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Augmentation of the Tear Film Lipid Layer by a New Artificial Tear Emulsion

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Introduction

- Meibomian Gland Disease (MGD) or posterior blepharitis, is known to involve an abnormality of the lipid layer of the tear film as a result of aberrant or reduced secretions of meibum from the meibomian glands.
- Most marketed artificial tear solution products are aqueous based lubricant eye drops containing water soluble polymers and viscosity enhancing agents.
- Recently, Alcon launched a novel artificial tear emulsion (ATE), SYSTANE® BALANCE, that contains the demulcent propylene glycol as well as polymer and lipid components.[1,2]. Based on these attributes this product is targeted towards patients with MGD.
- Previous work[1] has shown that this ATE can be activated in three steps when instilled on the ocular surface: (1) blinking (shear thinning); (2) pH shift (HPG-borate crosslinking); (3) dilution and cross-linking (release of lipid/oil). This study further characterizes interfacial mechanisms by which the ATE can deliver ocular surface protection and lipid components to the eye.

Methods and Materials

Materials
The ATE samples tested consisted of the following components: propylene glycol (demulcent) and sorbitol, a borate buffered solution, HPG, mineral oil and an anionic phospholipid, dimyristoylphosphatidylglycerol, (DMPG), and two emulsifiers to form colloidally stable oil droplets. The emulsion contains POLYQUAD® and the pH is at 7.0. Figure 1 represents a schematic of the ATE.

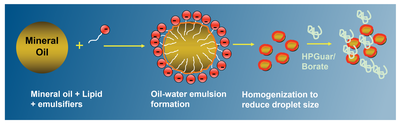

FIGURE 1: Schematic of ATE

Rheology and Friction methods have been previously published[1].

Langmuir Trough Studies
A teflon-surfaced Langmuir-Adam trough of 1000cm² available working area for gas/liquid interfacial film formation and compression was used for these studies.[3] 50-100 microliter volumes of ATE formulation were initially applied at < 1mg/m² and allowed to spontaneously spread promoting release of some emulsified and HPG-bound surface-active agents and oil components to the aqueous/air interface (Table 1).

TABLE 1. Surface Activity Results from Contact Angle Measurements on PTFE/Type C Film

Test Fluid	liquid/vapor surface tension [mN/m]	Maximum spreading pressure [mN/m]	Observations of test fluid on Pt wire
Distilled water	71.9	n/a	---
Marketed Emulsion Eye drop	48.4	20.3	- drains easily
ATE, No HPG pH 7.0 pH 8.0	69.0 71.3	6.2 4.8	- no drain-down - little drain-down
ATE pH 6.5 pH 8.0	71.9 58.8	6.7 8.1	- no drain-down - drains less easily
ATE, sorbitol removed pH 6.9 pH 8.0	64.0 56.9	8.6 11.6	- very slow drain - very slow drain

Infrared-transparent, highly optically polished and electrically semi-conductive germanium (Ge) internal reflection[4] prisms of 50x20x1 mm dimensions[2] were then lifted vertically and edge-wise through these films at rates of a few mm/minute to transfer representative film segments to the prisms as the available surface area was slowly diminished to keep surface film qualities constant[5]. Additional surface films of ATE were transferred after their compression to surface concentrations ranging from 1mg/m² to greater than 10mg/m², at which condition they were observed to show the grey-white interference colors of lipid-layer films observed clinically.

Mutliple Attentuated Internal Reflection InfraRed (MAIR-IR) Spectroscopy
MAIR-IR Spectra were recorded on a Perkin-Elmer Spectrum 100 infrared spectrophotometer, using specialized mirror optics[4] to obtain the film-free baselines of gas-plasma-cleaned high-surface-energy Ge prisms before their placement into the physiologic-saline-filled well of the Langmuir-Adam trough. After film formation and compression, these same prisms were slowly lifted through the films and the collected air dried samples nondestructively analyzed by the MAIR-IR technique, with a sensitivity ranging from single monolayer thicknesses (<1nm) to about 120nm (yellow-brown interference colors). Subsequent dips of the same prism into (from the air side) and back out of the still-large-area surface films on the Langmuir Trough were performed to see if the classical film build-up phenomenon noted for oil-free monolayers would occur[5].

Ellipsometry Studies
The highly polished, optically flat surfaces of the Ge prisms and their intrinsically high refractive indexes allowed ellipsometric data to be readily collected from the identical surface films whose MAIR-IR spectra were recorded, using Rudolph Research Ellipsometer Model #43702. Geometrical film thicknesses associated with the transferred layers' recorded optical thickness data were calculated, using pre-recorded Ge prism baseline values, for refractive index of 1.5, typical of hydrocarbon-dominated thin films, using a National Bureau of Standards-developed program[6]. The optical measurements also were non-contacting and nondestructive.

