lens-care solutions. Polymeric surfactants present in these lens-care solutions are more likely to adsorb irreversibly at water–air or water–oil interface. Previous

investigations [48] have shown that polymeric surfactant Pluronic is able to adsorb at the interface in the presence of double-tailed surfactant Aerosol OT™ (AOT, bis-ethylhexyl sulfosuccinate, sodium salt). The chemical structure of AOT is similar to that of phospholipids, except for the polar head group. We hypothesize that adsorption of Pluronics at the air–water interface is also taking place in the presence of lipids. We have also shown [24,26] that some components of lens-care solutions, for instance, Tetronic 1304 present in OptiFree lens-care solutions, were bound irreversibly and thus significantly altered the rheological properties of *ex vivo* extracted lipid films. Indeed, the results reported in Fig. 22a–c clearly show that components of diluted lens-care solutions were adsorbed irreversibly to the air–water interface. They remained bound to the air–water interface or to lipid films even after a total removal of lens-care solutions from the bulk solution during

washout. Table 5 demonstrates that rheological parameters of thick human tear-lipid films changed in the presence of lens-care solutions but to a different extent. The most pronounced effect was produced by diluted PureMoist and Revitalens solutions, whereas the effects of Clearcare and Biotrue solutions on elasticity modulus and relaxation time were less significant. The same trend was observed for the lipid films exposed to diluted lens-care solutions when we studied dynamic interfacial properties and recorded surface pressure *vs.* film thickness iso-cycles. As seen in Fig. 23a–d, at low surface pressure and for thinner films, the shape of the iso-cycles changes very dramatically as an outcome of lens-care solutions exposure. Those alterations are to some extent similar to the transformations observed in other studies [63] with meibum-lipid monolayers exposed to BAC-containing eye drops Travatan. However, these changes were not persistent and practically faded away after Clearcare and Biotrue solutions were displaced by a surfactants-free model tear electrolytes solution. That was not the case for the films exposed to PureMoist and Revitalens solutions, for which after the washout procedure, the changes in iso-cycles shape became less pronounced but did not disappear.

At elevated pressures, the alterations of iso-cycles are less evident; nevertheless, the changes were significant, as revealed by the film-compressibility data summarized in Table. 5.

The compressibility of initial lipid films was lower than that of the films exposed to lens-care solutions, both before and after washout. Note that exposure to all lens-care solutions did not significantly change the maximum surface pressure of lipid films. This fact indicates that surface-active components of lens-care solutions are not able to significantly displace human tear lipids when the films are exposed to lens-care solutions. The extent of compressibility changes correlates with surface activity of lens-care solutions as gauged by equilibrium surface tension or surface pressure of their diluted solutions. More pronounced alterations in dynamic properties of human tear lipids were induced by lens-care solutions that exhibited higher surface activity and higher dynamic surface pressures. The clinical implications of our results remain to be determined. However, these results suggest that prolonged exposure to lens-care solutions might be causally related to contact lens-induced dry eye symptoms.

5. Tear lipid layer structure: existing models and novel one based on thick-film interfacial behavior

One of the first ocular tear lipid-layer models based on what was available at that time regarding chemical composition of tear lipids was proposed in 1997 [10]. The authors hypothesized that "the more polar lipids with surfactant characteristics function as a structure upon which the functional stability of the more nonpolar lipids are dependent." [10] They also suggested that the interrelationships between lipid classes present, length of fatty acids and alcohols, their unsaturation and hydroxylation are important for maintaining proper thixotropic characteristics of the lipid layer as well as optimal barrier properties. They concluded that "the tear film lipid layer is composed of 2 phases: (1) a thin polar phase adjacent to the aqueous-mucin phase and (2) a thick nonpolar phase associated with both the polar phase and the air interface. The structural characteristics of the polar phase and the barrier functions of the nonpolar phase are a direct result of specific compositional parameters."

