Surface Chemistry Study of the Interactions of Pharmaceutical Ingredients with Human Meibum Films

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PURPOSE. To perform a surface chemistry study of the interactions between the benzalkonium chloride (BAC)-preserved eyedrops Travatan, the SofZia-preserved TravatanZ, and the Polyquad-preserved DuoTrav, and tear film (TF) constituents. The interactions of TF compounds with the individual preservatives, BAC, SofZia, and Polyquad, were also examined.

METHODS. Langmuir surface balance measurements were used to examine the interactions between the pharmaceuticals and films of human meibum and rabbit corneal cell lipid extracts. Surface pressure–area isocycles were used to assess the sample’s capability to compress and spread during dynamic area changes. The dilatational rheologic properties of human meibum films, pure and in the presence of preservatives, were probed by stress–relaxation studies. Lipid film morphology was monitored by Brewster Angle Microscopy. The viability of SofZia- and Polyquad-treated Statens Seruminstitut Rabbit Cornea (SIRC) cell cultures was also evaluated.

RESULTS. The interactions between BAC-preserved eyedrops and lipids resulted in impaired lipid spread, formation of discontinuous nonuniform surface layers, and increased subphase pressure–area hysteresis during compression/expansion. In contrast, TravatanZ, DuoTrav, and the individual preservatives SofZia and Polyquad proved to be safe to the lipid film structure and isothermal reversibility. The stress–relaxation experiments revealed that the viscoelastic properties of meibomian film are impaired by BAC, and remain unaffected by SofZia and Polyquad. SIRC cells’ viability and capability to form confluent cellular monolayer were also maintained after exposure to SofZia and Polyquad.

CONCLUSIONS. Surface chemistry studies present criteria for preclinical in vitro molecular scale characterization of the interactions between eyelash drop compounds and TF constituents. (Invest Ophthalmol Vis Sci. 2012;53:4605–4615) DOI: 10.1167/iovs.12-9907
Langmuir films were also probed. The lipid extract films mimic the cell membrane’s outer half,20 which is exposed to interactions with eyedrop constituents. In the in vitro surface chemistry results are correlated with recent clinical and in vivo findings4–6 on the impact of pharmaceuticals on tear film lipid layer (TFLL) spread and on TF stability and with our previous study 21 on the interactions of BAC with TF constituents.

Materials and Methods

Eyedrops and Preservative Systems

The eyedrops used were the travoprost formulations Travatan, TravatanZ, and DuoTrav preserved with BAC (0.015%), SofZia, and Polyquad (10–3%), respectively. These test formulations and the preservatives SofZia and Polyquad were provided by either Alcon Japan (Tokyo, Japan) or Alcon USA (Ft. Worth, TX).

Collection of Human Meibum

Meibum samples were collected from healthy volunteers working in the laboratory, three females (25–43 years old) and one male (33 years old). None of the volunteers wore glasses or contact lenses, or applied eye makeup prior to sample collection. All volunteers gave written informed consent. The collection procedures were in accordance to the tenets of the Declaration of Helsinki and approved by the Sofia University Ethics committee. Human Meibomian lipids were expressed from eyelids by applying pressure to the eyelids with two opposed cotton buds.22 Meibum was collected daily from each volunteer over a week's period to minimize the effect of day-to-day personal variations. The samples were collected from the lid margin with a platinum spatula, weighed, and dissolved in chloroform to a unified stock solution with a concentration of 1 mg lipid/mL. Prior experiments using the donor-specific lipid solutions obtained were kept at –80°C. Five meibum solutions were used: four individual donor-specific solutions and an equiweight meibomian mixture (see Supplement 1 and Supplementary Fig. S1, http://www.iovs.org/lookup/suppl/doi:10.1167/iovs.12-9907/-/DCSupplemental). The composition of meibum was examined by thin layer chromatography and revealed the presence mainly of wax and sterol esters in agreement with previous reports.23

Isolation of the Membrane Fraction of SIRC Cells

The plasma membrane fraction was obtained by the procedure of Evans with modifications.24

Langmuir Surface Balance Experiments

Surface pressure (π)-area (A) isotherms were measured using a computer-controlled Langmuir surface balance (Kibron, Helsinki, Finland) equipped with an automated trough (μTrough XL, area 225 cm², volume 40 mL; Hunter Automated Machinery Corp., Schaumburg, IL) by the Wilhelmy wire probe method (instrumental accuracy 0.01 mN/m).

