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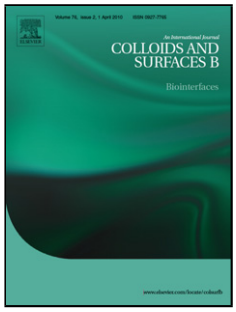

Title: Adherence of *Pseudomonas aeruginosa* onto surfactant-laden contact lenses

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Title: Adherence of *Pseudomonas aeruginosa* onto surfactant-laden contact lenses

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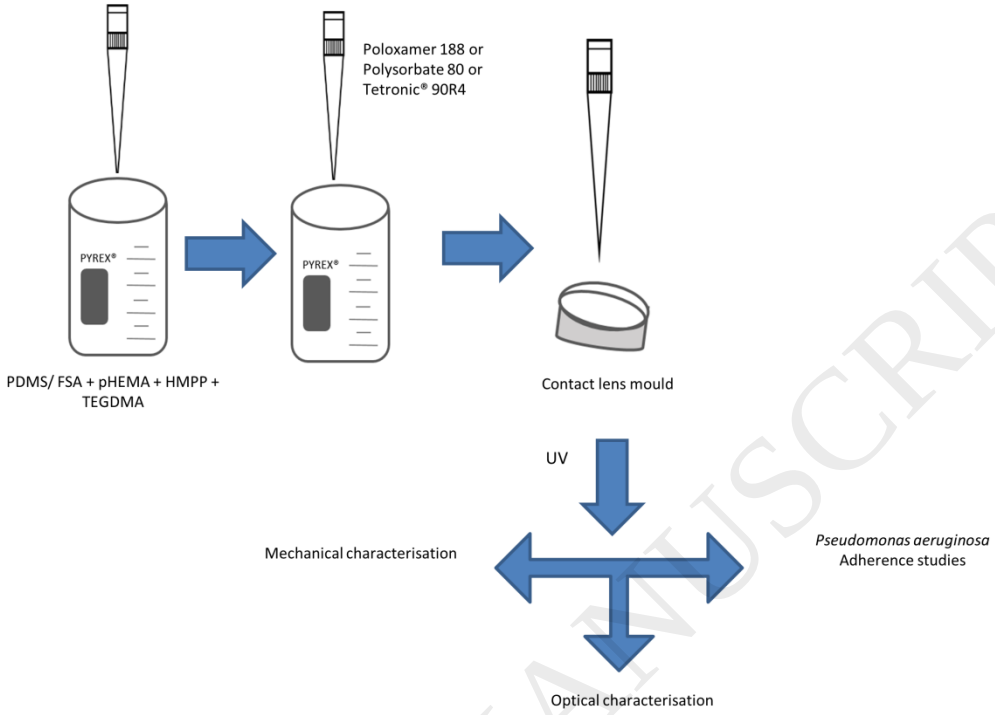
Graphical abstract



Graphical abstract showing the preparation and characterisation of surfactant-laden contact lenses. The lenses were prepared using Poly (dimethylsiloxane) with vinyl terminated (PDMS), and 2-hydroxyethyl methacrylate (pHEMA), Tetraethylene glycol dimethacrylate (TEGDMA) as cross- and 2-hydroxy-2-methylproiophenone (HMPP) as photoinitator.

Highlights

- Poloxamer 188, Polysorbate 80 and Tetronic® 90R4 were incorporated into CLs
- Surfactant-laden CLs had higher EWC but lower Young's modulus.
- Surfactant-laden CLs managed to minimize the adherence of *Pseudomonas aeruginosa*.

Abstract

There is an immense research interest to utilise contact lens (CLs) as a popular platform for ocular drug delivery. However, CLs are the major predisposing factors of bacterial keratitis which is

commonly caused by adhesion of microbes such as *Pseudomonas aeruginosa* and *Staphylococcus epidermidis*. The aim of the current study is to explore the effect of surfactants; Poloxamer 188, Polysorbate 80 and Tetronic® 90R4 (at 0.25% - 3% v/v) on the characteristics of CLs and on the adhesion abilities of *Pseudomonas aeruginosa* to the lenses' surfaces. CLs were formulated using a hydrophilic monomer; 2-hydroxyethyl methacrylate (HEMA) together with silicone-based polymer such as Poly dimethyl siloxane (PDMS) or 3,3,3-trifluoropropylsilane (FSA) then lenses were polymerized under UV light. The formulated CLs with surfactants were found to have an increased equilibrium water content (EWC) due to hydrophilic moiety present in surfactants. A relationship was deduced between EWC and surface contact angle of lenses containing surfactants; where an increased EWC was associated with a decrease in contact angle reflecting a more hydrophilic surfaces of CLs. Apart from the 3% Polysorbate 80 (p<0.0001) CLs, all other formulations had light transmission values over 80%. Lenses with surfactants were found to have lower bacterial ATP concentration than lenses without surfactants. Poloxamer 188 in FSA lenses reduced bacterial adhesion from $4.22x10^{-4}\pm1.30x10^{-4}$ pM to $1.03x10^{-4}\pm4.86x10^{-5}$ pM, a reduction by 75.59% when compared to the control lenses (p= 0.002). Moreover, 1% Tetronic® 90R4 in PDMS showed a reduction by 57.17% in ATP concentration. Polysorbate 80 in FSA exhibited the least bacterial adhesion with an average bacterial ATP concentration of $3.85x10^{-5}\pm2.61x10^{-5}$ pM; i.e 90.88% less bacterial ATP than control lenses (p= 0.001). Bioluminescence studies demonstrated a decrease in *Pseudomonas aeruginosa* adhesion to CLs containing surfactants without impairing the optical and mechanical characteristics of the lenses.

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Number of tables: 2
Number of figures: 6

List of abbreviations

µL microliter
ACLM Association of Contact Lens Manufacturers
AMD Age-related macular degeneration
AMP Adenosine monophosphate
ATP Adenosine triphosphate
BCLA British Contact Lens Association
Ca^{2+} Calcium ions
CLs Contact Lenses
CLMK Contact lens- related microbial keratitis
Dk Oxygen permeability
DSC Differential Scanning Calorimetry
E Young's modulus
EWC Equilibrium water content
FDA Food and Drugs Administration
FSA 3,3,3-trifluoropropylsilane

GMA Glycidyl methacrylate
HEMA 2-hydroxyethyl methacrylate
HMPP 2-hydroxy-2-methylproiophenone
MAA Methacrylic acid
Mg^{2+} Magnesium ions
min minute
MK Microbial keratitis
mL millilitre
MMA Methylmethacrylate
MPa megapascal
NVP N-vinyl pyrrolidone
OD_{600} Optical density
P80 Polysorbate 80
PEO Poly (ethylene oxide)
PF -127 Pluronic F-127
P188 Poloxamer 188
PBS Phosphate buffered saline
PDMS Poly(dimethoxysilane)
pHEMA Poly (2-hydroxyethyl methacrylate)
pM picomolar
PMMA Poly methylmethacrylate
POE Poly (oxyethylene)
PPO Poly (propylene oxide)
RGP Rigid gas permeable
RLU Relative Light Units
SCL Soft contact lens
SEM Scanning Electron Microscopy
TEGDMA Tetraethylene glycol dimethacrylate
TET Tetronic® 90R4
TGA Thermogravimetric Analysis
UV Ultraviolet

Keywords Bioluminescence, contact lenses, poloxamer, Polysorbate, surfactants, Tetronic

1. Introduction

Contact Lenses (CLs) are optical devices regulated through the Federal Food, Drug and Cosmetic Act by the US Food and Drug Administration (FDA) [1]. CLs replace eye glasses or spectacles to correct vision in myopia, hyperopia, and astigmatism cases. According to the Association of Contact Lens Manufacturers (ACLM), CLs market reached £226 million in 2014 [2] reflecting their increased popularity and high demand. The global market of contact lenses was USD 8.95 billion in 2015 and is estimated to maintain its high growth over the next decade [3].