Later, other tear-lipid film structures, similar to what was proposed by McCulley and Shine [10], have been suggested [67,68].

This model is based on extensive and detailed studies of human meibum compositions [67] and it emphasizes that OAHFA are the most surface-active amphiphilic substances found in meibum. This model totally excludes polar phospholipids from consideration since they were not found in meibum in detectable quantities. [67].

The other model, shown in Fig. 25, provides detailed schematics of highly-organized and ordered meibum-lipid layer with triglycerides acting as surfactants adsorbed at oil–air, and phospholipids adsorbed
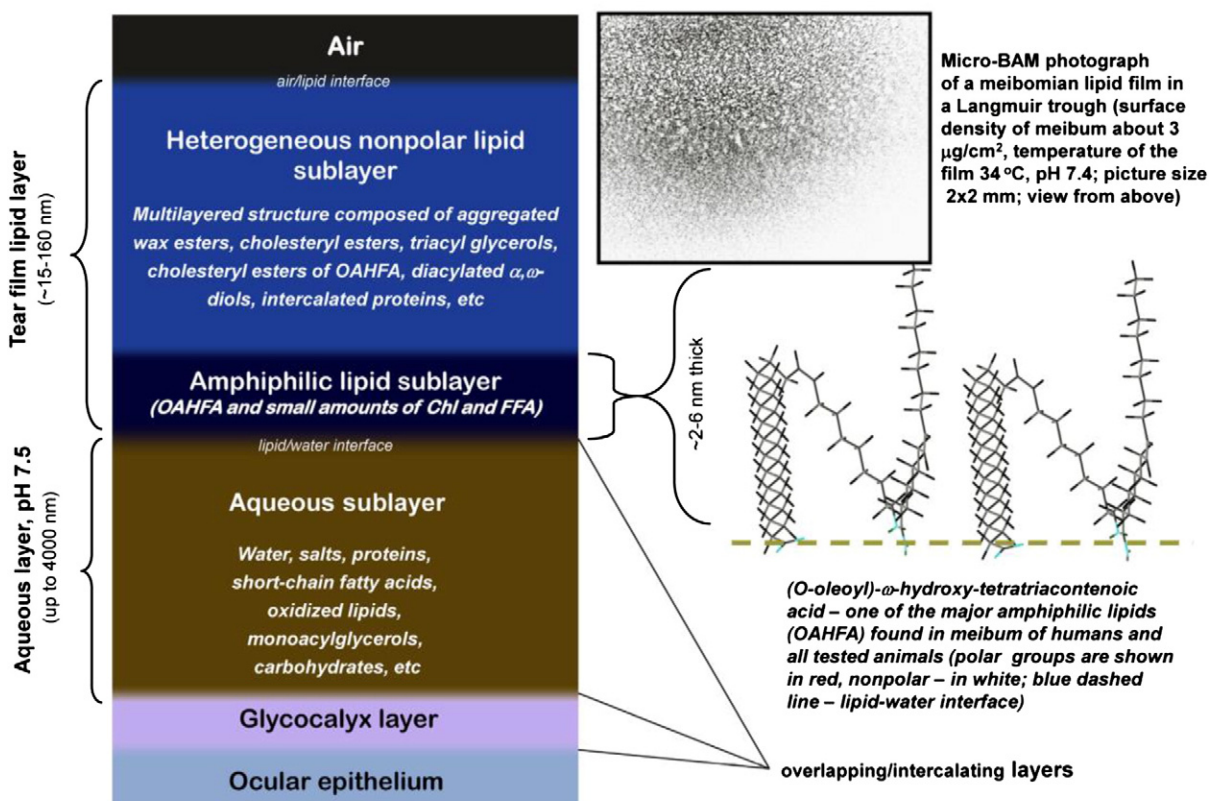