Human meibum dissolved in chloroform was spread (43 μL of 1 mg/mL) over the air/saline solution interface with a microsyringe (Hamilton Co., Reno, NV). An acrylic cover was put over the trough to protect the surface from dust and to suppress subphase evaporation. After 15 minutes to allow chloroform evaporation, film area compression was started using two symmetrically moving barriers. For the experiments where the effect of pure preservatives/whole eyedrop on meibum films is tested, prior to the compression the 250 μL pharmaceutical solution was injected into the saline solution below the lipid film to the designated concentration in the trough subphase. Fast dynamic compression-expansion isocycling of the film area was performed with the maximal possible barrier’s rate (140 mm/min) at which there was no leakage of the film. Ten consecutive cycles were performed with each sample. Normally between the first and third cycles the π(A) loops achieve stationary shape and those π(A) isocycles are presented and analyzed.

All isotherms were repeated at least three times; the difference between the repetitions did not exceed 2%. Corneal temperature usually ranges between 30 and 33°C and, at normal to cold weather conditions, the TF temperature can be lower due to heat exchange.25,26 The presented experiments were performed at 28°C.

The morphology of the films was observed by BAM (MicroBAM2; Nima Technology Ltd, UK).27

Analysis of the Dynamic Compression/Expansion Isocycles

The surface pressure-area curves obtained at compression and expansion of the film surface can strongly differ (loop 1, Fig. 1B) or...
closely overlap (loop 2, Fig. 1B) in their course. The discussed effects can be quantitatively evaluated by the integration of expansion/compression curves and calculation of the isotherm reversibility ($R_v$):

$$R_v = 100 \left( \frac{\int \pi \, dA}{\int \pi \, dA} \right)_{\text{expansion}} / \left( \int \pi \, dA \right)_{\text{compression}}$$  \hspace{1cm} (1)

For films of hydrophobic molecules such as the meibomian lipids, the magnitude of the hysteresis is determined by the kinetics with which the compressed surface films restore their structure and spread at the interface during area expansion.28 Highly reversible films (loop 2, Fig. 1B) restore their structure and spread at the interface with rate commensurable to that of the area expansion (achieved by the barriers of the Langmuir trough). Lower reversibility of the films (loop 1, Fig. 1B) reflects slower reorganization and spreading of the surface films compared with the rate of area expansion.

**Evaluation of the Adsorption of Preservatives and Whole Eyedrops at Pure Air/Water Interface and at Interface with Predeposited Meibomian Film**

A multiwell Teflon plate trough consisting of 15 individual circular wells, 1 mL volume each, was used in these experiments.29,30

To measure the adsorption of preservatives or whole eyedrop formulations at pure air/water interface solutions with desired concentrations, solutions were loaded into the wells, and their equilibrium surface pressures were recorded.

