BioNanoScience (2023) 13:1324–1338
https://doi.org/10.1007/s12668-023-01112-2

Tear Film Constituents and Medicines for Eyes Investigated as Langmuir Films

Juan Torrent-Burgués[1]

Accepted: 22 April 2023 / Published online: 6 May 2023

Abstract
The tear film has an important role on the quality of the vision process and is of importance in the fields of ophthalmology and optometry. The lipid layer, the outermost part of the tear film, can be investigated with the Langmuir technique, as well as the interaction of components of the aqueous part of the tear film with those of the lipid layer. Dysfunctions in the stability and composition of the tear film are the causes of eye diseases, as the dry eye syndrome. For the treatment of this disease, the artificial tears are used, and one type of them are the lipid tears, which can be investigated with the Langmuir technique. This technique can also be used to investigate the influence on the lipid layer of other artificial tear components or medicines instilled in the eye.
This review presents investigations where the Langmuir technique has been used in relation with the tear film. In particular, the surface properties of natural tears and of four commercial lipid-containing artificial tears have been reported and discussed in connection with their composition.

Keywords Tear film · Lipid layer · Langmuir film · Dry eye · Artificial tear · Lipid artificial tear

1 Introduction

The tear film [1] has a layered structure with three mean parts or layers. The inner layer adjacent to the corneal epithelium, the middle layer or aqueous layer and the outer layer or lipid layer [2, 3]. Even this lipid layer is a multilayer of several nanometers formed by several types of lipids, it can be investigated using the Langmuir technique, which can also be used to investigate the interactions of the lipid components with the components of the aqueous layer. These questions are considered in section 2 of this review.

The tear film presents dysfunctions or diseases and some of them, as the dry eye syndrome or ocular irritations, can be treated with artificial tears [4, 5]. One type of them are the lipid artificial tears which tray to repair or improve the lipid layer present in the outermost part of the tear film. These artificial tears are able to be studied using the Langmuir technique. These questions are considered in section 3 of this review.

Finally, some conclusions will be presented and a wide list of references for the reader to get more inside the subject.

2 The Tear Film

2.1 Structure and Composition

One model of the tear film considers three mean layers or phases [6], the mucin layer, in the inner part, the aqueous layer, and the lipid layer, in the outer part. Other models have been proposed [7, 8] where the separation between the aqueous and the mucin layer is not well defined. One of the mean function of the lipid layer is to preserve the aqueous layer from a fast evaporation.

The lipid layer is formed by a great variety of lipids [2, 3, 9–11]. The mucin layer is formed meanly by mucoglycoproteins (mucins) [3, 12]. The aqueous layer contains around 98% of water, and the rest are proteins (as lysozyme and IgG forming part of the immunologic system, lactoferrin and albumin) [3, 13], dispersed mucins, electrolytes

✉ Juan Torrent-Burgués
juan.torrent@upc.edu

[1] Department of Chemical Engineering. School of Optics and Optometry, Universitat Politècnica de Catalunya, Terrassa-, Barcelona, Spain

(as sodium, potassium, calcium and magnesium salts), glucose, vitamins (as vitamins A and C), and cells.

The aqueous layer is that with the most volume and thickness, around 90% of the total thickness of the tear film, established around 6–9 µm but with significant variations. The thickness of the mucin layer is around 0.8–1 µm and the thickness of the lipid layer is around 0.1 µm [14].

Some physical properties of the tear film are [15]: temperature≈32°C, pH≈7.4, volume≈5–10 µL, refractive index≈1.337, surface tension≈41 $mN \cdot m^{-1}$, viscosity≈0.9–1.3 mPa·s, and density≈1.004 $g \cdot cm^{-3}$.

Different techniques are used to evaluate clinically the tear film [16–21], being the most usual the Schirmer test, the height of the lacrimal meniscus, the break-up time (BUT) and the no-invasive break-up time (NIBUT), fluorescein staining or rose Bengal stain. More recently, the osmolarity test has been also used, especially related with the dry eye syndrome [18, 22–24].

2.2 The Lipid Layer

The lipid layer is a complex film and in its composition a lot of lipids have been found [25, 26], some of them polar (fatty acids, fatty amides, phospholipids) [27] and most of them non-polar (triglycerides, diesters, diacylglicerols, cholesterol and wax esters, hydrocarbons) [28–30]. Several compositions have been reported by several authors. Nichols et al. [31] studied the composition on fatty acids and fatty amides, which only represents around 2% of the total lipid content of the lipid layer. These authors found myristic, palmitic, stearic, and oleic acids, as well as myristamide, palmitamide, stearamide, oleamide, and erucamide, being oleamide [32] one of the most abundant which has been related with the dry eye syndrome. Butovich et al. [30] found that the content of oleamide is less than 0.1%, and also found that phospholipids and ceramides are in a low content, being the content of PC less than 0.01%. Borchman et al. [33] found that the composition of lipids in the tear film is different of that in the meibum. For instance, cholesterol and phospholipids represent around 15% in the tear film but only 0–7% in the meibum; meanwhile, cholesterol and wax esters represent around 45% in both. For a detailed lipid composition see references reported above, even composition usually changes from an author to another.

Grazing incidence X-ray diffraction and X-ray reflectivity were applied to meibum films at the air−water interface to identify its molecular organization [34]. The lipid layer interacts with lipocalins of the aqueous layer rendering stability to the tear film by reducing the surface tension [35]. Using several techniques, Rosenfeld et al. [36] proposed a refined structure for the tear-film lipid layer.

The lipid layer can be optically observed using interferometry, and some commercial instruments have been developed based in this principle [19, 37, 38]. The usual slit-lamp instrument can also be used to observe the lipid layer [39, 40]. The interferential patterns have been studied and related to thickness and type of lipid films [21, 41–45]. For images of these interferential patterns, see technical information from Tearscope-Keeler Ltd., and also Guillon and Godfrey [37]. A stroboscopic video color microscope has also been developed using interferometric principles and used for film imaging studies [46, 47] and for measuring the tear film lipid layer thickness [48]. A contemporary perspective on the structure-function relationship of the lipid layer of the tear film has been recently reviewed [49].

2.3 The Langmuir Film Technique Applied to Natural Tears

It is well known that when amphiphilic molecules, as fatty acids and phospholipids, are placed on water or aqueous solution, they spread forming a film, usually monomolecular, known as Langmuir film or monolayer. In the Langmuir technique, a trough is used to contain this film, provided of mobile barriers that permits to compress or decompress the film obtaining the surface pressure-area isotherms. The surface pressure (Eq. 1) is measured with an electrobalance using a Wilhelmy plate [50, 51]. From the isotherms, the compressibility modulus or its inverse, the elastic modulus (Eq. 2), can be calculated obtaining information on the physical states and elasticity of the film [52, 53].

$$\pi = \gamma_0 - \gamma \tag{1}$$

$$C_S^{-1} = -A\left(\frac{d\pi}{dA}\right)_T \tag{2}$$

Along the years, the Langmuir technique has been applied to a variety of compounds and systems, with several applications [50, 51, 54, 55]. In connection with Langmuir films, the Brewster angle microscopy (BAM) technique has been developed [56]. Recent applications of this technique have been reviewed [57]. Even the lipid layer of the tear is not a monolayer, its lipid nature permits to be studied with the Langmuir technique. Thus, the film formation on the water/air interface has been applied to study the behavior of Meibomian lipids secreted by the lipid glands, placed in the eyelids [58–65] or its interaction with lachrymal proteins [66–70] (see section 2.4). Tragoulias et al. [71] also applied this technique to study the surface activity of human tears and several of its components.

Kulovesi et al. [62] have studied the impact of lipid composition on the structural and dynamical properties of the tear lipid film using Langmuir films, X-ray diffraction, and coarse-grained molecular dynamics simulations. Georgiev et al. [58] applied surface relaxations as a tool to distinguish

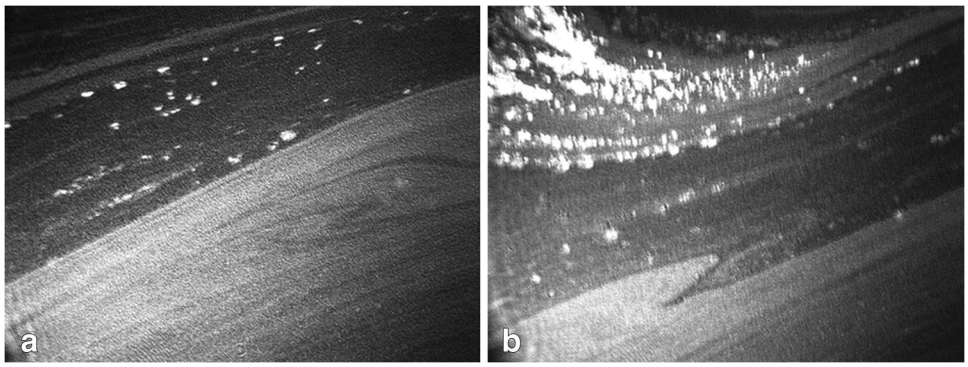

Fig. 1 BAM images, (**a**) and (**b**), of contaminated natural tears with cosmetics

the dynamic interfacial properties of films formed by normal and diseased meibomian lipids. The interaction of Meibomian lipids with polar lipids in Langmuir monolayers has been treated by Georgiev et al. [72] and by Rantamäki and Holopainen [73]. It has been pointed that the presence of anionic phospholipids (PS, PI, cardiolipin) in small amounts is necessary [11]. Bland et al. [74] investigated the role of specific tear film lipids connected to the dry eye syndrome. The molecular organization of the tear lipid layer has been simulated by Kulovesi et al. [75] using several techniques, including the Langmuir monolayer technique. Petrov et al. [64] also studied the organization of the tear lipid layer, with bovine meibomian tear film and simulated tear film, using surface pressure-area isotherms and fluorescence microscopy joined to grazing incidence x-ray diffraction (GIXD).

[76, 77], realized a combined clinical and Langmuir film study of natural tears. This study shows the applicability of the Langmuir technique to collected natural tears, which include the lipid components. Parameters obtained from clinical tests, as Schirmer test and tear film break-up-time (BUT), can be correlated with isotherm characteristics. Additionally, this study highlighted on the importance of not to use eye cosmetics prior to collect natural tears. Images obtained using a Brewster angle microscope showed the presence of excess of lipid components when eye cosmetics are used, Fig. 1, in comparison with BAM images obtained with natural tears without cosmetic contamination, Fig. 2. The effect of cosmetics has been pointed out by Guillon and Godfrey [37], which can break up the lipid layer and provoke dry eye, and stick to the contact lens surface producing a non-wetting effect.

Figure 3 presents surface pressure-area isotherms of collected natural tears spread on water subphase, showing in one case a good isotherm and in another case a weaker isotherm. Values of inverse of the compressibility modulus (inset in Fig. 3) indicate fluidity of the spread tear films, with slight differences in between both tear films. Figure 4 shows BAM images of collected natural tears spread on water surface, and in correlation with different tear patterns. As the thickness of the lipid tear film increases, the reflected light increases and the BAM image becomes brighter.