Since their introduction, there has been a stream of research work in using CLs as delivery vehicles to overcome the challenges imposed by conventional ophthalmic formulations. Such as poor bioavailability, short residence time due to drug loss through the nasolacrimal drainage. As a

potential drug delivery system, drug- embedded CLs enable continuous drug release over a longer period of time [4].

Although CLs are advantageous over conventional ophthalmic preparations, there are some drawbacks associated with contact lenses' use. Olivia *et al* [3] pointed out that lens fitting, poor hygiene and poor maintenance are the main disadvantages of CLs . With the increased number of CLs wearers over the past 20 years, there is an increase in the cases and incidences of severe eye complications such as ophthalmic infections, inflammations, abrasions and blindness.

Microbial keratitis (MK) also known as contact lens- related microbial keratitis (CLMK) is a corneal infection that can be caused by ocular surface disease, trauma, surgery and contact lens wear [4–6]. MK is associated with impaired vision, pain, red watery eyes and irritation. It is mainly caused by bacterial pathogens such as *Pseudomonas aeruginosa, Staphylococcus aureus* and *Staphylococcus epidermidis* [7]. Teo *et al* investigated the different types of complications associated with wearing contact lenses in hospitals in Singapore between 1999 to 2001 [8]. It was reported that 73% of soft CLs wearers suffered infective keratitis (25.6%), epithelial keratitis (24%) and allergic conjunctivitis (18.8%) [8]. According to Zimmerman *et al*, CLs break the normal physiology of the ocular epithelial mitosis, differentiation and exfoliation which then damage the corneal epithelium making it more susceptible to infections [5]. Zimmerman *et al* concluded that bacteria such as *P. aeruginosa* adheres onto the ocular surface, mainly the corneal epithelium, through receptor-binding mechanisms. Dutta *et al* [7] summarized similar findings, wherein bacteria adheres onto the corneal surface in two steps. The first step is a temporary adhesion through Van der Waals forces, followed by irreversible adhesion [7]. Over time, bacteria such as *P. aeruginosa* forms biofilms that can further develop into bacteria on the ocular surface [7]. Moreover, Willcox *et al* [9] reported that *P. aeruginosa* lipopolysaccharides gives the bacteria its characteristic hydrophobic surface that enables hydrophobic interactions with silicone-based CLs [7]. Other pathogens such as *Staphylococcus epidermidis* was studied and is believed to have similar adherence mechanism to *P. aeruginosa* [7]. *S. epidermidis* expresses polysaccharide adhesion, which plays a main role in its adherence and biofilm formation on the ocular surfaces. [7]

The adherence of bacterial strains can be reduced if the environment is made less hydrophobic. Surfactants, also known as surface active agents, are agents that are mainly used to reduce surface tension between two liquids or between a liquid and a solid. In the context of CLs, a surfactant can be used as a lubricant to minimise the initial discomfort caused by the insertion of CLs. In addition, it allows even distribution of tear volume over the lens and to act as a buffer between the lens and

finger in order to reduce contamination and avoid infection [10]. Table 1 summarizes some of the commonly used surfactants in ophthalmic formulations.

So far, no literature studies have evaluated the bacterial adhesions to surfactant-laden contact lenses. The aim of the current study was to incorporate three different surfactants; Poloxamer 188, Polysorbate 80 and Tetronic® 90R4 into CLs matrices and evaluate the physical, mechanical and optical properties of the lenses. The study assessed the effect of surfactants on the adhesion of *P. aeruginosa* to the CL's surfaces.

2. Materials

Poly (dimethylsiloxane) with vinyl terminated (PDMS), and 2-hydroxyethyl methacrylate (HEMA), Tetraethylene glycol dimethacrylate (TEGDMA, > 90%) as cross-linker and 2-hydroxy-2-methylproiophenone (HMPP, 97%) as photoinitator, surfactants; Poloxamer 188 (concentration of 10%), Polysorbate 80 (TWEEN® 80) and Ethylenediamine tetrakis (ethoxylate-block-propoxylate) tetrol (Tetronic® 90R4) all were purchased from Sigma-Alrich chemicals (Poole, Dorset). An ATP Bioluminescence Assay Kit HS II was purchased from Sigma-Aldrich (Roche, Germany). A clinical isolate of *Pseudomonas aeruginosa* NCTC00950 was kindly provided by the microbiology department. 3,3,3-trifluoropropyltrimethoxysilane (FSA) was purchased from Tokyo Chemical Industry (TCI, UK). All chemicals and materials were used in the form they were received.

3. Methodology

3.1 Formulation of contact lenses

Seven different formulations of CLs were prepared. All formulations contained around 95% HEMA as a hydrophilic backbone monomer, co-polymerised with a silicone-based polymer (either PDMS or FSA) at 3%. The cross-linker (TEGDMA) and photo initiator (HMPP) were used at 1%. Poloxamer 188 or Polysorbate 80 were added at a concentration of either 1% or 3%, while Tetronic® 90R4 was used at 0.25% or 1%. All CL's components were stirred for 30 minutes using a magnetic stirrer and 700µL of the CL's mixtures was injected into polypropylene moulds. The samples were then placed under a UV light at a height of 120mm, and left to polymerise for 72 hours.

3.2 Characterisation

3.2.1 Equilibrium water content (EWC)

EWC measures the maximum amount of water that can be absorbed into the polymer matrix [16]. After polymerisation, the prepared CLs were hydrated to determine the EWC. The lenses were

removed from the moulds and the dry weight of the CLs was recorded. CLs were soaked in 20mL of distilled water for 24 hours at room temperature. After 24 hours, the soaked lenses were dabbed on a lint free tissue and the weight of the soaked lenses was recorded. EWC was then calculated using the formula below, as adapted from [16]:

$$Equilibrium\ water\ content\ (EWC, \%) = \frac{Weight\ (wet) - Weight\ (dry)}{Weight\ (dry)}\ x\ 100\% \quad \textit{Equation 1}$$

3.2.2 Light transmittance

The hydrated CLs were cut into appropriate sizes (22mm length, 9mm width) to fit inside a UV-cuvette (Germany). Light transmittance (%) was measured using a UV-spectrophotometer (Genesys 10S, ThermoScientific) at a visible wavelength of 600nm as previously reported by [17].