To explore the adsorption of preservatives or whole eyedrop formulations to predeposited meibomian film, lipids dissolved in chloroform, were spread at the air/water interface and sufficient time was provided for chloroform evaporation and equilibration between the surface film and the subphase. Various amounts of meibum were deposited so that different initial surface pressure ($\pi_0$) values were achieved. Then 3 $\mu$L of eyedrop/preservative solution was injected in the subphase. The equilibrium increase in the surface pressure ($\Delta \pi$) after the injection of eyedrops was measured. The $\Delta \pi$ increments were plotted versus $\pi_0$ to measure the critical surface pressure ($\pi_{cr}$) at which the eyedrop constituents will no longer be able to penetrate into the lipid film.20

**Evaluation of the Ability of Eyedrop Components to Reside in or Be Squeezed Out from Meibomian Films during Dynamic Compression/Expansion Cycling**

In these experiments, the pharmaceutical components were injected into the saline solution beneath the lipid film precompressed to $\pi = 30$ mN/m (the TF surface pressure in an open eye31,32). Then the dynamic compression/expansion cycle was performed. Thus, it is possible to measure two parameters33,34 (Fig. 1C):

1. The insertion surface pressure, $\pi_{ins}$, at which during area expansion, the penetrant molecules insert into the film, registered as flattening of the expansion isotherm when the water-soluble compound adsorbs at the surface.
2. The squeezed-out surface pressure, $\pi_{sq}$, at which during area compression, the penetrant molecules are squeezed out of the lipid film, back in the subphase; this is registered as recovery of the original “lipidlike” steep slope of the compression $\pi(A)$.
trend due to the recovery of the lipid film, after the soluble compound is squeezed out in the saline solution.

Stress–Relaxation Studies via the Small Deformations Method

To gather information about the viscoelastic properties of meibum films, with or without preservatives included in the subphase, we monitored the relaxation of surface pressure after a small rapid compression deformation was applied to the surface film. In these experiments, the meibomian film preequilibrated with the subphase (saline, pure, or containing preservative solution) was instantaneously contracted with a small area change, \( \frac{\Delta A}{A_0} = \frac{5}{6} \) 1% (where \( A_0 \) is the initial area of the film and \( \Delta A \) is the area change). Then the relaxation of the surface pressure (see Fig. 7 for details) was registered; for the samples studied it usually took \( \leq 500 \text{ seconds} \) for complete relaxation. The surface pressure relaxation kinetics is described by the equation\(^7\)–\(^{14}\)

\[
\pi(t) - \pi_\infty = A_1 \exp(-t/\tau_1) + A_2 \exp(-t/\tau_2)
\]

where \( \pi(t) \) is the surface pressure in any moment \( t \), \( \pi_\infty \) is the maximal surface pressure at the start of the relaxation, immediately after the compression is performed; \( \tau_1 \) is the equilibrium surface pressure after completion of the relaxation; \( \tau_1 \) and \( \tau_2 \) are relaxation times for rapid and slow processes that take part in the total relaxation process; and \( A_1 \) and \( A_2 \) are constants that reflect the contribution of the fast relaxation time and of the slow relaxation time to the total surface pressure change (to the total relaxation process).

In the stress–relaxation experiments meibomian film prior deformation was compressed to surface pressure 25–30 mN/m. When preservative was injected into the subphase, below the precompressed film, half an hour was given for the lipid layer to equilibrate with subphase before the compression stress to be applied.

Cultivation of SIRC Cells, Treatment with SofZia or Polyquad, and Measurements of Cell Culture Confluence and of Cell Viability

SIRC cells were cultured in Dulbecco’s modified Eagle’s medium (DMEM), supplemented with 10% fetal bovine serum until 95–100% confluence is reached. The confluent cellular monolayers consisted of \( 5.8 \times 10^5 \) cells. The medium was removed and the cells were incubated at the desired concentrations of SofZia or Polyquad in phosphate-buffered saline for up to 5 minutes as described previously.\(^{30}\) After that, the preservative solution was swept out, and the cells were washed and reincubated in DMEM. The degree of confluence of SIRC cultures was monitored, before and after treatment with the pharmaceutical agents, and pictures were taken regularly with a microscope (Nikon ECLIPSE TS100-F; Nikon Instruments, Melville, NY) and photo documentation system. A magnification of \( \times 20 \) was used.

