Study of biocidal efficacy of contact lens disinfecting solutions against common ocular pathogens

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Abstract: Contact lens wearers are at great risk of developing microbial keratitis and other ocular infections. These infections are often associated with inadequate lens hygiene (incorrect usage and unhygienic maintenance). Therefore, contact lens care products should be able to sufficiently minimize the amount of pathogens that are responsible for these infections.

The present study was aimed at evaluating and comparing the disinfecting properties of three contact lens disinfecting solutions against potential bacterial and fungal pathogens. Three most widely used lens care solutions were tested according to the Stand Alone Test of ISO14729 [2001] against the standard strains of *Staphylococcus aureus* ATCC6538, *Pseudomonas aeruginosa* ATCC9027 and *Candida albicans* ATCC10231. In addition, effectiveness of multipurpose solution (MPSs) on conventional and silicon hydrogel lenses was investigated by biocide uptake studies.

The three contact lenses disinfecting solutions passed the minimum disinfection criteria recommended by the ISO14729 Stand-Alone Test. Biocide uptake study showed considerable uptake of OptiFree Express solution by silicone hydrogel lenses resulting in reduction of biocidal efficacy of the solution.

The present study also showed that the lens matrix significantly affect the disinfection efficacy of contact lens solutions by adsorbing the active biocide. This adsorbed biocide cause redness, corneal abrasions and inflammatory events in the eyes. Therefore, opticians should recommend a lens care solutions based on the composition and material of the lenses they prescribe to the lens wearer.

Key terms -Contact lens care solutions, microbial keratitis, ISO14729, MPSs.

I. Introduction

Worldwide, millions of people use contact lenses as an alternative to spectacles. It has been shown that contact lens wear, especially extended wear, is a major risk factor for microbial keratitis and corneal ulcers. Although the incidence rates of contact lens-related microbial keratitis is very low, this complication is an important health concern because a very large population is at risk of poor visual outcomes and blindness.

Investigations have documented that contact lens related microbial keratitis is most commonly caused by bacteria, such as *Pseudomonas aeruginosa* and *Staphylococcus aureus*, which are responsible for 90% of the cases. About 10% cases are associated with the amoeba *Acanthamoeba castellanii* and fungi like *Candida albicans* and *Fusarium solani*. [Emma B 2009]. Presence of microorganisms on contact lenses (CL) is associated with infiltrative keratitis (IK), an inflammatory condition, and microbial keratitis (MK) an eye infection that might ultimately lead to vision loss [Lucas A.D. 2009]. The factors involved in the occurrence of IK are not entirely understood, however it is hypothesized that continuous wear, presence of deposits, bacteria and toxins adhered to the lens are important etiological factors.

Contact Lenses:

Contact lenses are a visual aid placed on the cornea of eye to improve vision, beauty and for therapeutic purposes. There are two general types of contact lenses: hard lenses and soft lenses. Soft contact lenses are the best choice among all other options because they are more comfortable, they allow increased oxygen permeability and wettability [David I. 2001]. Commercially, soft contact lenses are made up of either

i. **conventional hydroge**l made up of hydrophilic polymer that give smooth and wettable surface, or
ii. A newer type of **silicone hydrogel** which are highly oxygen permeable and introduced for extended wear.

Contact lens care solutions

In order to minimize the risks associated with lens wear, use of disinfecting solutions is recommended. Products for contact lens disinfection by chemical means are intended to reduce microbial contaminants that introduced during lens wear/removal or storage. There are variety of commercially available contact lens disinfecting solutions present in the market [M Dutot 2009]. These solutions belong to two categories based on their principle biocide (table 01),

1) Hydrogen peroxide based solutions
2) Multipurpose solutions.

Contact lens wearers and eye care practitioners rely too much on lens care solutions to reduce microbial contamination. CL care products should ideally have a certain level of 'excess efficacy', or safety margin. Unfortunately, different CL care solutions do not provide minimum safety for the wearer and thus, had to be taken off from the market [Claudia H 2012.].