3.2.3 Young's modulus

TA.XT.plus Texture Analyser (Stable Microsystems Ltd, Surrey, UK) with A/MTG as tensile grip was used to determine the elasticity and the Young's modulus of the CLs. The hydrated lenses were cut in rectangular shapes with length, width and thickness of 25, 15 and 2 mm, respectively. CLs were placed between the clamps and stretched vertically at maximum distance of 20mm. Exponent software (Lite, Stable Micro Systems) calculated the stress (MPa) and strain (%) for each sample and equation (2) was used to calculate Young's modulus.

$$Young's\ modulus\ (E, MPa) = \frac{Stress\ (MPa)}{Strain(\%)} \quad \textit{Equation 2}$$

All measurements were carried out in triplicate and presented as mean value ± standard deviation.

3.2.4 Surface contact angle

Contact angle measurements were performed using KRUSS DSA30S, (KRÜSS GmbH, Borsteler Chaussee, Germany). The contact angle was measured using the static sessile drop method, after vertically dispensing droplets of deionized water of a specified volume (10µL) onto the CLs surface; using a high-tech optical camera, the angle that was created between the baseline of the drop (solid-liquid interface) and the tangent (liquid-air surface) was determined using Young-Laplace-Fit method [18,19].

3.3 Bioluminescence ATP Assay

Bacterial adherence to CLs was investigated through bioluminescence ATP method as reported by [20]. In this assay, *P. aeruginosa* NCTC00950 was studied primarily due to its most common cause of MK within CL wearers. This particular pathogenic microorganism has been discovered to adhere to the surface of CLs more easily compared to other pathogens.

3.3.1 Preparation of bacterial suspension

In a 100-ml flask containing nutrient broth, a single colony of *P. aeruginosa* (NCTC00950) was inoculated. Then the flask was incubated and continuously shaken at 37°C for 18 hours. After incubation, aliquots of 1ml were prepared in 2 Eppendorf tubes and centrifuged at room temperature (22°-25°C) at 4300g for 3 minutes. The supernatant was discarded and each pellet was re-suspended in 1mL of Ringers solution.

3.3.2 Calibration graph of bacterial count against optical density

To produce a calibration graph, the bacterial density, measured as optical density (OD_{600}) at different dilutions against bacterial count (Colony forming units per ml) (CFU/ml).

3.3.2.1 Measurement of optical density

One millilitre (1mL) of the bacterial suspension was transferred into a cuvette then the optical density was measured at 600nm. To achieve the bacterial concentration $2x10^8$ CFU/ml as reported by Kodjikian *et al* [20], different optical densities were measured using (ThermoSpectronic, UK) spectrophotometer [21]. To do this, 100µL of the bacterial suspension in the cuvette was replaced in each reading time with 100µL of Ringers solution until six different readings were obtained.

3.3.2.2. Bacterial count

In order to achieve bacterial concentration of $2x10^8$ CFU/ml as stated by Kodjikian *et al* (20), different optical densities of different bacterial dilutions were measured using a spectrophotometer (ThermoSpectronic, UK). For each individual OD_{600} reading, a serial dilution (neat to 10^{-6}) was prepared in ringer solution, 10µL aliquots of the resulting dilutions was inoculated onto a nutrient agar plate and incubated for 24 hours at 37°C. The number of colony forming units (CFU) were determined after incubation.

3.3.3 Calibration graph for standard ATP

Standard ATP was provided in the bioluminescence kit (Roche, Germany). The standard ATP was diluted with 990µL of dilution buffer (Roche, Germany). A serial dilution was carried out in the range of 10^{-6} to 10^{-14} M of ATP by adding 10µL of the resulting solution to a 90µL of the dilution buffer for each dilution. Then, 10µL of luciferase agent (Roche, Germany) was added and the luminescence was measured immediately using a luminometer (Infinite M200 Pro, Tecan) that is connected to (Magellan, Tecan) software to measure the relative light units (RLU).

3.3.4 Bioluminescence

The bacterial concentration was adjusted to $2x10^8$ CFU/mL with Ringers solution. One CL from each formulation was placed in a well plate (Thermo-Fisher Scientific, UK). Two millilitres (2mL) of bacterial suspension was transferred into each well. Plates were incubated and continuously shaken in the prepared bacterial suspension at 37°C for three different time points; 0.5 hour, 6 hour, 16 hour. After incubation, the lenses were rinsed with Ringers solution three times to remove unbound bacteria. This process was repeated three times and data is presented as mean ± SD.

For a highly sensitive and quantitative detection of ATP, a kit (ATP Bioluminescence Assay Kit HS II, Roche Diagnostics, Germany) was used. After incubation and washing with Ringers solution, lenses were soaked in 500µL of the cell lysis reagent (provided in the kit) for 1 minute to enable the release of the bacterial ATP. After 1 minute, the samples were centrifuged at 8000 rpm for 3 minutes. 160µL of the supernatant was mixed with 40µL of Luciferase reagent (Bioluminescence Assay Kit HS II, Roche, Germany). Then, the light emitted by the mixture was quantified using a luminometer (Infinite M200 Pro, Tecan) and analysed using (Magellan, Tecan) software and the measurements were recorded as RLU. The bacterial ATP concentration was determined using the log-log calibration graph of RLU and bacterial ATP concentration was expressed as picomolar (pM= 10^{-12} M) of ATP.

3.4 Statistical analysis

The results were statistically compared using statistical software GraphPad Prism 7. An unpaired t-test was used to determine the *p* value between two samples. One-way analysis of variance (ANOVA) to compare three samples for every test variable and a *p* value <0.05 was considered statistically significant.

4. Results and Discussion

4.1 Equilibrium water content (EWC) and surface contact angle (°)

One ideal property identified by Caló *et al* [16] is high water content also known as EWC. According to ElShaer *et al* , for a typical hydrogel comprising of poly 2-hydroxyethyl methacrylate (pHEMA) only, the water uptake should be more than 38% which gives flexibility and permeability to oxygen [22]. EWC is an important property of CLs as it describes the comfort for wearers. Generally, the water content of the CLs increased when formulated with surfactants (Table 2). In the present study all formulations with surfactants yielded EWC higher than 30% (Table 2). Each surfactant was used at two different concentrations where Poloxamer 188 at 3% has increased EWC in PDMS lenses to

34.33±1.81% (p>0.05) from 31.42 ± 0.72 of the control lenses. An increase from 29.79 ± 1.04% to 33.42±0.52 % was also observed in FSA lenses (p< 0.05).

Similarly, P80 at 3% resulted in a higher EWC compared to the lower concentration in both PDMS and FSA (P80 at 3% yielded the highest EWC out of all the formulations for both PDMS and FSA). TET 1% increased EWC from 30.38± 0.54% (0.25% TET) to 32.89±0.30% in FSA (p= 0.005). An increase of 2.51% was also observed with PDMS but was found to be statistically insignificant (p= 0.64).

EWC increases as the concentration of the surfactant increases because water content is determined by the length and concentration of the hydrophilic moiety present in the formulation [23]. This is could be attributed to the additional –OH groups available for hydrogen bonding in the surfactant structure such that of PEO groups present in Poloxamer 188 and Tetronic® 90R4 and the oxylated sorbitol in Polysorbate 80 (Table 2) [23]. However, the increase in EWC did not exceed 10% in all formulations. Kapoor *et al* investigated the use of Brij 700 at 8% w/v in hydrogels and reported an increase in EWC by 21.8% over pure pHEMA hydrogels. This suggests that the results of the present study were possibly influenced by the low concentration of surfactants in the matrix which did not exceed 3% v/v [23].