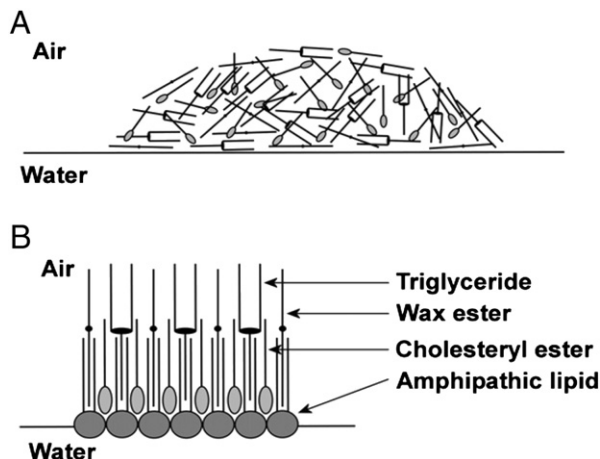**Fig. 24.** Tear film structure [66]. © Elsevier.

Fig. 25. Tear lipid layer schematics [67]. © Elsevier.

at oil–water interfaces. This model also illustrates the transformation of drop-containing random mixture of nonpolar-lipids deposited at air–water interface (A), which then gets re-organized into a film spread over the aqueous surface if amphiphilic phospholipids are present (B). The authors also [68] compare the properties of tear lipids and lung surfactants and conclude that both tear lipids and lung surfactant are thixotropic fluids.

The authors suggested that hydrophobic lipids deposited at the hydrophilic air–water interface are not able to spread as a uniform layer and thus form lipid aggregates or droplets. They also hypothesized that the "amphipathic phospholipids form a monolayer, thereby turning the interface hydrophobic and offering a suitable surface for the nonpolar lipids to spread on." Note that the schematic emphasizes the increased order of hydrophobic lipids and does not demonstrate the real molecular organization of the lipid layer [68].

This model was based on the then-current knowledge regarding the composition of human meibum, essentially poor or totally devoid of polar lipids such as phospholipids and shingomyelines. These polar components were lately found to be an essential part of human-tear lipidoms [28–30]. However, the authors [68] emphasize that "polar lipids, in particular phospholipids, are most likely a crucial component of the tear film." They also report some unpublished data indicating that they found quite abundant quantities of polar amphiphilic species in tear-fluid samples.

Our major disagreement with this and previous models is based on the thermodynamically-derived concept of spreading coefficient that was formulated almost 100 years ago [69].

The spreading coefficient for liquid a to spread upon liquid b is:

$$S = \gamma_b - (\gamma_a + \gamma_{ab}), \qquad (5)$$

where a and b are two immiscible liquids, γ_b is surface tension of liquid b, γ_a is surface tension of liquid a, and γ_{ab} is interfacial tension between these two liquids. The value of this spreading coefficient must be positive for liquid **a** to spread into thin film over the surface of liquid b, otherwise it forms a lens floating on top of the liquid b surface or goes down if **a** density is higher than density of substrate b.

For instance, according to [69], the spreading coefficient of oleic acid on water is 24 mN/m, and for chloroform it is 13 mN/m. Both organic liquids readily spread on pure water. However, if the surface tension of aqueous phase reduces to 47 mN/m or lower, neither of these organic fluids can spread on aqueous substrate. Thermodynamically-speaking, for tear-lipid layer to spread over the surface of tear-aqueous phase and form stable 40–100 nm thick film, the former must have positive spreading coefficient. Spreading coefficient will be positive only if the sum of surface and interfacial tensions ($\gamma_{lipid–air} + \gamma_{l–aq}$) is lower than surface tension of tear-aqueous γ_{aq}, which is 35–40 mN/m, as

determined in our studies described above (Fig. 11). Note that for all tear-lipid samples we studied, either extracted from worn lenses or from Schirmer strips, the static minimum surface tension was 22 ± 1 mN/m at ocular temperature 35.5 °C. These tear-lipids are likely to have positive spreading coefficient on aqueous protein solutions, as it was found [53] that the interfacial tension of phospholipids at oil–water interface can be reduced to 3–10 mN/m.

