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A Combined Clinical and Langmuir Film Study of Natural Tear Films

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Abstract: The Langmuir technique has been applied to the study of natural tears and the results obtained from surface pressure-area isotherms have been correlated with those of clinical tests (the Schirmer and the TFBUT tests). The population studied only included people aged over 45. Natural tears were collected from Schirmer strips, which were placed in contact with the water subphase of a Langmuir trough for recording the surface pressure-area isotherms. These isotherms have been classified as good or weak according to the characteristics obtained. From the isotherms, characteristic values such as π_{limit}, $C_S^{-1}{}_{max}$ and π_{max} were also obtained. The analysis from the Chi-squared test indicates dependence between the Schirmer test and the type of isotherms ($P<0.05$). In the case of the TFBUT test and the type of isotherms the value of $P>0.05$ indicates that the independence cannot be discarded with the significance criteria imposed. Working with the same criteria of significance, we obtain moderate or good Pearson correlation coefficients with statistical significance ($P<0.05$) between the values of the Schirmer test and the π_{limit}, $C_S^{-1}{}_{max}$ and π_{max} values. On the other hand, we obtain low Pearson correlation coefficients with no statistical significance ($P>0.05$) between the values of the TFBUT test and the π_{limit}, $C_S^{-1}{}_{max}$ and π_{max} values.

Keywords: Natural Tears, Surface Pressure-Area Isotherm, Elastic Modulus, Schirmer Test, Tear Film Break-Up-Time (TFBUT)

1. Introduction

The Langmuir technique is a powerful tool to study polar lipids (fatty acids, phospholipids …) at the air/water interface. The tear film presents a lipid layer at the outermost part, and this lipid layer is described as a multi-layered lipid film that contains a fraction of polar lipids as well as non-polar lipids [1]. The Meibomian glands, located in the eyelid margins, secrete mainly the lipid layer. The Langmuir technique has been applied to the study of Meibomian lipids [2-6], their interaction with proteins [7-10], to the study of lipid-containing artificial tears [11] and to the study of the instability and breakup of model tear films [12]. The study of natural tear films is of great importance in the fields of ophthalmology and optometry. The tear film is involved in optical and physiological functions [13-15], and its quality ensures the correct accomplishment of these functions. Dysfunctions in the secretion, stability and composition of the tear film are one of the causes of the disease (or syndrome) known as dry eye [16]. Moreover, the quality of the tear film is crucial for the prescription of contact lenses.

For the assessment of the quality of the tear film, several clinical methods are of common use in the ocular surface field such as the Schirmer or the tear film break-up time (TFBUT) tests [16-20]. The Schirmer test evaluates aqueous tear secretion and then is related with the tear volume and the TFBUT test assesses the tear film stability.

The study of Langmuir behaviour of natural tear films has been done directly using Meibomian lipids, collected from eyelids or from contact lenses and dissolved in chloroform [4-6]. Hagedorn et al. [4] have studied the Langmuir behaviour of Meibomian lipids deposited on contact lenses. Petrov et al. [5] have studied the Langmuir behaviour of bovine Meibomian lipids collected from lid borders. Mudgil and Millar [6] have studied the isotherms of human Meibomian lipids collected from human eyelids.

In our study, we use the whole tear film extract collected

with a Schirmer strip, a specific paper strip, and for that, the tear film components in the strip are lipids, proteins, electrolytes and others. Between them, the most surface-active components are lipids. In addition, our study permits a more direct correlation between the clinical tests of the tear film of individuals and the corresponding Langmuir behaviour. Thus, in this work, we propose to study the characteristics of the tear film collecting it with a Schirmer strip and transferring the strip content to a Langmuir trough. After transfer, the surface pressure-area isotherms, π-A, are registered and some characteristics of them will be correlated with clinical tests results. Among these characteristics the inverse of the compressibility modulus, or elastic modulus (Eq. 1), will be used; the greater the value of C_s^{-1}, the more rigid the film will be.

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

2. Materials and Methods

2.1. Materials

Schirmer strips were of standard Wathman 41 paper (size 5 mm x 35 mm) from GECIS. These strips are folded at 5 mm from the end in order to place it on the lid margin, and on it there is a millimetric scale. Powder free nitrile gloves were used for strip manipulation. Water was ultrapure Milli-Q (18.2 MΩ·cm). 2% sodium fluorescein was used for the TFBUT test.

2.2. Population

Tested eyes were from people pending cataract surgery, and the inclusion criteria for the study were that these people do not present apparent ocular pathology, do not wear contact lenses and have not had any previous surgery. People were asked not to use eye cosmetics on the day the tests were performed. People were classified in two groups: people ≤ 65 years old and people > 65 years old, but also a statistical analysis was performed joining both groups.

2.3. Techniques and Equipment

2.3.1. Schirmer I Test (SCH)

Schirmer strips were placed on the temporal side of the lower lid margin, leaving 5mm of the Schirmer strip inside the conjunctival sac, during 5 min, without anaesthesia and with the eyes closed [21]. After removal of the strip, the impregnated length is measured in mm and the strip is kept in a sterile tube for further experiments and placed in a fridge at 4ºC.

2.3.2. TFBUT Measurement

For tear film break-up time (TFBUT) measurements, preservative free 2% sodium fluorescein was instilled in the eye, in the superior bulbar conjunctiva [17]. After 1 minute, the patient had to stop blinking. The time passing between the last blink and the moment of breaking of the tear, observed with a bio-microscope incorporating a blue cobalt filter, was taken as the TFBUT value. Three measurements were made in each eye.