Table 01: Composition of commercially available contact lens care solutions [Manal M 2016]

Solutions	Characteristics
Hydrogen Peroxide Based System	• 3% H_2O_2 as principle disinfectant • Preservative-free • Recommended for contact lens wearers who are allergic to preservatives used in multipurpose solutions.
Multipurpose Solution	• All-in-one care system used to clean, rinse, disinfect, and store soft contact lenses. • "no-rub" solutions - eliminating the need to mechanically rub the lenses to remove lens deposits. • Utilizes PHMB or quaternary ammonium compounds as principle disinfectant.

Studies in the past indicated that several multipurpose solutions and hydrogen peroxide systems do not adequately disinfect the high concentrations of microbial load.

Regulatory approval of lens care solutions requires demonstration of antimicrobial efficacy by fulfilling the criteria set by International Organization for Standardization (ISO) 14729 Stand-alone test. The ISO Stand-alone test determines the inherent microbicidal efficacy of contact lens care solutions by challenging them with standard microorganisms associated with ocular infections and monitoring the reduction in viable cells. In accordance with this Test criteria, a disinfecting solution must be able to reduce the starting concentration of bacteria by three logarithmic units (99.9%) and fungi by one logarithmic unit (90%) at the manufacturer's minimum recommended disinfection (MMRD) time [ISO14729].

The goal of this laboratory-based study is to evaluate and compare the microbicidal activity of the three contact lens disinfecting solutions available in India, when inoculated with standard ATCC strains of *P. aeruginosa, S. aureus* and *C. albicans* based on the ISO 14729 Stand-alone procedure for disinfecting products.

Being true that the incidence of IK and MK could be reduced by using MPS it is also true that some formulations are not compatible with some lens materials and as a result affect the corneal integrity, causing discomfort, inflammatory events and eventually discontinuation of lens wear. So, clinicians should pay special attention to compatibility between lens materials and MPS when prescribing a disinfecting solution [Livia Santosa 2011]. CL materials or lens cases are capable of taking up the biocide present at the MPS thus leading to the overall reduction of its disinfection efficacy [Lucas A.D. 2009]. The most recent ISO and FDA standards ignore these facts, which may very well contribute to the past infection outbreaks and product recalls. Currently, new standards are being formulated, so improvements are expected in the way disinfection is assessed.

In the present work it was hypothesized that different MPS formulations exhibit different scores of disinfection on contaminated CL. Therefore three lens care solutions were tested against 2 CL materials contaminated with one Gram-positive, one Gram-negative and one fungal strain. The aim is to assess the influence of the MPS formulation and the influence that CL physico-chemical properties might exert upon disinfection.

II. MATERIALS AND METHODS:

Contact lens care solutions:

The CL care solutions listed in Table 02 were used according to the respective manufacturer's instructions. The CL care solutions were assessed before their stated expiration dates.

Contact lenses:

Table 03 details the contact lenses used in the study. Two types of lenses were purchased from commercial source and removed from their packaging under aseptic conditions and stored under sterile conditions.

Microorganisms

The test organisms *Pseudomonas aeruginosa* (ATCC 9027), *Staphylococcus aureus* (ATCC 6538), *Candida albicans* (ATCC 10231) were obtained from Bhavans research center. The test organisms are grown according to EN ISO 14729 (2001) protocol.

Chemical and Media:

The growth media used are; Soyabean casein agar medium for bacteria and Sabourauds medium for fungi from Bhavans College, Andheri.

Dulbecco's Phosphate Buffered Saline without calcium chloride &magnesium chloride (DPBS), Dey Engley neutralizing broth were used as dilution and neutralizing medium. All the media and chemicals were obtained from department of microbiology, Bhavans College.

Type	Contact lens material	Brand	Water content
Conventional hydrogel lenses	Hilafilcon B	Bausch & Lomb U4	59%
Silicone hydrogel lenses	Lotrafilcon B	Alcon	33%

Table 02: Characteristic properties of the contact lenses used in the present study.

