THE INTERACTION OF HYDROPHILIC POLYMERS WITH TEAR FILM COMPONENTS IN DRY EYE SYNDROME THERAPY – MAKING THE RIGHT CHOICE

Petar Eftimov[1*], Nadezhda Stefanova[1], Nikola Peev[2], Georgi Georgiev[2]

[1] – SU St.Kliment Ohridski, Faculty of Biology, 1164 Sofia, 8 Dragan Tsankov Blvd.

[2] - SU St. Kliment Ohridski, Faculty of Physics, 1164 Sofia, 5 James Bourchier Blvd.

Abstract

The need for the use of quality ophthalmological compositions for supplementary therapy of the dry eye syndrome (DES) is ever increasing. This study aims at elucidation of biophysical interactions between four prospective hydrophilic polymers – gellan gel (GG), carboxymethyl-celulose (CMC), hyaluronic acid (HA) and cross-linked hyaluronic acid (CHA) and human meibum (lipid component of the tear film). Various therapeutically accepted concentrations of the aforementioned polymers mixed with the meibum from healthy volunteers were evaluated by measurement of the film pressure. Ferning patterns of evaporated micro drops (V=3μl) from the polymers were observed and compared. The wettability of the polymers was evaluated by axisymmetric drop-shape analysis (ADSA). In lower concentrations (0.0001% and 0.001%) CMC outperformed the rest of the polymers, but in the higher concentrations (0.01%, 0.05% and 0.1%) it generated lower film pressures. Complex intermolecular rearrangements of the meibum may be responsible for that behavior. The results will contribute in getting more correct understanding of the action of some medications, used for treatment of DES and for corrections in calculating the precise concentration of their active compounds.

Keywords: *hydrophilic polymers, dry eye syndrome, meibum, film pressure, ferning pattern*

Introduction

Dry eye syndrome, is often characterized by decreased stability of the tear film and drying of the ocular surface, which leads to a painful sensation and exposure of the eye for invasion of pathogens. According to numerous studies [1, 2, 3] it affects the quality of life of 10-30% of the human population worldwide, thus being the major current ophthalmic public health disease. Although surgical approaches exist, the most widespread therapy remains the supplementary therapy with eye drops. It is accepted that some viscous polymer additives can improve the distribution and adhesion of the drop by interacting with the eye's mucosal layer [4, 5], but their concentration should be meticulously calculated, to prevent some side effects like blurred vision, reflex blinking, and resistance to the eyelid's movements [6]. Our goal was to investigate the interactions between four prospective hydrophilic polymers – gel (GG), carboxymethyl-celulose (CMC), hyaluronic acid (HA), cross-linked hyaluronic acid (CHA) and human meibum (lipid component of the tear film), as well as to shed a light on the choice of the correct concentration of those polymers in some commercially available or prospective eye-drops.

Materials and Methods

Human tears and meibomian glands secretions (MGS) were collected from 5 healthy volunteers (age 22 - 38), using 5 μl glass microcapillary tubes (Blaubrand® IntraEND, Wertheim, Germany). The collection time was limited to a maximum of 3 min resulting in collection volumes between 3 and 12 μl. The contact with the bulbar conjunctiva or the lid margin was minimized to prevent the reflex tearing [7]. MGS obtaining procedure was performed as described in previous publications [8, 9]. The tested polymers (table 1) were purchased by Santen Pharmaceutical Co., Ltd., Osaka, Japan.

* Corresponding author: 8, Dragan Tzancov Blvd., Sofia, Bulgaria, zip 1164; e-mail: peftimov@uni-sofia.bg, tel./fax: +35928656 641

Name	Abbreviation	CAS number	Concentrations, %
Hyaluronic acid	HA	9004-61-9	0.0001, 0.001, 0.01
Cross-linked hyaluronic acid	CHA	N/A	0.0001, 0.001, 0.01, 0.05
Carboxy-methyl cellulose	CMC	9000-11-7	0.0001, 0.001, 0.01, 0.05, 0.1
Gellan gel	GG	71010-52-1	0.0001, 0.001, 0.01, 0.05

Table 1. List of used hydrophilic polymers and their corresponding testing concentrations

We used three different methods for evaluation of the collected samples:

1. Langmuir balance experiments

We used a computer-controlled Langmuir surface balance (Kibron, Helsinki, Finland) equipped with a µTrough XL (area 225 cm^2, volume 40 ml) and the Wilhelmy wire probe method (instrumental accuracy 0.01 mN/m [10]) to measure the surface pressure (π) vs. area (A) isotherms. The MGS samples (45µg dissolved in chloroform) were spread over buffered NaCl (pH=7.4) with a Hamilton micro-syringe. In order to prevent ozone induced oxidation of the films [11], we protected the sample with acrylic cover and supplied it with air that had been processed by an ozone destructing unit (Ozone Solutions ODS-3P, Hull, Iowa). After the evaporation of the chloroform, we performed at least 10 iso-cycles before introducing the tested polymer in the subfase. Five subsequent measurements were made and used for analyzing the polymer/MGS interactions. Selected polymers were tested within the pharmaceutically relevant scale, with regards to normal human tear turn-over rate.

2. Ferning pattern analysis

We analyzed microphotographs of the crystalline structures after complete evaporation of droplets from the different concentrations of the tested polymers in human tears (V=3 µl), using STEPanizer software. The presence of specific, branched structures i.e. ferning patterns was scaled according to Rolando's classification [12]: type I, characterized by a dense crystallization composed of multibranched crystals, without spacing between them; Type II, characterized by dense crystallization, shorter branches, and less and more spaced-out ramifications; Type III, characterized by rare ramifications, much larger and incomplete, and ample space between them; and Type IV, characterized by the absence of crystallizations and the presence of clumps and mucus.

3. ADSA – axisymmetric drop shape analysis

To evaluate the wettability of the polymers, we analyzed the contact surface between a micro-drop of whole tears (V=3 µl) and a mucine-coated glass plate, by measuring the contact angle on the air-polymer solution-plate interface (fig.1). We used HO-IAD-CAM-01B contact-angle meter (Holmarc Optomechatronics), with build-in software, dedicated for the aforementioned analysis.

All the experiments were performed at 35°C, which is in agreement with the consensus mean temperature of the central cornea in humans as obtained by infrared thermography [13].

KyPlot platform was used for statistical analysis of the data.

Results and Discussion

We tested all the polymers in physiologically relevant concentrations (see table 1.). The surface pressure/area (π/A) isotherms, showed particular concentration-depending pattern (fig. 2). Hyaluronic acid (HA), as well as carboxy-methyl cellulose (CMC), outperformed the rest of the tested polymers, with peak efficiency at 0.01% concentration. The complicated interactions of tested polymers with multi-compositional MGS may result in observed decline of the surface pressure in the highest concentrations (0.05% and 0.1%), where they were applied.

Ferning pattern analysis revealed, that all the tested polymers could form type I ferning pattern, but at different concentration points: HA and CHA – at 0.01% (fig. 3, fig.4), while GG and CMC – at 0.05% (fig. 5, fig. 6). As these analyzes were performed with whole human tears, we consider the higher concentrations of GG and CMC to be more relevant, despite the demonstrated lower values of π, when tested with MGS only.

Contact angle measurements outlined CHA as the best performing hydrophilic polymer in terms of wettability at all the tested concentrations. It maintained lowest values of the static contact angle at concentrations of 0.01% and 0.05%, with no significant difference between them (table 2 and fig. 7).

	Contact angles [°]			
Concentration [%]	HA	CHA	CMC	GG
0,0001	43,54 ± 1,22	33,61 ± 0,96	39,82 ± 1,69	42,41 ± 1,47
0,001	41,55 ± 1,47	27,73 ± 0,55	32,43 ± 0,99	40,43 ± 1,84
0,01	41,79 ± 1,51	25,30 ± 0,95	33,12 ± 1,34	29,96 ± 1,03
0,05	N/A	25,97 ± 0,97	30,23 ± 1,00	31,24 ± 1,23
0,1	N/A	N/A	37,05 ± 1,20	N/A

Table 2. Static contact angle values of tested hydrophilic polymers.

This result agrees with the data from ferning pattern and Langmuir experiments, and reveals the statistically significant effect on the wettability of CHA vs. HA ($p < 0.01$), which could be related with its increased stability [14].