2.3.3. Langmuir Experiments

The isotherm experiments have been done in a NIMA Teflon trough, model 1232D1D2 (area 1200 cm^2), with two movable barriers and using a Wilhelmy plate to measure the surface pressure. The linear velocity of the barriers was 1.5 cm/min, which means 30 cm^2/min in the trough used. The Teflon trough and barriers were cleaned with chloroform and ultrapure water. Experiments were conducted at room temperature of 23°C. Water was used as subphase because in previous studies with polar lipids, see for instance ref. [22], no significant influence of a 0.9% NaCl phosphate buffered subphase was observed. For the spreading of the tear components onto the water, the Schirmer strip was placed in contact with water during 30 seconds.

Previously to the full study, a test for the Langmuir technique was done with the Schirmer strip applied to the healthy eyes of a young person (i.e. values of the SCH≥ 10 mm and TFBUT≥ 10 s, according to the grading scheme explained in section 3.1), and the isotherm obtained is shown in Fig. A1 in Appendix.

2.4. Statistical Analysis

The data were analysed with the Minitab 17 statistical program. The Pearson Chi-squared statistical test was applied to contingence tables, imposing the significance criteria that values of probability (P values) lower than 0.05 indicate that the hypothesis of independence between variables can be discarded. The Pearson correlation coefficient was applied to test the correlation between two variables.

3. Results

The study was conducted in an adult population, which was subdivided into people aged 65 or less (range 45-65), and people aged more than 65 (range 66-92). For the first case, 23 eyes were studied, and 21 eyes for the second. The age of 65 was selected as the division as it is the usual retiring age. The gender distribution was 60% males and 40% females in both cases. Nevertheless, a global study including both populations has also been discussed. As the sample corresponds to an adult population, several eyes could present dry eye disease, as will be commented.

Table 1. *Results of the Schirmer (SCH) and the tear film break-up time (TFBUT) tests.*

A) Eye (Age>65)	SCH (mm)	TFBUT (s)	B) Eye (Age≤65)	SCH (mm)	TFBUT (s)
1	6	7	1	2	5
2	7	8	2	1	4
3	5	5	3	3	7
4	10	3	4	7	7

A) Eye (Age>65)	SCH (mm)	TFBUT (s)	B) Eye (Age≤65)	SCH (mm)	TFBUT (s)
5	15	3	5	6	5
6	12	3	6	9	5
7	6	4	7	22	7
8	4	3	8	9	4
9	6	4	9	24	3
10	10	4	10	28	8
11	14	7	11	6	3
12	1	5	12	7	4
13	24	3	13	19	3
14	6	0	14	19	4
15	5	3	15	3	4
16	8	3	16	12	3
17	6	0	17	1	3
18	0	4	18	12	5
19	18	3	19	29	5
20	6	8	20	2	3
21	4	5	21	0	3
			22	1	5
			23	7	5
Mean	8.2	4.0	Mean	10.0	4.6
Standard deviation	5.7	2.1	Standard deviation	9.1	1.5
Maximum	24	8	Maximum	29	8
Minimum	0	0	Minimum	0	3
Median	6	4	Median	7	4

3.1. Clinical Trials

Table 1 presents the results of the Schirmer and TFBUT tests for both populations, A) people >65 years old (21 eyes) and B) people ≤65 years old (23 eyes).

The Schirmer and the TFBUT are common tests for the diagnosis of dry eye [20]. The term dry eye includes a wide spectrum of alterations of the ocular surface and cut-off values of the clinical tests have been proposed enabling a distinction to be made between healthy and affected eyes. The grading scheme used in this work to classify the severity of the dry eye disease has been proposed in references [18, 19]. In the case of Schirmer I test, the cut-off values for each level are: healthy eye (SCH≥ 10 mm); moderate dry eye (5 mm ≤SCH<10 mm); severe dry eye (SCH<5 mm). For the TFBUT test, the cut-off values are: healthy eye (TFBUT≥ 10 s); moderate dry eye (5 s ≤TFBUT<10 s); severe dry eye (TFBUT<5 s). According to this grading scheme, the eyes of the over 65 population (Table 1) are distributed thus: 33.3% healthy eyes, 47.6% moderate dry eyes and 19% severe dry eyes for the Schirmer test, and 33.3% moderate dry eyes and 66.7% severe dry eyes for the TFBUT test. In the case of the 65 or under population (Table 1) the distribution is: 34.8% healthy eyes, 30.4% moderate dry eyes and 34.8% severe dry eyes for the Schirmer test, and 47.8% moderate dry eyes and 52.2% severe dry eyes for the TFBUT test. In this work we are going to consider each clinical test separately in order to correlate each one with the Langmuir results.

Data from different studies [16] show that the prevalence of dry eye disease lies in the range of 5-30% in the population aged 50 years and older, and that the number of women affected with dry eye appears to exceed that of men. If we consider that a person presents dry eye disease if he presents clinical signs in at least one of the two clinical tests, our results show a greater prevalence of dry eye disease, but this could be due to the specific studied population, that is, people pending cataract surgery.

3.2. Langmuir Experiments

Fig. 1 shows typical π-A isotherms obtained with the method described in section 2.2. In most of the cases a good isotherm (G) is obtained (Fig. 1A), but in some cases the increase in the surface pressure is low or very low (Fig. 1B). As a criterion, the later situation has been fixed for a maximum value of surface pressure (π_{limit}) less than 4 mN/m, and is called a weak isotherm (W). A good isotherm allows calculation of the elastic modulus, as is presented in the inset in Fig. 1A. As will be see in the experimental data, there is a gap between the isotherms with a π_{limit} less than 4 mN/m and the isotherms with a π_{limit} more than 8 mN/m, which justifies in part our criterion. Usually the elastic modulus plot reaches a maximum, which is characterised by a pair of values corresponding to the surface pressure, π_{max}, and the elastic modulus, $C_s^{-1}{}_{max}$. The global of all π-A isotherms is presented in Supplementary data.