These results and as seen from Fig. 13c, given that tear lipids spread into uniform thick films on aqueous sub-phase and these films are stable for several hours, prompted us to put forward an improved tear-lipid layer model shown in Fig. 26.

This schematic, though simplified and not to scale, differs from previous models [67,68] because besides the polar lipid monolayer at the oil–water interface, like shown in schematics of Fig. 25, it assumes that there is an inverted bilayer at the *air–oil* interface. This bilayer is formed by most surface-active polar species, namely, double-tailed polar lipids such as phospholipids, sphingomyelins, and ceramides, comprising ~8–16 mol% of total lipids. As a result, this bilayer endows tear-lipid films with surface tension as low as 22 mN/m, which we have observed for all human tear-lipid samples. The non-polar lipids, comprising 84–92% of total, serve to support and separate these two (mono- and bilayer) membranes and provide a reservoir containing some dissolved extra polar lipids, which are more soluble in non-polar oils than in water. The other conceivable function of non-polar constituents is to provide appropriate rheological bulk properties, thus keeping lipid-films more uniform in density and thickness, and more stable. The existence of inverted bilayer has not been proven experimentally, yet is theoretically plausible. The packing parameters for the double-tailed molecules, forming the inverted bilayers, are close to 1 (one), so that their shape facilitates or forces them to be arranged into lamellar membranes and bilayers. The presence of inverted-bilayer membrane, with high water-transport resistance, at the top of tear-lipid films would also explain why in vivo tear-lipid films retard evaporation [20,70], whereas meibum films of ocular thickness are found to be unable to reduce evaporation in vitro [71]. This schematic is tentative, it is based on current knowledge regarding tear-lipid composition and interfacial properties, thus it is simplified and inescapably imperfect. Additional physical–chemical and structural studies are required to prove that this schematic indeed represents real human tear-lipid layer structure in vivo.

6. Conclusions

We demonstrated that sessile bubble with bulk-phase exchange technique is particularly suitable for investigating dynamic interfacial properties of microgram-sized biological samples. A 20 nm or thicker films of human tear-lipids have been proven to exert a surface pressure
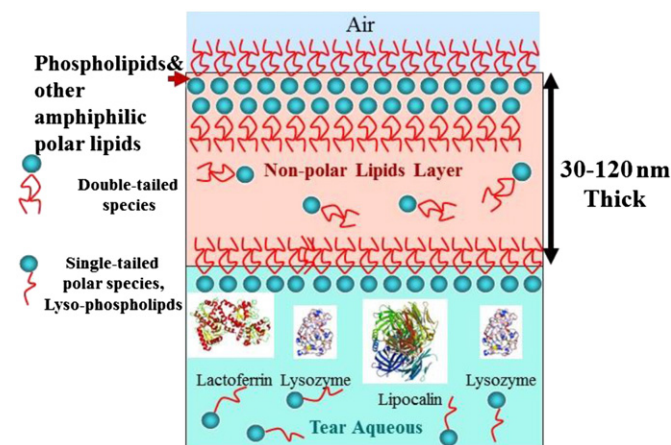

Fig. 26. Suggested tear-lipid layer schematics with inverted double-tailed species bylayer.

as high as 50 ± 1 mN/m. These tear-lipids films exhibit visco-elastic behaviors when subjected to small and uniform radial dilatational deformations. These findings were valid for both lipids extracted from worn contact lenses and from whole tears collected using Schirmer strips. The minimum static tension of human tear-lipid films in all cases studied was significantly lower than the surface tension previously reported for whole human tears and meibum-lipid monolayers.

