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Lipid artificial tears at a mimetic ocular interface

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ABSTRACT

We studied the behaviour of three lipid tear products, commercialised by the same brand, as Langmuir films at the air/liquid interface to simulate the ocular environment. No significant differences were observed in the surface behaviour of two of them disclosing the same composition, but commercialised for different applications. The interaction of several subphases, namely sodium chloride, glucose, albumin and lysozyme present in the natural tear, with the lipid films was assessed at room temperature and the temperature of human tear using surface pressure-area isotherms and elastic modulus plots. There is a notable influence of sodium chloride and the proteins albumin and lysozyme on the surface pressure-area isotherm of the lipid Langmuir films. Albumin shifted this isotherm to lower areas while an opposite shift was caused by lysozyme. These studies could be useful for the formulation of new lipid-containing artificial tears, and for increasing the confidence of the customers in commercial eye care formulations.

1. Introduction

Dry eye condition (Patel and Blades, 2003) is one of the most extended ocular conditions counting with millions of patients world-wide yearly. Among the most important causes for this condition are the wearing of contact lenses (CL) (Nichols and Sinnott, 2006), meibomian gland dysfunction, pregnancy, Sjögren syndrome, vitamin A deficiency, omega-3 fatty acid deficiency, LASIK surgery, and certain medications (Kanellopoulos and Asimellis, 2016). Hydrophilic coating on CL partially reduce the dryness syndrome caused by CL wearing (Hoyo et al., 2019), whereas the dry eye syndrome caused by other conditions is mitigated by the use of artificial tears (Geerling et al., 2011; Jones et al., 2017).

The lipid layer of the tear is comprised of polar lipids, mainly phospholipids, fatty acids and fatty amides, forming a nanometre layer in contact with the aqueous layer of the tear, and non-polar lipids, mainly cholesterol esters, triglycerides and waxes, which form the tear outermost layer (Butovich, 2008; Millar and Schuett, 2015). On the other hand, the aqueous layer of the tear contains sodium chloride, glucose, albumin, lysozyme and lactoferrin, between others. Albumin and lysozyme are part of the eye antimicrobial and immunologic defence mechanisms, lowering also the surface tension.

Most of the marketed artificial tears include, besides of isotonic saline solution that only increases the tear volume, other components, such as viscous or wetting agents, in order to extend the residence time of the tear conferring user comfort (Moshirfar et al., 2014; Murube et al., 1998). These artificial tears are usually dispensed as drops and several studies have been reported with this subject (Doughty and Glavin, 2009; McCann et al., 2012; Nilforoushan et al., 2005; Ridder et al., 2005; Urzua et al., 2012; Wang et al., 2010). Lipid artificial tears are prescribed for restitution of the lipid layer of the natural tears (Benelli, 2011; Korb et al., 2005; Peters and Millar, 2002; Scaffidi and Korb, 2007), and the Langmuir technique can be used to study characteristics of these lipid tears (Kulovesi et al., 2010; Torrent-Burgués, 2016). Some of these tears use lecithin (phosphatidylcholines) in the form of liposomes. The lipid layer of the natural tears is secreted meanly by Meibomian glands, which is also susceptible to be studied using the Langmuir technique (Bland et al., 2019; Guaus et al., 2017; Guaus and Torrent-Burgués, 2018; Hagedorn et al., 2015; Kaercher et al., 1995; Mudgil and Millar, 2011; Petrov et al., 2007; Rantamäki and Holopainen, 2017; Yoshida et al., 2019).

A limited number of works report on the application of the Langmuir technique for analysing the interaction of Meibomian lipids with lachrymal proteins (Miano et al., 2005; Millar et al., 2009; Mudgil et al., 2006; Mudgil and Millar, 2008), and the effect of a preservative as benzalconium chloride on tear films (Georgiev et al., 2011).

In a previous work (Torrent-Burgués, 2016) we validated the applicability of the technique for analysing the behaviour of several lipid-containing tear products, using water as the subphase. The present work extends this approach for assessing the interaction of commercial

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lipid-containing artificial tears with components from the aqueous layer of natural tears, such as sodium chloride, glucose and proteins. The improvement of the knowledge of such interactions will render better commercial artificial tears. For the practical relevance of this work, the surface behaviour of three lipid tear products, commercialised by the same brand, however with similar compositions, has been investigated.