Trade name	Brand	Composition	MMRDT*	Neutralizer
BioTrue	Bausch & Lomb	Polyaminopropyl biguanide + polyquaternium, poloxamine, sodium borate, NaCl	4 hrs	Dey-Engley Neutralizing Broth
Optifree express	Alcon	Polyquad (0.001%) + MAPD (0.0005%), sodium citrate, NaCl, sodium borate, PEG	6 hrs.	Dey-Engley Neutralizing Broth
AO Sept	Ciba Vision	3% H_2O_2, NaCl, phosphonic acid	6 hrs	Platinum neutralizing disc. .

Table 03: Characteristic properties of the contact lens disinfecting solutions used in the present study.

III. RESEARCH METHODOLOGY

Stand Alone Test:

The test solutions were first subjected to sterility test as per the protocol of Indian Pharmacopoea. The sterility test was performed to rule out the possibility of inherent microbial contamination and false results. Multipurpose solutions -BioTrue, OptiFree express and hydrogen peroxide based-AO Sept solution were inoculated in standard media and examined for seven days for visible turbidity. Appropriate controls were kept to avoid the false positive results and were examined under the same incubation conditions as test.

The Stand Alone Test was performed in accordance with EN ISO 14729 (2001). 0.1 ml of broth culture (for bacteria 10^9 CFU/ml, for fungi 10^8 CFU/ml) was transferred into 10 ml CL care solution and incubated at 25°C for manufacturer's recommended minimum disinfection time (MMRDT). After disinfection, the active biocide was neutralized for 30 minutes at room temperature by transferring 1 ml of CL care solution to 9 ml of inactivation solution i.e. Dey-Engley Neutralization Solution. Afterwards serial dilutions were subjected to viable count on appropriate media according to EN ISO 14729 by standard pour plate technique, and plates were incubated at respective incubation condition.

Effect of lens material on disinfection efficacy of lens care solutions:

The unworn contact lenses were first subjected to agar sandwich method to check their sterility. Then the contact lenses were stored in 3ml of multipurpose solutions for 12 hrs in polypropylene screw-capped lens cases. After, 12hrs, the solution was pooled, 3ml aliquot was used for HPLC analysis to determine the concentration of active biocide. Remaining solution was subjected to Stand-alone test for biocidal efficacy testing with no lens control [Fig 01].