The complicated nature of tear film interactions with the environment is hard to reproduce in a single model system, thus a multilevel analytical approach is needed for better understanding and reproducing these interactions in health and disease. The combination of Langmuir surface balance experiments, tear ferning and contact angle studies allows comprehensive and simultaneous analysis of both lipid interaction and mucomimetic properties of the tested polymers.

The presented study is part of the continuous efforts for achieving more clear and correct perspective on the contemporary non-surgical treatment of DES. It is in accordance with the pharmaceutical companies needs to narrow the preclinical selection or postproduction monitoring of ophthalmological solutions – an expensive and time-consuming process.

Statement for Potential Conflicts of Interest

The authors declare no conflicts of interest.

Acknowledgements

The study was supported via National Program "Young Scientists and Postdoctoral Students", grant number РД-22-876/8.04.2019 and by Santen S.A.S, France.

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Figure legends:

Fig.1 ADSA – static contact angle measurement. Increased value of the contact angle ϴ corresponds to lower contact surface of the droplet, i.e. lesser wettability.

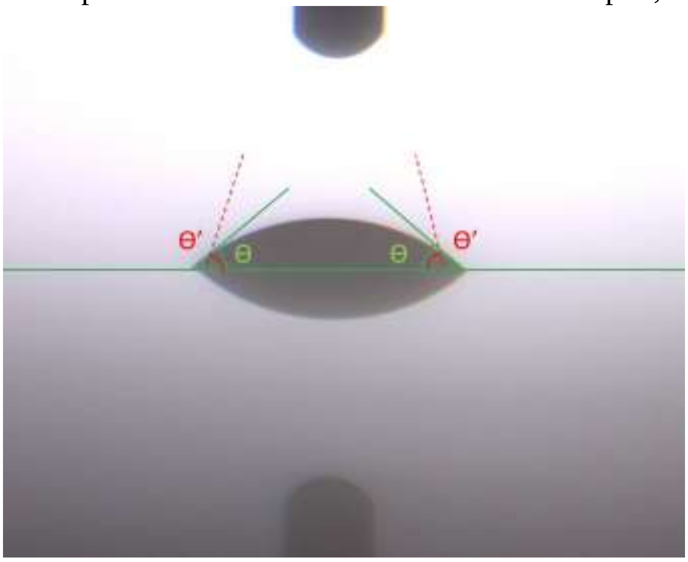

Fig.2 Comparison of the (π/A) isotherms, of the tested polymers at different concentration points.

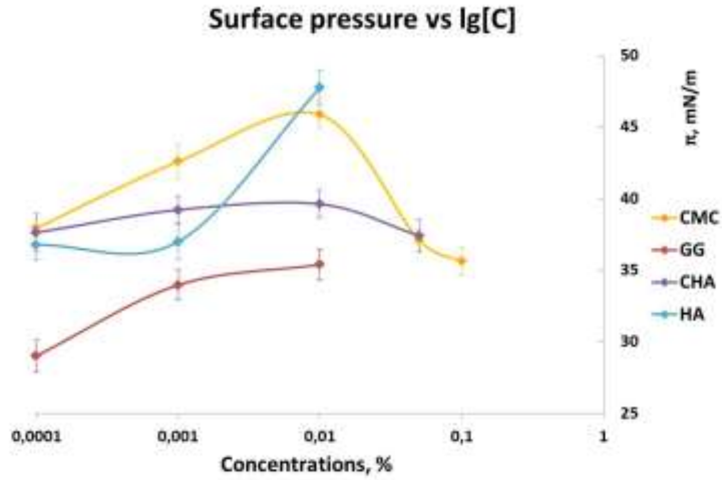

Fig.3 Ferning pattern of hyaluronic acid (HA). A – 0,0001%, B – 0,001%, C – 0,01%

Fig.4 Ferning pattern of cross-linked hyaluronic acid (CHA). A – 0,0001%, B – 0,001%, C – 0,01%, D – 0,05%.