Contact Potential Studies
The intrinsic electrical conductivity of the germanium prisms allowed further nondestructive, non-contacting determination of thin films' electrical dipole potentials, using Vibrating Reed equipment custom-constructed in accord with schematics published by the US Naval Research Laboratory[7].

Results and Discussion

Several important mechanistic characteristics of the ATE were explored in this presentation. The data showed that the ATE possesses a variety of interfacial properties that can provide ocular surface protection and deliver lipid components at an air/water interface. The following studies describe the predominant features of the ATE.

Rheology and Friction
Previous work has shown that the viscoelasticity of HPG solutions can be controlled by pH and sorbitol changes[1]. This effect will influence the shear thinning and lubrication properties of the ATE.

ATE: pH and Sorbitol Effects

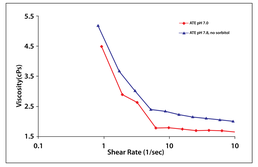

FIGURE 2. Steady State Flow (SSF)-Viscosity as a Function of Shear Rate for ATE at pH 7.0 and 7.8 With and Without Sorbitol.

The viscosity decreased as a function of shear rate indicating the ATE is shear thinning at both pH 7 and pH 7.8 (without sorbitol). When the pH was increased from 7.0 to 7.8 and sorbitol was removed from the ATE, the viscosity increased. The ATE was formulated to have a low viscosity and shear thin when mixed with the tear film to achieve effective spreading, adhesion and minimal blurring upon contact with the ocular surface. Upon mixing with the tear film and following pH adjustment to physiological, the ATE containing HPG/borate and the demulcent will crosslink and viscoelasticity will predominate.

Coefficient of Friction (Cof) Screening

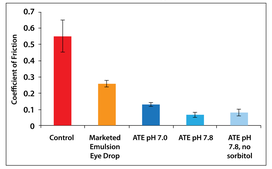

FIGURE 3. Average Coefficient of Fiction Data: After 2 minutes following application to pericardium tissue for saline control, marketed emulsion eye drop and the ATE's.

The Cof data showed significant differences between the test solutions following the post-rinse cycles. The shows the ATE displayed excellent tissue adhesion characteristics. The following order in decreasing Cof (improving lubrication) was observed following the application on the tissue substrate without rinsing : saline control>marketed emulsion eye drop>ATE, pH 7.0>ATE, pH 7.8=ATE, pH 7.8, no sorbitol. Increasing the pH and removing sorbitol decreased the Cof compared to the saline control by approximately 90%.

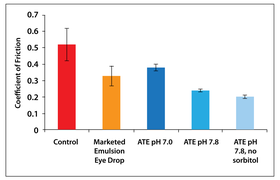

FIGURE 4. Average Coefficient of Friction Data: After 2 minutes and 2 rinse cycles following application to pericardium tissue for saline control, marketed emulsion eye drop and the ATE's.

Figure 4 shows the Cof data following the 2 rinse cycles showed similar trends to the pre-rinse Cof's indicating the ATE was protecting the tissue surface and had improved adhesion characteristics compared to the saline control and the marketed emulsion eye drop. Increasing the pH and removing sorbitol decreased the Cof compared to the saline control by approximately 75%.

Microscopy
Figure 5 shows a micrograph representation of an ATE film on a glass substrate that was semi-dried and subsequently diluted with saline to show the mineral oil and demulcent film adhesion to HPG and subsequent release via dilution.

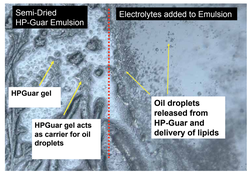

Figure 5: Light Microscopy of Emulsion (ATE) Drop on Mineral Oil: Spreading and Intermixing Characteristics

MAIR-IR Spectroscopy Characterization
As illustrated by the collected MAIR-IR spectra in Figure 6, the first-spread high-surface-area films of ATE comprise ingredients representative of –predominantly-- the mineral oil fraction of the artificial tear emulsion, but also retain HPG and surfactant components throughout compression and re-expansion cycles based on IR spectral comparisons with the individual emulsion ingredients.

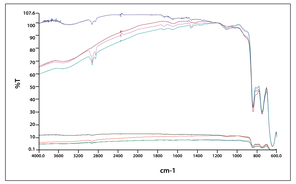

FIGURE 6: Infrared Spectra of ATE Films Transferred to Germanium Prism from Langmuir Trough Under Different Surface Area Conditions

Ellipsometry and Contact Potential
The Ellipsometry and Contact Potential data for the initially spread high-surface-area films showed their thicknesses to be less than would be associated with closely packed monolayers of the surface-active ingredients (Table 2), but consistent with the previously reported net negative surface potentials associated with the anionic lipid contributions to the zeta potentials of the emulsion droplets[1]. Multiple dips of the film-coated prism through the still-spread remaining film area on the Langmuir Trough did not accumulate more material on the sampling prism, indicating the retained two-dimensional (2-D) liquid nature of the spread film as contrasted with 2-D solids characteristic of classical lipid layers. Thus, the ATE-formed films do not irreversibly collapse into coagulated threads as do natural surface films of lipids and glycoproteins, potentially providing a therapeutic benefit for people with diminished production of these natural tear components.