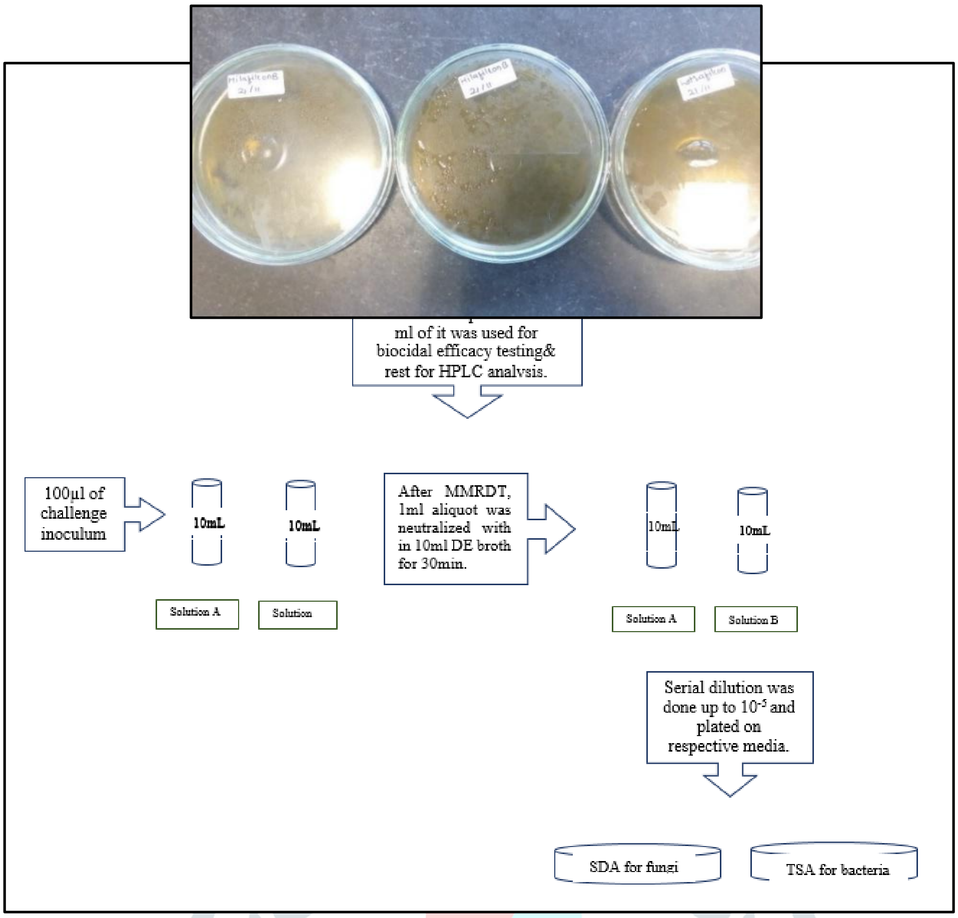


Fig 01: Protocol for determining PHMB uptake and its effect on biocidal efficacy against test organisms after soaking periods. Solution A- BioTrue Solution B- OptiFree Express [Charles R. 2012]

Fig 02: Contact lens sterility test by agar sandwich method.(Unworn contact lenses were placed on agar surface under aseptic condition and small amount of molten agar was poured over the lens surface.)

For chemical analysis high-performance liquid chromatography was performed as defined by Lucas et al with slight modification. The UV detector was set at 254 nm. Separation was performed on a LC-8 column (5 cm×4.6mm, 5micro metre). The mobile phase component was 100% deionized water held isocratically for 4min, then an immediate change to 50:50 water: a solution composed of acetonitrile (76%), water (9.5%),triethylamine (5%), and formic acid (9.5%), pH adjusted to 3.1 with formic acid for the next 2min, then a linear gradient back to 100% water at 10min. The flow rate was 2ml/min and the injection volume was 0.25 ml. the analysis was performed under the guidance of Ms. Pallavi Kapre, Instrument Operator at SAIF IIT Bombay.

IV. CALCULATIONS AND STATISTICAL ANALYSIS

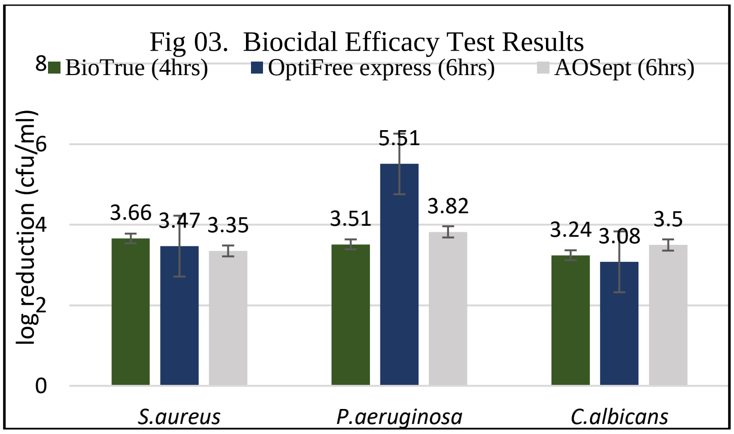
Log reduction values were calculated by determining CFU counts, after which the mean log value for each lens care solution was subtracted from its mean log value at baseline to obtain the log reduction, as follows:

Log reduction = Log10 (mean baseline CFU) – Log (mean lens care solution CFU)

Log reductions among PHMB/PQ-1 (BioTrue), PQ-1/MAPD (Optifree Express) and H2O2 based (AO Sept) solution were compared using an analysis of variance. Statistical significance between solutions was determined using a two-sided, two sampled t-test with a type I error rate of 0.05.