2.4 Interactions of Aqueous Tear Components with the Lipid Layer

It is important, in order to understand the structure and alterations of the lipid layer, to study the interactions of aqueous components of the tear with lipids. The composition of the aqueous layer of the natural tears has been indicated previously in section 2.1. It has been observed that lachrymal proteins have surface activity and that contribute to the decrease of the surface tension of the tear film and to its stability. In the formation of the lipid layer, the presence of ions must

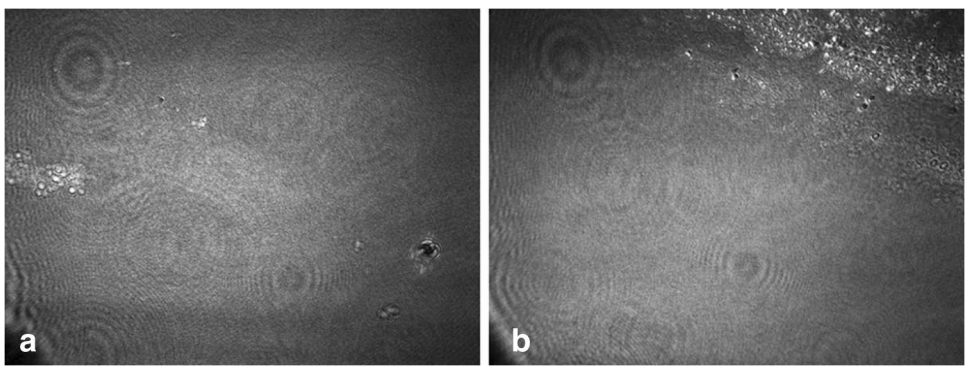

Fig. 2 BAM images, (**a**) and (**b**), of non-contaminated natural tears

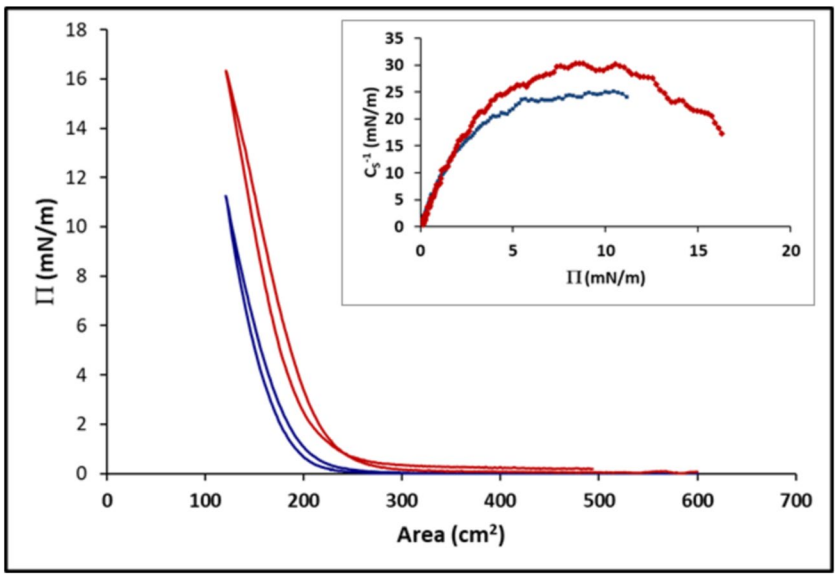

Fig. 3 Surface pressure-area isotherms, and inverse of the compressibility modulus (inset), for collected natural tears

be also considered [11] since its presence can stabilize the formation of the monolayer of polar lipids.

Bartkowiak et al. [78] studied the interaction of mucin with phospholipids, using the Langmuir technique. The authors found that mucin molecules interact with PC headgroups forming a more stable film. Millar et al. [79] investigated the interaction of ocular mucin with Meibomian lipids. Mudgil and Millar [68] studied the interaction of lipocalin with a bovine Meibomian lipid film, while Millar et al. [67] studied the adsorption of human tear lipocalin to human Meibomian lipid films. Mudgil et al. [69] studied the interaction of lysozyme with phospholipid and Meibomian lipid films, and Nishimura et al. [80] studied the effect of lysozyme on DPPC and cholesteryl myristate films. Miano et al. [81] studied the interaction of lactoferrin with DPPC as a model of the preocular tear film outer interface. The role of lactoferrin in the tear film has been reviewed by Flanagan and Willcox [82]. Miano et al. [66] studied the insertion of tear proteins (lactoferrin, human serum albumin, lysozyme, sIgA and lipocalin) in a Meibomian lipid film from bovine eyelids. Millar and Schuett [26] reviewed the effect of proteins on the tear film lipid layer, and Svitova and Lin [70] reviewed the interactions of human tear-lipid films with model-tear proteins in vitro.

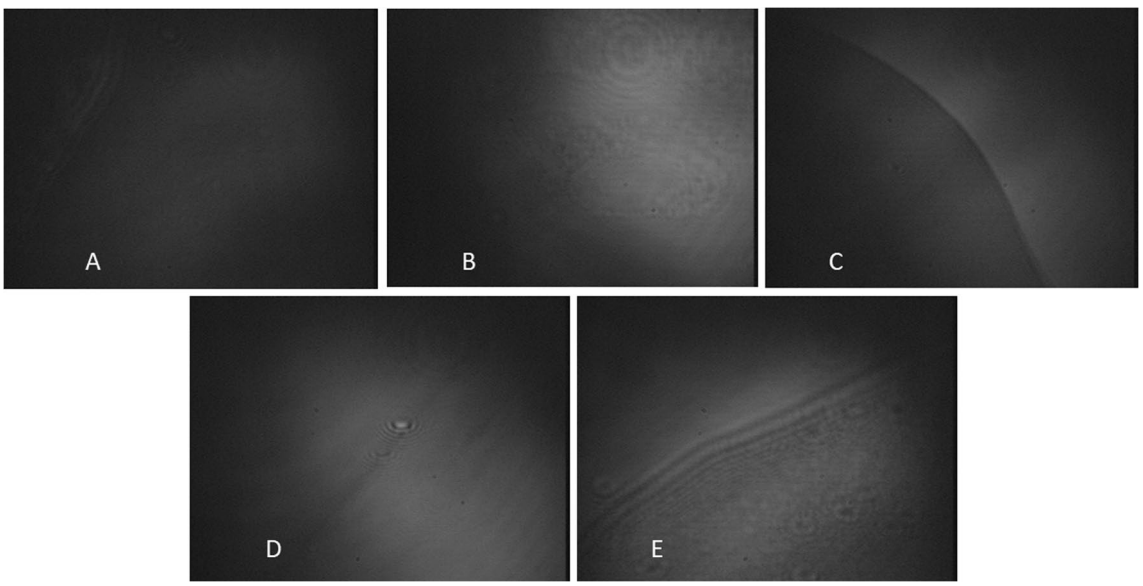

Fig. 4 BAM images of natural tears corresponding to different patterns. **A** Open meshwork, **B** closed meshwork, **C** fluid or wave, **D** amorphous, **E** colored

Torrent-Burgués and Raju [83] studied the interaction of lysozyme with several lipids, all of them present in the lipid layer of the tear film. They observed strong influence of lysozyme on the surface pressure-area isotherms and on the elastic modulus of the Langmuir films. The influence was greater on fatty acids, as oleic acid or stearic acid, than on phospholipids, as DPPC or POPC, or cholesterol. The interaction of lipocalins of the aqueous layer with the components of the lipid layer has been studied by Gasymov et al. [35]. Interpretation of these effects are based on electrostatic and hydrophobic interactions. For that, the presence and distribution of charges in the biomolecules are essential, as well as the values of isoelectric points (IEP) and the pH of the medium. Guckeisen et al. [84] found that the IEP of proteins at the air/liquid interface is close to that in the bulk solution.

Other studies that investigate the interactions of proteins with lipid films are also of interest in respect to understand the interaction of human tear proteins with the tear lipid layer [85–97]. Toimil et al. [98] studied with the Langmuir technique the interaction of human serum albumin with phospholipid monolayers. Calvez et al. [99, 100] analyzed the contribution of phospholipid monolayers to the binding of proteins, and proposed a new approach to determine factors that governs protein binding onto lipid monolayers. Stefaniu et al. [101] reviewed the applicability of Langmuir monolayers as models to study interactions of lipids with proteins and drugs, between others.

3 Tear Film Diseases

3.1 The Dry Eye Syndrome

The dry eye syndrome [102] is a common disease which affects a lot of people and is getting more frequent due to external factors as pollution, screens of devices, air conditioning, or the use of contact lenses. Actually, the dry eye syndrome is classified as a multifactorial disease of the tear film and it can provoke red eye, itchiness, sensation of tired eye, and discomfort or alter the visual quality or the tear stability [5, 103], and even can damage the ocular surface.

The dry eye syndrome can be classified as secretion lack or as evaporative. The first one (the former) is due to gland dysfunction [104]; meanwhile, the second one (the later) is due to an excess of evaporation of the aqueous component due to poor quality in the lipid layer [105, 106], but also to a poor flicker, poor wetting of the ocular surface or distortion of the tear film caused by contact lenses [107, 108] or environment factors [109, 110]. Contributions of evaporation and other mechanisms to tear film thinning and break-up were revised by King-Smith et al. [111]. The evaluation of the dry eye is performed with the methods or techniques commented previously in section 2.1 and also reported in other works [112, 113].

One of the usual treatments for the dry eye is the use of artificial tears, which are commented in the next section. Other solution is the use of polymer inserts [114] or the use of medicaments. Nichols et al. [115] reviewed the use of four approved dry eye medications in the USA and Europe, the vehicle used to deliver the medication and new treatment options for dry eye disease with novel vehicles.

3.2 Artificial Tears

It exist different types of artificial tears [116–118] and several studies have been reported on them [119–131]. One type of them are the lipid artificial tears, which tray to repair or improve the lipid layer present in the outermost part of the tear film, and some studies have been reported in literature [48, 119, 132–136]. In these studies, the artificial tears are used as eye drops. The lipids in these tears are present as liposomes [137].

Benelli [119] studied Systane® lubricant eye drops, Systane Ultra®, and Systane Balance®, which provide symptomatic relief to patients with dry eye. Scaffidi and Korb [135] used two tears, Refresh Dry Eye (Allergan, Inc., Irvine, CA, USA) and Soothe, and concluded that the application of a lipid emulsion eye-drop will increase the lipid layer thickness (LLT). Korb et al. [133] used Soothe and Systane and also concluded that the application of lubricant eye drops increase the LLT. Peters and Millar [134] used three artificial tear fluids: buffered saline alone, one with proteins and mucins, and one containing proteins, mucins and lipids. They concluded that the tear break-up time (BUT) was improved by the presence of phospholipids, in especial by phosphatidylinositol (PI). Steven et al. [136] found that BUT and other clinical signs of Meibomian gland disease improved with the application of semifluorinated alkane eye drops. Rohit et al. [138] studied the effect of lipid supplements on tear lipid biochemistry and their influence on lens wearers, and results suggest a potential role for lysophospholipids and OAHFAs in modulating symptoms during contact lens wear.