The viability of cells in the presence of SofZia or Polyquad was evaluated by MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] colorimetric assay.\(^{35}\)

RESULTS

Adsorption of Preservatives and Whole Eyedrops at Pure Air/Water Interface and at Interface with Predeposited Meibomian Film

The adsorption of compounds contained in eyedrops, at the pure air/water interface, was estimated to compare the surface activity of the formulations (Fig. 2, top left panel). BAC-preserved Travatan displayed higher surface activity compared with that of TravatanZ and DuoTrav. The maximal \( \pi \) reached by the eyedrop with BAC was 43 mN/m and is similar to the
Surface pressure (39–40 mN/m) of concentrated (‡10/C02%) BAC solutions. In contrast with that of TravatanZ and DuoTrav the surface pressure remained < 28 mN/m. Because both SofZia and Polyquad solutions showed p < 3 mN/m at the highest pharmaceutical concentrations (SofZia stock solution and 0.001% Polyquad) the surface activity displayed by TravatanZ and DuoTrav can be clearly attributed to the surface activity of the emulsifiers used as inactive ingredients in the formulations.

The adsorption of compounds present in the whole eyedrops to air/water interface covered with predeposited meibum film with initial surface pressure p0 was also measured (Fig. 2, top right panel). For this purpose, eyedrops were injected into the subphase and if formulations’ ingredients penetrated the surface film, an increase of the surface pressure (Dp) was registered. The eyedrop concentration achieved in the subphase corresponded to a surface activity of 10 mN/m (i.e. < than the minimal p0 of the predeposited meibum film used), which ensured that if penetration occurred, it was not due to nonspecific adsorption of compounds at the interface but was due to specific interaction of ingredients with meibomian lipids. Also because the eyedrop concentration implemented in the penetration studies corresponded to strong dilution (<10% of the initial compositions; Fig. 2, top left panel) of eyedrops, the measurements allowed emulation of the long-term effects of trace amounts of the pharmaceuticals at the ocular surface.

By the linear extrapolation of Δp(p0) dependence (Fig. 2, top right panel) the so-called critical surface pressure pCr was obtained: that is, the minimal surface pressure of the predeposited lipid film at which and above which pharmaceutical constituents will not be able to be inserted in the interfacial film. The compounds’ insertion in films by biologically relevant lipids at physiologic values of surface pressure, approximately 30 mN/m for tears in an open eye, is considered to correlate with the constituents’ capability to incorporate and alter the integrity of membranes in vivo.

It can be seen (Fig. 2, bottom panel) that for BAC-preserved Travatan pCr was 37 mN/m, whereas for TravatanZ and DuoTrav it was 23 and 18 mN/m, respectively. Experiments performed to test the penetration of eyedrop components into whole human tears, using the pendant drop technique, confirmed the same trend: Travatan increased the surface pressure of tears, whereas TravatanZ and DuoTrav showed no effect (data not shown).

Compression Isotherms and Dynamic Isocycles of Human Meibum, Pure and in the Preservatives and Whole Eyedrops

A typical surface pressure–area compression/expansion isocycle for human meibum over a saline subphase is shown in Figure 3. The shape of the isocycle was similar to that presented by other authors. The BAM (Fig. 3, bottom row) revealed that even at low surface pressures (between 0.5 and 10 mN/m) meibum formed a continuous but rough film, which is characteristic of lipid multilayers. The films were composed of bright lipid aggregates that contained interspersed areas resembling “black stripes,” with a lower lipid amount. When the surface pressure was increased above 10–11 mN/m, the “black stripes” closed and the lipid film became more uniform and dense. The isocycles of human meibum over pure saline are denoted with solid lines, whereas in the presence of eyedrops they are denoted with dashed lines. Identical results were obtained with the four donor-specific meibum solutions.