V. RESULTS

The mean log reduction data at the manufacturer's recommended disinfection time for each of the multipurpose solutions and hydrogen peroxide based solution against standard strains of *S.aureus*, *P. aeruginosa* and *C. albicans* is summarized in table 04. The primary criteria of the Stand Alone Test recommended in EN ISO 14729 [ISO14729] require a reduction of ≥ 3 log units for bacteria and ≥ 1 log unit for fungi. On the basis of data obtained, both the multipurpose solutions and H_2O_2 based disinfecting solution fulfil the minimum reduction criteria set by the ISO14729. All the test solutions exceeds the minimum 3 log reduction criteria for bacteria and 1 log reduction criteria for fungi. There were differences in the biocidal activity of lens care solutions against the test organisms as MAPD and Polyquaternium based Opti Free Express showed almost 100% reduction of *Pseudomonas aeruginosa*. PHMB and Polyquaternium based BioTrue solution showed consistent biocide efficacy against all the three test organisms. H_2O_2 based AO Sept solution showed greater efficacy against *C. albicans* when compared with the other two solutions [Fig 03].



The results of biocide uptake study was presented in table 05. Hydrogen peroxide based AO Sept solution was not included in this study as it contains H_2O_2 as the principle biocide which neutralizes immediately by in-built neutralization system (catalase or platinum disc) present in lens case and converted into H_2O and oxygen. The test was carried out twice in duplicates and mean log reduction was calculated for each combination.

	BioTrue (4 hrs)	OptiFree Express (6hrs)	AOSept (6hrs)
S.aureus	3.66 ± 0.30	3.47 ± 0.07	3.35 ± 0.12
P.aeruginosa	3.51 ± 0.05	5.51 ± 0.05	3.82 ± 0.05
C.albicans	3.24 ± 0.53	3.08 ± 0.39	3.50 ± 0.05

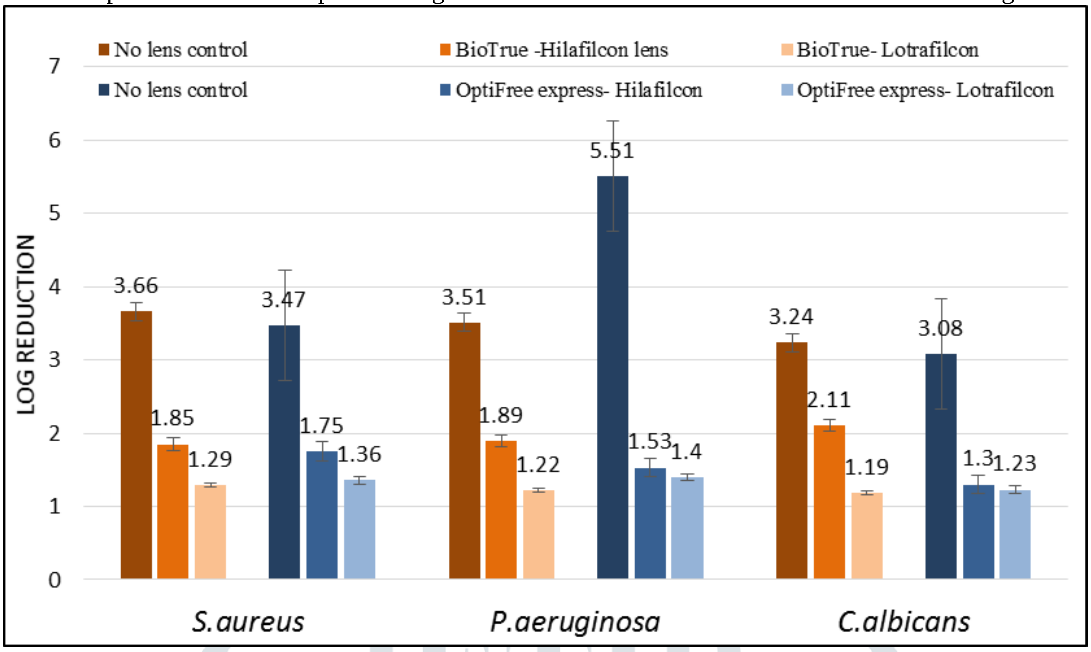
Table 04. Log reduction at MMRDT with standard deviation

From the data presented in table 05, it was confirmed that, in presence of contact lenses, significant reduction in disinfection efficacy was observed, although this effect differ with different combination.