Several lipid artificial tears are present in the market and commercialized by several companies. In the composition of some of these lipid tears occurs, as a principal item, a phospholipid component that is soy lecithin, which is composed mainly by phospholipids as phosphocholine or also named phosphatidylcholine (PC). Soy lecithin is obtained from soy grains by mechanical extraction or chemically using hexane. It has a great content of PC, specially refined lecithin, but also other lipids are present as phosphatidylserine (PS) and phosphatidylinositol (PI). The fatty acid chains present in these PC are mainly palmitic or stearic, in C1, and oleic or linoleic, in C2. For example, POPC is

palmitoyloleoylphosphatidylcholine (M=760.1), or DLPC that is dilinoleoylphosphatidylcholine (M=782.1) (see Fig. 5), but an average soy polar extract PC is that reported with M=775.0 and phospholipid composition wt/wt%: PC 45.7, PE 22.1, PI 18.4, PA 6.9, unknown 6.9 (Avanti Polar Lipids, https://avantilipids.com/). Refined lecithin is used mostly for pharmaceutical applications and research.

In commercial lipid artificial tears, other components are also present and have been detailed for some of them in the next section. Glycosaminoglycans have also been used in artificial tears, as hyaluronic acid or chondroitin sulfate. Ceridório et al. [139] have studied how chondroitin sulfate interacts with headgroups in phospholipid monolayers, and Zander et al. [140] studied the influence of hyaluronan on DPPC.

3.3 Lipid Artificial Tears and the Langmuir Film Technique

The behavior of several lipid artificial tears has been studied in a few works using the Langmuir technique due to the presence of phospholipid components. Dinslage et al. [132] prepared lipid tears containing phospholipids and realized a clinical and Langmuir film study. The Langmuir technique has been applied to study the behavior of commercial lipid artificial tears [141] presented in dispensers for spray applications, Innoxa, Opticalm, Optrex and Zero, which composition is shown in Table 1. From the surface pressure-area isotherms, the inverse of the compressibility modulus or elastic modulus, C_s^{-1}, is obtained from eq. 2, which give information on the compressibility and fluidity of the film.

Figure 6 shows similar behavior of the lipid tears Optrex and Zero but a different behavior for the Opticalm one. Figure 1 in reference Torrent-Burgués [141] shows that the tear

Table 1 Composition of four commercial lipid-containing artificial tears

Composition in 1mL of purified water	Innoxa (a)	Opticalm (b)	Optrex (c)	Zero (d)
Soy lecithin	10 mg	10 mg	10 mg	*
Sodium chloride	8 mg	2.8 mg	8 mg	*
Ethanol	8 mg	8 mg	8 mg	*
Phenoxyethanol	5 mg	-	5 mg	*
Vitamin A-palmitate	0.25 mg	0.25 mg	0.25 mg	*
Vitamin E	0.02 mg	0.02 mg	0.02 mg	*

a, Innoxa (Optimyst). Manufacturer: Optima Pharmazeutische GmbH. Distributor: Omega Pharma España.

b, Opticalm (Lipomyst). Manufacturer: Medena AG. Distributor: Omega Pharma.

c, Optrex (Actimist). Manufacturer: Optima Medical Swiss AG. Distributor: Reckitt Benckiser Healthcare.

d, Zero. Manufacturer: Optima Medical Swiss AG. Distributor: DISOP. *Quantitative composition not specified

Innoxa presents a behavior more similar to Optrex. Analyzing the composition of these tears, which is similar even a bit different for Opticalm, the different behavior of Opticalm seems more related to the raw material of soy lecithin as it is manufactured by different companies.

Values of elastic modulus indicate fluidity of the films, corresponding to the liquid expanded state (LE) [53, 141]. These values, which are lower compared with others reported in literature for PCs ([141] and references cited therein), indicate the presence of several unsaturations in the PCs of soy lecithin, especially for Opticalm.

Figure 7 shows the behavior of the tear Zero at two temperatures (23 and 32°C) and in two subphases (water and NaCl 0.9%). It can be seen a notable influence of the saline

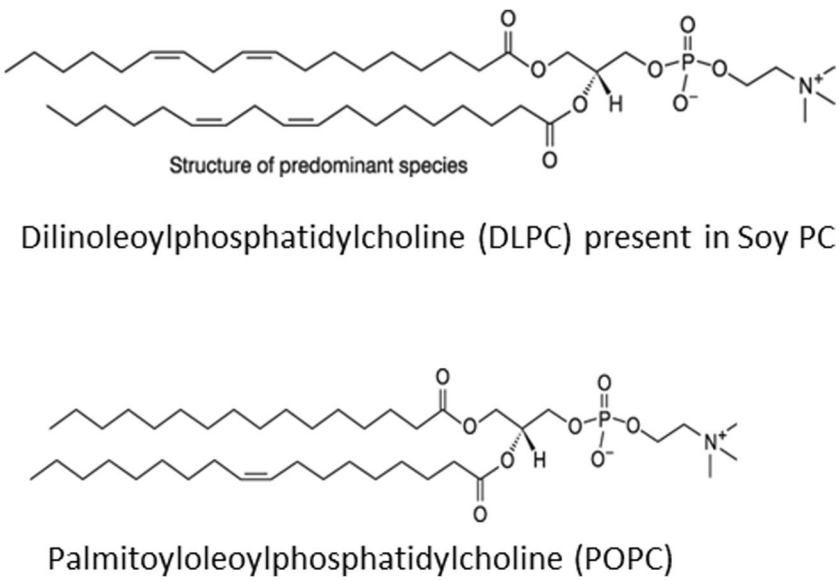

Fig. 5 Molecular structure of some phospholipids

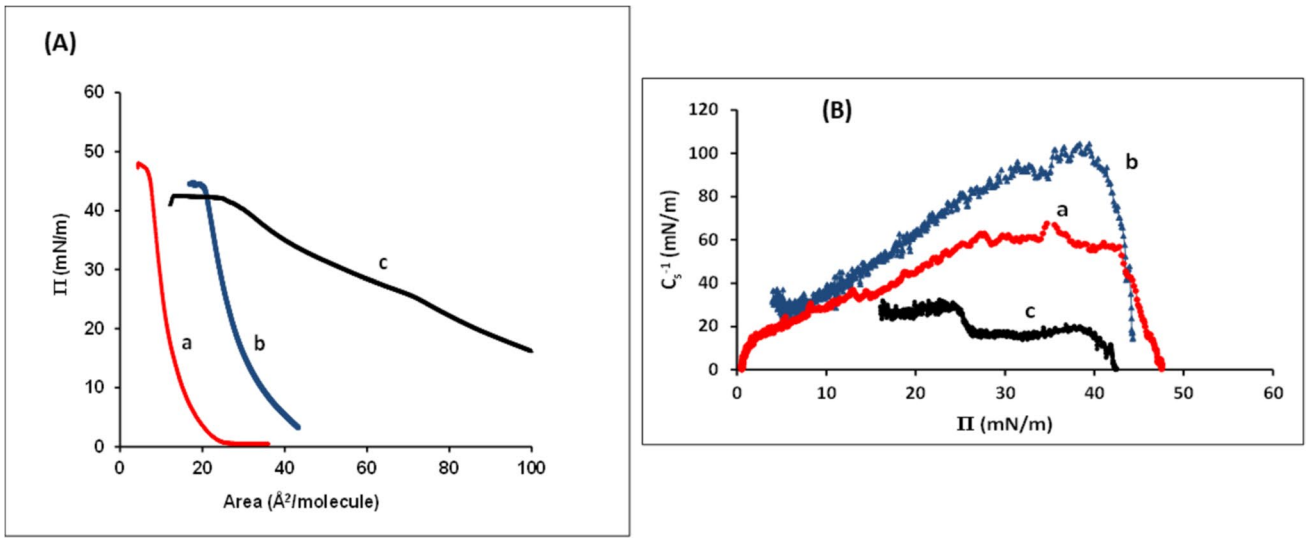

Fig. 6 **A** Surface pressure-area isotherms in water subphase at 23°C for three lipid-containing artificial tears: (a, red) Optrex, (b, blue) Zero, (c, black) Opticalm. **B** Inverse of the compressibility modulus for isotherms of Fig. 6(A)

subphase, shifting the isotherms to higher areas. The influence of temperature shows a behavior according with it is expected: a temperature increase expands the Langmuir film. Studies done with different samples indicate that slightly different results can be obtained from the same lipid brand depending on the industrial batch for preparing the tear, as can be seen comparing isotherm b in Fig. 6A and isotherm a in Fig. 7A, which corresponds to different samples.

Another result from the isotherms, where it has been assumed for molecular discussion that the main phospholipid component of soy lecithin is POPC, is that the low value of area per molecule attained at the collapse indicates that the liposomes remains partially forming a bilayer on the subphase, it means they do not spread totally as a monolayer. This fact influence on the isotherm position, which will depend on the amount of lipid spread. For more information, see reference Torrent-Burgués [141].

The presence of the lipid artificial tear film can be observed by BAM (Fig. 8), but due to its fluidity and probably to a refractive index similar to that of the subphase, it cannot be distinguished clearly and only big aggregates appears bright.

The behavior of different Optrex tears, see composition in Table 2, has also been investigated by the author's group. These investigations show no significant differences in the Langmuir film behavior of these three Optrex tears

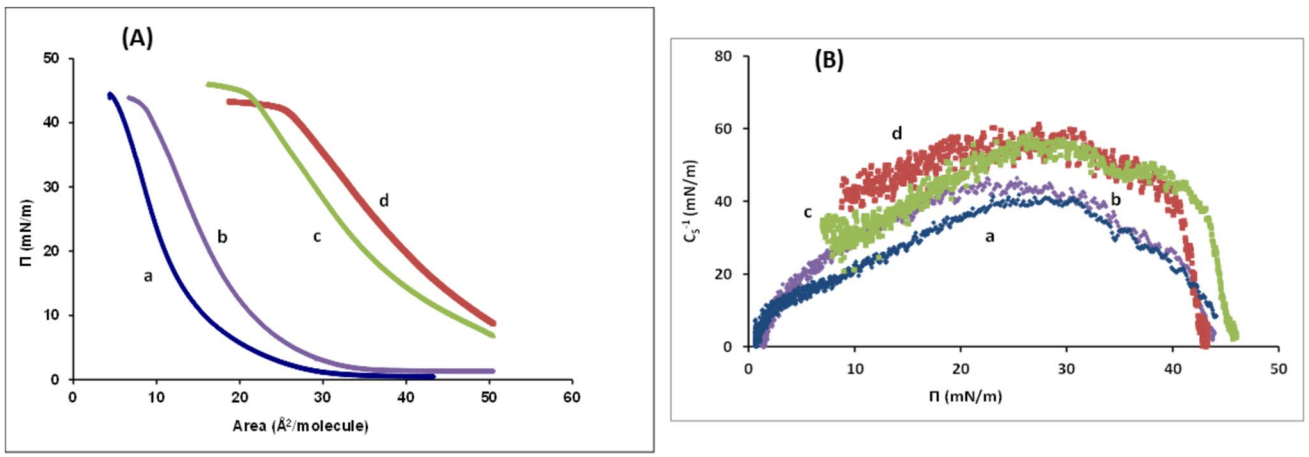

Fig. 7 **A** Surface pressure-area isotherms for the zero lipid-containing artificial tear at two temperatures and in two subphases: (a, blue) water 23°C, (b, violet) water 32°C, (c, green) NaCl 0.9% 23°C, and (d, red) NaCl 0.9% 32°C. **B** Inverse of the compressibility modulus for isotherms of Fig. 7(A)