Even though tears collected with the Schirmer strip contain proteins and other tear components, as well as lipids, the shape of the π-A isotherms is that of typical lipids, and the surface pressure after the Schirmer strip contacts water remains at zero with open barriers. This fact can be explained, even though proteins are surface active, by the low amount of the tear film collected, which is around a few microliters, in the same order of the tear film volume. After contacting water with the strip, these soluble proteins diffuse to the whole water subphase and the protein concentration attained is very low and does not interfere appreciably with the lipids extended on the surface. We have estimated that this protein concentration is around 0.0001-0.0002 g/L. Thus, we have also done tests with a lysozyme solution at a greater concentration of 0.001 g/L and, on one hand, we have

obtained a surface tension of 72.1 mN/m, close to that of water, and, on the other hand, the lysozyme isotherm always present a zero value in the surface pressure during compression. This behaviour is maintained after 140 min indicating no significant changes in lysozyme adsorption at the air-water interface at this level of protein concentration. We have also tested the isotherm of the phospholipid DPPC (dipalmitoylphosphatidylcholine) on this lysozyme subphase and we have not observed any change with respect to the DPPC isotherm on water subphase.

3.2.1. Population > 65 Years Old

In Table 2 the main characteristics obtained from the π-A isotherms and elastic modulus plots, of the population > 65 years old, are presented.

Considering the isotherms and the elastic modulus plots (Fig. A2 in Appendix), it is observed that even though notable differences have been obtained between the values of π_{limit} in the isotherms, all of them reach a maximum in the elastic modulus plots that is located around 8-9 mN/m of surface pressure (X axis) and around 29-31 mN/m of elastic modulus (Y axis). Analysing the values of the elastic modulus, and its maximum values, the physical states of the film can be obtained [23, 24]. Thus, for C_s^{-1} between 0-12.5 mN/m the gas phase (G) is present, for C_s^{-1} between 12.5-100 mN/m it is the liquid expanded state (LE), for C_s^{-1} between 100-250 mN/m it is the liquid condensed state (LC), and for C_s^{-1} >250 mN/m it is the solid State (S). With this criterion, the studied films correspond to an LE state, which indicates the presence of unsaturated lipids and non-polar lipids.

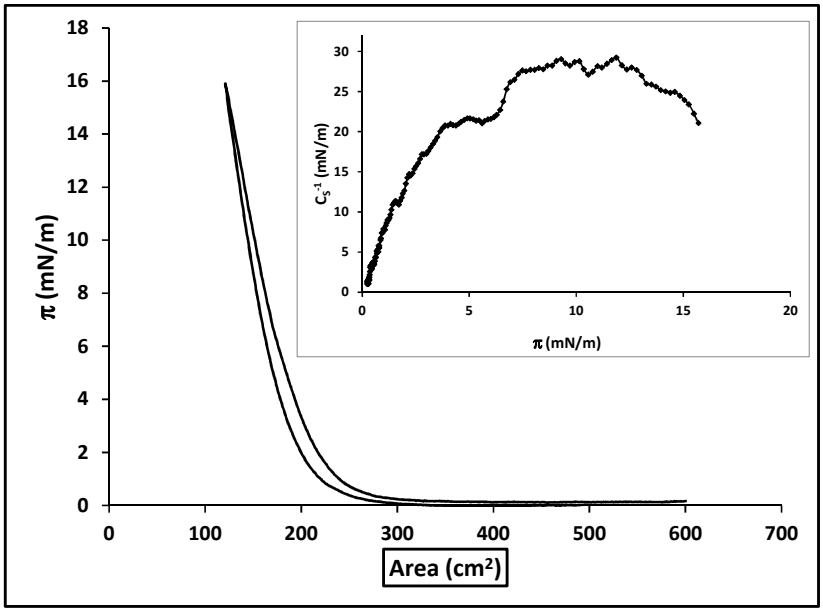


Figure 1A. *A good (G) π-A isotherm corresponding to eye number 4 of population > 65. Inset: The corresponding elastic modulus from the isotherm, obtained applying Eq. (1).*

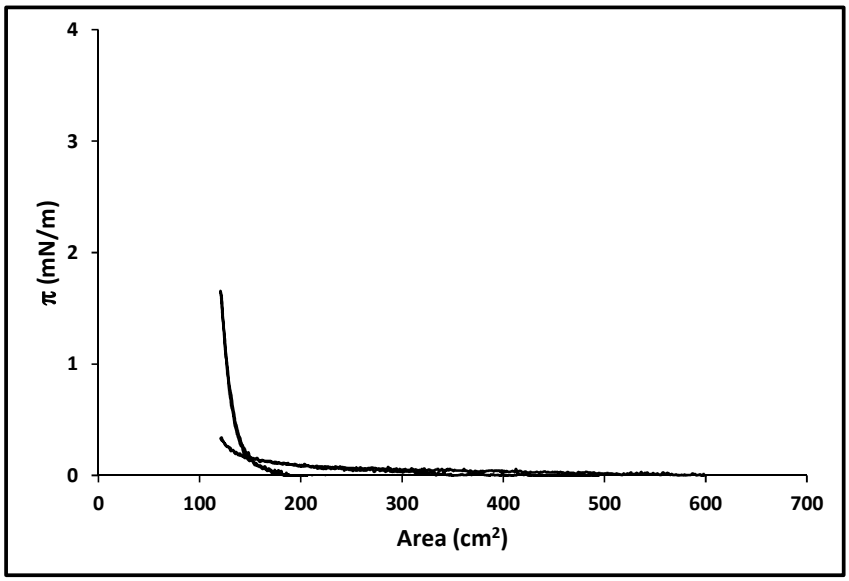


Figure 1B. *Two examples of weak (W) π-A isotherms corresponding to eyes numbers 12 and 23 of population ≤ 65.*