The Lotrafilcon lens affect the log kill efficacy of BioTrue and OptiFree Express solutions more than the Hilafilcon lens. For example, in case of BioTrue solution, the log kill efficacy against *S. aureus* was 1.85 in presence of Hilafilcon lens and 1.29 in presence of Lotrafilcon lens. The log kill efficacy against *P. aeruginosa* was 1.89 in presence of Hilafilcon lens and 1.22 in presence of Lotrafilcon lens. Similar results were observed with respect to *C. albicans* also.

The log kill efficacy of OptiFree express solution against *S. aureus* was 1.75 in presence of Hilafilcon lenses whereas 1.36 in presence of Lotrafilcon lenses. Upon comparing these results with the control data (Fig 04), it was confirmed that the contact lenses can take up the active biocide and thus reduces the efficacy of MPSs significantly.

Fig 04: Statistical representation of comparative log reduction efficacies of MPS before and after soaking in contact lenses



In Figure 4 it is clearly observed that the log kill efficacy of both solutions decreases when they were soaked in contact lenses. Also, among the two lens materials tested, the Lotrafilcon lens which is a type of silicone hydrogel polymer showed greater reduction in the biocidal efficacy then the Hilafilcon lens which is a type of conventional hydrogel polymer. The HPLC analysis also showed the greater uptake of PHMB by Lotrafilcon lenses. HPLC results of BioTrue solution are depicted in the figures below. The concentration of active biocide was relatively compared and it was observed to be lesser in solutions exposed to lotrafilcon lenses than solution exposed to Hilafilcon lenses.

	BioTrue	**OptiFree express**
	Hilafilcon lens	
S.aureus	1.85±0.37	1.75±0.31
P.aeruginosa	1.89±0.18	1.53±0.014
C.albicans	2.11±0.04	1.30±0.106
	Lotrafilcon lens	
S.aureus	1.29±0.38	1.36±0.47
P.aeruginosa	1.22±0.155	1.40±0.22
C.albicans	1.19±0.50	1.23±0.466
	Control (without lenses)	
S.aureus	3.66 ± 0.30	3.47 ± 0.07
P.aeruginosa	3.51 ± 0.05	5.51 ± 0.05
C.albicans	3.24 ± 0.53	3.08 ± 0.39

Table 05: Log reduction value (with SD) of MPS after exposure to contact lenses for 12 hrs soaking period and control log reduction values.