We demonstrated that the dynamic behavior of thick films formed by meibum lipids and by tear lipids collected from the same person differ significantly. We hypothesize that these differences stem from the fact that polar phospholipids, sphingomyelines, and other double-tailed amphiphilic species, comprising 8–16% of human tear-lipids, act as an impelling cause for tear-lipid films spreading over tear-aqueous, and because these surfactants significantly reduce tension at both water–oil and oil-air interfaces.

We also showed that the interfacial rheological characteristics of these tear-lipid films are altered by interactions of the lipids with the model proteins similar to the proteins found in human tears. Proteins are found to adsorb irreversibly at tear lipids–aqueous interfaces.

The exposure of human tear-lipid films to diluted lens-care solutions causes significant alterations in interfacial dynamic and rheological properties; in most cases these changes are irreversible. Surface-active components of lens-care solutions adsorb and irreversibly bind to thick human tear-lipid films. The interactions of these substances with lipids change lipid-film interfacial dynamics significantly. After exposure to lens-care solutions, gel-like human tear-lipid films become less viscous and less elastic and behave similar to condensed liquid films. Clinical implications of these findings suggest the possibility of human tear-film destabilization upon prolonged exposure to lens-care solutions.

Despite alterations in dynamic interfacial properties, the static surface tension of multilayered human tear-lipid films remained the same (22 ± 2 mN/m) indicating that neither proteins nor lens-care solutions are able to displace thick lipid films from the air–water interface.

These original experimental studies deliver new findings essential for elucidating the mechanisms of tear-lipid films spreading and stability, of lipid–protein, and lipid lens-care solution interactions. The results emphasize the importance of the lipid–protein interactions because of significant alterations in rheological and dynamic interfacial properties of thick human tear-lipid films caused by these interactions. Our systematic studies of human tear-lipid interfacial properties made available sufficient experimental evidence to put forward a new model for ocular tear-lipid layer structure, where the double-tailed amphiphilic molecules form inverted bilayer membrane at air–lipid interface, whereas the monolayers of numerous surface-active species maintain low tension at oil-aqueous interface. Therefore, these two interfacial membranes act in unison to provide positive spreading coefficient for lipid films to spread on tear-aqueous sub-phase in a broad range of lipid-film thicknesses.