2. Materials and methods

2.1. Materials

Sodium chloride (Reagent Plus ≥99 %), glucose (ACS Reagent), bovine serum albumin (BSA, ≥98 %) and lysozyme from chicken egg white were purchased from Sigma Aldrich. Phosphate buffer solution (PBS) tablets from Fisher BioReagents and MilliQ® quality ultrapure water were used for the subphase preparation. The artificial lipid tear sprays were provided by Optima Medical Swiss AG, and designated as "red", "green" and "yellow" according to the packaging colour (Table 1). Optrex-yellow and Optrex-red have exactly the same composition, whereas Optrex-green only differs in the presence of pro-vitamin B5. The soy lecithin component is in liposomal form.

2.2. Subphase preparation

A saline solution was prepared with NaCl to obtain a physiological concentration of 0.153 M (0.9 % wt/wt). The PBS solution was prepared by dissolving 5 tablets in 1 L of milliQ water, obtaining a final PBS composition of 0.01 M phosphate buffer, 0.0027 M potassium chloride and 0.137 M sodium chloride. Glucose, BSA and lysozyme were dissolved in PBS solution to obtain concentration values similar to those of the natural tear film: glucose 1 g L^{-1} (Miano et al., 2005), BSA 0.2 g L^{-1} (Li et al., 2010) and lysozyme 0.2 g L^{-1} (Li et al., 2010; Slack, 2004).

2.3. Techniques and equipment

Langmuir compression isotherms were acquired on a KSV-NIMA Teflon trough, equipped with two movable barriers. The surface pressure was measured using a pressure sensor with a Wilhelmy plate (10 mm × 50 mm filter paper; Whatman 1). The linear velocity of the barriers was 2 cm.min^{-1}, which corresponds to an area change of 15 cm^2. min^{-1}. The trough was placed on a vibration-isolated table and enclosed in an environmental chamber. The temperature was controlled at 23 ± 1 °C or 32 ± 1 °C. Prior each experiment, the trough and barriers were cleaned twice with chloroform, allowing complete chloroform evaporation, and once with MilliQ® quality water before the subphase addition.

A 5−7 μL aliquot of lipid tear was spread on the surface of the subphase solution with a Hamilton microsyringe. The experiment started after 15 min equilibration time to allow lipid spreading. Every Surface pressure-Area (π–A) isotherm was performed in triplicate showing satisfactory reproducibility. 1-palmitoyl-2-oleoyl-glycero-3-phosphocholine (POPC) as the mean component of the artificial tear is used as a representative only for molecular area extrapolation.

2.4. Data analysis

The inverse of the compressibility modulus, or elastic modulus, (C_s^{-1}) provides information about the elasticity and the compressibility of the corresponding monolayer and is used for physical state identification. C_s^{-1} is obtained from the π-A isotherms calculated according to Eq. 1, where A is the mean area per molecule (Å^2·molecule^{-1}), π the surface pressure (mN m^{-1}) and T the absolute temperature (K).

$$C_s^{-1} = -A\left(\frac{d\pi}{dA}\right)_T \quad (1)$$

3. Results and discussion

3.1. Interfacial mimetic tear inorganic subphase

The behaviour of three Optrex tear products has been studied first in water subphase, and then the influence of 0.9 % sodium chloride without pH control, and in a PBS buffered subphase has been assessed.

The surface pressure-area isotherms corresponding to the three tear products, on water subphase at 23 °C (Fig. 1A) show a similar behaviour and collapse pressure of ≈ 45 mN m^{-1} with only slight area shifts between them. The elastic modulus plots (Fig. 1B), however, present the maximum of C_s^{-1} below 50 mN m^{-1} indicating a fluid film in the liquid expanded state (Vitovič et al., 2006). In contrast, significant differences were previously observed among spray lipid tears commercialised by different companies (Torrent-Burgués, 2016). The artificial tears studied in the current work are commercialised by the same company as three different products with the same or a very similar composition. Thus, the small differences in the isotherms can be attributed to the experimental errors and particularly to the fact that these are commercial products and deviation in their behaviour may be due to differences in the industrial batch preparations in terms of concentration or consistency of the raw material from different providers.