Table 2. *Main characteristics from the π-A isotherms and elastic modulus plots obtained for population > 65 years old.*

Eye N°	Isotherm	π_{limit} mN/m	$C_s^{-1}{}_{max}$ mN/m	π_{max} mN/m
1	W	0	0	0
2	G	18.87	26.72	8.25
3	W	0.10	0.64	0.05
4	G	15.24	31.88	9.16
5	W	0.75	6.82	0.75
6	G	9.72	27.16	8.76
7	W	0.09	0.92	0.09
8	W	0.16	0.79	0.16
9	W	0.34	11.73	0.22
10	G	9.40	28.17	8.87
11	G	22.82	32.29	10.25
12	W	0	0	0
13	G	20.50	28.98	7.80
14	G	8.51	29.22	8.23
15	W	0	0	0
16	W	1.44	12.40	1.40
17	G	8.06	40.26	6.49
18	W	0	0	0
19	G	10.25	33.70	9.32
20	G	14.27	30.00	12.18
21	W	0.20	0.67	0.18
Mean		6.70	17.98	4.61
Standard deviation		7.80	14.62	4.57
Maximum		22.82	40.26	12.18
Minimum		0	0	0
Median		1.44	12.4	1.4

Table 3. *Main characteristics from the π-A isotherms and elastic modulus plots obtained for population ≤65 years old.*

Eye N°	Isotherm	π_{limit} mN/m	$C_s^{-1}{}_{max}$ mN/m	π_{max} mN/m
1	W	0.13	1.44	0.02
2	W	0.30	0.87	0.17
3	W	0	0	0
4	W	3.82	22.83	3.74
5	G	13.87	34.14	8.53
6	G	20.27	29.70	10.53
7	G	17.89	28.77	10.10
8	G	15.70	29.23	11.84
9	G	22.64	28.95	8.80
10	G	22.16	27.27	8.54
11	W	0	0	0
12	W	1.66	13.55	1.44
13	G	10.90	26.68	9.27
14	W	3.76	17.63	3.59
15	W	0.18	0.60	0.07
16	W	0.34	1.45	0.31
17	W	0	0	0
18	G	22.42	32.52	8.88
19	G	22.72	33.98	8.22
20	W	0	0	0
21	W	0.28	0.78	0.26
22	W	0.34	1.06	0.17
23	W	0.33	1.97	0.09
Mean		7.81	14.49	4.11
Standard deviation		9.39	14.19	4.52
Maximum		22.72	34.14	11.84
Minimum		0	0	0
Median		1.66	13.55	1.44

3.2.2. Population ≤65 Years Old

In Table 3 the main characteristics obtained from the π-A isotherms and elastic modulus plots, of the population ≤ 65 years, are presented.

Considering the isotherms and the elastic modulus plots (Fig. A3 in Appendix), it is observed that even though notable differences have been obtained between the values of π_{limit} in the isotherms, all of them reach a maximum in the elastic modulus plots that is located around 8.5-9.5 mN/m of surface pressure (X axis) and around 29-31 mN/m of elastic modulus (Y axis). Analysing the values of the elastic modulus, and its maximum values, and with the criterion exposed formerly [23,24], the studied films correspond to an LE state, which indicates the presence of unsaturated lipids and non-polar lipids. This result is very similar to that obtained for the population of > 65 years old, indicating that the typology of lipids present in the tear film does not change significantly in the two populations.

The results reported in our study are not directly comparable with those of the literature [4-6] since the lipids are collected in a different way and for a different purpose. As has been commented in the introduction, Hagedorn et al. [4], Petrov et al. [5], Mudgil and Millar [6] collected Meibomian lipids from eyelids or from contact lenses, while our lipids came from the tear film. Nevertheless, the isotherms obtained in our study are comparable with those reported in the other cited studies, and in all cases, the isotherms do not reach a collapse. These can be explained because the amount of lipid spread is not enough and also because the lipid composition contains non-polar lipids which tend to form multilayers instead of a compact monolayer. We have made an estimation of the inverse of the compressibility modulus (C_s^{-1}) at π=10 mN/m for the Meibomian lipids from the isotherm in Fig. 2A published by Mudgil et al. [7] and we obtained a value around 24 mN/m, while for the phospholipids used in that study higher values were obtained. Comparing these values with our values in Figures S1, S2 B and S3 B, it is seen that at =10 mN/m our values range between those of Meibomian lipids and phospholipids. We think that this indicates we have a mixture of Meibomian lipids and phospholipids, which are always present in tears [9].

3.3. Discussion

3.3.1. Population > 65 Years Old

We analyse if there is dependence between the number of good (G) and weak (W) isotherms and both the Schirmer and the TFBUT tests results, for the population > 65 years old. The results of Tables 1 and 2 are distributed in the contingency Table 4. The rows in Table 4 show the eyes classified in three grading levels, healthy eyes, moderate dry eyes and severe dry eyes, according to the cut-off values explained in section 3.1 and the columns show the eyes classified according to the type of isotherm. The Pearson Chi-squared statistical test is applied to the sets in Table 4, to assess the dependence or independence between them. The Chi-squared test gives a value of P=0.019 between the Schirmer test and the type of isotherms, indicating dependence between them with statistical significance, while

between the TFBUT test and the type of isotherms the value of P=0.757 indicates independence between both variables.

