Novel Soft Contact Lens Disinfection with Sodium Chlorite and Hydrogen Peroxide.

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Introduction

Preferences for soft contact lens disinfection regimens have evolved over the last four decades in an attempt to improve on inherent limitations:

Disinfection method	Major Drawbacks
Heaters	Safety, Baking of protein deposits
Chemical disinfection with a saline rinse	Ocular irritation, lens coloration
Hydrogen peroxide with a neutralisation step	Handling risk
Current "no-rinse" or "all-in-one" chemical disinfection systems.	Efficacy, Allergic sensitisation

The current chemical systems are generally well tolerated, but chronic use of certain disinfectants may result in a reduction of comfort due to sensitisation to the preservative and disruption of the corneal epithelial layer.

Proposal

A sodium chlorite based alternative is proposed due to its unique characteristics to generate a potent disinfectant:

- **Chlorine Dioxide**

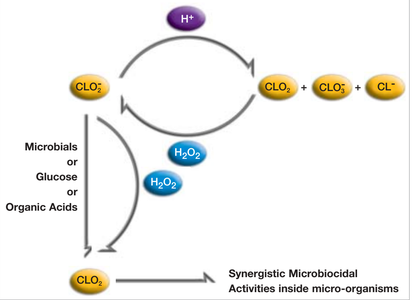
which is very effective towards killing Gram + and Gram - Bacteria, Yeasts and Fungi and then breaksdown into components of natural tears:

- **Salt** - **Water** - **Oxygen**

Sodium chlorite has been used safely for many years as a treatment for municipal drinking water. The sodium chlorite molecule is unstable, and can be activated by acidic cellular components, resulting in the generation of chlorine dioxide, a well-known, potent disinfectant. However, the resulting chlorine dioxide is even more unstable than the sodium chlorite starting material.

The chlorite/peroxide formulation is based on synergisitc microbiocidal activities of chlorite/H_2O_2. A schematic of the synergisitic reaction mechanism is presented in Figure I.

Figure I: Schematic Representation of Synergistic Microbiocidal Action

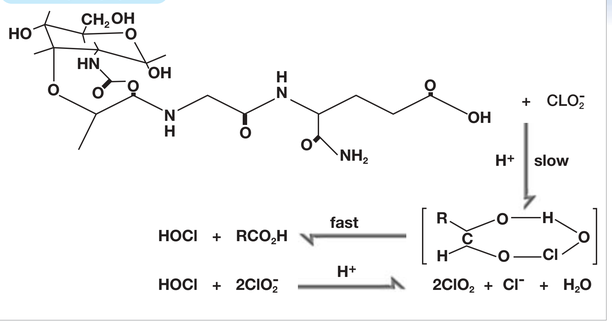


Chemistry

The following figures represent the on-site production of chlorine dioxide from the interaction of the chlorite ion with microbial membranous glycopeptides. Such a reaction can also occur deep inside microorganisms in the presence of H_2O_2.

Postulated Reaction Mechanism for Antimicrobial Activities

Muramyl Dipeptide



Key to the chlorite/peroxide disinfection activity is to utilize the transient species of ClO_2 generated from the interation between chloite ions and acidic microbial components, and to enhance the production of ClO_2 by creating an acidic environment inside microbial organisms which is effected by employing a small amount of H_2O_2. This not only does not disturb the stability of chlorite, but maintins stability through a constant reconversion as discussed further below.

It has been well known that sodium chlorite alone does not decompose to $2ClO_2$ at neutral pH or in alkaline solution. However, when chlorite is in contact with acidic impuities or acidic components in micro-organisms at neutral pH, it is converted to ClO_2. In acidic solution, on the other hand, it readily produces chlorous acid which can disporportionate to ClO_2, ClO_3^-, Cl^- as indicated in equation 1:

$$ClO_2^- \xrightleftharpoons{H^*} (HClO_2) \longrightarrow ClO_2 + ClO_3^- + CL^{-*} \quad ....(1)$$

It should be noted that a trace amount of a higher oxidation state species, ClO_3^- is generated which is known to be harmless to humans.

The microbiocidal activity of ClO_2 is well known as a powerful bactericidal and fungicidal agent which has been used for disinfecting drinking water.
ClO_2 generated at the surface of and inside of microorganisms is mainly responsible for the observed microbiocidal activities of chlorite. To enhance the microbiocidal activities of chlorite, a trace amount of H_2O_2 is employed.