Amongst all surfactants, TET gave the least water content which is possibly due to the conformation of PEO and PPO groups in the surfactant [24,25]. Chen *et al* explained that Tetronic® 90R4 is the reverse form of the sequential Tetronic® where the hydrophobic PPO group surrounds the hydrophilic PEO, restricting the interaction of PEO with water molecules [25].

Another property that is also linked to the water content of CLs is the surface wettability, which governs patient comfort. Wettability, measured as surface contact angle, is the balance between cohesive and adhesive forces on the surface of the material in which cohesive forces arise between similar states of molecules and adhesive forces arise from different states [26]. Hydrophobicity of the surface of contact lenses is one of the key factors described by Dutta *et al* [7] that is believed to govern bacterial adherence [7]. One of the objectives of this study was to make the surface of CLs more hydrophilic by achieving a lower value of surface contact angle. As described by Campbell *et al* a contact angle value higher than 90° defines a hydrophobic surface [21].

Figure 2 shows how surface contact angle was assessed using the sessile drop method. As soon as the liquid is dropped on the surface, the baseline (in blue) and shape line (in green) were set before calculation of contact angle was carried out.

In the present study, the contact angle values for lenses with surfactants are lower than those listed in Table 2. As shown, there is a relationship between EWC and surface contact angle, where an increase in EWC is associated with a lower contact angle, defining a greater wettability and a more hydrophilic surface.

Increasing concentration of P188 from 1% to 3% v/v decreased the contact angle in PDMS CLs from 40.93±1.65° to 31.40°±0.70° respectively (p<0.0001) and from 45.33±2.59° to 27.90±1.30° in FSA CLs (p<0.0001). Similarly, a lower contact angle was observed with higher concentration of P80. This suggests more hydrophilic surfaces of CLs upon incorporation of surfactants. Analogous to the results reported in the present study is the findings of Troster *et al* [27] where contact angles of surfactants solutions on PMMA-based solid materials were found to be of lower values due to the strong interactions of surfactants such as P188 and P80 with the polymer material. Conferring to the report of Jiao [11], the decrease in surface contact angle of lenses when used with P188 and P80 can also be explained by the differences in their hydrophilic-lipophilic balance (HLB) that characterises the hydrophilicity of the surfactants. Wu *et al* [24] reported that the positions of PPO and PEO in the non-ionic surfactants could impact their HLB value. For instance the sequential pluronic P188 (HLB= 29) is more hydrophilic than the reverse-sequential forms because of the position of PEO groups in the surfactant.

TET at 0.25% yielded the highest contact angle in all test lenses, both in PDMS (control vs TET 0.25%, p= 0.0006) and FSA (control vs TET 0.25%, p= 0.98). Ketelson *et al* reported relatively lower values of contact angles for Tetronic based pHEMA-MAA CLs [28]. In Ketelson *et al* studies Tetronic® 904, which is the sequential form of Tetronic® 90R4, showed a contact angle of about 20°. It is believed that the differences in position isomerism and surface tension caused the dissimilarities of contact angle values [28]. The surface tension of the sequential TET is relatively lower (39.04 mN/m) than the reverse sequential form (42.86 mN/m), which shows that sequential TET has better ability to reduce surface tension compared to the reverse form. Moreover, the position isomerism of the reverse Tetronic® 90R4 potentially shows steric hindrance effect due to the outward position of hydrophobic PPO as suggested by Wu *et al* [24].

4.2 Light transmittance

Similar to any drug delivery system, there are requirements for CLs to become an ideal platform, as summarised by Calo *et al* [16]. Lens transparency is one of the most important properties of a CL where a desirable transparency value is expected to be above 95%[16]. Figure 3a shows various CLs

prepared immediately after polymerisation. When hydrated over 24 hours, the hard lenses swell and expand because of the absorption of water into the polymer matrix.

The percentage light transparencies against the various CLs formulations are summarized in Figure (2b). With the exception of 3% Polysorbate, the results are in line with the findings reported by Fuentes et al [29] where transmission of silicone-based contact lenses ranged between 80 to 99%. P80 at 1% had transmission above 80% for both PDMS and FSA (p= 0.23). Nonetheless, increasing the concentration of P80 decreased the transparency of the PDMS lenses to 31.27% and to 58.04% for FSA lenses (Figure 2b) and the lenses looked turbid. This turbidity could be attributed to the immiscibility of P80 with the other components in the lenses' matrix. Tejwani et al reported that Polysorbate 80 at concentrations higher than 2% (w/w) is immiscible with hydrophilic solvents such as polyethylene glycol (PEG) 200, 300 and 400 [30].

Looking at poloxamer 188 and TET formulations, light transmission was not impaired when compared to the control CLs (Figure 2b). It can be concluded that the use of surfactants does not compromise the transparency of lenses. Similar results were reported by Kapoor *et al* where pure pHEMA hydrogels showed light transmittance of 98.9% and above 99% for surfactant-laden hydrogels consisting of non-ionic Brij surfactant when used at 2-8% w/v [23]. Nonetheless, when high concentrations of poloxamer 188 and TET (beyond 3% and 1% v/v respectively) were used the lenses lost their transparency.

4.3 Young's modulus

CLs should possess good mechanical characteristics in order to provide better comfort for the patient and enable good adhesion to the corneal epithelium [16,23]. Commonly, Young's modulus (E) is the parameter used in evaluating the elastic deformation of contact lenses under tension [16,26]. A stiff contact lens has a high Young's modulus while a flexible contact lens has a low Young's modulus.

The value of Young's modulus is commonly calculated as the ratio of stress (in megapascal, MPa) to strain (in percentage, %) [26]. A low value of Young's modulus may increase comfort for wearers, nonetheless it can cause corneal astigmatism [31]. The reported literature values of the commercially available lenses is ranging between 0.36 ± 0.050 MPa for Etafilcon A lenses and can exceeds 1.74 MPa for Lotrafilcon A lenses [32]. Furthermore, a high water content is not desirable as this will yield CLs with low mechanical property leading to deformity and breaking as reported by

[31]. In order to achieve a more desirable balance between water content and Young's modulus, hydrophobic polymers such as PMMA or silicone-based polymer are incorporated into CLs.

In the present study, increasing the surfactant concentration reduced Young's modulus measurement for both PDMS and FSA containing lenses (Figure 2b). Interestingly, the addition of 1% of P188 has increased the Young's modulus of lenses in comparison to the control lenses but these are not statistically significant (PDMS vs P188 1%, p= 0.40, FSA vs P188 1%, p=0.52).

It was found that Young's modulus was decreased by 2.6% to 0.0037±0.00028 MPa for PDMS lenses (p= 0.82) with increasing concentration of P188 surfactants. Similar result was observed in FSA lenses where Young's modulus declined by 23% when P188 concentration increased to 3% v/v (p= 0.74).