Figure 4. Compression/expansion p(A) trends of meibomian films (by equiweight mixture of donor-specific meibum samples) immediately after injection of eyedrop aliquots in the trough subphase beneath lipid film compressed to p = 30 mN/m. BAC-preserved Travatan inserted in the film and strongly perturbed the p(A) trends, whereas for TravatanZ and DuoTrav p(A) values are observed at subphysiologic surface pressures during film expansion. The p(A) isotherms of human meibum over pure saline are denoted with solid lines, whereas in the presence of eyedrops they are denoted with dashed lines. Identical results were obtained with the four donor-specific meibum solutions.
injected into the subphase below the lipid film precompressed to $\pi = 30$ mN/m (the tear's surface pressure in an open eye). The interaction was evaluated by measurement of: (1) the insertion ($\pi_{\text{ins}}$) and squeezed-out ($\pi_{\text{sq}}$) surface pressures of pharmaceutical ingredients to the lipid film; (2) the changes in $\pi(A)$-isocycles shape/reversibility; and (3) in the morphology of the eyedrop-treated meibomian film.

BAC-preserved Travatan was inserted in the lipid film at 30 mN/m immediately after inclusion into the subphase and strongly perturbed the compression and expansion $\pi(A)$ trends (Fig. 4). The result is consistent with the previous findings of instantaneous adsorption of BAC to the lipid layer.\textsuperscript{21} In agreement with the data on $\pi_{c}$ (Fig. 2) TravatanZ and DuoTrav did not influence meibum at 30 mN/m and were inserted only after the films were expanded to surface pressures of $\leq 23$ mN/m (Fig. 4, bottom row).

Further compression/expansion of the meibum over the subphase containing eyedrops resulted in refinement of the

**Figure 5.** Compression/expansion $\pi(A)$-isocycles of meibomian films (by equiweight mixture of donor-specific meibum samples) over eyedrop-containing subphase. BAC-preserved Travatan strongly impairs the shape and the reversibility of the isocycle loops, whereas in the presence of TravatanZ and DuoTrav the characteristics of the isocycle are maintained and clear $\pi_{\text{ins}}$ and $\pi_{\text{sq}}$ values are observed at subphysiological surface pressures. Bottom panel: Statistically significant difference between the $R_v$ values for films over Travatan containing subphase compared with films over subphases of pure saline solution and of BAC-free eyedrops' solutions; ***$P < 0.001$ is determined by paired $t$-test with KyPlot. $R_v$ for each meibomian sample is averaged from triplicate measurements; SD $\pm 1.5\%$.
surface film composition, achieving a stationary distribution of the lipid and the eyedrop compounds between the surface and the bulk and establishment of steady shape of the isocycle loops (Fig. 5). It was not possible to squeeze out the ingredients of Travatan in the subphase during the cycle, and the mixed film isotherm reversibility was remarkably lowered (Fig. 5). Thus within the examined \( p \) range, the pharmaceutical molecules remained stably and irreversibly incorporated in the surface film and it was not possible to identify the \( p_{sq} \) value.

BAM images (Fig. 6) showed that the film structure became very patchy and discontinuous, consisting of bright thick lipid aggregates separated by "dark lakes" of soluble compounds accumulated at the surface. The observed effects are analogous to those of pure BAC solutions as previously reported by us. 21 In contrast, the mixed film in the presence of TravatanZ/DuoTrav maintained high isotherm (98%) reversibility and \( p_{ins} \) and \( p_{sq} \) values (the surface pressure values at which the slope of the \( A[\pi] \) trends changes from steep to flat at expansion and compression, respectively) were registered at approximately 20 mN/m, and the mixed films containing meibum/eyedrop ingredients maintained high isotherm reversibility (Fig. 5) and a continuous and rough meibumlike surface structure.