Due to its neutrality and small size, hydrogen peroxide can easily penetrate the microorgaisms' membranes, and its subsequent oxidative interation with microorganism components produces altered membrane components as well as its own biocidal activities which in turn activates ClO_2^- to generate ClO_2. Since H_2O_2 can easilly reduce ClO_2 to ClO_2^- via a single electron transfer, even if ClO_2 is formed during a long term storage period, H_2O_2 can effectively convert ClO_2 to ClO_2^- and maintain the stability of chlorite ions as indicated in equation 2:

$$2ClO_2 + H_2O_2 \xrightleftharpoons{RT} 2ClO_2^- + O_2 \quad .....(2)$$

It should be also noted that sodium chlorite produces sodium and oxygen when exposed to light as indicated in equation 3, which would occur when contact lenses are taken out of their storage case and placed in the eye.

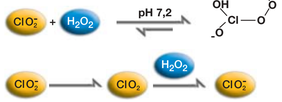
$$2NaClO_2 \xrightarrow{hv} NaCl + 3O_2 + 2NaClO_3 \quad .... (3)$$

A proposed overall reaction mechanism is presented in Figure II.

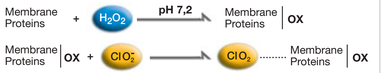
Figure II

Summary of Synergistic Antimicrobial System H_2O_2 + CLO_2^-

Stability of Chlorite Solution by H_2O_2:



Membrane Penetration:



Antimicrobial Efficacy Testing of Chlorite/Peroxide Complex

PURPOSE:
To determine the disinfection efficacy of the Chlorite/ H2O2 complex disinfecting solution.

MATERIALS AND METHODS:
The Antimicrobial effectiveness test methodology of the United States Pharmacopeia 25 (USP) was used in all the in-vitro testing. Working cultures of ATCC strains of *P.aeruginosa*, *S.aureus*, *E.coli*, *S. marcescens*, *C. albicans* and *A. niger*, and human resistant strains were prepared as per the USP.

Sterile saline suspensions of the five organisms are prepared in separate sterile culture tubes, to contain 10^5 - 10^6 colony forming units (cfu)/ mL. of solution. The initial concentration of viable microorganisms in each test preparation is estimated based on the concentration of microorganisms in each of the standardized inoculum as determined by the plate-count method.

Twenty (20) mL. of product sample is transferred aseptically to 5 sterile capped bacteriological tubes, Each product tube is inoculated with one of the standardized microbial suspensions, using a ratio equivalent to 0.10 mL. of inoculum to 20 mL. of product. and mixed.

The inoculated containers are incubated at 22.5°C ± 2.5°C. The culture tubes are sampled at 1, 2, 3, 4, 6 and 24 hours, 7 and 14 days. Determine by the plate-count procedure the number of colony forming units (cfu) present in each test preparation for the applicable intervals.The neutralizer of the specific antimicrobial is incorporated in the plate count or the appropriate dilution prepared for plating.

Using the calculated concentration of cfu per mL. present at the start of the test, we calculated the change in $\log_{10}$ values of of the concentration of cfu per mL. for each microorganism at the applicable test intervals, and expressed the changes in terms of log reductions in Table 1 and 2.

RESULTS:

TABLE 1. Log reduction of ATTC strains of microorganisms

ATTC Bacteria	S. aureus	P. aeruginosa	E. coli	S. marcescens	C. albicans	A. niger
Log Initial inoculum	6.09	5.65	5.96	6.40	5.54	5.48
Log reduction- 2 hrs	3.60	4.67	5.12	4.83	-----	-----
Log reduction- 3 hrs	6.09	5.65	5.96	5.90	-----	-----
Log reduction- 4 hrs	6.09	5.65	5.96	6.40	-----	-----
Log reduction- 7 days	-----	-----	-----	-----	5.54	2.32
Log reduction- 14 days	-----	-----	-----	-----	-----	5.48

TABLE 2. Log reduction of human resistant strains

Human resistant Bacteria	S. haemolyticus	P. aeruginosa
Log Initial inoculum	7.03	6.35
Log reduction- 2 hrs	2.43	6.35
Log reduction- 4 hrs	7.03	6.35

DISCUSSION:

The results of the chlorite/peroxide complex antimicrobial testing, shows a 6 log reduction of ATTC strains of bacteria, *S. aureus*, *P. aeruginosa*, *E. coli* and *S. marcescens* in 3 hours. In addition a 7.0 log reduction of S. haemolyticus human resistant strain in 4 hours and 6.34 log reduction of human resistant strain of P. aeruginosa in 2 hours.