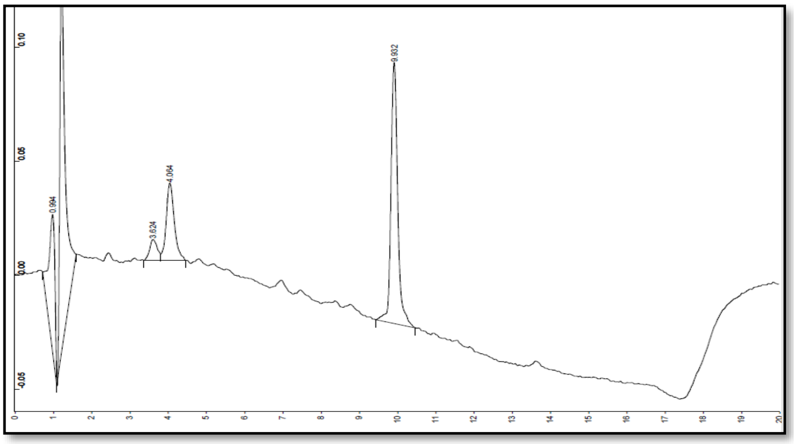


Fig 05 : HPLC chromatogram of BioTrue solution

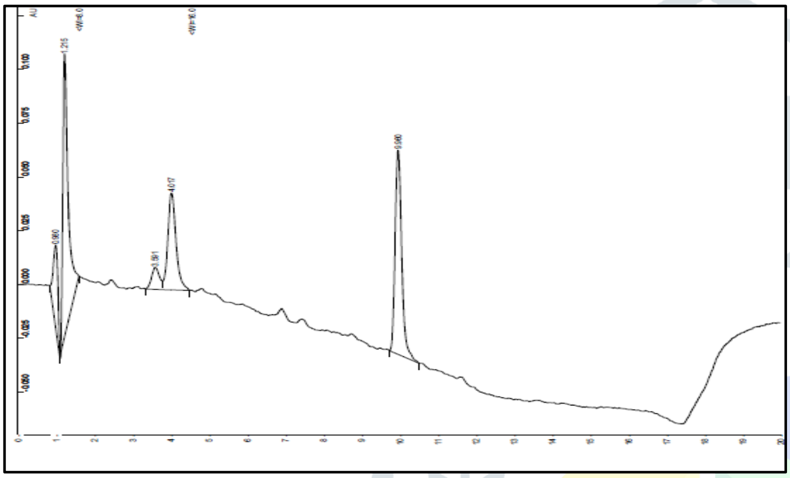


Fig 06 : HPLC chromatogram of BioTrue solution exposed to Lotrsfilcon lens

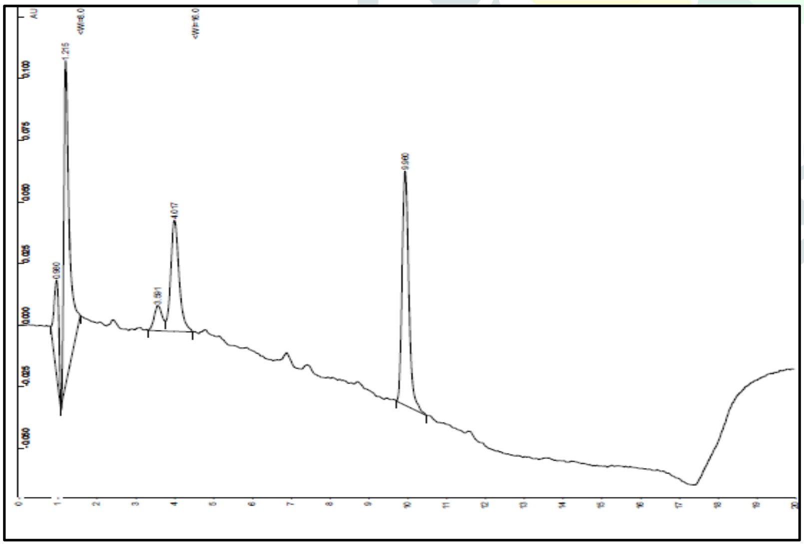


Fig 07 : HPLC chromatogram of BioT rue solution exposed to Hilafilcon lens

VI. DISCUSSION

The eye is innately resistant to microbial infections and these infections usually do not occur except after trauma or injury to the eye. The use of a contact lens can predispose the eye to corneal epithelial micro-trauma. It is known that microorganisms might be transferred easily from the contact lens to the eye. The care system bottles themselves can easily become contaminated and become a source of microbes that may adhere to the lens, cause an inflammatory reaction, or infect the cornea. All types of care solutions, including hydrogen peroxide, have been shown to become contaminated, even in experienced and compliant users, including unopened factory sealed bottles [M Dutot 2009]. Thus the sterility of contact lens care solutions under study, was checked in accordance with the protocol of Indian Pharmacopoea. All the test solutions showed no traces of microbial growth, hence considered as sterile.

Many studies in the past have determined the disinfecting efficacy of Polyhexamethylene biguanide- or Polyquad-based multipurpose solutions (MPSs) and hydrogen-peroxide-based CL care solutions according to the primary criteria of the Stand Alone Test of the EN ISO 14729. The results of the present study showed that all the three solutions passed the primary stand-alone test for their manufacturer's recommended disinfection time (MMRDT). BioTrue and AO Sept solutions showed consistent efficacy against all the test organisms, whereas Optifree express solution showed highest activity against *P. aeruginosa* with almost 100% log reduction. The findings of the present study do not support the findings of Lever and Roya et al and Marsha Oberholzer who showed that Opti-Free Express MPS failed the primary stand-alone test [Claudia H. 2012].