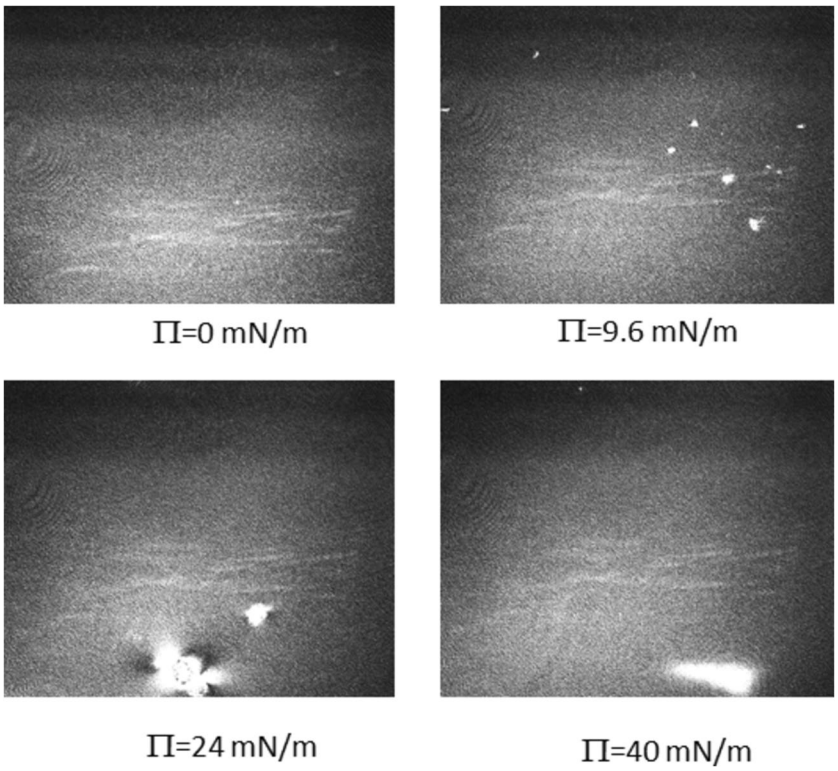

Fig. 8 BAM images for Langmuir films of the tear Innoxa, at different surfaces pressures

(see Fig. 9), which seems reasonable due to the similar composition they present. There is a subphase influence when comparing water, 0.9% saline solution and phosphate buffer solution (PBS). The influence of temperature was also studied: a temperature increase expands the Langmuir film and fluidizes it. More interesting are the effect due to the presence of proteins in the subphase at pH=7.4, with a marked difference in between bovine serum albumin (BSA) and lysozyme (Fig. 10). BSA produces a shift of the Optrex tear isotherm to lower areas and close to that of BSA alone; meanwhile, lysozyme produces a shift to higher areas and with a marked increase in the surface pressure. An explanation to this different behavior is related to the different isoelectric point (IEP) of both proteins, IEP≈4.8 for BSA and IEP≈11 for lysozyme, that leads to a negative charge for BSA and a positive charge for lysozyme at pH=7.4.

Weisenberger et al. [48] performed a clinical study using Systane Complete®, which contains lipids, and Systane Ultra® eye drops. This study found that Systane Complete increases the lipid layer thickness and benefit subjects with dry eye symptoms. In the author's laboratory a physicochemical study allowed to obtain some properties of Systane Complete: surface tension of 43.9 mN/m and pH of 7.23 at 23°C. The presence of the lipid components was observed with BAM (Fig. 11). Interactions of some eyedrop formulations with human meibum lipids have been reported by Eftimov et al. [142] using the Langmuir technique and BAM.

Table 2 Composition for 1 mL of purified water of three Optrex lipid tears

Optrex yellow (for itchiness and tearing)	Optrex green (for tired and annoying eyes)	Optrex red (for dry and irritated eyes)
10 mg soy lecithin	10 mg soy lecithin	10 mg soy lecithin
8 mg sodium chloride	8 mg sodium chloride	8 mg sodium chloride
8 mg ethanol	8 mg ethanol	8 mg ethanol
5 mg phenoxiethanol	5 mg phenoxiethanol	5 mg phenoxiethanol
0.25 mg vitamin A palmitate	0.25 mg vitamin A palmitate	0.25 mg vitamin A palmitate
0.02 mg vitamin E	0.02 mg vitamin E	0.02 mg vitamin E
	5 mg pro-vitamin B5 (dexapantenol)	

3.4 The Influence of Instilled Medicines on the Tear Film

The tear film can be altered by the instillation of medicines and artificial tears [115, 137, 143–145], especially for the action of some components, but also for the action of medicines dispensed orally or by other methods that can affect the gland secretion of the natural tear components. When a drop of an artificial tear or a pharmacological product, with a volume around 20–40 L, is instilled in the eye, with a tear volume of around 10 L, obviously, the tear film is perturbed, not only by the enormous quantity of instilled liquid but also because the structure of the tear is momentarily altered, which can provoke alterations in the vision quality. But at each blind new tear is supplied by the eye glands and the tear structure, composition and functionality is restored or improved with the afforded active ingredients. The application of an lipid artificial tear as a spray, which is done with the closed eyes, do not perturb the tear film but the quantity of artificial tear that reach the tear film is reduced. Usually, the sprayed volume in each application is around 100 L, and as the usual concentration of PC is 10 mg/mL, the amount of PC is around 1 mg, which is enormous if all of it will reach the tear film. But the eyelids acts as a barrier and thus the amount that reach the tear film and incorporates in the lipid layer is small.

Cell membranes can be affected by a drug, and this effect can be studied by the Langmuir technique and by insertion experiments of drugs into lipid films. The effect of drugs is a wide field and is out of the target of this work, but some references and comments will be done here. A list of common drugs used in ophthalmology can be found [146–148]. Ophthalmic drugs are used for three main purposes: diagnostic, therapeutic, or prophylactic. Previous sections have treated the case of artificial tears. Sometimes, inserts are used to deliver drugs to the eye [114, 149], but viscous drops, gels, and ointments are also available. Preservatives in eye-drops, as benzalconium chloride, polidronium, cetrimide, biguanides, chlorhexidine, or polyhexanide [150], or surfactant agents in contact lens solutions can also affect the cell membranes of the corneal or conjunctival epithelia. Mishra et al. [137] reviewed the use of liposome preparations for ophthalmic drug delivery. These formulations are mainly composed of phosphatidylcholine (PC) and other constituents such as cholesterol and lipid-conjugated hydrophilic polymers.

Sanfilippo et al. [151] presented a study in vitro on bacterial infections of the ocular surface commonly treated empirically with broad spectrum antibiotics. For glaucoma treatment or glaucoma prevention, some prescribed medicaments are instilled on the eye. Ocular surgery, as cataract surgery, which usually use eye drops before and after surgery, can

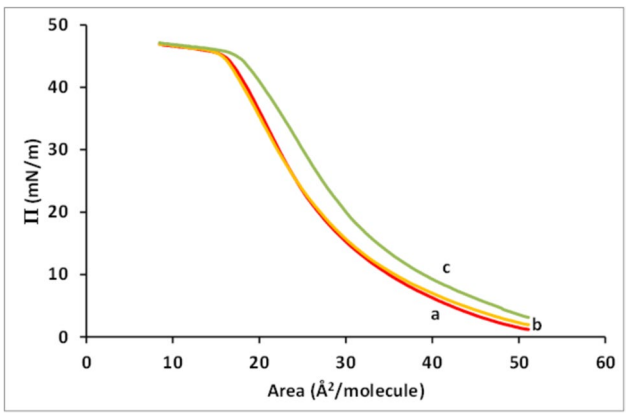

Fig. 9 Surface pressure-area isotherms for three Optrex tears (see composition in Table 2) in NaCl 0.9% subphase at 23°C: Optrex red (a, red), Optrex yellow (b, yellow), Optrex green (c, green)

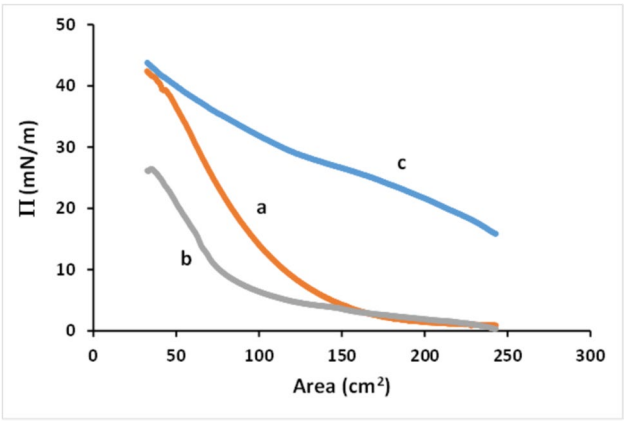

Fig. 10 Surface pressure-area isotherms for the red Optrex tear in PBS (a, orange), PBS+BSA (b, grey) and PBS+Lysozyme (c, blue) subphases, pH=7.4, at 23°C.

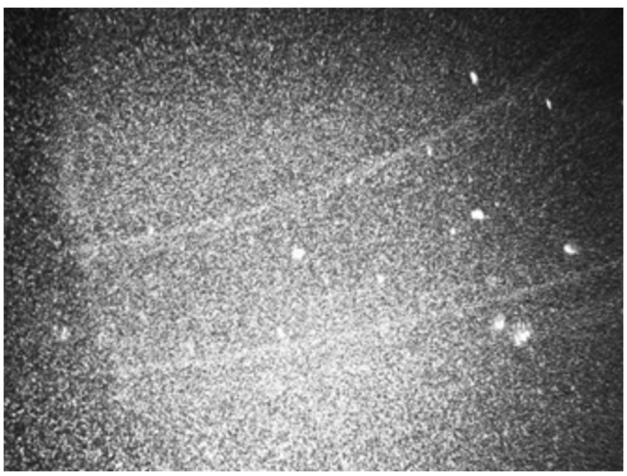

Fig. 11 BAM image when a drop of Systane Complete® is spread on water

also provoke changes in the tear film [152, 153]. Fangueiro et al. [154] studied epigallocatechin gallate lipid nanoparticles for ocular instillation for the future treatment of several diseases, such as dry eye, age-related macular degeneration, glaucoma, diabetic retinopathy and macular edema. The use of antimicrobial peptides in ocular surface has been reviewed by Mohammed et al. [155]. Studies published about the interaction of some drugs and antimicrobials with mimetic biomembranes using the Langmuir technique can be useful in ophthalmology. Nano-formulations with antimicrobial activity has been developed recently ([156–158], ; [159]; and references cited therein) and its effect on biomimetic membranes has been studied using the Langmuir technique. Caseli et al. [160] investigated, using the Langmuir technique, the influence of the components of a dye solution on model membranes.