Viscoelasticity of Human Meibum, Pure and in the Presence of Preservatives, Accessed via Stress–Relaxation Experiments

Figure 7A presents a typical transient of surface pressure relaxation; Figure 7B depicts the stress–relaxation data for meibomian film over saline subphase, pure and in the presence of preservatives, in the \( \{\pi(t) - \pi_{\infty}\}/(\pi_{\max} - \pi_{\infty}) \) versus \( t \) coordinates as required by equation 2. The fitting of the experimental data by the equation produced numerical values for the relaxation times, \( \tau_1 \) and \( \tau_2 \), and for the constants, \( A_1 \) and \( A_2 \), which are presented in the Table.

The meibomian films of the equiweight meibum mixture over saline subphase, pure and in the presence of SofZia or Polyquad, showed fast relaxation time \( \tau_1 \approx 2 \) seconds, and slow relaxation times \( \tau_2 > 200 \) seconds. The contribution of the fast elastic relaxation to the total change of surface pressure was given by the constant \( A_1 > 0.6 \), and of the slow relaxation by \( A_2 < 0.4 \) (i.e., the fast processes were predominant in the rheology behavior of the film). A higher value of \( A_1 \) compared with \( A_2 \) was observed for all meibum samples. The results agree with the prevalence of the elastic over the viscous modulus at \( \leq 29^\circ C \) reported previously. 37 In contrast to other preservatives in the presence of BAC, the value of \( A_2 \) became almost twice higher than \( A_1 \) (i.e., the slow processes were prevalent for the viscoelastic properties of the film; Fig. 7, bottom panel).

Impact of SofZia and Polyquad on the SIRC Cells’ Viability and on Membrane Extract Surface Properties

The capacity of SIRC cells to form confluent cellular monolayers was maintained in the presence of preservatives (Fig. 8). The viability of the cells as determined by the MTT method, \( 5.8 \times 10^{5} \) cells per sample, was not impaired (data not shown). The performance of the SIRC lipid extract film also remained unaffected by the preservatives.

These findings clearly demonstrate the advantages of SofZia and Polyquad in comparison with BAC that, as reported in our previous studies, 21 disrupts the integrity and the performance of meibum and impairs the corneal cells viability even at extremely low subclinical concentrations.

DISCUSSION

Surface Behavior of Pure Human Meibum Films

Brewster Angle Microscopy images (Fig. 3) agree with the published literature 27 that meibum spontaneously forms multilayer film (instead of monolayer) when spread at the...
air/water interface. Normally, lipid monolayers at low surface pressure are in a liquid-expanded phase that does not modify the Brewster angle of the surface significantly; only at further compression, when high surface pressures are achieved, are condensed lipid phases observed. In contrast, human meibum even at low $p$ (0.5–10 mN/m) forms rough continuous films at the air/water interface, which become more uniform when $p$ increases and preserve high lateral elasticity for the whole $p$ range.\(^{21,37–39}\)

The very high isotherm reversibility, $R_v \approx 98\%$, of meibum and tears indicates rapid spread and recovery of the film structure during dynamic area changes. The data agree with in vivo studies,\(^{40}\) showing rapid TFLL spreading that closely follows the upward movement of the eyelid in “healthy” eyes. In contrast to multilayers, monolayers of disaturated phosphatidylcholines with acyl chains longer than C14 (the major phospholipids of the biomembranes) and of tissue lipid extracts (like pulmonary surfactant lipids) are much less reversible and $R_v$ can reach values as low as 45%.$^{41}$

Meibomian lipids’ performance at the air/water surface resembles that of triglycerides/cholesterol esters mixtures (i.e., compositions partially similar to meibum), which are also reported$^{12}$ to form noncollapsible and reversible multilayers. A key feature of multilayers enriched with hydrophobic lipids (such as the wax and sterol esters in meibum) is that during area contraction the polar lipid molecules, instead of being compressed to a condensed phase and then forced to collapse (the typical scenario leading to loss of material during monolayer cycling$^{45}$), migrate to the upper layer of the film, normally consisting mainly of nonpolar lipids. Then the so-formed upper stratum of the multilayer acts as an interfacial reservoir from which polar lipids rapidly spread back onto the surface during film expansion.$^{42}$