Working with the same criteria of significance, we obtain, on one hand, moderate or high Pearson correlation coefficients with statistical significance (P<0.05) between the values of the Schirmer test and the π_{limit}, $C_S^{-1}{}_{max}$ and π_{max} values (see Table 6). On the other hand, we obtain low Pearson correlation coefficients with no statistical significance (P>0.05) between the values of the TFBUT test and the π_{limit}, $C_S^{-1}{}_{max}$ and π_{max} values (see Table 6).

These results indicate that the Langmuir technique works well when the volume of tear secretion is sufficient (acceptable Schirmer test values), which allows us to obtain acceptable isotherms and values derived from them.

3.3.2. Population ≤65 Years Old

We analyse if there is dependence between the number of good (G) and weak (W) isotherms and both the Schirmer and the TFBUT tests results, for the population ≤ 65 years old. The results of Tables 1 and 3, are distributed in the contingency Table 5 following the same cut-off criteria explained in Table 4. The Pearson Chi-squared statistical test is applied to the sets in Table 5, to assess the dependence or independence between them with the same significance criteria. A value of P=0.009 between the Schirmer test and the type of isotherms is obtained, indicating dependence between them with statistical significance. In the case of the TFBUT test and the type of isotherms the value of P=0.147 indicates that the independence between both variables cannot be discarded with the significance criteria imposed.

Working with the same criteria of significance, we obtain, on one hand, moderate or high Pearson correlation coefficients with statistical significance (P<0.05) between the values of the Schirmer test and the π_{limit}, $C_S^{-1}{}_{max}$ and π_{max} values (see Table 6). On the other hand, we obtain low Pearson correlation coefficients with no statistical significance (P>0.05) between the values of the TFBUT test and the π_{limit}, $C_S^{-1}{}_{max}$ and π_{max} values (see Table 6).

These results match with those obtained for population >65 years old, but better correlations are observed for population ≤ 65 years old than for population > 65 years old.

3.3.3. Combining Both Populations, People ≤65 and People > 65 Years Old

With the aim of analysing in depth the correlation between the Langmuir results and the clinical tests, on one hand we combined both populations, people ≤ 65 and people > 65 years old, and on the other hand, we considered only the eyes with good (G) isotherm results in the combined population. The Pearson correlation coefficients and the corresponding P values are presented in Table 6.

When we consider the combined population, we obtain moderate or high Pearson correlation coefficients with statistical significance (P<0.05) between the values of the Schirmer test and the π_{limit}, $C_S^{-1}{}_{max}$ and π_{max} values, and low Pearson correlation coefficients with no statistical significance (P>0.05) between the values of the TFBUT test and the π_{limit}, $C_S^{-1}{}_{max}$ and π_{max} values. This result reinforces our previous consideration indicating that the Langmuir technique works well when the volume of tear secretion is sufficient.

If we consider only the good (G) isotherms (a joined-sample of 24 eyes), we obtain moreover Pearson correlation coefficients with statistical significance between π_{limit} and π_{max} values and the values of the TFBUT test (see Table 6). It seems to indicate that the TFBUT test can only be correlated with the isotherms when the latter are good enough. One of the limits of the present study is that the populations studied present low values of the TFBUT test corresponding to moderate or severe dry eye disease, as was shown in section 3.1, and consequently there is a limited variability in the studied eyes.

Table 4. *Contingency table: A) between isotherm and Schirmer test, and B) between isotherm and TFBUT, for people>65. (n_i=number of eyes of each type of isotherm and grading level of Schirmer test (Table A) or grading level of TFBUT test (Table B), n_s= number of eyes of each grading level of Schirmer test, n_B= number of eyes of each grading level of TFBUT Test, n_{ti}= number of eyes of each type of isotherm).*

		ISOTHERMS		
A		**Good**	**Weak**	**Total Percentage %**
TOTAL sample, n_t=21		**n_i % n_i/n_s**	**n_i % n_i/n_s**	
	SCH<5	0	4	
	n_s=4	0	100	100
	5 ≤SCH<10	4	6	
SCHIRMER TEST / mm	n_s=10	40.0	60.0	100
	SCH≥ 10	6	1	
	n_s=7	85.7	14.3	100
		10	11	
n_{ti} % n_{ti}/n_t		47.6	52.4	100

		ISOTHERMS		
B		**Good**	**Weak**	**Total Percentage %**
TOTAL sample, n_t=21		**n_i**	**n_i**	
		% n_i/n_B	**% n_i/n_B**	
	TFBUT<5	7	7	
TFBUT TEST / s	n_B=14	50.0	50.0	100
	5≤TFBUT<10	3	4	
	n_B=7	43.9	57.1	100
		10	11	
n_{ti} % n_{ti}/n_t		47.6	52.4	100

Table 5. *Contingency table: A) between isotherm and Schirmer test, and B) between isotherm and TFBUT, for people≤65. (n_i=number of eyes of each type of isotherm and grade of Schirmer test (Table A) or grade of TFBUT test (Table B), n_s= number of eyes of each grade of Schirmer test, n_B= number of eyes of each grade of TFBUT Test, n_{ti}= number of eyes of each type of isotherm).*

		ISOTHERMS		
A		**Good**	**Weak**	**Total Percentage %**
TOTAL sample, n_t=23		**n_i**	**n_i**	
		% n_i/n_s	**% n_i/n_s**	
SCHIRMER TEST / mm	SCH<5	0	8	
	n_s=8	0	100	100
	5 ≤SCH<10	3	4	
	n_s=7	42.9	57.1	100
	SCH≥ 10	6	2	
	n_s=8	75.0	25.0	100
n_{ti} % n_{ti}/n_t		9	14	
		39,1	60.9	100