The presence of surfactants and preservatives in the eye drops can alter the surface tension of the tear film and/or modify the epithelial cells and its environment [144]. Surfactants can alter meanly the lipid layer, preservatives can alter meanly the epithelium and the mucin layer. Souza et al. [161] studied the biocide action of poly(hexamethylene biguanide), a common component in contact lens solution, using Langmuir monolayers of dipalmitoylphosphatidylglycerol. Sandez-Macho et al. [162] studied the interaction of poloxamine block copolymers, usual components in contact lens solutions, with lipid membranes. Di Pascuale et al. [143] observed the changes in the tear lipid layer using interferometry after instillation of emulsion eye drops. Other works have also used the Langmuir technique to study interactions of drugs with lipid and phospholipid films, which could be useful for the reader [163–176]. Zhang et al. [177] studied, using the Langmuir technique, the interaction between *Lycium barbarum* polysaccharide (LBP) and model lipids (PC, PE, PS, and PI) of the human retinal pigment epithelial cells (RPEs), located at the outermost layered structure of the retina. Oxidative stress damages retinal cells. The LBP are a type of exogenous biological macromolecule that can be used to treat the disease and play an important role in the thickness of the retina and the tightness of retinal cell connections. For that, LBP has been used to treat oxidative damage of RPE cells.

4 Conclusions

The Langmuir technique can be used to investigate the following: (a) the lipid layer of the tear film and its interaction with components of the aqueous part of the tear film; (b) lipid tears, containing lipid components, used for the treatment of dry eye syndrome; and (c) the influence of other artificial tear components or medicines instilled in the eye on the lipid layer.

Parameters obtained from clinical tests, as Schirmer test and tear film break-up-time (BUT), can be correlated with isotherm characteristics. BAM images show on the importance of not to use eye cosmetics prior to collect natural tears.

Lachrymal proteins have surface activity and contribute to the decrease of the surface tension of the tear film and to its stability. The effects of proteins on lipid layers are very important, and can be interpreted based on electrostatic and hydrophobic interactions. The presence of BSA and lysozyme proteins in the subphase, at pH=7.4, shows a marked difference in between them. BSA produces a shift of the Optrex artificial tear isotherm to lower areas and close to that of BSA alone; meanwhile, lysozyme produces a shift to higher areas and with a marked increase in the surface pressure. An explanation to this different behavior is related to the different IEP of both proteins, that leads to a negative charge for BSA and a positive charge for lysozyme at pH=7.4.

The surface behavior of several studied lipid-containing artificial tears shows similarities in between some of them but important differences in respect to others. As the reported composition of these tears is similar, the different behavior seems more related to the raw material of soy lecithin, as different companies manufacture it.

Acknowledgements The author thanks PhDs E. Guaus and J. Hoyo, and MD C. Serés, for collaborating along several years.

Funding Open Access funding provided thanks to the CRUE-CSIC agreement with Springer Nature.

Declarations

Ethical Approval Not applicable.

Competing Interests The authors declare no competing interests.

References

1. Korb, D. R., Craig, J., Doughty, M., Guillon, J. P., Smith, G., & Tomlinson, A. (2002). *The tear film: Structure, function and clinical examination*. Butterworth-Heinemann.
2. McCulley, J. P., & Shine, W. (1997). A compositional based model for the tear film lipid layer. *Transactions of the American Ophthalmological Society, 45*, 79–93.
3. Ohashi, Y., Dogru, M., & Tsubota, K. (2006). Laboratory findings in tear fluid analysis. *Clinica Chimica Acta, 369*, 17–28.
4. Behrens, A., Doyle, J. J., Stern, L., Chuck, R. S., & McDonnell, P. J. (2006). Dysfunctional tear syndrome: A Delphi approach to treatment recommendations. *Cornea, 25*, 900–907.
5. Craig, J. P., et al. (2017). TFOS DEWS II report executive summary. *The Ocular Surface, 15*, 1–11.
6. Wolff, E. (1954). *Anatomy of Eye and Orbit* (4th ed., p. 207). Blakiston Co..
7. Chen, H. B., Yamabayashi, S., Ou, B., Tanaka, Y., Ohno, S., & Tsukahara, S. (1997). Structure and composition of rat precorneal tear film. A study by an in vivo cryofixation. *Investigative Ophthalmology & Visual Science, 38*, 381–387.
8. Holly, F. J., & Lemp, M. A. (1971). Wettability and wetting of corneal epithelium. *Experimental Eye Research, 11*, 239–250.
9. Bron, A. J., Tiffany, J. M., Gouveia, S. M., Yokoi, N., & Voon, L. W. (2004). Functional aspects of the tear film lipid layer. *Experimental Eye Research, 78*, 347–360.
10. Cwiklik, L. (2016). Tear film lipid layer: A molecular level view. *Biochimica et Biophysica Acta, 1858*, 2421–2430.
11. McCulley, J. P., & Shine, W. E. (2001). The lipid layer: The outer surface of the ocular surface tear film. *Bioscience Reports, 21*, 407–418.
12. Gipson, I. K. (2004). Distribution of mucins at the ocular surface. *Experimental Eye Research, 78*, 379–388.
13. Tsai, P. S., Evans, J. E., Green, K. M., Sullivan, R. M., Schaumberg, D. A., Richards, S. M., Dana, M. R., & Sullivan, D. A. (2006). Proteomic analysis of human meibomian gland secretions. *The British Journal of Ophthalmology, 90*, 372–377.
14. Ruskell, G. L., & Bergmanson, J. P. G. (2007). Anatomy and physiology of the cornea and related structures. In A. J. Phillips & L. Speedwell (Eds.), *Contact Lenses* (5th ed.). Butterworth-Heinemann.
15. Slack, S.M., 2004. Properties of biological fluids, pg. 813 in Biomaterials science, 2nd, Ratner, B.D. et al.. Elsevier.
16. Isreb, M. A., Greiner, J. V., Korb, D. R., Glonek, T., Mody, S. S., Finnemore, V. M., & Reddy, C. V. (2003). Correlation of lipid layer thickness measurements with fluorescein tear film break-up time and Schirmer's test. *Eye, 17*, 79–83.
17. Nichols, K. K., Mitchell, G. L., & Zadnik, K. (2004). The repeatability of clinical measurements of dry eye. *Cornea, 23*, 272–285.
18. Savini, G., Prabhawasat, P., Kojima, T., Grueterich, M., Espana, E., & Goto, E. (2008). The challenge of dry eye diagnosis. *Clinical Ophthalmology, 2*, 31–55.
19. Tong, L., & Teng, L. S. (2018). Review of literature on measurements of non-invasive break up times, lipid morphology and tear meniscal height using commercially available hand-held instruments. *Current Eye Research, 43*, 567–575.
20. Wang, J., Palakuru, J. R., & Aquavella, J. V. (2008). Correlations among upper and lower tear menisci, noninvasive tear break-up time, and the Schirmer test. *American Journal of Ophthalmology, 145*, 795–800.
21. Yokoi, N., & Komuro, A. (2004). Non-invasive methods of assessing the tear film. *Experimental Eye Research, 78*, 399–407.
22. Benelli, U., Nardi, M., Posarelli, C., & Albert, T. G. (2010). Tear osmolarity measurement using the TearLabTM Osmolarity System in the assessment of dry eye treatment effectiveness. *Contact Lens & Anterior Eye, 33*, 61–67.
23. Sullivan, B., Zmina, S., Senchyna, M., Williams, R., Chen, C., Garst, S., Harvey, E., Hayes, J., Solomon, M., Gamble, L., Davis, F., Knaack, A., Berg, M., Kosheleff, V., & Lemp, M. (2009). A novel nanoliter tear collection and analysis platform - The TearLab™. *Lacrimosa, 1*, 100–103.
24. Tomlinson, A., Khanal, S., Ramaesh, K., Diaper, C., & McFadyen, A. (2006). Tear film osmolarity: Determination of a referent for dry eye diagnosis. *Investigative Ophthalmology & Visual Science, 47*, 4309–4315.
25. Butovich, I. A., Millar, T. J., & Ham, B. M. (2008). Understanding and analyzing meibomian lipids—A review. *Current Eye Research, 33*, 405–420.
26. Millar, T. J., & Schuett, B. S. (2015). The real reason for having a meibomian lipid layer covering the outer surface of the tear film - A review. *Experimental Eye Research, 137*, 125–138.
27. Shine, W. E., & McCulley, J. P. (2003). Polar lipids in human meibomian gland secretions. *Current Eye Research, 26*, 89–94.
28. Butovich, I. A. (2009). The Meibomian Puzzle: Combining pieces together. *Progress in Retinal and Eye Research, 28*, 483–498.
29. Butovich, I. A. (2008). On the lipid composition of human meibum and tears: Comparative analysis of nonpolar lipids. *Investigative Ophthalmology & Visual Science, 49*, 3779–3789.
30. Butovich, I. A., Uchiyama, E., & McCulley, J. P. (2007). Lipids of human meibum: Mass-spectrometric analysis and structural elucidation. *Journal of Lipid Research, 48*, 2220–2235.
31. Nichols, K. K., Ham, B. M., Nichols, J. J., Ziegler, C., & Green-Church, K. B. (2007). Identification of fatty acids and fatty acid amides in human Meibomian gland secretions. *Investigative Ophthalmology & Visual Science, 48*, 34–39.
32. Torrent-Burgués, J. (2011). Oleamide and oleamide-lipid mixed monolayers. *BioNanoScience, 1*, 202–209.
33. Borchman, D., Foulks, G. N., Yappert, M. C., Tang, D., & Ho, D. V. (2007). Spectroscopic evaluation of human tear lipids. *Chemistry and Physics of Lipids, 147*, 87–102.
34. Leiske, D. L., Miller, C. E., Rosenfeld, L., Cerretani, C., Ayzner, A., Lin, B., Meron, M., Senchyna, M., Ketelson, H. A., Meadows, D., Srinivasan, S., Jones, L., Radke, C. J., Toney, M. F., & Fuller, G. G. (2012). Molecular structure of interfacial human meibum films. *Langmuir, 28*, 11858–11865.
35. Gasymov, O. K., Abduragimov, A. R., Prasher, P., Yusifov, T. N., & Glasgow, B. J. (2005). Tear lipocalin: Evidence for a scavenging function to remove lipids from the human corneal surface. *Investigative Ophthalmology & Visual Science, 46*, 3589–3596.
36. Rosenfeld, L., Cerretani, C., Leiske, D. L., Toney, M. F., Radke, C. J., & Fuller, G. G. (2013). Structural and rheological properties of meibomian lipid. *Investigative Ophthalmology & Visual Science, 54*, 2720–2732.
37. Guillon, J. P., & Godfrey, A. (2007). Tears and contact lenses. In A. J. Phillips & L. Speedwell (Eds.), *Contact Lenses* (5th ed., pp. 111–127). Butterworth-Heinemann.
38. Markoulli, M., Duong, T. B., Lin, M., & Papas, E. (2018). Imaging the tear film: A comparison between the subjective Keeler Tearscope-Plus™ and the objective Oculus® Keratograph 5M and LipiView® Interferometer. *Current Eye Research, 43*, 155–162.
39. Edwards, K., Sherman, J., Portello, J. K., & Rosenfield, M. (2009). Examination of the anterior segment of the eye. In M. Rosenfield, N. Logan, & K. Edwards (Eds.), *Optometry: Science, Techniques and Clinical Management* (2ng ed.). Butterworth-Heinemann Chapter 17.
40. Josephson, J. E. (1983). *Preocular tear film layer. Optometry and Vision Science*, 883–887.