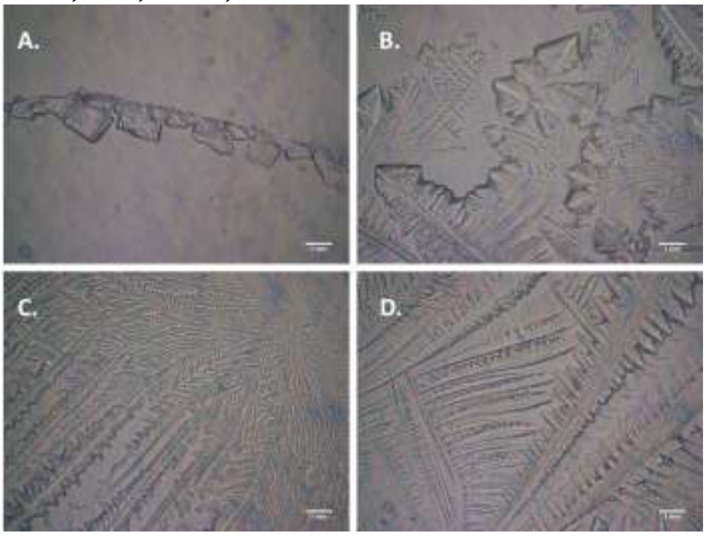

Fig.5 Ferning pattern of gellan gel (GG). A – 0,0001%, B – 0,001%, C – 0,01%, D – 0,05%.

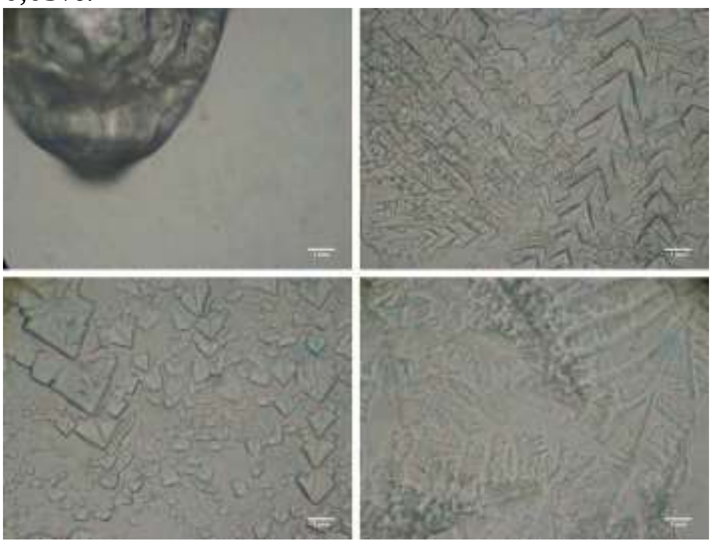

Fig.6 Ferning pattern of carboxy-methyl cellulose (CMC). A – 0,0001%, B – 0,001%, C – 0,01%, D – 0,05%, E -0,1%.

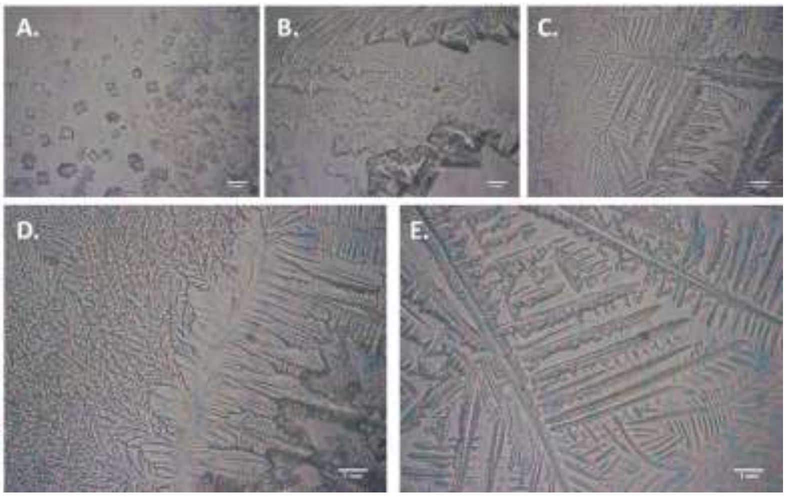

Fig.7 Static contact angles of the selected polymers for all of the tested concentrations.