Analogously, there was a decrease in the Young's modulus of lenses when P80 concentration was increased. However, the values were not statistically significant in both PDMS (1% vs 3%, p=0.54) and FSA (1% vs 3%, p= 0.74). TET at its lowest concentration gave the highest Young's modulus in both PDMS and FSA (Figure 3b). Subsequent increase in TET concentration decreased Young's modulus in PDMS (1% vs 3%, p=0.20) and FSA (1% vs 3%, p=0.0.007) lenses.

Generally, addition of higher concentration of surfactants yielded lower Young's modulus values. These results are mainly governed by the polymeric structure formed with surfactants to which the hydrophilic moieties of surfactants influence the mechanical property of lenses. CLs with lower Young's modulus devoid any mechanically induced complications such as superior epithelial arcuate lesion (SEAL) and contact lens induced papillary conjunctivitis (CLIPC) mucin balls, and conjunctival flaps [32]. Besides, CLs with high modulus will have a tight fit and fluting edge [32]. Nevertheless, wearers might find surfactant laden CLs difficult to handle, also these lenses will have excessive movement in the eye [32].

4.4 Bacterial adhesion assay

4.4.3 Bacterial adhesion assay

Bioluminescence is an established method for quantifying microbial contamination to surfaces, based on the assumption that bacterial cells have adenosine triphosphate (ATP) [20,33]. This method requires an optical biosensor to enable optical measurement using a luminometer where ATP is considered extremely effective [33]. Stollenwerk *et al* confirmed that bioluminescence ATP assay is a very sensitive method that avoids false low results [34]. The concept of bioluminescence

involves a chemical reaction between bacterial ATP and luciferase enzyme to form adenosine monophosphate (AMP) with pyrophosphate (PP_i) and emits light at 562 nm, shown in the chemical equation below [35]:

$$ATP + D\text{-}Luciferin + O_2 \xrightarrow[Mg^{2+}]{Luciferase} Oxyluciferin + PP_i + AMP + CO_2 + Light$$

A correlation between the bacterial count (CFU/ml) and optical density (OD_{600}) was established with a coefficient of determination (R^2= 0.9885), and linear equation of y=15.63x+6.22 [21]. In the present study, the corresponding optical density was 0.123 OD_{600}. This was used as reference when preparing bacterial suspensions for all time points.

A linear log-log calibration curve was recorded for bioluminescence intensity (RLU) against different ATP molar concentrations (M) where coefficient of determination (R^2) of 0.9872 was obtained as shown in Figure 3.

4.4.3.1 Effect of Poloxamer 188 on bacterial adhesion to CLs

In comparison to the controls, lenses with Poloxamer 188 had less number of bacteria bound to their surfaces at all time points (0.5h, 6h and 16h) as shown in Figure 4 (a&b). In PDMS lenses, 3% P188 bound the least bacteria with an average ATP concentration of $1.79x10^{-4}$±$3.99x10^{-4}$ pM after 16-hour incubation. However, the two concentrations of P188 used had no significant difference at the three time points (1% vs 3%, 0.5h: *p*= 0.74, 6h: *p*= 0.53, 16h: *p*= 0.24). Both concentrations of Poloxamer 188 at 1% and 3% gradually bound more bacteria from 0.5-hour to 16-hour incubation. Nonetheless, these concentrations were lower than that of the control (1%: *p*= 0.40, 3%: *p*= 0.13).

Looking at Figure 4b, FSA lenses with 3% Poloxamer 188 had the least number of bacteria on their surfaces after 16 hours. The number of *Pseudomonas aeruginosa* decreased by 75.59% compared to the control (FSA control vs P188 3%, *p*= 0.002). Nonetheless there was no significant difference between the two concentrations of P188 at 16 hours (1% vs 3%, *p*= 0.49). Similar results were reported earlier by [36]. The study investigated the adhesion of *Staphylococcus epidermidis* on silicone sheets soaked in varying concentrations of Poloxamer 188 solution. At 20% P188, the bacterial adhesion decreased to 3.02% from 22.2% in the control sheets [36]. The reduction in bacterial adhesion in the presence of P188 can be attributed to the ability of PPO groups to be

adsorbed onto the surface of the silicone-based contact lens allowing the two PEO groups to project outwards into the polymer matrix [25]. The hydrophilic PEO provides a 'steric hindrance', thus preventing the hydrophobic surface of *P. aeruginosa* from attaching to the surface of the lens [7,36].

4.4.3.2 Effect of Polysorbate 80 on bacterial adhesion to CLs

Interestingly, the pattern of bacterial adhesion on lenses with Polysorbate 80 in PDMS is different to FSA lenses. Figure 5a shows the bacterial adhesion to PDMS lenses with P80 where there is an increase in bacterial ATP concentration over time for all lenses. However, lenses with P80 had less bacteria attached to their surfaces compared to the control lenses (Figure 5a). At all-time points, least bacteria were bound to 3% P80 compared to the control and at 1% P80 (0.5h: p=0.03, 6h: p= 0.004, 16h: p= 0.41). Between the two concentrations of P80, there is no significant difference on bacterial adhesion at all time points (0.5h: p=0.07, 6h: p= 0.67, 16h: p= 0.83).

On the other hand, Figure 5b shows bacterial adhesion on FSA formulations. Bacterial adhesion on FSA alone increased over time but this was not the case in the presence of P80. At 0.5h, all formulations had nearly the same bacterial ATP concentration bound. After 6 hours, lenses with P80 had less bacterial binding in both concentrations but not statistically significant (p=0.10). At 16 hours, there is a statistically significant decrease of bacterial ATP concentration with P80 at $3.85 \times 10^{-5} \pm 2.61 \times 10^{-5}$ pM in comparison to control with an average bacterial ATP concentration of $4.22 \times 10^{-4} \pm 1.30 \times 10^{-4}$ pM, resulting in a decrease of 90.88% (p= 0.001).

P80 is a non-ionic surfactant that is commonly used as an emulsifier in cosmetics and food industry [37]. Toutain-Kidd *et al* [38] reported that P80 reduces the adhesion abilities of *P. aeruginosa* (PA14) by 45% when tested on the surfaces of medical devices including CLs [38]. Moreover, it was previously established that P80 has the ability to enhance the permeation of bacterial cell hence improve the activity of antibiotics [38].

In the present study, it is believed that the reduction in bacterial adhesion is mainly due to the disruption of biofilm formation of the bacteria over time [38]. The bacteria itself does not overexpress the enzyme Lipase A (Lip A) on its surface and prevents permanent bacterial growth as demonstrated in Figure 5b [38].

4.4.3.3 Effect of Tetronic® 90R4 on bacterial adhesion to CLs

Generally, the bacterial adhesion to both PDMS and FSA lenses increases with time. However, it is evident from Figures 6a and 8b that bacterial adhesion in presence of Tetronic® 90R4 is less than that of the control lenses.

In PDMS lenses at 0.5 hours (Figure 6a), control lenses had more bacterial binding compared to lenses with TET at 0.25% and 1% but these were statistically insignificant (control vs TET 0.25%: p= 0.11, control vs TET 1%: p= 0.10). After 6 hours, more bacteria were bound to the control lenses compared to the test lenses (control vs TET 0.25% vs TET 1%, p= 0.001) and the high concentration of TET (1%) managed to keep the number of bound bacteria down at 16 hours (control vs TET 1%, p= 0.001).