The isotherms are referred to the area per molecule of the 1-palmitoyl-2-oleoyl-glycero-3-phosphocholine (POPC) molecule for comparison purposes. POPC is a phosphatidylcholine model, which presents a collapse at an area of 70 Å^2·molecule^{-1} and surface pressure of 46 mN m^{-1} (Domènech et al., 2005). The low area per molecule at the collapse of the different artificial tears observed in Fig. 1 (≈7 Å^2/molecule) indicates that the liposomes in the preparation, even spread onto the surface, may go inside the subphase and remain partially

Table 1
Composition for 1 mL of Optrex lipid tears.

Optrex-yellow itchiness and tearing	**Optrex-green** tired and annoying eyes	**Optrex-red** 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
Purified water	Purified water	Purified water
	5 mg pro-vitamin B5 (dexapantenol)	

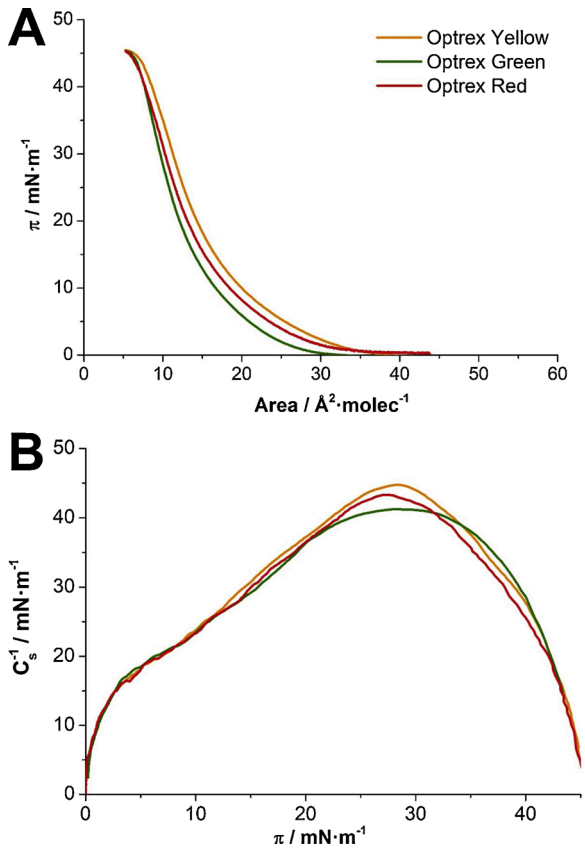

Fig. 1. A) Surface pressure-area isotherms for three Optrex artificial tears (see Table 1) in water subphase at 23 °C. B) Elastic modulus vs. surface pressure for the isotherms of Fig. 1A.

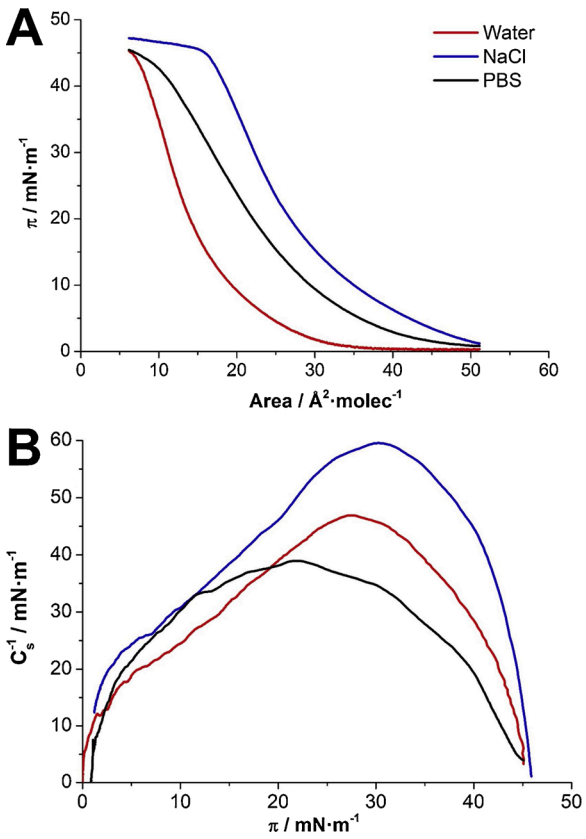

Fig. 2. A) Surface pressure-area isotherms for the Optrex-red artificial tear at 23 °C in different subphases: water, saline and PBS. B) Elastic modulus vs. surface pressure for the isotherms of Fig. 2A.

dispersed there, that is, the liposomes forming the spray don't extend completely onto the subphase.