In addition the formulation, shows a 5.54 log reduction of ATTC strains of yeast, *C. albicans* in 7 days and a 2.32 log reduction of ATTC strains of a mold, *A. niger* in 7 days.

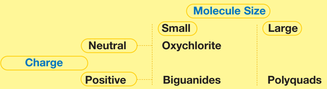
CONCLUSION:

The Chlorite/Peroxide Complex is very effective as a broad spectrum antimicrobial agent.

Discussion

The chlorite/peroxide complex is a very active anti-microbial agent and is safe and non-irritating to the corneal epithelium. The complexed molecule is self-stabilised in the bottle or lens case, but rapidly degrades in the ocular environment into salt, water and oxygen. As such, it is a non-toxic alternative and may be instilled directly into the eyes and thus used for other functions such as rinsing, storage and lubricating.

Large or Small Molecule, Positive or Neutral Charge?



In the case of a lens polymer with a negative ionic charge, biguanide molecules penetrate into the lens and become strongly bound to the polymer matrix, gradually increasing in concentration, This high concentration in the lens is exposed to the eye on an ongoing basis, gradually causing discomfort and irritation.

In the case of a large molecule like polyquad, it may not get into the polymer matrix, however it is still positively charged, and thus attaches itself to the surface of the Lens and accumulates gradually causing discomfort and irritation. In addition, a high molecular weight material has more difficulty achieving anti-microbial activity inside the lens.

The ClO_2^- / H_2O_2 compound is a very small molecule. It does not have any charge, it is neutral, so it can get into the lens and clean and disinfect the inside as well as the surface of the lens, but because it does not bind to the polymer matrix it does not accumulate into the lens, it goes in and out like the tear film that goes in and out of the lens.

Safety - Chlorite

Chlorine dioxide and the chlorite ion are oxidising agents which serve as effective biocides.[1,2]

The major concern regarding exposure to chlorite and chlorine dioxide would be the oxidative stress they may induce on the body. In order to validate the use of chlorite and chlorine dioxide as a preference over chlorine for municipal drinking water supplies, the literature reports numerous animal and human safety studies in the following areas:[3,4]

Animal studies
- metabolic studies
- subchronic
  - oral
  - inhalation
- chronic
- mutagenicity
- oncogenicity
- reproductive
- developmental

Human studies
- Clinical studies
- Epidemiological studies

Chlorine dioxide and chlorite are rapidly reduced following ingestion and chronic human studies have showed no negative effects at ingesting 500ml at 5ppm daily.[5]
At a typical concentration of 1ppm ClO_2^- in drinking water, and that the normal adult drinking 2 liters of water daily, the estimated daily intake of ClO_2^- via drinking water can be calculated as:

$$\frac{1\text{ mg } ClO_2^-}{\text{liter}} \times \frac{2\text{ liters}}{\text{day}} = \frac{2\text{ mg } ClO_2^-}{\text{day}}$$

Even estimating an assumption via ocular/nasal pathways of 5ml of solution (both wells of a flat lens case) the estimated daily intake would be:

$$\frac{4\text{ mg } ClO_2^-}{\text{liter}} \text{ or } \frac{0{,}004\text{ mg } ClO_2^-}{\text{ml}} \times \frac{5\text{ ml}}{\text{day}} = \frac{0{,}02\text{ mg } ClO_2^-}{\text{day}}$$

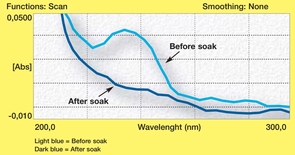
This dose is only 1/100th of a typical ingestion in chlorite treated drinking water.

1) Rovito C et al. Antimicrobial activity of chlorine dioxide and sodium hypochlorite in water disinfection. Ind. Conserve 1985; 60(3): 209-12.
2) Noss CI, Olivieri VP. disinfecting capabilities of oxychlorine bcompounds. Appl Environ Microbiol 1985;50(5): 1162-4
3) Couri D Abdel-Rahman MS, Bull RJ. Toxicological of chlorine dioxide, chlorite and chlorate. Environ health perspect 1982;46:13-7.
4)Abdel-Rahman MS, Couri D, Bull RJ. Toxicity of chlorine dioxide in drinking water. J Environ Pathol Toxicol Oncol 1985;6(1):105-13
5) Lubbers JKR, Chauhan S, Miller JK, Bianchine JR. The effects of chronic administration of chlorine dioxide, chlorite and chlorate to normal healthy adult male volunteers. J environ Pathol Toxicol Oncol 1984;5(4-5):229-38

Lens Absorption Study with PHMB

Etafilcon-A lens (Power = -2,0, BC = 8,8. Dia. = 14,0)

Etafilcon-A lens were soaked in 2,5 mls of 1 ppm PHMB, 0,90 NaCl solution for 22 hrs. Absorbance spectrum of solution taken before and after soak.