In FSA lenses at 0.5 hours (Figure 6b), all lenses had relatively similar bacterial ATP concentration on their surfaces. After incubation of 6 hours, the number of bacteria on control lenses and lenses with TET (0.25%) gradually increased whilst the number of bound bacteria on TET (1%) declined. Similar to PDMS formulations, at 16-hour incubation, all FSA lenses increased bacterial adhesion where control lenses yielded the highest bacterial ATP concentration at $4.22 \times 10^{-4} \pm 1.30 \times 10^{-4}$ pM.

Although Tetronic® 90R4 is the reverse sequential form of Tetronic, there is a great reduction in bacterial adhesion of *P.aeruginosa* to Tetronic® containing CLs. This is possibly because of the conformational changes that happen when the reverse sequential Tetronic is exposed to a higher temperature as explained by [25]. During the bacterial adhesion studies, all lenses were incubated at 37°C. At room temperature (22-25°C), the PPO chains in the structure are positioned on the surface of the lens, making it more hydrophobic [25]. On the other hand, increasing the temperature to 37°C enables the PPO groups to undergo transition and to change their aggregation behaviour, allowing PEO groups to be pulled outwards onto the surface of the CLs. This subsequently prevents bacteria from attaching onto the surface of the contact lens [25].

5. Conclusion

Poloxamer 188, Polysorbate 80 and Tetronic® 90R4 were successfully incorporated in contact lenses at concentrations ranging between 0.25- 3%. Hydration tests showed that the EWC of CLs with surfactants increased above 30%. Whilst Young's modulus decreased by increasing the surfactant concentration. This can be attributed to the increased EWC of hydrogels, making the material flexible which is favourable for CLs. All CLs except for 3% Polysorbate 80 lenses showed light transmittance above 80% demonstrating low opacity and high lens transparency, which is desirable for contact lens wearers. Bacterial adhesion assay proved that lenses with surfactants had lower *P. aeruginosa* ATP concentration than lenses without surfactants.

In Summary, incorporating surfactants into CLs matrices can play a key role in improving the characteristics of CLs whilst minimizing the attachment of bacteria onto their surfaces hence lower risks of MK.

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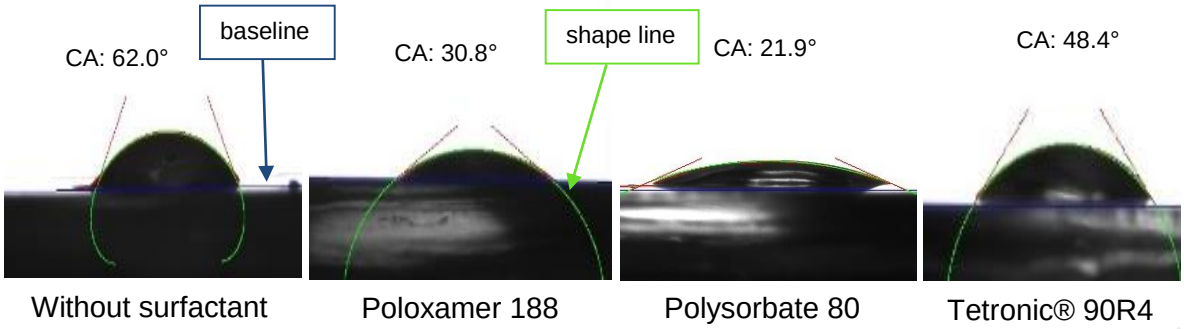
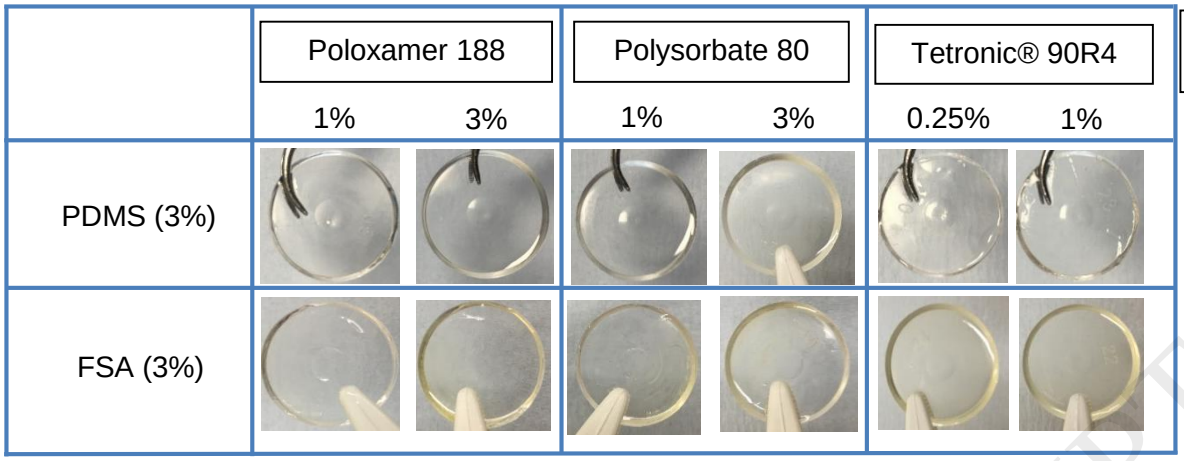
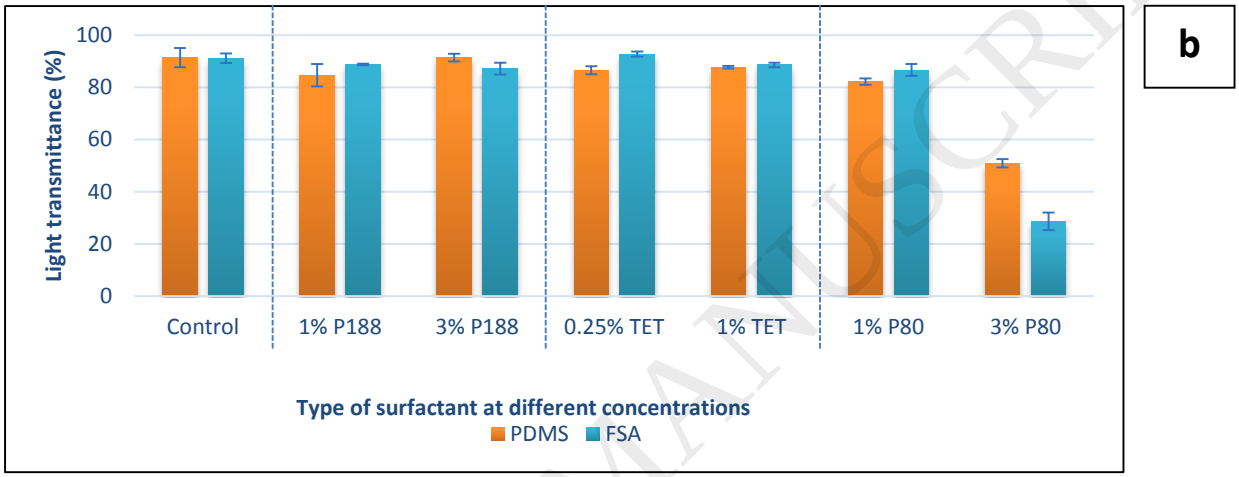