The three tears had a similar behaviour at 23 °C in both saline and PBS subphase (Figs. 2 and S1). Artificial tear Optrex-red, shown as representative in Fig. 2, demonstrates that PBS and more intensively NaCl produce an expansion of the layer, shifting the isotherms to higher molecular areas. NaCl also induces the increase of the collapse area ≈17 $Å^2$·molecule^{-1}. The Optrex-red layer is in liquid expanded state in the three subphases, although NaCl induces a higher elastic modulus increase, indicating a slight rigidity effect due to this salt. Gurtovenko and Vattulainen (2008) and Redondo-Morata et al. (2012) reported that sodium ions had a strong effect on phosphatidylcholine bilayers, increasing the lateral interactions between phospholipid molecules. Molecular dynamic simulations of the effect of NaCl and KCl on POPC and POPE phospholipid bilayers under physiological conditions indicated strong interactions for POPC and Na^+ ions leading to considerable membrane compression, and less influence of K^+ ions (Gurtovenko and Vattulainen, 2008). Redondo-Morata et al. (2012) using force spectroscopy concluded that the charge and size of ions, and the type of phospholipid must be considered in their interactions, and a stronger influence of K^+ ions than of Na^+ ions on DLPC and DPPC bilayers was found. Bockmann et al. (2003) demonstrated that sodium chloride alters strongly the structural and dynamic properties of a neutral lipid bilayer. Zasadzinski et al. (2010), working with lung surfactants (LS), also observed similar shift of their isotherms to higher areas in presence of NaCl. The PC composition of commercial lipid-containing artificial tears is not well known; thus, it is difficult to correlate their behaviour with previous findings on well-established systems using a single model molecule. Nevertheless, the expansion observed in the present work in the π-A isotherms in NaCl subphase could be due to a greater extension of the PC liposomes induced by the presence of the sodium ions.

The isotherms and the corresponding elastic modulus plots in Fig. 3 of the tree Optrex tears were obtained on water subphase at 32 °C, the temperature of the human tear. The shape of the isotherms is similar for the three tear products and the physical state is liquid expanded. Supplementary Fig. S2 shows again the influence of the subphase on one of the Optrex tear (the Optrex-red) at 32 °C. A shift caused by PBS and the saline subphase to higher areas of the isotherms was observed. The values of the elastic modulus correspond to a liquid expanded state, however those of the saline solution are slightly higher, following the same tendency as at 23 °C.

Comparing the effect of temperature using the same Optrex tear and the same subphase, a shift of the isotherms to higher areas was observed, coupled to a slight decrease of the elastic modulus at higher temperature. The effect on the collapse pressure was insignificant. Comparing the combined effect of the subphase and the temperature, it is seen that the effect of the subphase is more accused than that of the temperature, in the narrow temperature range studied.

3.2. *Interfacial mimetic tear organic subphase*

Based on the similar behaviour of the three commercial tear products observed in the previous experiments, and the minor differences in their interfacial behaviour at the studied temperatures, Optrex-red was selected as a representative artificial tear to be further investigated at 23 °C in PBS subphase containing different organic molecules from the natural tears. The PBS subphase was used to maintain a pH similar to that of the tear film and also to provide a buffering effect in front for the tear proteins.

The Optrex isotherm in presence of glucose (Fig. 4) is similar to that obtained in PBS, collapses at similar surface pressure and shows minor shift to larger molecular areas. The C_s^{-1} curves are also similar, with