References

[1] Castner DG, Ratner BD. Biomedical surface science: foundations to frontiers. Surf Sci 2002;500:28–60.
[2] Yañez-Soto B, Mannis MJ, Schwab IR, Li JY, Leonard BC, Nl A, et al. Interfacial phenomena and the ocular surface. Ocul Surf 2014;12:3,178–201.
[3] Dilly PN. Structure and functions of tear film in advances in experimental medicine and biology; lacrimal gland, tear film, and dry eye syndromes. basic science and clinical relevance Sullivan DA ed., 350; 1994. p. 239–47 (New York).
[4] Holly F, Lemp MA. Formation and rupture of the tear film. Exp Eye Res 1973;15:515–25.
[5] Holly FJ. Physical–chemistry of the normal and disordered tear film. Trans Ophthalmol Soc 1985;104:374–80.
[6] Craig JP, Tomlinson A. Importance of the lipid layer in the human tear film stability and evaporation. Optom Vis Sci 1997;74:8–13.
[7] Rieger G. The importance of the precorneal tear film for the quality of optical imaging. Br J Ophthalmol 1992;76:157–8.
[8] Tiffany JM, Winter N, Bliss G. Tear film stability and tear surface tension. Curr Eye Res 1989;8:507–15.
[9] Nagoyova B, Tiffany JM. Components responsible for the surface tension of human tears. Curr Eye Res 1999;19:4–11.
[10] JP MC, Shine WE. A compositional based model for the tear film lipid layer. Trans Am Ophthalmol Soc 1997:79–93 (XCV).
[11] King-Smith PE, Fink BA, Hill RM, Koelling KW, Tiffany JM. The thickness of the tear film. Curr Eye Res 2004;29:4–5 357–368.
[12] McCulley JP, Shine WE. The lipid layer: the outer surface of the ocular surface tear film. Biosci Rep 2002;21:407–18.
[13] Zhoua L, Zhaoc SZ, Koha SK, Chena L, Vazd C, Tanavded V, et al. In-depth analysis of the human tear proteome. J Proteome 2013;75(75:13):3877–85.
[14] Saraygord-Afsharia N, Naderi-Manesha H, Naderib M. Increasing proteome coverage for gel-based human tear proteome maps: towards a more comprehensive profiling. Biomed Chromatogr 2015;29:1056–67.
[15] Fullard RJ, Snyder C. Protein levels in nonstimulated and stimulated tears of normal human subjects. Invest Opthamol Vis Sci 1990;31:1119–26.
[16] Butovich IA, Millar TJ, Ham BM. Understanding and analyzing meibomian lipids. Curr Eye Res 2008;33:405–20.
[17] Butovich IA, Uchiyama E, Di Pascuale MA, McCulley JP. Liquid chromatography-mass spectrometric analysis of lipids present in human meibomian gland secretions. Lipids 2007;42:765–76.
[18] Andrews JS. Human tear film lipids. I. Composition of the principal non-polar component. Exp Eye Res 1970;10:223–7.
[19] Butovich IA. On the lipid composition of human meibum and tears: comparative analysis of nonpolar lipids. Invest Ophthalmol Vis Sci 2008;49:3779–89.
[20] Peng C-C, Cerretani C, Braun RJ, Radke CJ. Evaporation-driven instability of precorneal tear fil. Adv Colloid Interf Sci 2014;206:250–64.
[21] Li W, Graham AD, Selvin S, Lin MC. Ocular surface cooling corresponds to tear film thinning and breakup. Optom Vis Sci 2015;92(9):e248–56.
[22] Bron AJ, Tiffany JM, Gouveia SM, Yokoi N, Voon LW. Functional aspects of the tear film lipid layer. Exp Eye Res 2004;78:347–60.
[23] Millar TJ, Schuett BS. The real reason for having a meibomian lipid layer covering the outer surface of tear film. Exp Eye Res 2015;137:125–38.
[24] Svitova TF, Lin MC. Interfacial rheological properties of ex vivo human-tear lipids: the effect of lysozyme and multi-purpose lens-care solutions. Optom Vis Sci 2010;87:10–20.
[25] Svitova TF, Lin MC. Racial variations in interfacial behavior of lipids extracted from worn SCL. Optom Vis Sci 2013;90:1361–9.
[26] Svitova TF, Lin MC. Lens-care-solution-induced alterations in dynamic interfacial properties of human tear-lipid films. CLAE 2014;37:5,368–76.
[27] King-Smith PE, Reuter KS, Braun RJ, Nichols JJ. Tear film breakup and structure studied by simultaneous video recording of fluorescence and tear film lipid layer images. Invest Ophthalmol Vis Sci 2013;54:4900–9.
[28] Lam SM, Tong L, Yong SS, Li B, Chaurasia SS, Shui G, et al. Meibum lipid composition in Asians with dry eye disease. PLoS One 2011;6, e24339.
[29] Lam SM, Tong L, Duan X, Petznick A, Wenk MR, Shui G. Extensive characterization of human tear fluid collected using different techniques unravels the presence of novel lipid amphiphiles. J Lipid Res 2014;55:289–98.
[30] Rohit A, Willcox MDP, SHJ B, Mitchell TW, Stapleton F. Clinical and biochemical tear lipid parameters in contact lens wearers. Optom Vis Sci 2014;91:12,1384–90.
[31] Saville JT, Zhao ZJ, Willcox MDP, Ariyavidana MA, Blanksby SJ, Mitchell TW. Identification of phospholipids in human meibum by nano-electrospray ionisation tandem mass spectrometry. Exp Eye Res 2011;92:238–40.
[32] Brown SHJ, Kunnen CME, Duchoslav E, Dolla NK, Kelso MJ, Papas EB, et al. A comparison of patient matched meibum and tear lipidomes. Invest Opthamol Vis Sci 2013;54:7417–24.
[33] Dean AW, Glasgow BJ. Mass spectrometric identification of phospholipids in human tears and tear lipocalin. Invest Opthamol Vis Sci 2012;53:1773–82.
[34] Kulovesi P, Telenius J, Koivuniemi A, Brezesinski G, Rantamaki A, Viitala T, et al. Molecular organization of the tear fluid lipid layer. Biophys J 2010;99:2559–67.
[35] Kulovesi P, Telenius J, Koivuniemi A, Brezesinski G, Vattulainen I, Holopainen JM. The impact of lipids composition on the stability of the tear fluid lipid layers. Soft Matter 2012;8:5826–34.
[36] Pucker AD, Nichols JJ. Analysis of meibum and tear lipids. Ocul Surf 2012;10(4):230–50.
[37] Pucker AD, Haworth KM. The presence and significance of polar meibum and tear lipids. Ocul Surf 2014;13(1):26–42.
[38] Nagarajan R. Molecular packing parameter and surfactant self-assembly: the neglected role of the surfactant tail. Langmuir 2002;18:31–8.
[39] Svitova TF, Lin MC. Interfacial properties of whole human tears, meibomian lipids, and tear lipids extracted from Schirmer strips. ARVO; 2012(E-Abstract 85/617).
[40] Miano F, Calcara M, Millar TJ, Enea V. Insertion of tear proteins into meibomian lipids film. Colloids Surf B 2005;44:49–55.
[41] Tragoulias ST, Anderton PJ, Dennis GR, Miano F, Millar TJ. Surface pressure measurements of human tears and individual components indicate that proteins are major contributor to the surface pressure. Cornea 2005;24(2):189–200.
[42] Millar TJ, Tragoulias ST, Anderton PJ, Ball MS, Miano F, Dennis GR, et al. The surface activity of purified ocular mucin at the air–liquid interface and interactions with meibomian lipids. Cornea 2006;25(1):91–100.
[43] Mudgil P, Torres M, Millar TJ. Adsorption of lysozyme to phospholipid and meibomian lipid monolayer film. Colloids Surf B 2006;48:128–37.
[44] Miano F, Zhao X, Lu JR, Penfold J. Coadsorption of human milk lactoferrin into the dipalmitoylglycerophosphatidylcholine phospholipid monolayer spread at the air/water interface. Biophys J 2007;92:1254–62.
[45] Green-Church KB, Butovich I, Willcox M, Borchman D, Paulsen F, Barabino S, et al. The International workshop on Meibomian gland disfunction: report of the