Figure 1. Images of contact angle measurement of contact lenses with and without surfactant using the sessile drop method





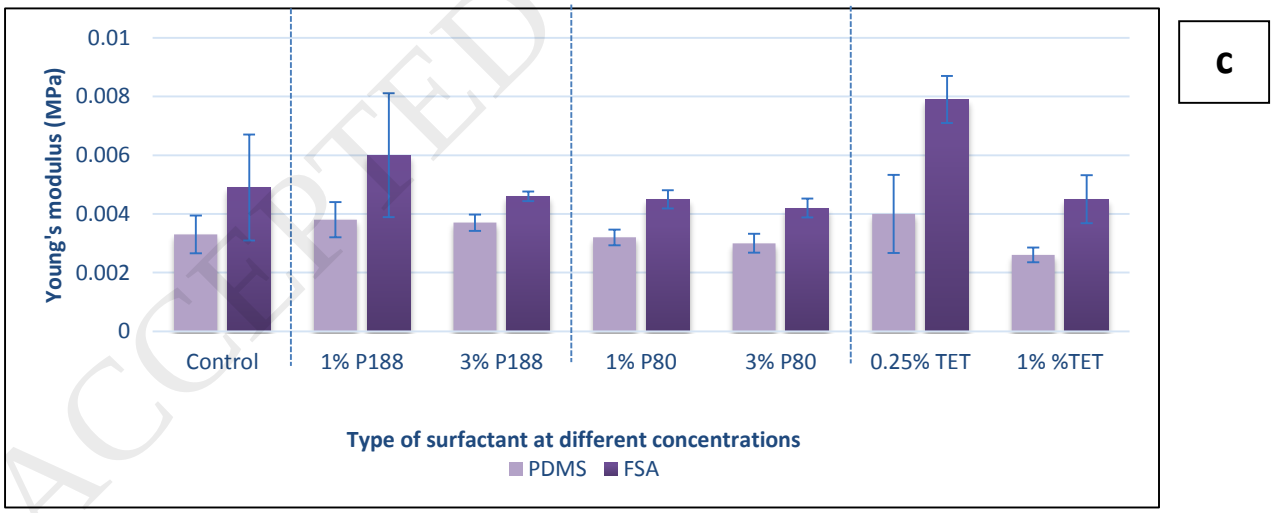


Figure 2. Images of the contact lenses formulated with different type of surfactants at different concentrations showing percentage Light transmittance against type and concentration of surfactants used (a), light transmittance (%)against type and concentration of surfactants used (b) and Young's modulus (MPa) against types and concentration of surfactants used (c). (The data is presented as Mean ± SD, where n=3)

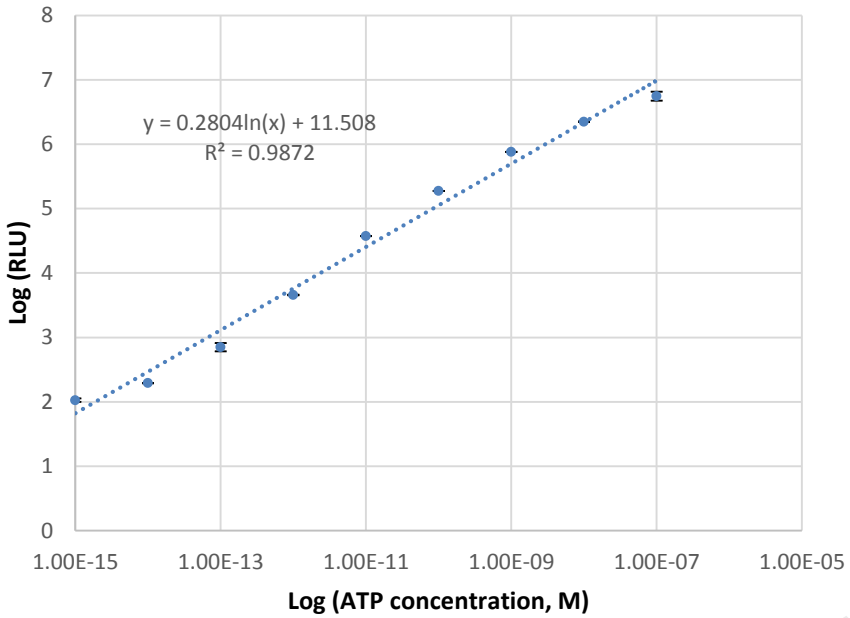
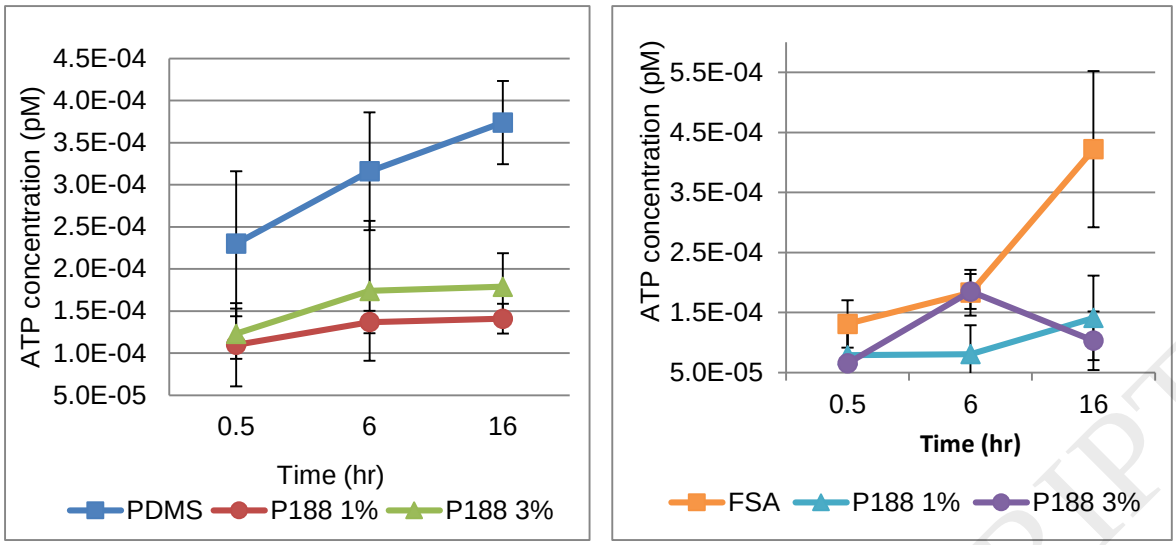