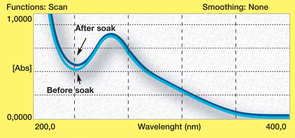


Light blue = Before soak
Dark blue = After soak

Lens Absorption Study with Chlorite/Peroxide Complex

Etafilcon-A lens (Power = -2,0, BC = 8,8. Dia. = 14,0)

Etafilcon-A lens were soaked in 2,5 mls ClO_2^- / H_2O_2 formula for 22 hrs. Absorbance spectrum of solution taken before and after soak.



Light blue = Before soak
Dark blue = After soak

Results of Lens Absorption Study

1. PHMB uptake by Etafilcon-A lenses is 2,0 mg out of 2,5 mg available in 2,5 mls solution after soaking for 22 hrs.
2. No uptake of the chlorite/peroxide complex with Etafilcon-A lenses is observed.

Safety – Hydrogen Peroxide

Hydrogen peroxide is well known as an antiseptic in medical use and as an antimicrobial agent in contact lens care products typically at a starting concentration of 3% or 30,000 ppm. At this concentration, a neutralisation via a platinum catalyst or catalase enzyme is required to reduce the oxidative activity before instilling in the eye.

Catalase, superoxide dismutase and glutathione peroxidase enzymes are naturally present in tear film and in particular in the heavily vascularized palpebral conjunctiva. These enzymes quickly reduce residual H_2O_2 into water and oxygen, which do not create allergic sensitivities. Many studies have measured the capacity of these enzymes to neutralise externally applied H_2O_2.

One study demonstrated an ocular sensitivity threshold at 210 ppm[1], while another study found this to be 400-800ppm, with a resolution of conjunctival hyperaemia within two minutes, indicating the rapid reduction of the hydrogen peroxide molecule.[2] Another study demonstrated a robust decay curve for lenses soaked in 50 to 150ppm with no decrease in the eye's neutralisation ability with four repeated installations per day.[3]

The cornea acts as a refracting surface and membrane barrier and several studies have measured the effects of hydrogen peroxide on rabbit corneas. One study showed that peroxide concentrations of 235ppm for 10 minutes caused no change in stromal swelling or epithelial light scatter.[4] Another study demonstrated that hydrogel lenses soaked in 680ppm H_2O_2 did not penetrate the corneas, while up to 270ppm in 0.8ml solution would be held on the epithelial side for up to one hour.[5]

Hydrogen peroxide is used at 100ppm in the chlorite/peroxide complex, not for its stand-alone oxidative activity (which would require 300 times this concentration), but for the synergistic effect to enhance chlorine dioxide production and consistently reconvert back into chlorite in a closed system.

Literature studies clearly show that 100 ppm is well below the threshold for ocular sensitivity and quickly reduced in the ocular environment.

1) Chalmers RL, Mc Nally JM: Ocular detection threshold for hydrogen peroxide: drops vs. lenses. ICLC 1988;15:351-357.
2) Paugh JR, Brennan NA, Efron N: Ocular response to hydrogen peroxide. Am J Optom Physiol Opt 1988;65:91-98.
3) Mc Kenney CD, Roth L, Scott G: In-vivo ocular H202 neutralization after repeated H202 exposure. Invest Ophthalmol Vis Sci 1992;34Suppl):1294.
4) Wilson GS, Chalmer RL: Effect of H202 concentration and exposure time on stromal swelling: An epithelial perfusion model. Optom Vis Sci 1990;67:252-255.
5) Riley MV, Kast M: Penetration of hydrogen peroxide from contact lenses or tear-side solutions into the aqueous humor. Optom Vis Sci 1991;68:546-551.

Biographical Sketch of Authors

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Hampar Karageozian is an ophthalmic industry scientist and developer of a number of widely used ophthalmic pharmaceutical and contact lens care products. H. Karageozian was employed by Allergan, Inc. from 1970 to 1992, where he ultimately served as senior vice president of optical research and development. Mr. Karageozian holds a M.S. Degree from the Massachusetts Institute of Technology and a B.Sc in pharmacy and pharmaceutical chemistry from the American University of Beirut. He also holds a M.B.A. degree form the University of California, Irvine.

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