Thus the multilayer structure might well be responsible for the characteristic surface properties (high lateral elasticity and high reversibility) of meibomian lipids. It renders meibum film noncollapsible and highly resistant to the loss of material during cycling. Possible physiologic consequences are that the film can maintain its performance at the air/tear surface for an extended period of time and there is no need for the meibomian glands to constantly secrete new lipids at the interface. These assumptions agree well with the clinical observation$^{44}$ that the in vivo TFLL turnover rate is very slow and full exchange of TFLL takes more than 8 hours.

**Surface Behavior of Human Meibum Treated with BAC-Preserved Eyedrops**

The penetration of ingredients from BAC-preserved Travatan breaks the integrity of the meibomian layer. The disruption of the lipid film continuous structure hampers the rapid reorganization of the molecules during cycling, which is reflected by the decrease of isotherm reversibility; analogous effects were previously reported in Langmuir surface balance studies$^{21,45,46}$ for the impact of BAC on meibomian film performance. These detrimental effects can be explained$^{37}$ by the inherent property of polar penetrant molecules, such as BAC, to spontaneously repel and separate in distinguished film regions when brought into contact with hydrophobic lipids, such as meibum,$^{48}$ at the interface, in turn leading to formation
of heterogeneous and discontinuous surface films. The essential prerequisite for a stable TF is the presence of continuous insoluble lipid multilayer, which not only ensures that the lipid film reorganizes promptly in response to compression/expansion, but also enhances the formation of tangentially immobile surfaces that increase the TF resistance to thinning, so called Gibbs–Marangoni effect, and suppresses the aqueous tear evaporation.

Thus BAC-induced heterogeneity/solubilization will obstruct these key properties of the TFLL.

Penetration studies revealed that BAC adsorbs to both, the predeposited lipid film equilibrated with the subphase (Fig. 2) and to meibum during dynamic isocycling (Figs. 4, 5), at surface pressures ≥ physiologic values. As once incorporated in the meibum, BAC does not get squeezed out (Figs. 5, 6) in the subphase at 30 mN/m (the surface pressure of TF in the open eye) it can be expected that after the preservative molecules get inserted in the tear film lipid layer they might stably reside in it for an extended period of time.

Since the lipid layer turnover is on the scale of hours, this could be the cause for long-term adverse effects. The in vitro results correlate well with a detailed clinical study, which reports a decrease of TF noninvasive break-up time in eyes treated with BAC-preserved timolol and with in vivo findings showing TF instability and corneal damage in rabbit eyes treated with BAC.

**Surface Behavior of Human Meibum Treated with TravatanZ and DuoTrav: Effect of the BAC, SofZia, and Polyquad on the Viscoelasticity of Meibum in Stress–Relaxation Experiments**

In contrast to BAC, the novel compounds SofZia and Polyquad, and the formulations preserved with them, did not impair the meibum/SIRC lipid extract surface properties. This can be explained by the fact that the new preservatives are designed to specifically interact with the membranes of bacteria and their mechanism is not based on a nonspecific, strong, detergent action.

The rest of the TravatanZ/DuoTrav components displayed good miscibility with meibum as even after their penetration in the film, the lipid-layer integrity and isotherm reversibility were maintained. The result is to be expected because the active ingredients (travoprost and timolol maleate, respectively) and the emulsifiers in TravatanZ/DuoTrav are of lipophilic character and thus are compatible with the lipid layer, whereas the remaining eyedrop ingredients are readily water soluble and with limited surface activity. Also as the pharmaceutical compounds were squeezed out of the surface film at subphysiologic surface pressures, it can be expected that the TravatanZ/DuoTrav impact on tear film lipid layer performance at the ocular surface will be short term if any.