TABLE 2. Thin Film Elipsometry and Contact Potential Data

Analytical Technique	1st Transfer	2nd Transfer	3rd Transfer	4th Transfer	5th Transfer
Thin Film Ellipsometry (3 separate areas on Ge) average ± s.d.	12 ± 2 Angstroms	10 ± 2 Angstroms	9 ± 3 Angstroms	16 ± 7 Angstroms	14 ± 3 Angstroms
Contact Potential (difference from baseline of clean Ge prism)	-0.297 mV	-0.267 mV	-0.243 mV	-0.253 mV	-0.241 mV

The formation of oil-dominated thin surface films under the initially spread conditions is consistent with surfactant-increased spreading pressure for the hydrocarbon layer, as first discovered and utilized by N.K. Adam[8]. The surfactant molecules remain bound at the original aqueous/air interface, directing their carbon-long side chains into the surrounding oil phase and anchoring that oil layer into a clear uniform film as the surface area available is diminished in a fashion mimicking eye blinking. Oil films without such surfactant anchorage retract over the aqueous surface to produce light-scattering separated droplets.

At the greatest compression, the dominantly hydrophilic HPG components of the spread ATE films are mostly forced into the aqueous sub-phase where they provide effectively greater anterior layer thickness and resistance to rupture by structuring the boundary water phase into a higher-refractive-index gel, as natural tear film mucins may also function[9].

Since the oil-rich surface layer does not cross-link or coagulate during the compression event, it remains available to re-spread to cover larger surface areas again as the eyelids (or Langmuir Trough sliding barrier) return to their original positions. This is an important mechanistic finding as it relates to the delivery, spreading and substantivity of lipid components at air/water interfaces such as the tear film.

Clinical Support for Enhanced Lipid Layer Thickness[2]
40 patients were enrolled in a double masked, randomized, contralateral eye study designed to evaluate lipid layer thickness (LLT) after a single drop of SYSTANE® BALANCE was administered. OPTIVE™ Lubricant Eye Drops (Allergan) was used as the active control. LLT was determined using white light interferometry and quantified as described by Korb and Greiner[10]. To qualify, patients had to give a positive response to the question "Do you *ever feel your eyes?*" and show a baseline LLT of ≤ 75nm in both eyes not varying by more than ± 15nm over the course of a 10-min baseline observation period. One drop of the assigned test article was administered per eye per randomization. LLT was assessed at 1, 5, 15 and 60 min post drop instillation.

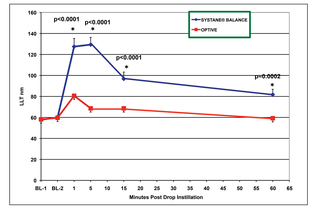

FIGURE 7: Mean Lipid Layer Thickness (LLT) Post Dose

A thicker LLT should retard evaporation and help stabilize the tear film. Results from this clinical study (Figure 7) showed significant differences in LLT at times: 1 minute ($p<0.0001$), 5 minutes ($p<0.0001$), 15 minutes ($p<0.0001$) and 60 minutes ($p=0.0002$) in favor of SYSTANE® BALANCE vs OPTIVE lubricant eye drops.

Conclusions

- The novel combination of Hydroxypropyl Guar (HPG)/borate, demulcent (PG), anionic phospholipid and mineral oil into an emulsion that releases these components when instilled into the eye provides function to increase tissue-on-tissue lubricity and apparent lipid-layer thickness persisting through numerous blinking cycles.
- Langmuir Trough-based laboratory studies of ATE-formed surface films at the physiologic saline/air interface show the mechanism of the apparent lipid-layer augmentation effect to be interfacial anchorage of otherwise non-surface-active hydrocarbon (mineral oil) to the original aqueous/air interface by the added long-hydrocarbon-side-chain surfactants, the oil layer thickening and thinning as the available surface area is decreased and increased.
- Water structuring by the surface-film-bound HPG beneath the oil-rich air-phase film is a further benefit of this ingredient beyond its friction-reduction effects.
- Mechanistically, the ATE composition functions to both protect the ocular surface and deliver lipophilic components as demonstrated clinically through increased lipid layer thickness. These properties may be especially useful for patients with MGD.

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