		ISOTHERMS		
B		**Good**	**Weak**	**Total Percentage %**
TOTAL sample, n_t=23		**n_i**	**n_i**	
		% n_i/n_B	**% n_i/n_B**	
TFBUT TEST / s	TFBUT<5	3	9	
	n_B=12	25,0	75.0	100
	5≤TFBUT<10	6	5	
	n_B=11	54.5	45.5	100
n_{ti} m% n_{ti}/n_t		9	14	
		39.1	60.9	100

Table 6. *Pearson correlation coefficients, r, and P values for several pairs of magnitudes.*

	Population	**r**	**P**
π_{limit}-Schirmer	> 65	0.6107	0.0033
π_{limit}-Schirmer	≤ 65	0.7590	2.7E-05
π_{limit}-Schirmer	Combined	0.7069	8.1E-08
π_{max}-Schirmer	> 65	0.5176	0.0162
π_{max}-Schirmer	≤ 65	0.6783	0.0004
π_{max}-Schirmer	Combined	0.5958	2.0E-05
$C_S^{-1}{}_{max}$-Schirmer	> 65	0.5487	0.0100
$C_S^{-1}{}_{max}$-Schirmer	≤ 65	0.7094	0.0001
$C_S^{-1}{}_{max}$-Schirmer	Combined	0.6173	8.1E-06
π_{limit}-TFBUT	> 65	0.2088	0.3636
π_{limit}-TFBUT	≤ 65	0.3162	0.1416
π_{imit}-TFBUT	Combined	0.2617	0.0862
π_{max}-TFBUT	> 65	0.0614	0.7915
π_{max}-TFBUT	≤ 65	0.2681	0.2161
π_{max}-TFBUT	Combined	0.1423	0.3567
$C_S^{-1}{}_{max}$-TFBUT	> 65	0.1731	0.4529
$C_S^{-1}{}_{max}$-TFBUT	≤ 65	0.3249	0.1303
$C_S^{-1}{}_{max}$-TFBUT	Combined	0.0275	0.8595
π_{limit}-TFBUT	Combined G isotherms	0.5842	0.0086
π_{max}-TFBUT	Combined G isotherms	0.4730	0.0408
$C_S^{-1}{}_{max}$-TFBUT	Combined G isotherms	0.3380	0.1569

4. Conclusions

The present study indicates the boundary conditions in which the Langmuir technique can be applied to obtain physicochemical information about the lipid layer of the tear film, when collecting the latter through a Schirmer strip. The study has established under which conditions the results obtained from the isotherms can correlate with the results obtained using two common clinical tests, the Schirmer test and the TFBUT test, which assess the tear film. The Langmuir technique is indicated to study the cases in which the tear secretion is enough to impregnate the Schirmer strip with enough tear volume. In these cases, a posterior study of the correlation between the values of isotherm parameters and the values of TFBUT test could give information about the stability of the film. This kind of information could determine if the lipid film presents different characteristics (different values of $C_S^{-1}{}_{max}$, π_{max} or π_{limit},), but in all cases an accurate selection of the population would be necessary to assure a distribution of TFBUT values in all the three grading scales of the dry eye symptom for the TFBUT test. In future studies we will try to analyse a wider population in order to extend the conclusions of the present work and to find more

physicochemical supports for the clinical values of the Schirmer and TFBUT tests. Also, in future studies the Langmuir study will be done at the temperature of 32ºC, which is the temperature of the anterior segment of the eye.

Appendix

π-A isotherms and the corresponding elastic modulus plots.