After small instantaneous compression of the film is performed to establish a new equilibrium some molecular reorientation, adsorption/desorption, respreading, and structural rearrangement processes are necessary, which are not completed instantly. All processes on the scale of the short relaxation time, $\tau_1$, can be described mainly by elasticity, whereas the slower processes, on the scale of the long relaxation time $\tau_2$, by viscosity.

The analysis of the stress–relaxation results revealed that SofZia and Polyquad did not alter the viscoelasticity of meibum and that the fast processes remain dominant for the rheology performance of meibum. In contrast, BAC solution changed the balance and the slow processes seem to be accelerates the formation of tangentially immobile surfaces that increase the TF resistance to thinning, so called Gibbs–Marangoni effect, and suppresses the aqueous tear evaporation.

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<th>$\tau_1$ (s)</th>
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<td>Equiweight mixture</td>
<td>2.01</td>
<td>0.66</td>
<td>201.20</td>
<td>0.34</td>
</tr>
<tr>
<td>+ BAC</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sample 1</td>
<td>0.74</td>
<td>0.17</td>
<td>227.43</td>
<td>0.83</td>
</tr>
<tr>
<td>Sample 2</td>
<td>5.45</td>
<td>0.22</td>
<td>198.62</td>
<td>0.78</td>
</tr>
<tr>
<td>Sample 3</td>
<td>3.59</td>
<td>0.25</td>
<td>275.83</td>
<td>0.65</td>
</tr>
<tr>
<td>Sample 4</td>
<td>0.64</td>
<td>0.36</td>
<td>155.70</td>
<td>0.64</td>
</tr>
<tr>
<td>Equiweight mixture</td>
<td>1.42</td>
<td>0.34</td>
<td>162.15</td>
<td>0.66</td>
</tr>
</tbody>
</table>

$\tau_1$ and $\tau_2$, relaxation times; $A_1$ and $A_2$ constants. The fittings were with $R^2 \geq 0.98$. Results are summarized for meibomian films by the five compositions used (four donor-specific samples and an equiweight mixture) over a variety of subphases. Each of the data points is averaged from triplicate measurements; SD is ±1.5% (see Fig. 8).

**Tolerability of SofZia and Polyquad to SIRC Cells’ Viability and to Membrane Extract Films**

SofZia and Polyquad proved to be safe to SIRC cell culture viability and confluence in contrast to our previous findings with BAC. The results agree with the findings of Baudouin et al., who reported impaired viability of corneal epithelium cells cultured with BAC-preserved eyedrops and high compatibility of the cells with SofZia- and Polyquad-containing pharmaceuticals. The adverse effects of BAC are frequently attributed to its cationic charge, which enables it to electrostatically aggregate with the anionic polysaccharide moieties of the glycocalyx and to its surface activity, which allows the preservative molecules to insert into the tear film lipid layer and in cell membranes and to perturb their integrity. In the current work, Polyquad, which is cationic like BAC, but...
maintained in the presence of saline solution, SofZia solution, or 0.001% Polyquad solution. Both the surface properties of SIRC membrane extract film and the confluence and viability of SIRC cell culture monolayer are fully preserved in the presence of saline solution, SofZia solution, or 0.001% Polyquad solution.

is not surface active, does not impair the properties of meibum or the survival of SIRC cells. Thus the precise control of the detergent properties of eyedrop ingredients is of key importance for the design of optimal pharmaceuticals.

**General Comments on the Interactions of Pharmaceutical Ingredients with Films of Tear Film Compounds**

The capability of whole eyedrops and of individual pharmaceutical ingredients to penetrate the meibomian film and to alter its structure integrity, isothermal reversibility and rheology behavior correlate well with clinical and in vivo studies. Thus surface chemistry techniques provide information complementary to the established clinical approaches and present criteria for molecular scale profiling of eyedrop compounds and for preclinical in vitro evaluation of their interactions with tear film constituents.

**Acknowledgments**

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**References**

Interactions of Pharmaceuticals with Human Meibum Films