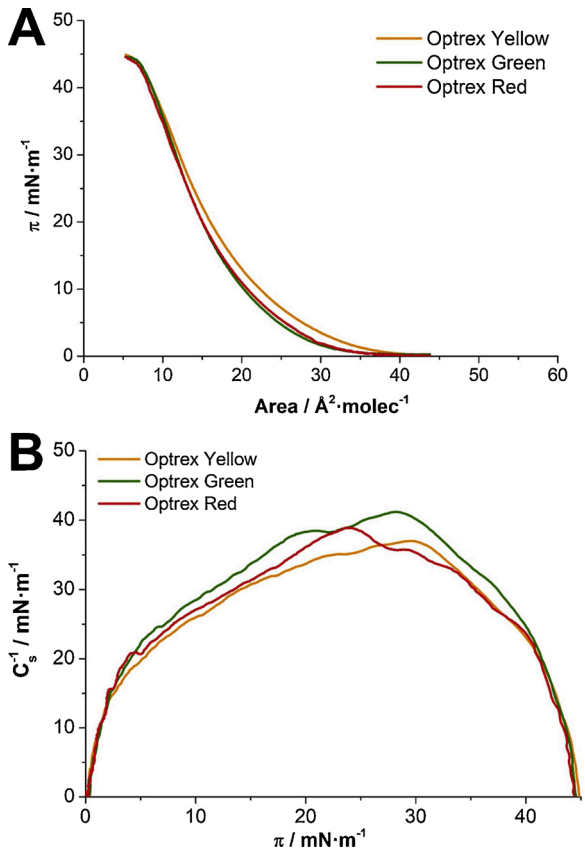

Fig. 3. A) Surface pressure-area isotherms for three Optrex artificial tears (see Table 1) in water subphase at 32 °C. B) Elastic modulus vs. surface pressure for the isotherms of Fig. 3A.

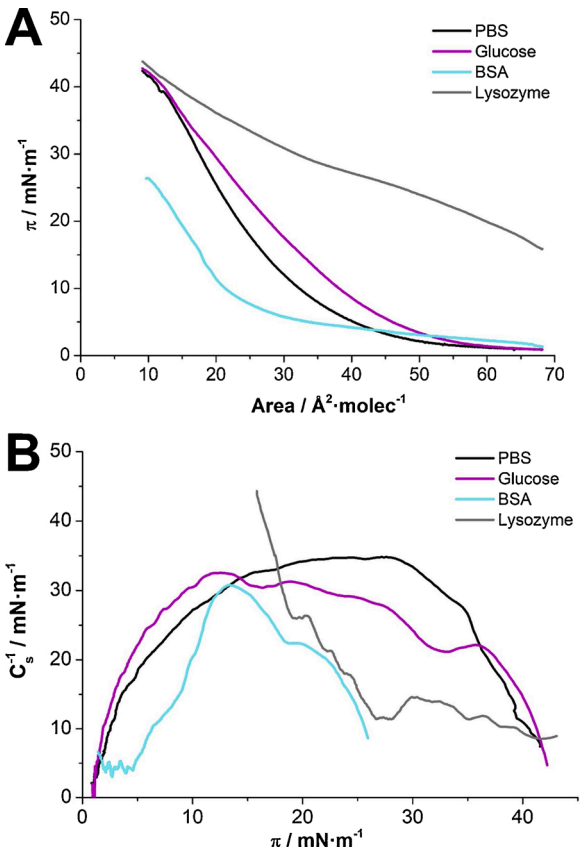

Fig. 4. A) Surface pressure-area isotherms for the Optrex-red at 23 °C in different subphases: PBS, PBS + glucose, PBS + BSA and PBS + Lysozyme. B) Elastic modulus vs. surface pressure for the isotherms of Fig. 4A.

slightly higher values in the initial zone of the isotherm and slightly lower in the final zone, both corresponding to a LE state. Therefore, the interactions of glucose with the Optrex surface active components is weak and produces a minimum expansion in the film.

High surface activity of BSA alone (Fig. S3) has been observed as well by Miano et al. (2005). BSA strongly influences the Optrex isotherm (Fig. 4) which shape is similar to the neat BSA. The isotherm is shifted to lower molecular areas and surface pressures, including a notable reduction in the collapse pressure. The isotherm of lysozyme alone (Fig. S3) indicates the high surface activity of lysozyme. The presence of lysozyme induces a shift to larger molecular areas in the Optrex isotherm (Fig. 4) and shows an initial higher surface pressure of ≈ 17 mN m^{-1}. It is of particular relevance that the presence of lysozyme induces lower C_s^{-1} upon scaling the compression. The compression effect exerted by the barriers can be assimilated to the compression effect exerted by the eyelids, concluding that lysozyme fluidises the lipid layer during blinking. These results confirm that proteins strongly interact with the polar phospholipids of Optrex tear, as also observed for the natural tear (Miano et al., 2005; Millar and Schuett, 2015; Svitova and Lin, 2016). This interaction stabilises the tear film and reduces the tear surface tension.