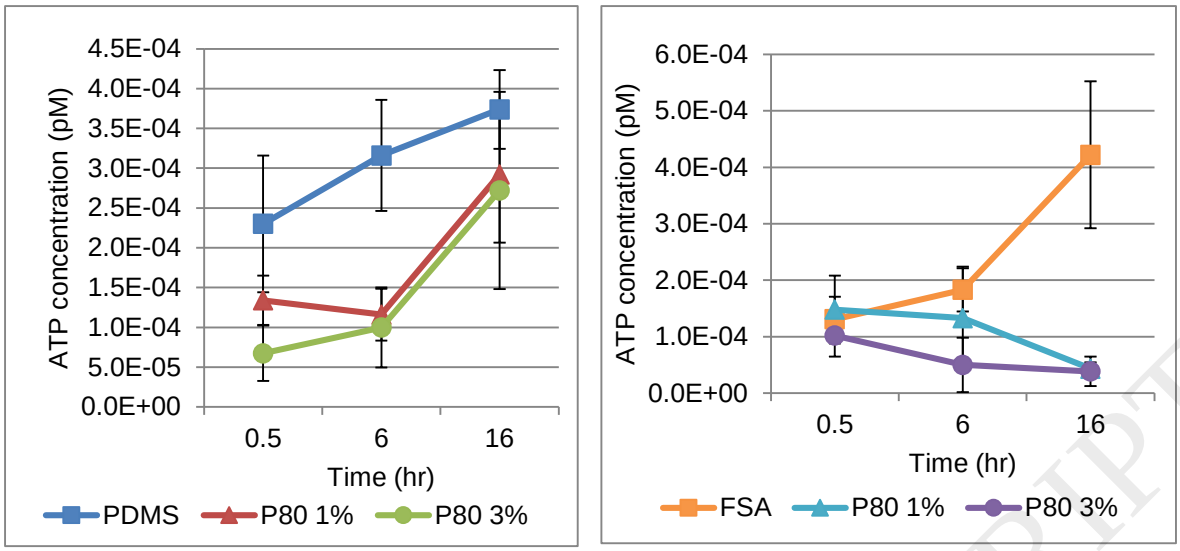
Figure 3. Bioluminescence calibration curve showing a log-log relationship between bioluminescence intensity (RLU) and ATP concentration (M) (The data is presented as Mean ± SD, where n=3)



(a) PDMS + Poloxamer 188

(b) FSA + Poloxamer 188

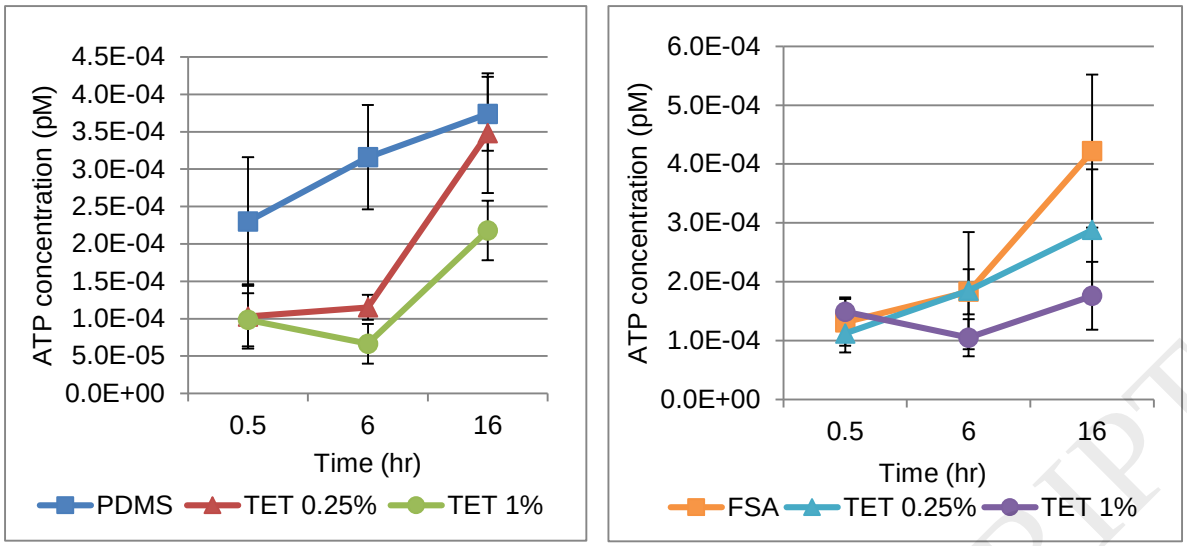
Figure 4:- ATP concentrations (pM) of Pseudomonas aeruginosa on the surface of Poloxamer 188 CLs measured by bioluminescence at three different timepoints (0.5h, 6h, 16h) for both PDMS (a) and FSA (b) formulations (n=3) (The data is presented as Mean ± SD, where n=3).



(a) PDMS + Polysorbate 80

(b) FSA + Polysorbate 80

Figure 5. ATP concentrations (pM) of Pseudomonas aeruginosa on the surface of Polysorbate 80 CLs measured by bioluminescence at three different time points (0.5h, 6h, 16h) for both PDMS (a) and FSA (b) formulations (The data is presented as Mean ± SD, where n=3)



(a) PDMS + Tetronic® 90R4

(b) FSA + Tetronic® 90R4

Figure 6:- ATP concentrations (pM) of Pseudomonas aeruginosa on the surface of Tetronic® 90R4 CLs measured by bioluminescence at three different timepoints (0.5h, 6h, 16h) for both PDMS (a) and FSA (b) formulations. (The data is presented as Mean ± SD, where n=3)

Table 1: Examples of non-ionic surfactants used in ophthalmic formulations.

Surfactant	Marketed product	Maximum concentration used (%)	References
Brij 35	Not provided	0.011	[11]
Cremophor EL	Not provided	0.02	[11]
Pluronic 17R4	ClearCare®		[12]
Poloxamer 185	Not provided	0.5-9	[13]
Poloxamer 188	Neosporin	0.1	[11,13]
Poloxamer 237	Complete Moisture Plus, Complete® MPS Easy Rub™	1.0	[12,14,15]
Poloxamer 407	Solo-Care Aqua, Renu MoistureLocm Focus Aqua	1.0	[12,14,15]
Poloxamine	ReNu	0.5	[14]
Polysorbate 80	Refresh endure, Restasis, Blephamide, Viva ultra tears	0.0013	[11]
Tetronic 1304	Opti- Free Express		[15]
Tyloxapol	Alrex, Azopt, Lotemax, Nevanac	0.0081	[11]

Table 2. Effect of surfactant type and concentration on the equilibrium water content and contact angle of CLs. (The data is presented as Mean ± SD, where n=3)

Formulations		Surfactant (%v/v)	Contact angle (°)	EWC (%)
Control		**0**	63.83 ± 1.47	31.42 ± 0.72
PDMS	**P188**	**1**	40.93 ± 1.65	32.45 ± 0.09
		3	31.40 ± 0.70	34.33 ± 1.81
	P80	**1**	27.57 ± 1.07	31.04 ± 3.17
		3	17.53 ± 0.85	35.18 ± 0.81
	TET 90R4	**0.25**	48.23 ± 0.74	31.05 ± 1.03
		1	36.73 ± 1.70	31.41 ± 0.65
Control		**0**	59.63 ± 2.11	29.79 ± 1.04
FSA	**P188**	**1**	45.33 ± 2.59	30.69 ± 1.25
		3	27.90 ± 1.30	33.42 ± 0.52
	P80	**1**	41.83 ± 0.21	34.46 ± 1.09
		3	39.80 ± 0.85	37.01 ± 1.37
	TET 90R4	**0.25**	59.60 ± 1.90	30.38 ± 0.54
		1	47.70 ± 1.21	32.89 ± 0.30