41. Guillon, J. P., & Guillon, M. (1993). Tear film examination of the contact lens patient. *Optician, 206*, 21–29.
42. Guillon, M., Styles, E., Guillon, J. P., & Maissa, C. (1997). Pre-ocular tear film characteristics of nonwears and soft contact lens wearers. *Optometry and Vision Science, 74*, 273–279.
43. Guillon, J. P. (1998). Non-invasive Tearscope Plus routine for contact lens fitting. *Contact Lens & Anterior Eye, 21*, S31–S40.
44. de la Jara Percy, L. (1997). Técnicas no invasivas en la evaluación de la película lagrimal. *Franja Visual, 8*(34), 12–15.
45. Yokoi, N., Takehisa, Y., & Kinoshita, S. (1996). Correlation of tear lipid layer interference patterns with the diagnosis and severity of dry eye. *American Journal of Ophthalmology, 122*, 818–824.
46. Braun, R. J., King-Smith, P. E., Begley, C. G., Li, L., & Gewecke, N. R. (2015). Dynamics and function of the tear film in relation to the blink cycle. *Progress in Retinal and Eye Research, 45*, 132–164.
47. Fogt, J. S., Kowalski, M. J., King-Smith, P. E., Epitropolous, A. T., Hendershot, A. J., Lembach, C., Maszczak, J. P., Jones-Jordan, L. A., & Barr, J. T. (2016). Tear lipid layer thickness with eye drops in meibomian gland dysfunction. *Clinical Ophthalmology, 10*, 2237–2243.
48. Weisenberger, K., Fogt, N., & Swingle Fogt, J. (2021). Comparison of nanoemulsion and non-emollient artificial tears on tear lipid layer thickness and symptoms. *Journal of Optometry, 14*, 20–27.
49. Georgiev, G. A., Eftimov, P., & Yokoi, N. (2017). Structure-function relationship of tear film lipid layer: A contemporary perspective. *Experimental Eye Research, 163*, 17–28.
50. Petty, M. C. (1966). *Langmuir-Blodgett films An Introduction*. Cambridge University Press.
51. Ulman, A. (1990). *An introduction to ultrathin organic films*. Academic Press.
52. Davies, J. T., & Rideal, E. K. (1961). *Interfacial phenomena* (pp. 217–281). Academic Press.
53. Vitovic, P., Nikolelis, D. P., & Hianik, T. (2006). Study of calix[4]resorcinarene-dopamine complexation in mixed phospholipid monolayers at the air/water interface. *Biochimica et Biophysica Acta, 1758*, 1852–1861.
54. Ariga, K. (2020). Don't forget Langmuir-Blodgett films 2020: Interfacial nanoarchitectonics with molecules, materials, and living objects. *Langmuir, 36*, 7158–7180.
55. Peterson, I. (2000). Langmuir monolayers. In T. H. Richardson (Ed.), *Functional organic and polymeric materials: molecular functionality--Macroscopic reality*. Wiley.
56. Hönig, D., & Möbius, D. (1991). Direct visualization of monolayers at the air-water interface by Brewster angle microscopy. *The Journal of Physical Chemistry, 95*, 4590–4592.
57. Daear, W., Mahadeo, M., & Prenner, E. J. (2017). Applications of Brewster angle microscopy from biological materials to biological Systems. *Biochimica et Biophysica Acta, 1859*, 1749–1766.
58. Georgiev, G. A., Yokoi, N., Ivanova, S., Tonchev, V., Nencheva, Y., & Krastev, R. (2014). Surface relaxations as a tool to distinguish the dynamic interfacial properties of films formed by normal and diseased meibomian lipids. *Soft Matter, 10*, 5579–5588.
59. Hagedorn, S., Drolle, E., Lorentz, H., Srinivasan, S., Leonenko, Z., & Jones, L. (2015). Atomic force microscopy and Langmuir-Blodgett monolayer technique to assess contact lens deposits and human meibum extracts. *Journal of Optometry, 8*, 187–199.
60. Kaercher, T., Hönig, D., & Möbius, D. (1995). Meibomian lipid layers seen through BAM. *Orbit, 14*, 17–24.
61. Kaercher, T., Hönig, D., & Möbius, D. (1993). Brewster angle microscopy. A new method of visualizing the spreading of Meibomian lipids. *International Ophthalmology, 17*, 341–348.
62. Kulovesi, P., Telenius, J., Koivuniemi, A., Brezesinski, G., Vattulainence, I., & Holopainen, J. M. (2012). The impact of lipid composition on the stability of the tear fluid lipid layer. *Soft Matter, 8*, 5826–5834.
63. Mudgil, P., & Millar, T. J. (2011). Surfactant properties of human Meibomian lipids. *Investigative Ophthalmology & Visual Science, 52*, 1661–1670.
64. Petrov, P. G., Thompson, J. M., Abdul Rahman, I. B., Ellis, R. E., Green, E. M., Miano, F., & Winlove, C. P. (2007). Two-dimensional order in mammalian pre-ocular tear film. *Experimental Eye Research, 84*, 1140–1146.
65. Yoshida, M., Yamaguchi, M., Sato, A., Tabuchi, N., Kon, R., & Iimura, K.-i. (2019). Role of endogenous ingredients in meibum and film structures on stability of the tear film lipid layer against lateral compression. *Langmuir, 35*, 8445–8451.
66. Miano, F., Calcara, M., Millar, T. J., & Enea, V. (2005). Insertion of tear proteins in a Meibomian lipids film. *Colloids and Surfaces, B: Biointerfaces, 44*, 49–55.
67. Millar, T. J., Mudgil, P., Butovich, I. A., & Palaniappan, C. K. (2009). Adsorption of human tear lipocalin to human Meibomian lipid films. *Investigative Ophthalmology & Visual Science, 50*, 140–151.
68. Mudgil, P., & Millar, T. J. (2008). Adsorption of apo- and holotear lipocalin to a bovine Meibomian lipid film. *Experimental Eye Research, 86*, 622–628.
69. Mudgil, P., Torres, M., & Millar, T. J. (2006). Adsorption of lysozyme to phospholipid and Meibomian lipid monolayer films. *Colloids and Surfaces, B: Biointerfaces, 48*, 128–137.
70. Svitova, T. F., & Lin, M. C. (2016). Dynamic interfacial properties of human tear-lipid films and their interactions with model-tear proteins in vitro. *Advances in Colloid and Interface Science, 233*, 4–24.
71. Tragoulias, S. T., Anderton, P. J., Dennis, G. R., Miano, F., & Millar, T. J. (2005). Surface pressure measurements of human tears and individual tear film components indicate that proteins are major contributors to the surface pressure. *Cornea, 24*, 189–200.
72. Georgiev, G. A., Kutsarova, E., Jordanova, A., Krastev, R., & Lalchev, Z. (2010). Interactions of Meibomian gland secretion with polar lipids in Langmuir monolayers. *Colloids and Surfaces B: Biointerfaces, 78*, 317–327.
73. Rantamäki, A. H., & Holopainen, J. M. (2017). The effect of phospholipids on tear film lipid layer surface activity. *Investigative Ophthalmology & Visual Science, 58*, 149–154.
74. Bland, H. C., Moilanen, J. A., Ekholm, F. S., & Paananen, R. O. (2019). Investigating the role of specific tear film lipids connected to dry eye syndrome: A study on O-acyl-ω-hydroxy fatty acids and diesters. *Langmuir, 35*, 3545–3552.
75. Kulovesi, P., Telenius, J., Koivuniemi, A., Brezesinski, G., Rantamäki, A., Viitala, T., Puukilainen, E., Ritala, M., Wiedmer, S. K., Vattulainen, I., & Holopainen, J. M. (2010). Molecular organization of the tear fluid lipid layer. *Biophysical Journal, 99*, 2559–2567.
76. Guaus, E., & Torrent-Burgués, J. (2018). The Langmuir technique applied to the study of natural tears. *BioNanoScience, 8*, 559–565.
77. Guaus, E., Fàbregas, C., Pérez, M., & Torrent-Burgués, J. (2017). A combined clinical and Langmuir film study of natural tear films. *International Journal of Ophthalmology and Visual Science, 2*, 5–14.
78. Bartkowiak, A., Rojewska, M., & Prochaska, K. (2019). Study of mucin interaction with model phospholipid membrane at the air-water interface. *Colloids and Surfaces A: Physicochemical and Engineering Aspects, 578*, 123587.
79. Millar, T. J., Tragoullas, S. T., Anderton, P. J., Bali, M. S., Miano, F., Dennis, G. R., & Mudgil, P. (2006). The surface activity of