subcommittee on tear film lipids and lipid–protein interaction in health and disease. Invest Opthamol Vis Sci 2011;52:1979–93.

[46] Bachman WG, Wilson G. Essential ions for maintenance of the corneal epithelial surface. Invest Ophthalmol Vis Sci 1985;26(11):1484–8.

[47] Schurch S, Bachofen H, Possmayer F. Surface activity in situ, in vivo, and in the captive bubble surfactometer. Comp Biochem Physiol A 2001;129:195–207.

[48] Svitova TF, Weatherbee M, Radke CJ. Dynamics of surfactant sorption at the air/water interface: continuous-flow tensiometry. J. Colloid Interf. Science 2003; 261(1):170–9.

[49] Svitova TF, Radke CJ. AOT and Pluronic F68 co-adsorption at fluid/fluid interface: a continuous flow tensiometry study. Ind Eng Chem Res 2005;44(5):1129–38.

[50] Vollhard D, Fainerman VB. Progress in characterization of Langmuir monolayers by consideration of compressibility. Adv Colloid Interf Sci 2006;127:83–97.

[51] Guzman E, Ritacco H, Ortega F, et al. Adsorption kinetics and mechanical properties of ultrathin polyelectrolyte multilayers: liquid-supported versus solid-supported films. J Phys Chem B 2009;113:7128–37.

