Some factors influencing the stability of Sterilox®, a super-oxidised water

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IN BRIEF

- This article studies the stability of Sterilox solutions and some factors that might cause changes in chlorine concentration and pH
- Sterilox was found to be stable for a 14-day period when stored in ideal conditions
- Sunlight exposure accelerates the decomposition rate of chlorine
- The presence of air in the bottle does not influence chlorine decomposition

RESEARCH

Super-oxidised waters, particularly Sterilox®, have been suggested for the disinfection of dental unit water lines and dental impression materials owing to their antimicrobial efficacy. One of the previously suggested characteristics is their short shelf life. The purpose of this investigation was to understand the effect of storage conditions on Sterilox®'s stability. Eight bottles (four completely full, four half-full) of freshly prepared solution were divided into four groups and subsequently stored by being either exposed to or protected from sunlight. The chlorine concentration was monitored using chlorine test strips until the concentration reached zero, or until the thirteenth week. Statistically significant differences between the groups exposed to sunlight and the non-exposed groups ($p < 0.001$) were found. The mean loss of chlorine per day for the non-exposed samples was 1.01 mg/L, whilst the mean for the exposed samples was 2.42 mg/L. The presence of air did not affect the chlorine decomposition in the bottles. The results of this investigation indicate that when the solution is exposed to sunlight, the decrease of chlorine starts at day 4, whilst for the groups sheltered from sunlight, the process started after day 14. Therefore, Sterilox® solutions appear to be more stable than previously surmised.

INTRODUCTION

Super-oxidised waters (also known as electrochemically activated solutions) are produced from saline solutions following electrolysis by passing over titanium electrodes.[1] In dentistry they have been proposed for the removal of bacteria from dental unit water supplies[1] and disinfection of common impression materials,[2] and their antimicrobial and cleaning effectiveness in root canals have been studied.[3,4]

Sterilox® is a super-oxidised water currently used to decontaminate endoscopes. Its main component is hypochlorous acid (HOCL),[5,6] the recommended concentration of which has been suggested to be between 144 mg/L[5,6] and 240 mg/L,[7] whilst the manufacturer suggests 200 mg/L chlorine concentration in the solution (personal communication). Its pH has been described as 5-6.5,[5,6] 6.3,[7] and between 2 and 14, but this can be controlled between 2.7 and 6.8.[8] Meanwhile, the manufacturer suggests a pH of between 5 and 7.[9]

The decomposition and evaporation of chlorine solutions leads to a loss of chlorine, and hence to a loss of antimicrobial activity.[10] It has been suggested that Sterilox® solutions are inherently unstable and are highly antimicrobial when freshly generated.[5,6,11] However, the effects of time and storage conditions on Sterilox®'s chlorine concentration have not been reported in the literature. A different super-oxidised water, Microcyn (Oculus Innovative Sciences, California, USA), according to its manufacturer, has a neutral pH (between 6.2 and 7.8), lower active chlorine (51-85 ppm) and a one-year shelf life.[12]

A direct association between pH and stability of hypochlorous acid solutions has previously been shown; the stability decreases dramatically with the change from alkaline to acidic.[13] A further factor which has been suggested to influence the stability of chlorine solutions is their concentration: higher concentrations are more stable.[10]

The purpose of this study was to investigate the effects of time, sunlight and head air on the chemical stability (chlorine concentration and pH) of a freshly prepared Sterilox® solution.

MATERIALS AND METHODS

A freshly prepared and electrochemically activated solution (Aquatine Alpha Electrolyte, Sterilox Dental, Ilkley, West Yorkshire) was tested for chlorine concentration using 25-500 mg/L chlorine test strips (Merckoquant, Merck, Darmstadt, Germany) and for pH using non-bleeding pH indicator strips pH 0-14 (Merckoquant, Merck, Darmstadt, Germany). The solution was then dispensed into eight 250 ml plastic transparent bottles, in duplicates, as follows: two bottles containing 250 ml solution protected completely from sunlight by storage in a light-proof cupboard (group 1), two bottles containing 125 ml solution, also protected completely from sunlight by storage in a light-proof

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cupboard (group 2), two bottles containing 250 ml solution exposed to direct sunlight (group 3) and two bottles containing 125 ml solution, also exposed to direct sunlight (group 4). The bottles were closed with a screw cap-type lid. The groups were randomly allocated to the storage conditions, but kept in the same room at a constant temperature of 21°C ± 2°C.