purified ocular mucin at the air-liquid interface and interaction with Meibomian lipids. *Cornea, 25*, 91–100.
80. Nishimura, S. Y., Mata Magana, G., Ketelson, H. A., & Fuller, G. G. (2008). Effect of lysozyme adsorption on the interfacial rheology of DPPC and cholesteryl myristate films. *Langmuir, 24*, 11728–11733.
81. Miano, F., Zhao, X., Lu, J. R., & Penfold, J. (2007). Coadsorption of human milk lactoferrin into the dipalmitoylglycerolphosphatidylcholine phospholipid monolayer spread at the air/water interface. *Biophysical Journal, 52*, 1254–1262.
82. Flanagan, J. L., & Willcox, M. D. P. (2009). Role of lactoferrin in the tear film. *Biochimie, 91*, 35–43.
83. Torrent-Burgués, J., & Raju, R. (2019). Effect of lysozyme subphase and insertion on several lipid films. *Advanced Materials science, 4*, 1–7.
84. Guckeisen, T., Hosseinpour, S., & Peukert, W. (2019). Isoelectric points of proteins at the air/liquid interface and in solution. *Langmuir, 35*, 5004–5012.
85. de Paula Ayoub, F., & Caseli, L. (2017). Controlling the molecular architecture of lactase immobilized in Langmuir-Blodgett films of phospholipids to modulate the enzyme activity. *Colloids and Surfaces B: Biointerfaces, 150*, 8–14.
86. Blume, A., & Kerth, A. (2013). Peptide and protein binding to lipid monolayers studied by FT-IRRA spectroscopy. *Biochimica et Biophysica Acta, 1828*, 2294–2305.
87. Boisselier, E., Demers, E., Cantin, L., & Salesse, C. (2017). How to gather useful and valuable information from protein binding measurements using Langmuir lipid monolayers. *Advances in Colloid and Interface Science, 243*, 60–76.
88. Dopierała, K., Krajewska, M., & Prochaska, K. (2019). Binding of -lactalbumin to oleic acid monolayer and its relevance to formation of HAMLET-like complexes. *International Dairy Journal, 89*, 96–104.
89. Elderdfi, M., & Sikorski, A. F. (2018). Langmuir-monolayer methodologies for characterizing protein-lipid interactions. *Chemistry and Physics of Lipids, 212*, 61–72.
90. Finot, E., Markey, L., Hane, F., Amrein, M., & Leonenko, Z. (2013). Combined atomic force microscopy and spectroscopic ellipsometry applied to the analysis of lipid–protein thin films. *Colloids and Surfaces B: Biointerfaces, 104*, 289–293.
91. Fragneto, G., Alexandre, S., Valleton, J.-M., & Rondelez, F. (2013). Competition for space between a protein and lipid monolayers. *Colloids and Surfaces B: Biointerfaces, 103*, 416–421.
92. Gorbenko, G. P., Ioffe, V. M., & Kinnunen, P. K. J. (2007). Binding of lysozyme to phospholipid bilayers: Evidence for protein aggregation upon membrane association. *Biophysical Journal, 93*, 140–153.
93. Krajewska, M., Dopierała, K., & Prochaska, K. (2020). Lipid–protein interactions in Langmuir monolayers under dynamically varied conditions. *The Journal of Physical Chemistry B, 124*, 302–311.
94. Maiti, K., Bhattacharya, S. C., Moulik, S. P., & Panda, A. K. (2013). Effect of bovine serum albumin on the functionality and structure of catanionic surfactant at air–buffer interface. *Materials Science and Engineering: C, 33*, 836–843.
95. Miller, R., Fainerman, V. B., Makievski, A. V., Krägel, J., Grigoriev, D. O., Kazakov, V. N., & Sinyachenko, O. V. (2000). Dynamics of protein and mixed protein surfactant adsorption layers at the water fluid interface. *Advances in Colloid and Interface Science, 86*, 39–82.
96. Ohtsuka, I., & Yokoyama, S. (2005). Penetration of bovine serum albumin into dipalmitoyl-phosphatidylglycerol monolayers: direct observation by atomic force microscopy. *Chemical & Pharmaceutical Bulletin, 53*, 42–47.
97. Suárez-Germà, C., Domènech, O., Montero, M. T., & Hernández-Borrell, J. (2014). Effect of lactose permease presence on the structure and nanomechanics of two-component supported lipid bilayers. *Biochimica et Biophysica Acta, 1838*, 842–852.
98. Toimil, P., Prieto, G., Miñones, J., Jr., Trillo, J. M., & Sarmiento, F. (2012). Monolayer and Brewster angle microscopy study of human serum albumin-Dipalmitoylphosphatidylcholine mixtures at the air-water interface. *Colloids and Surfaces B: Biointerfaces, 92*, 64–73.
99. Calvez, P., Demers, E., Boisselier, E., & Salesse, C. (2011). Analysis of the contribution of saturated and polyunsaturated phospholipid monolayers to the binding of proteins. *Langmuir, 27*, 1373–1379.
100. Calvez, P., Bussieres, S., Demers, E., & Salesse, S. (2009). Parameters modulating the maximum insertion pressure of proteins and peptides in lipid monolayers. *Biochemie, 91*, 718–733.
101. Stefaniu, C., Brezesinski, G., & Möhwald, H. (2014). Langmuir monolayers as models to study processes at membrane surfaces. *Advances in Colloid and Interface Science, 208*, 197–213.
102. Patel, S., & Blades, K. J. (2003). *The dry eye*. Butterworth-Heinemann.
103. Hom, M. M., & Bruce, A. S. (2009). Prelens tear stability: Relationship to symptoms of dryness. *Optometry, 80*, 181–184.
104. Viso, E., Gude, F., & Rodríguez-Ares, M. T. (2011). The association of Meibomian gland dysfunction and other common ocular diseases with dry eye. *Cornea, 30*, 1–6.
105. Albietz, J. M. (2000). Prevalence of dry eye subtypes in clinical optometry practice. *Optometry and Vision Science, 77*, 357–363.
106. Cerretani, C. F., Ho, N. H., & Radke, C. J. (2013). Water-evaporation reduction by duplex films: Application to the human tear film. *Advances in colloid and interface science, 197–198*, 33–57.
107. Nichols, J. J., & Sinnott, L. T. (2006). Tear film, contact lens, and patient-related factors associated with contact lens–related dry eye. *Investigative Ophthalmology & Visual Science, 47*, 1319–1328.
108. Pult, H., Murphy, P. J., & Purslow, C. (2009). A novel method to predict the dry eye symptoms in new contact lens wearers. *Optometry and Vision Science, 86*, E1042–E1050.
109. González-Méijome, J. M., Parafita, M. A., Yebra-Pimentel, E., & Almeida, J. B. (2007). Symptoms in a population of contact lens and noncontact lens wearers under different environmental conditions. *Optometry and Vision Science, 84*, E296–E302.
110. Nagda, N. L., & Hodgson, M. (2001). Low relative humidity and air cabin quality. *Indoor Air, 11*, 200–214.
111. King-Smith, P. E., Nichols, J. J., Nichols, K. K., Fink, B. A., & Braun, R. J. (2008). Contributions of evaporation and other mechanisms to tear film thinning and break-up. *Optometry and Vision Science, 85*, 623–630.
112. Khanal, S., Tomlinson, A., McFadyen, A., Diaper, C., & Ramaesh, K. (2008). Dry eye diagnosis. *Investigative Ophthalmology & Visual Science, 49*, 1407–1414.
113. Labbé, A., Brignole-Baudouin, F., & Baudouin, C. (2007). Méthodes d'évaluation de la surface oculaire dans les syndromes secs. *Journal français d'ophtalmologie, 30*, 76–97.
114. Wander, A. H. (2011). Long-term use of hydroxypropyl cellulose ophthalmic insert to relieve symptoms of dry eye in a contact lens wearer: Case-based experience. *Eye & Contact Lens, 37*, 39–44.
115. Nichols, K. K., Evans, D. G., & Karpecki, P. M. (2020). A comprehensive review of the clinical trials conducted for dry eye disease and the impact of the vehicle comparators in these trials. *Current Eye Research, 46*(5), 609. https://doi.org/10.1080/02713683.2020.1836226
116. Moshirfar, M., Pierson, K., Hanamaikai, K., Santiago-Caban, L., Muthappan, V., & Passi, S. F. (2014). Artificial tears potpourri: A literature review. *Clinical Ophthalmology, 8*, 1419–1433.

117. Murube, J., Paterson, A., & Murube, E. (1998). Classification of Artificial Tears I. *Advances in Experimental Medicine and Biology, 438*, 693–701.
118. Murube, J., Murube, A., & Zhuo, C. (1998). Classification of Artificial Tears II. *Advances in Experimental Medicine and Biology, 438*, 705–715.
119. Benelli, U. (2011). Systane® lubricant eye drops in the management of ocular dryness. *Clinical Ophthalmology, 5*, 783–790.
120. Doughty, M. J., & Glavin, S. (2009). Efficacy of different dry eye treatments with artificial tears or ocular lubricants: A systematic review. *Ophthalmic and Physiological Optics, 29*, 573–583.
121. Grene, R. B., Lankston, P. B. A., Mordaunt, J. M. S., Harrold, M. B. S. M., Gwon, A., & Jones, R. (1992). Unpreserved carboxymethylcellulose artificial tears evaluated in patients with Keratoconjunctivitis Sicca. *Cornea, 11*, 294–301.
122. Markoulli, M., Sobbizadeh, A., Tan, J., Briggs, N., & Coroneo, M. (2018). The effect of optive and optive advanced artificial tears on the healthy tear film. *Current Eye Research, 43*, 588–594.
123. McCann, L. C., Tomlinson, A., Pearce, E. I., & Papa, V. (2012). Effectiveness of artificial tears in the management of evaporative dry eye. *Cornea, 31*, 1–5.
124. Nepp, J., Schauersberger, J., Schild, G., Jandrasits, K., Haslinger-Akramian, J., Derbolav, A., & Wedrich, A. (2001). The clinical use of viscoelastic artificial tears and sodium chloride in dry-eye syndrome. *Biomaterials, 22*, 3305–3310.
125. Nilforoushan, M. R., Latkany, R. A., & Speaker, M. G. (2005). Effect of artificial tears on visual acuity. *American Journal of Ophthalmology, 140*, 830–835.
126. Ousler, G. W., Michaelson, C., & Christensen, M. T. (2007). An evaluation of tear film breakup time extension and ocular protection index scores among three marketed lubricant eye drops. *Cornea, 26*, 949–952.
127. Paugh, J. R., Nguyen, A. L., Ketelson, H. A., Christensen, M. T., & Meadows, D. L. (2008). Precorneal residence time of artificial tears measured in dry eye subjects. *Optometry and Vision Science, 85*, 725–731.
128. Ridder, W. H., III, Tomlinson, A., & Paugh, J. (2005). Effect of artificial tears on visual performance in subjects with dry eye. *Optometry and Vision Science, 82*, 835–842.
129. Springs, C. L. (2010). Novel hydroxypropyl-guar gellable lubricant eye drops for treatment of dry eye. *Advances in Therapy, 27*, 1–10.
130. Urzua, C. A., Vasquez, D. H., Huidobro, A., Hernandez, H., & Alfaro, J. (2012). Randomized double-blind clinical trial of autologous serum versus artificial tears in dry eye syndrome. *Current Eye Research, 37*, 684–688.
131. Wang, T.-J., Wang, I.-J., Ho, J.-D., Chou, H.-C., Lin, S.-Y., & Huang, M.-C. (2010). Comparison of the clinical effects of carbomer-based lipid-containing gel and hydroxypropyl-guar gel artificial tear formulations in patients with dry eye syndrome: A 4-week, prospective, open-label, randomized, parallel-group, noninferiority study. *Clinical Therapeutics, 32*, 44–52.
132. Dinslage, S., Stoffel, W., Diestelhorst, M., & Krieglstein, G. (2002). Tolerability and safety of two new preservative-free tear film substitutes. *Cornea, 21*, 352–355.
133. Korb, D. R., Scaffidi, R. C., Greiner, J. V., Kenyon, K. R., Herman, J. P., Blackie, C. A., Glonek, T., Case, C. L., Finnemore, V. M., & Douglass, T. (2005). The effect of two novel lubricant eye drops on tear film lipid layer thickness in subjects with dry eye symptoms. *Optometry and Vision Science, 82*, 594–601.
134. Peters, K., & Millar, T. J. (2002). The role of different phospholipids on tear break-up time using a model eye. *Current Eye Research, 25*, 55–60.
135. Scaffidi, R. C., & Korb, D. R. (2007). Comparison of the efficacy of two lipid emulsion eyedrops in increasing tear film lipid layer thickness. *Eye & Contact Lens, 33*, 38–44.
136. Steven, P., Augustin, A. J., Geerling, G., Kaercher, T., Kretz, F., Kunert, K., Menzel-Severing, J., Schrage, N., Schrems, W., Krösser, S., Beckert, M., & Messmer, E. M. (2017). Semifluorinated alkane eye drops for treatment of dry eye disease due to meibomian gland disease. *Journal of Ocular Pharmacology and Therapeutics, 33*, 678–685.
137. Mishra, G. P., Bagui, M., Tamboli, V., & Mitra, A. K. (2011). Recent applications of liposomes in ophthalmic drug delivery. *Journal of drug delivery, 2011*, 1–14.
138. Rohit, A., Willcox, M. D. P., & Stapleton, F. (2016). Effects of lipid supplements on tear biochemistry in contact lens wearers. *Optometry and Vision Science, 93*, 1203–1209.
139. Ceridório, L. F., Caseli, L., & Oliveira, O. N., Jr. (2016). Chondroitin sulfate interacts mainly with headgroups in phospholipid monolayers. *Colloids and Surfaces B: Biointerfaces, 141*, 595–601.
140. Zander, T., Florian Wieland, D. C., Raj, A., Wang, M., Nowak, B., Krywka, C., Dédinaité, A., Claesson, P. M., Garamus, V. M., Schreyer, A., & Willumeit-Römer, R. (2016). The influence of hyaluronan on the structure of a DPPC-bilayer under high pressures. *Colloids and Surfaces B: Biointerfaces, 142*, 230–238.
141. Torrent-Burgués, J. (2016). Langmuir films study on lipid-containing artificial tears. *Colloids and Surfaces, B: Biointerfaces, 140*, 185–188.
142. Eftimov, P., Yokoi, N., Tsuji, K., Peev, N., & Georgiev, G. A. (2022). Langmuir trough study of the interactions of tear mimetic eyedrop formulations with human meibum films. *Applied Sciences, 12*, 12095.
143. Di Pascuale, M. A., Goto, E., & Tseng, S. C. G. (2004). Sequential changes of lipid tear film after the instillation of a single drop of a new emulsion eye drop in dry eye patients. *Ophthalmology, 111*, 783–791.
144. Georgiev, G. A., Yokoi, N., Koev, K., Kutsarova, E., Ivanova, S., Kyumurkov, A., Jordanova, A., Krastev, R., & Lalchev, Z. (2011). Surface chemistry study of the interactions of benzalkonium chloride with films of meibum, corneal cells lipids, and whole tears. *Investigative Ophthalmology & Visual Science, 52*, 4645–4654.
145. Moon, J. W., Lee, H. J., Shin, K. C., Wee, W. R., Lee, J. H., & Kim, M. K. (2007). Short term effects of topical cyclosporine and viscoelastic on the ocular surfaces in patients with dry eye. *Korean Journal of Ophthalmology, 21*, 189–194.
146. Dougthy, M. J. (2009). Basic pharmacology relevant to the action of drugs on the eye. In M. Rosenfield, N. Logan, & K. Edwards (Eds.), *Optometry: Science, Techniques and Clinical Management* (2nd ed.). Butterworth-Heinemann Chapter 6.
147. Hopkins, G. (2009). Therapeutic drugs. In M. Rosenfield, N. Logan, & K. Edwards (Eds.), *Optometry: Science, techniques and clinical Management* (2nd ed.). Butterworth-Heinemann Chapter 8.
148. Lam, C. S. Y. (2009). Diagnostic drugs. In M. Rosenfield, N. Logan, & K. Edwards (Eds.), *Optometry: Science, techniques and clinical management* (2nd ed.). Butterworth-Heinemann Chapter 7.
149. Koffler, B. H., McDonald, M., & Nelinson, D. S. (2010). Improved signs, symptoms, and quality of life associated with dry eye syndrome: Hydroxypropyl cellulose ophthalmic insert patient registry. *Eye & Contact Lens, 36*, 170–176.
150. Williams, L., & Stapleton, F. (2007). Microbiology, lens care and maintenance. In A. J. Phillips & L. Speedwell (Eds.), *Contact Lenses* (5th ed.). Butterworth-Heinemann.