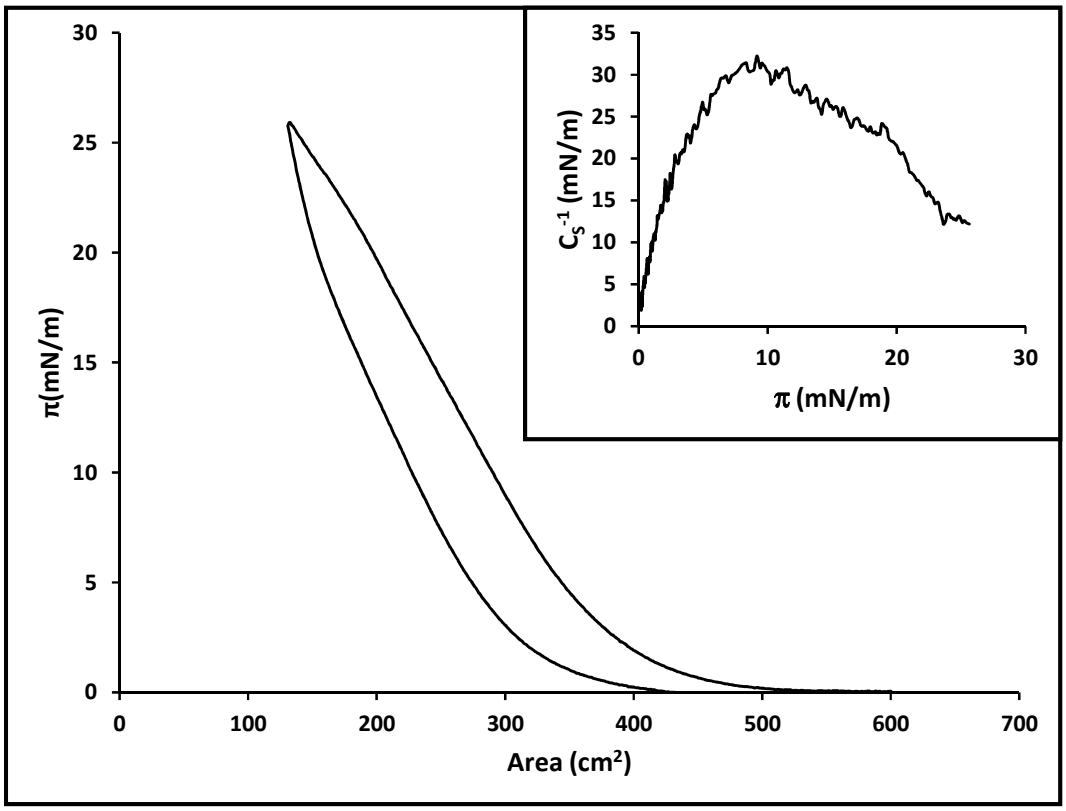
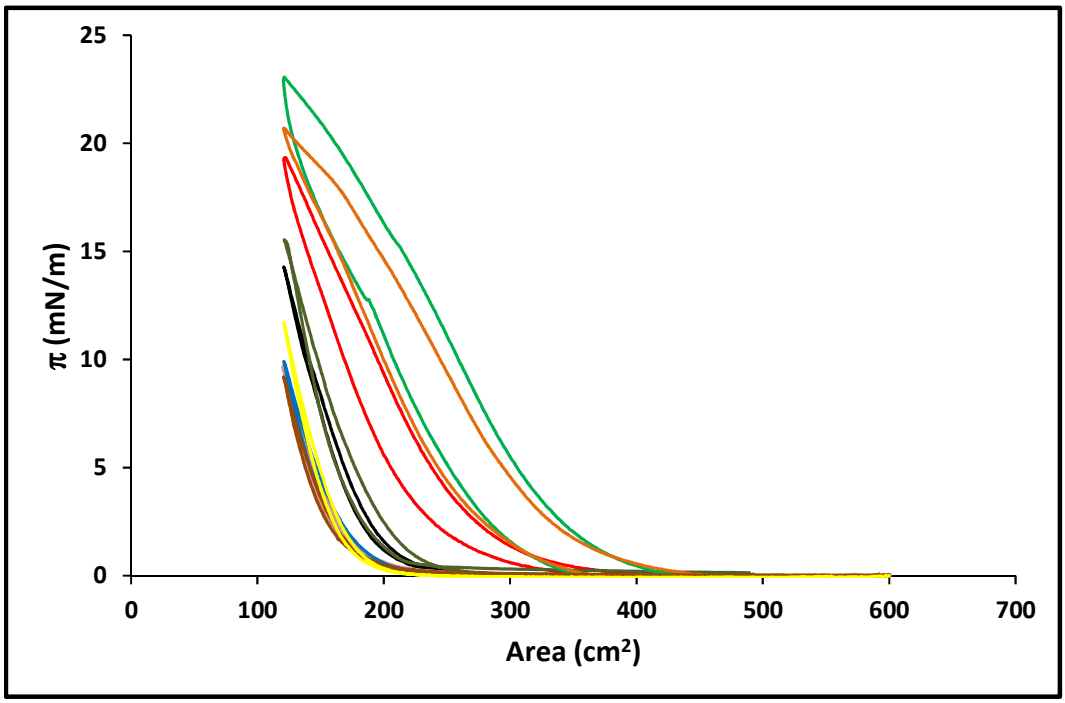
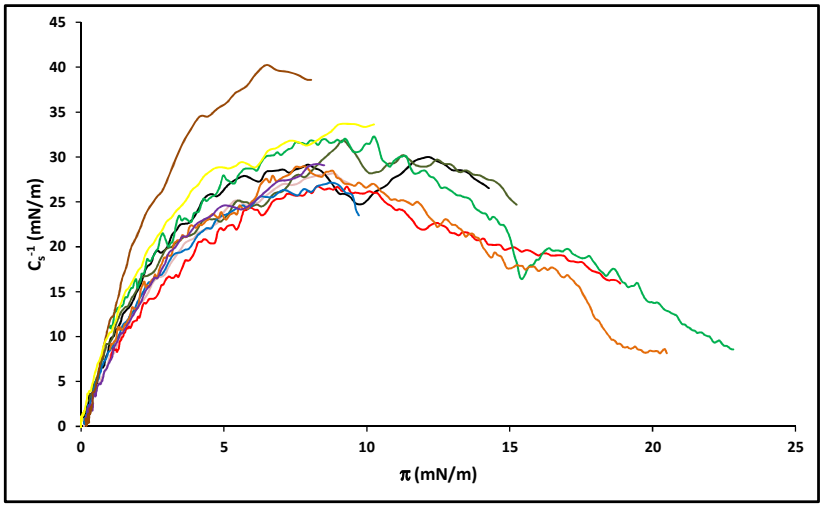


Figure A1. *π-A isotherm and the corresponding elastic modulus plot for a healthy eye.*

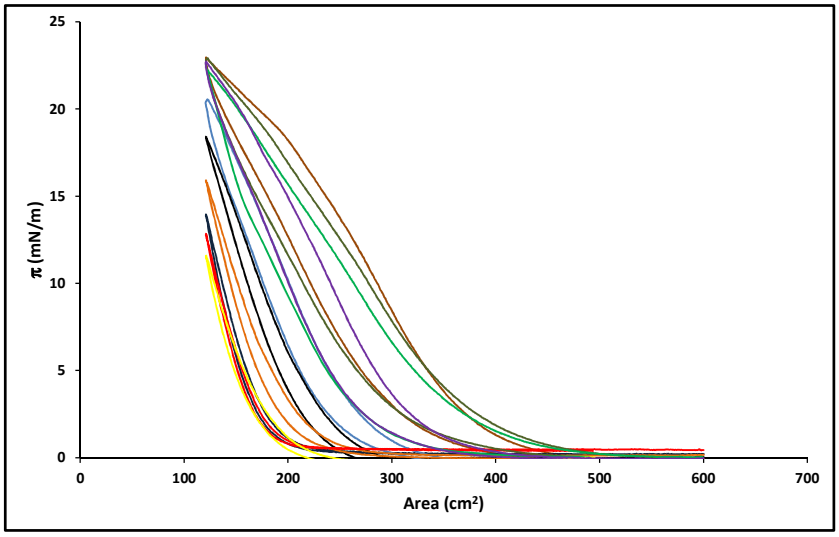


A)