Chlorine concentration and pH were measured daily during the first week, on days 10 and 14, and then at weekly intervals, either up to the 13th week or until the chlorine concentration value reached zero, whichever was the sooner.

Two observers blinded to the experimental groups visually assessed the chlorine and pH indicator strips. Where the observers were unable to agree on the findings, discussions were held until they agreed on a result.

The statistical approach for the findings, considering the chlorine concentration in mg/L and the variables exposure to light, volume of solution and time (days), was a multiple linear regression model at $\alpha = 0.05$.

RESULTS

Results are presented in Figure 1. These show the decrease of chlorine starting at day 4 for the groups exposed to sunlight and at day 14 for the groups protected from sunlight. The volume of air left above the Sterilox® solution in the storage containers (ie 0 ml *versus* 125 ml) did not affect the rate of chlorine decomposition. However, there were statistically significant differences between the groups exposed to sunlight and the non-exposed groups ($p < 0.001$). The mean loss of chlorine/day for the non-exposed samples was 1.01 mg/L, whilst the mean for the exposed samples was 2.42 mg/L. Therefore, the loss of chlorine was statistically greater when Sterilox® was exposed to sunlight. As for pH, this remained unchanged throughout the duration of the assays with a value of 5 for all samples.

DISCUSSION

Paper strips were used to measure chlorine concentration. This is a more clinically representative process than the titration technique as it is a simple and repeatable procedure which does not require specific laboratory equipment and skills. In the present study, the counts were consistent and the duplicates were almost identical.

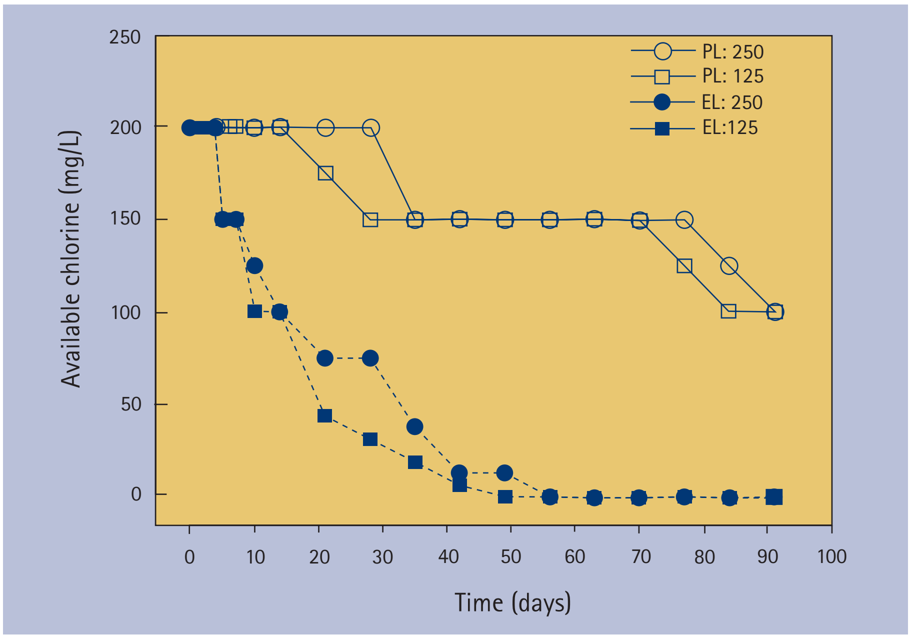

Fig. 1 Graph displaying the chlorine concentration in mg/L amongst groups considering the time of exposure in days. PL 250: Protected from sunlight, 250 ml. PL 125: Protected from sunlight, 125 ml. EL 250: Exposed to sunlight, 250 ml. EL 125: Exposed to sunlight, 125 ml

The different amounts of solution in the bottle were tested to replicate clinical usage and storage conditions.