It must be noted that the ISO standard test is performed under well-controlled laboratory conditions while in practice, the use of lens care solutions has been associated with contact lens user noncompliance/malpractice, presence of organic soil, wearing of used contact lenses or bacterial biofilms that would further reduce disinfecting efficacy of MPDS. Especially the influence of the tear fluid and its components is an important factor for simulating more realistic conditions. Thus, different studies have reappraised the effect of organic load on test results, for example, 0.2% bovine albumin, 0.2% mucin or even more contaminated conditions with 1% albumin and 0.1% mucin [Claudia H 2012].

The uptake of preservatives from lens care products into the contact lens matrix was discussed in the literature as early as 1976 [Livia S. 2011]. In 1997, it was shown that uptake of PHMB by Hydro curve lenses decreased bactericidal activity after 4 hours of storage and had no activity after 3 days.12 More recently, Rosenthal et al. reported that due, in part, to direct uptake of the disinfectant by the lens, the antifungal activity was reduced in products containing biguanides (alexidine and PHMB) and was unable to kill *F. solani* after storing etafilcon A contact lenses. The present study also evidences that lens materials significantly reduced the MPS's ability to kill test organisms and had significantly less microbicidal activity than fresh solution taken directly from the bottle (control) (Table 05).

Such observations are attributed to different degrees of biocide uptake onto the lens matrix [Livia S. 2011]. The fact is that when a biocide and lens material interact, lenses sequester the biocide within it matrix and system loses efficacy. ISO and FDA standards virtually ignore the biocide uptake phenomena albeit its importance on disinfection efficacy. The conventional hydrogels lenses have greater propensity for biocide uptake due to electrostatic interactions with the biocides, whereas the silicone hydrogel lens materials being hydrophobic in nature, establish attractive forces with the nonpolar heads of the biocides, and sequester them [David I. 2001]. In the present study, the Lotrafilcon lenses treated with MPS solution showed lesser log reduction than the Hilafilcon lenses. This confirms the fact that the silicone hydrogels lens materials take up the active biocides at a greater extent than the conventional lenses and support the previous findings.

Another important finding of the study was comparative efficacies of multipurpose solutions. Both BioTrue and Opti free express solution are dual disinfection systems. It was observed that lenses treated with BioTrue solution showed higher rates of log reduction than the lenses treated with Optifree express solution. The possible reason for this is the probability of MAPD biocide to be sequestered by lens matrix is more than PAPB because of its fatty acid coupled cationic group. Lastly it was observed that the Optifree express solution treated Lotrafilcon lenses showed the highest survival rates of organisms, suggesting the incompatibility of this combination.

In addition to the reduced biocidal efficacy as a result of uptake by the lens matrix, another concern is the potential for corneal epithelial cell damage and loss as a result of subsequent release of the biocide (PHMB) onto the ocular surface once the lenses are placed back into the eye [Tomislav K. 2008]. Although our study did not examine in vivo effects, Pritchard et al. compared one MPS containing Polyquad with two PHMB-based solutions and found significantly more corneal staining with PHMB solutions when used in conjunction with alphafilcon a lenses. Corneal epithelial cell loss or damage provides a breach through which microbes may enter In vitro studies have shown that currently marketed MPS can damage the ocular surface and have negative effects on human corneal epithelial viability and barrier function [Gorbet M.B.2011]

VII.CONCLUSION:

A proper use of MPS helps to control the microbial load and should reduce the chances of developing any of these ocular events. However the present study suggests that the MPS formulation as well as the CL material affects the disinfection efficacy of a contaminated CL, so special care must be taken when combining a certain CL material with a MPS. Taken collectively, these findings underscore the need for a more critical performance evaluation of multipurpose contact lens solutions. The current ISO Standard oversimplifies the complex multifactorial interplay of critical elements such as pathogen and solution, lens material with solution and organism, lens material with solution and ocular tissue, and consumer compliance. As a result, the current testing recommended in the Standard results in an optimistic perspective of the performance of these products. The ISO committee should consider adding "real world soaking experiments" to the standard to better quantify the effect OF various contact lens materials have on the biocidal efficacy of MPSs.

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