Comparing the effect due to the presence in the subphase of glucose, BSA or lysozyme, the presence in the subphase of glucose induces a negligible effect whereas BSA and lysozyme induces large and opposite influence in the Optrex isotherm (Fig. 4). The main lipid component of the Optrex tear is soya lecithin that is phosphatidylcholine (PC), a zwitterion with positively charged choline group. On the other hand, according to the reported isoelectric points (Guckeisen et al., 2019) (IEP), BSA (IEP ≈5) at pH 7.4 presents a negative charge, while lysozyme (IEP ≈11) at pH 7.4 is positively charged. It could be expected that the positive choline group interacts with the negative charge of BSA forming a BSA-PC complex, which induces to the Optrex tear film behaviour close to that of BSA. Kundu et al. (2012) reported the formation of a complex BSA-DPPC and the formation of a unique layer of BSA under the lipid monolayer. On the contrary, the positive charge of lysozyme may provoke repulsive interactions with the choline group and consequently an expansion of the Optrex tear film. A similar expansion was observed on POPC using lysozyme subphases (Torrent-Burgués and Raju, 2019). Toimil et al. (2012) studied the interaction of human serum albumin (HSA) with DPPC at the air-water interface, however using HSA-DPPC mixed monolayers on water subphase. The results obtained in their work cannot be compared to the present work as the approach and substances are used are different. Zasadzinski et al. (2010) reported that BSA inhibited the adsorption of lipid suspensions on the interface, which is in agreement with the similar behaviour of the BSA isotherm and the isotherms of the lipid artificial tear in presence of BSA. These authors claimed a competitive adsorption between lipids and surface-active substances, such as BSA. Nevertheless, further studies are needed to comprehensively interpret these results.

Miano et al. (2005) found that all tear proteins adsorbed on the interface, being lipocalin the most surface active and inserted into the lipid layer upon the entire range of surface pressures exerted by the Meibomian lipid mixture. Lactoferrin, lysozyme and IgA were also inserted into the lipids whereas albumin insertion was lower. These observations are in line with the results obtained in the current work. Mudgil et al. (2006) found that lysozyme penetrated a Meibomian lipid film, and the palmitoyllinoleylphosphatidylglycerol (PLPG), stearyloleoylphosphatidylserine (POPS) and phosphatidylethanolamine from bovine brain (PE) lipid films up to a surface pressure of 20 mN/m, but not a dipalmitoylphosphatidylcholine (DPPC) film at pressures ≥ 10 mN/m. Penetration becomes more difficult as the rigidity of the phospholipid monolayer increases. The surface behaviour of the herein

studied lipid-containing artificial tears indicates the presence of unsaturation in the lipid composition yielding a less rigid film, which allows the insertion of lysozyme and explains the film expansion observed (Fig. 4). Mudgil and Millar (2008) observed adsorption of lipocalin onto bovine Meibomian lipid films, which induced an increase of the film stability and consequently a higher surface pressure upon compression of the film.

Millar et al. (2009) carried a similar study using a human Meibomian lipid film and observed that lipocalin binds more slowly to it than lysozyme or lactoferrin. Both works highlight the different adsorption behaviour of lipocalin depending on the nature of the lipids. The similar results obtained in our study with three different artificial tears confirms that regardless the different commercial name, the lipid nature and content is very similar in these products.

For future studies the interaction of lipid-containing artificial tears with Meibomian lipids would be of interest. In this sense, Georgiev et al. (2010) studied the interactions of Meibomian gland secretion with polar lipids in Langmuir monolayers. It would also be of interest to study the interaction of lipid-containing artificial tears with other ocular proteins, such as lipocalin, due to its binding action. Such studies will contribute to state-of-the art in the formulation of novel lipid-containing artificial tears.

4. Conclusions

Different commercial Optrex lipid tears with the same or similar composition showed similar Langmuir behaviour. The fluidity of the Langmuir films built from these lipid tears is high, with low values of the elastic modulus. The subphase composition and the temperature influence the surface behaviour of the Optrex lipid tears. Proteins, such as BSA and lysozyme, have stronger influence over the isotherm of Optrex tear than glucose, being the effect of BSA opposite to that of lysozyme. Lysozyme fluidises notably the film at higher surface pressures. In light of the similar surface behaviour of the three Optrex lipid tears using the Langmuir technique, it seems of interest to perform a clinical study to find out whether these artificial tears have a different performance in vivo.

Author contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Declaration of Competing Interest

The authors report no declarations of interest.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.chemphyslip.2021.105087.

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