The chlorine decomposition rate depends on the solution's pH, concentration, temperature, presence of impurities in the solution and exposure to sunlight.[10,13]

Chlorine decomposition rates and sunlight

In our investigation, the bottles were exposed to direct sunlight on a windowsill around the northern hemisphere equinox in southern England. It is difficult to quantify the amount of sunlight as this would vary in intensity on a temporal and geographical basis, therefore sunlight exposure in different conditions might cause a different rate of decrease in chlorine concentration. A previous investigation using diffused fluorescent light found this to be an important factor in causing chlorine loss in electrolysed oxidised water in similar experimental conditions.[13] That investigation tested a pH 2.5-2.6 53-56 mg/L chlorine concentration super-oxidised water in a sealed jar and showed that approximately 60% of the chlorine was lost after 1400 hours in diffused light. About 40% was lost in the solutions that were protected from light.[13] Our study revealed a statistically significant difference in the loss of chlorine when there was exposure to sunlight. The solutions were stable only until day 4 under sunlight exposure, whilst solutions in non-exposed bottles were stable until day 14. Sterilox®'s prolonged stability may be clinically relevant and therefore allow for greater use, even if the renewal of the solution is easy.

Another study tested the action of direct sunlight *versus* diffused sunlight on the decomposition of chlorine solutions and found that direct sunlight greatly accelerated the decomposition of the solution at a rate that was three to four times faster.[14] It is worth noting that the solutions were kept at a much higher temperature (42-45°C) than in our assays. Also, *in vitro* models involving specimens exposed to sunlight might subsequently experience an increase in temperature, resulting in acceleration in the rate of chlorine decomposition.

Chlorine decomposition rates and head space

Chlorine loss occurs as a result of the evaporation of chlorine gas dissolved in the solution in addition to HClO decomposition.[13] In 'closed' conditions, as in our experiments, it has been suggested that the primary mechanism of chlorine loss could be the self-decomposition of chlorine species in the solution because chlorine evaporation is normally limited.[13,15]

The presence of 'head space' in the 'half-full' bottles and the fact that the containers were opened during sampling might have speeded up the process as repeated opening of the bottles might have increased the evaporation and loss of chlorine gas into the environment, especially in the 'half-full' bottles. One must bear in mind that it will only be possible to use the solution if the bottle is opened, therefore this procedure has clinical relevance.

Chlorine decomposition rates and pH

The influence of pH on chlorine solution stability has been explained by the fact that with an increase in pH the equilibrium in the solution will shift towards the formation of HClO and therefore a consequent decrease in volatile chlorine gas, resulting in a reduction of chlorine evaporation.[13] However, this seems to be important in the presence of a solution exposed to air, while in sealed solutions and higher pH, the chlorine loss will depend on the self-decomposition of the chlorine species.[13] pH increase also influences the dissociation of HClO to hypochlorite ions (OCl^-), a less antimicrobial form, therefore reducing its disinfecting efficacy.[10]

Regarding pH changes in super-oxidised waters, a previous investigation showed similar results to our assays with the pH almost unchanged during storage for a two-month period.[13] This trend was also observed in our study.

Clinical and experimental applications

Previous publications suggest different cut-off points for the use of Sterilox® after production: 5 hours,[5] 24 hours.[16] It has also been suggested that 'the solution needs to be generated on site, its pH and redox potential confirmed, and all old disinfectant replaced every 24 hours'.[16] Sterilox® was considered to be more suitable to a centralised service[17] and one of the suggested advantages is that it can be generated on site.[13] However, this might require the availability and maintenance of multiple Sterilox® activator machines instead of the delivery of the activated solution from a centralised source.

Regular confirmation of the microbial activity of Sterilox® by biological tests or by determination of chlorine levels has been suggested.[16] The authors agree with this suggestion, given that preliminary investigations showed that different Sterilox® generators produced inconsistent chlorine concentration solutions (data not shown).

Furthermore, it would be ideal if any further investigations on chlorine-containing solutions could include chlorine concentration analysis as part of the experimental methodology. More importantly, this needs to be carried out regularly in clinical conditions in order to confirm that the solution fulfils the required criteria. However, the 'ideal' pH, chlorine concentration and redox potential for Sterilox® and other super-oxidised waters have yet to be established, if indeed these exist, considering that they might also influence super-oxidised waters' toxicity and corrosive action against metals.

The study suggests that, if stored protected from sunlight, Sterilox® solutions are stable for at least a two-week period. The solution thus needs to be replaced less frequently than previously thought.

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