[52] Freer EM, Svitova T, Radke CJ. The role of interfacial rheology in reservoir mixed wettability. J Pet Sci Eng 2003;39:137–45.

[53] He Q, Zhang Y, Lu G, Miller R, Mohwald H, Li JB. Dynamic adsorption and characterization of phospholipids and mixed phospholipids/protein layers at liquid/liquid interfaces. Adv Colloid Interf Sci 2008;140:67–76.

[54] Ramirez P, Stocco A, Munos J, Miller R. Interfacial rheology and conformations of triblock copolymers adsorbed onto the water–oil interface. J Colloid Interface Sci 2012; 378(1):135–43.

[55] Raju SR, Palaniappan CK, Ketelson HA, Davis GW, Millar TJ. Interfacial dilatational viscoelasticity of human meibomian lipid films. Curr Eye Res 2013;38(8):817–24.

[56] Zang H, Wang YE, Zuo YY. On the low surface tension of lung surfactants. Langmuir 2011;27:8351–8.

[57] Cerrano E. Ricerche fisico-chimiche sulle lacrime in relazione alla practica dei collirii. Arch Ital Biol 1910:347–459 (1iv).

[58] Miller D. Measurements of the surface tension of tears. Arch Ophthalmol 1969;82: 368–71.

[59] Lee S, Kim DH, Needham D. Equilibrium and dynamic interfacial tension measurements at microscopic interfaces using a micropipet technique. 2. Dynamics of

[60] Borchman D, Foulks GN, Yappert MC, Milliner SE. Differences in human meibum lipid composition with meibomian gland dysfunction using NMR and principal component analysis. Invest Ophthalmol Vis Sci 2012;53:337–47.

[61] Leiske DL, Raju SR, Ketelson HA, Millar TJ, Fuller GG. The interfacial viscoelastic properties and structures of human and animal meibomian lipids. Exp Eye Res 2010;90: 598–604.

[62] Svitova T, Lin MC. Thick human tear lipid films: effect of lipids interaction with model tear proteins on interfacial properties. ARVO; 2014(E-Abstract 41/0029).

[63] Georgiev GA, Yokoi N, Ivanova S, Krastev R, Lalchev Z. Surface chemistry study of the interactions of pharmaceutical ingredients with human meibum films. Invest Ophthalmol Vis Sci 2012;53:4605–15.

[64] Bergmanson IPG, Barbeito R. Clinical assessment of ocular response to a multipurpose contact lens-care solution. Ophthalmic Physiol Opt 1995;15(6):535–44.

[65] Carnt N, Jalbert I, Stretton S, Naduvilath T, Papas E. Solution toxicity in soft contact lens daily wear is associated with corneal inflammation. Optom Vis Sci 2007;24: 309–15.

[66] Georgiev GA, Kutsarova E, Jordanova A, Krastev R, Lalcheva Z. Interactions of meibomian gland secretion with polar lipids in Langmuir Monolayers. Colloids Surf B 2010;78:317–27.

[67] Butovich IA. Tear film lipids. Exp Eye Res 2013;117:4–27.

[68] Rantamäki AH, Telenius J, Koivuniemi A, Vattulainen I, Holopainen JM. Lessons from the biophysics of interfaces: lung surfactant and tear fluid. Prog Retin Eye Res 2011; 30:204–15.

[69] Harkins W, Films FA. The spreading of liquids. J Am Chem Soc 1922;44(12):2665–85.

[70] Cedarstaf TH, Tomlinson A. A comparative study of tear evaporation rates and water content of soft contact lenses. American. Am J Optom Physiol Optic 1983;60(3): 167–74.

[71] Cerretani CF, Ho NH, Radke CJ. Water-evaporation reduction by duplex films: application to the human tear film. Adv Colloid Interf Sci 2013;197:33–57.