151. Sanfilippo, C. M., Morrissey, I., Janes, R., & Morris, T. W. (2016). Surveillance of the activity of aminoglycosides and fluoroquinolones against ophthalmic pathogens from Europe in 2010–2011. *Current Eye Research, 41*, 581–589.
152. Oh, T., Jung, Y., Chang, D., Kim, J., & Kim, H. (2012). Changes in the tear film and ocular surface after cataract surgery. *Japanese Journal of Ophthalmology, 56*, 113–118.
153. Venincasa, V. D., Galor, A., Feuer, W., Lee, D. J., Florez, H., & Venincasa, M. J. (2013). Long-term effects of cataract surgery on tear film parameters. *The Scientific World Journal, 2013*, 643764.
154. Fangueiro, J. F., Andreani, T., Fernandes, L., Garcia, M. L., Egea, M. A., Silva, A. M., & Souto, E. B. (2014). Physicochemical characterization of epigallocatechin gallate lipid nanoparticles (EGCG-LNs) for ocular instillation. *Colloids and Surfaces B: Biointerfaces, 123*, 452–460.
155. Mohammed, I., Said, D. G., & Dua, H. S. (2017). Human antimicrobial peptides in ocular surface defense. *Progress in Retinal and Eye Research, 61*, 1–22.
156. Fernandes, M. M., Ivanova, K., Francesko, A., Rivera, D., Torrent-Burgués, J., Gedanken, A., Mendonza, E., & Tzanov, T. (2016). Escherichia coli and Pseudomonas aeruginosa eradication by nano-penicillin G. Nanomedicine: Nanotechnology. *Biology, and Medicine, 12*, 2061–2069.
157. Fernandes, M. M., Francesko, A., Torrent-Burgués, J., Carrión-Fité, F., Heinze, T., & Tzanov, T. (2014). Sonochemically processed cationic nanocapsules - Efficient antimicrobials with membrane disturbing capacity. *Biomacromolecules, 15*, 1365–1374.
158. Fernandes, M. M., Francesko, A., Torrent-Burgués, J., & Tzanov, T. (2013). Effect of thiol-functionalisation on chitosan antibacterial activity: Interactions with a bacterial membrane model. *Reactive and Functional Polymers, 73*, 1384–1390.
159. Hoyo, J., Ivanova, K., Guaus, E., & Tzanov, T. (2019). Multifunctional ZnO NPs-chitosan-gallic acid hybrid nanocoating to overcome contact lenses associated conditions and discomfort. *Journal of Colloid and Interface Science, 543*, 114–121.
160. Caseli, L., Sousa-Martins, D., Maia, M., Lima-Filho, A. A. S., Rodrigues, E. B., & Belfort, R., Jr. (2013). An intraocular dye solution based on lutein and zeaxanthin in a surrogate internal limiting membrane model: A Langmuir monolayer study. *Colloids and Surfaces B: Biointerfaces, 107*, 124–129.
161. Souza, A. L., Ceridório, L. F., Paula, G. F., Mattoso, L. H. C., & Oliveira, O. N., Jr. (2015). Understanding the biocide action of poly(hexamethylene biguanide) using Langmuir monolayers of dipalmitoyl phosphatidylglycerol. *Colloids and Surfaces B: Biointerfaces, 132*, 117–121.
162. Sandez-Macho, I., Casas, M., Lage, E. V., Rial-Hermida, M. I., Concheiro, A., & Alvarez-Lorenzo, C. (2015). Interaction of poloxamine block copolymers with lipid membranes: Role of copolymer structure and membrane cholesterol content. *Colloids and Surfaces B: Biointerfaces, 133*, 270–277.
163. Basak, U. K., Datta, A., & Bhattacharyya, D. (2015). Stability and softening of a lipid monolayer in the presence of a pain-killer drug. *Colloids and Surfaces B: Biointerfaces, 132*, 34–44.
164. Corvis, Y., Barzyk, W., Brezesinski, G., Mrabet, N., Badis, M., Hecht, S., & Rogalska, E. (2006). Interactions of a fungistatic antibiotic, griseofulvin, with phospholipid monolayers used as models of biological membranes. *Langmuir, 22*, 7701–7711.
165. Costalonga, B. L. P., da Silva, R. C., Caseli, L., & Molina, C. (2012). Interaction of chlorhexidine with biomembrane models on glass ionomer by using the Langmuir–Blodgett technique. *Colloids and Surfaces B: Biointerfaces, 97*, 57–61.
166. Fernández-Botello, A., Comelles, F., Alsina, M. A., Cea, P., & Reig, F. (2008). A monolayer study on interactions of docetaxel with model lipid membranes. *The Journal of Physical Chemistry B, 112*, 13834–13841.
167. Flasiński, M., Gawryś, M., Broniatowski, M., & Wydro, P. (2016). Studies on the interactions between parabens and lipid membrane components in monolayers at the air/aqueous solution interface. *Biochimica et Biophysica Acta, 1858*, 836–844.
168. Geraldo, V. P. N., Pavinatto, F. J., Nobre, T. M., Caseli, L., & Oliveira, O. N., Jr. (2013). Langmuir films containing ibuprofen and phospholipids. *Chemical Physics Letters, 559*, 99–106.
169. Karewicz, A., Bielska, D., Gzyl-Malcher, B., Kepczynski, M., Lach, R., & Nowakowska, M. (2011). Interaction of curcumin with lipid monolayers and liposomal bilayers. *Colloids and Surfaces B: Biointerfaces, 88*, 231–239.
170. Korchowiec, B., Korchowiec, J., Orlof-Naturalna, M., Regnouf de Vains, J.-B., & Rogalska, E. (2016). Two antibacterial nalidixate calixarene derivatives in cholesterol monolayers: Molecular dynamics and physicochemical effects. *Colloids and Surfaces B: Biointerfaces, 145*, 777–784.
171. Kotecka, K., & Krysinski, P. (2015). Effect of tetracycline antibiotic on the monolayers of phosphatidylcholines at the air–water interface. *Colloids and Surfaces A: Physicochemical and Engineering Aspects, 482*, 678–686.
172. Matyszewska, D., & Bilewicz, R. (2015). Interactions of daunorubicin with Langmuir–Blodgett thiolipid monolayers. *Electrochimica Acta, 162*, 45–52.
173. Mircheva, K., Gonnet, M., Balashev, K., Ivanova, T., Boury, F., & Panaiotov, I. (2014). Properties of -carotene and retinoic acid in mixed monolayers with dipalmitoylphosphatidylcholine (DPPC) and Solutol. *Colloids and Surfaces A: Physicochemical and Engineering Aspects, 460*, 209–218.
174. Morandi, S., Puggelli, M., & Caminati, G. (2008). Antibiotic association with phospholipid nano-assemblies: A comparison between Langmuir–Blodgett films and supported lipid bilayers. *Colloids and Surfaces A: Physicochemical and Engineering Aspects, 321*, 125–130.
175. Olechowska, K., Mach, M., Hąc-Wydro, K., & Wydro, P. (2019). Studies on the interactions of 2-hydroxyoleic acid with monolayers and bilayers containing cationic lipid: Searching for the formulations for more efficient drug delivery to cancer cells. *Langmuir, 35*, 9084–9092.
176. Wu, X., Chantemargue, B., Di Meo, F., Bourgaux, C., Chapron, D., Trouillas, P., & Rosilio, V. (2019). Deciphering the peculiar behavior of β-Lapachone in lipid monolayers and bilayers. *Langmuir, 35*, 14603–14615.
177. Zhang, Z., Hao, C., Qu, H., & Sun, R. (2019). Studies on the dynamic adsorption process of Lycium barbarum polysaccharide in the POPC/DPPC monolayers. *Colloids and Surfaces B: Biointerfaces, 178*, 38–43.

Note: Figures presented in the paper are original from the author.