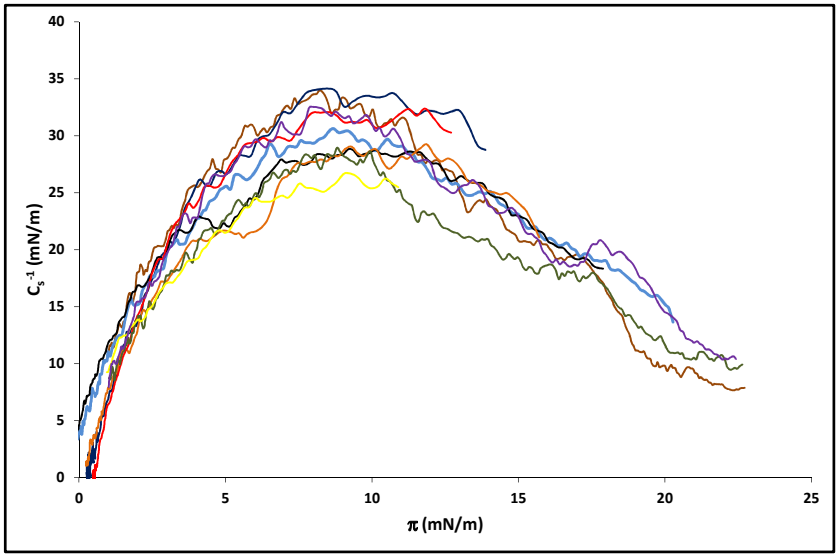


B)

Figure A2. *A) π-A good isotherms for eyes >65, B) The corresponding elastic modulus plots.*



A)



B)

Figure A3. *A) π-A good isotherms for eyes ≤65, B) The corresponding elastic modulus plots.*

References

[1] P. Kulosevi, J. Telenius, A. Koivuniemi, G. Brezesinski, A. Rantamäki, T. Viitala, E. Puukilainen, M. Ritala, S. K. Wiedmer, I. Vattulainen, J. M. Holopainen, Biophys. J. 99 (2010) 2559-2567.

[2] T. Kaercher, D. Hönig, D. Möbius, Internat. Ophthalmol. 17 (1993) 341-348.

[3] T. Kaercher, D. Hönig, D. Möbius, Orbit 14 (1995) 17-24.

[4] S. Hagedorn, E. Drolle, H. Lorentz, S. Srinivasan, Z. Leonenko, L. Jones, J. Optom. 8 (2015) 187-199.

[5] P. G. Petrov, J. M. Thompson, I. B. Abdul Rahman, R. E. Ellis, E. M. Green, F. Miano, C. P. Winlove, Exp. Eye Res. 84 (2007) 1140-1146.

[6] P. Mudgil, T. J. Millar, Invest. Ophthalmol. Vis. Sci. 52 (2011) 1661–1670.

[7] P. Mudgil, M. Torres, T. J. Millar, Colloids Surf. B 48 (2006) 128-137.

[8] F. Miano, M. Calcara, T. J. Millar, V. Enea, Colloids Surf. B 44 (2005) 49-55.

[9] T. F. Svitova, M. C. Lin, Adv. Colloid Interf. Sci. 233 (2016) 4-24.

[10] T. J. Millar, P. Mudgil, I. A. Butovich, C. K. Palaniappan, Invest. Ophthalmol. Vis. Sci. 50 (2009) 140-151.

[11] J. Torrent-Burgués, Colloids Surf. B 140 (2016) 185-188.

[12] M. Saad-Bhamla, C. Chai, N. I. Rabiah, J. M. Frostad, G. G. Fuller, Invest. Ophthalmol. Vis. Sci. 57 (2016) 949-958.

[13] B. Milder, The lacrimal apparatus. In Adler's Physiology of the Eye, 8th Ed., R. A. Moses, W. M. Hart Eds, 1987, St Louis, Mosby, pp. 15-35.

[14] D. R. Korb, The tear film: structure, function and clinical examination, Butterworth Heineman, 2002.

[15] A. J. Bron, J. M. Tiffany, S. M. Gouveia, N. Yokoi, L. W. Voon, Exp. Eye Res. 78 (2004) 347–360.

[16] DEWS, Report of the Dry Eye Workshop, Ocul. Surf. 5 (2007) 75-92 and 93-107.

[17] P. Cho, M. Yap, Optom. Vis. Sci. 70 (1993) 152-156.

[18] M. A. Lemp, Int. Ophthalmol. Clin. 13 (1973) 97-102.

[19] A. Behrens, J. J. Doyle, R. S. Chuck, Cornea 25 (2006) 900-907.

[20] G. Savini, P. Prabhawasat, T. Kojima, M. Grueterich, E. Espana, E. Goto, Clinical Ophthalmology 2 (2008) 31-55.

[21] T. A. Saleh, B. McDermott, A. K. Bates, P. Ewings, Eye 20 (2006) 913-915.

[22] J. Torrent-Burgués, BioNanoSci. 1 (2011) 202-209.

[23] J. T. Davies, E. K. Rideal, Interfacial Phenomena, Academic Press, N. Y. 1993.

[24] P. Vitovic, D. P. Nikolelis, T. Hianik, Biochim. Biophys. Acta 1758 (2006) 1852-1861.