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The shelf-life of sodium hypochlorite irrigating solutions

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Abstract

Background: Sodium hypochlorite (bleach) solutions are widely used as irrigants in endodontics. Their tendency to deteriorate is worsened by environmental influences which could cause loss of available chlorine content.

Methods: This study measured the loss of available chlorine concentration in a range of sodium hypochlorite solutions under conditions which mimic clinical usage and storage. Domestic bleach, both undiluted and diluted with demineralised water or hard water, was tested, along with Milton, for initial concentration of available chlorine, and then retested after varying periods and modes of storage to measure loss of chlorine concentration. The types of storage were: (1) in closed plastic bottles which were opened daily and agitated; (2) in open plastic bottles; (3) in syringes exposed to sunlight; (4) in syringes kept in the dark; (5) in open stainless steel bowls; (6) in closed stainless steel bowls; and (7) when heated to 50°C. Not all solutions were tested under all storage conditions.

Results: Of the solutions opened daily, undiluted domestic bleach was the most stable and Milton was the least stable. Initially, diluted bleach left open deteriorated rapidly but deterioration slowed with time. Solutions in syringes exposed to sunlight showed the most rapid loss of chlorine content. Heated bleach lost nearly 5 per cent of its strength in six hours. Diluted bleach surprisingly, increased its chlorine concentration in open bowls probably due to evaporation of water. Dilution of bleach with hard tap water did not significantly affect shelf-life. Preloading of diluted bleach into syringes appears to be a sound technique if the syringes are stored away from light.

Conclusions: This study reinforces the need for sodium hypochlorite to be stored in closed opaque containers. Constant opening of containers appears to cause greater loss in chlorine concentration of diluted bleach solutions, perhaps because a lower concentration of sodium hydroxide allows the pH to drop more rapidly.

Key words: Sodium hypochlorite, domestic bleach, endodontics, shelf-life, clinical usage, open containers, syringes.

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INTRODUCTION

Biomechanical instrumentation and cleaning of root canals require the use of an irrigant to suspend and flush pulpal and dentinal debris from root canals.[1] Ideally, an endodontic irrigant should be sterile and should also be capable of destroying the micro-organisms found in a necrotic pulp, as well as dissolving both vital and necrotic pulpal tissue.[2-4] Sodium hypochlorite solutions, which are bactericidal, virucidal and which dissolve protein, have been widely used for this purpose.[5-7] The concentration of sodium hypochlorite solutions used in endodontics may vary from 0.5-5.25 per cent available chlorine.[8] While sodium hypochlorite may be prepared and packaged commercially for therapeutic use,[1] it is common in many countries for domestic bleach (diluted or undiluted) to be used as an endodontic irrigant.[8]

Sodium hypochlorite is a strong oxidising agent.[9] The level of available chlorine is the critical factor affecting the activity of sodium hypochlorite solutions.[5,10] These solutions are unstable.[11] Available chlorine concentration deteriorates with time, exposure to light[5,12] and heat[8,13] and on contact with air,[5,12] metals, metallic ions[10] and organic materials.[1] While Velvart[14] concluded that cold storage did not appear to improve the shelf-life of two per cent solutions, other authors[8,11] demonstrated much better shelf-life under refrigeration, and concluded dilute solutions were more stable than more concentrated solutions. While the bactericidal activity is greater at pH values as low as 6, high pH levels are necessary for effective shelf-life.[6,11] Sodium hydroxide is usually present in the solutions to enhance alkalinity.

While the individual influences of environmental factors on the stability of sodium hypochlorite have been well documented, the influence of many of the factors which are likely to be encountered in clinical endodontic practice in combination have not been examined. The most pertinent of these influences are combinations of time, temperature and exposure to light and air. In view of the recorded instability of sodium hypochlorite solutions and of the wide range of potential storage conditions and methods of dispensing these solutions in different dental practices, further examination of shelf-life under combinations of storage and dispensing conditions which could accelerate the loss of available chlorine concentration was considered warranted.

The activity of sodium hypochlorite solutions can be assessed by determining the amount of available chlorine in each solution. There are a number of methods to measure available chlorine, including tissue solubility;[10] N-diethyl-p-phenylenediamine ferrous ammonium sulphate analysis;[5] titration against sodium arsenite;[11] and iodometric titration with a variety of end-point detection procedures, which include titration against sodium thiosulphate and amperometric titration against phenylarsine.[15] While the accuracy of iodometric titration against sodium thiosulphate has been questioned,[11] because breakdown products such as chlorates and chlorites could potentially give artificially high readings, it is the simplest and most widely used test for available chlorine; does not have the disposal problems or toxicity of arsenic compounds;[15] and was used in this study.

This study examined hypochlorite solutions commonly used for endodontic irrigation in Australia. Solutions assessed included an infant sanitiser and a common brand of domestic bleach, both undiluted and diluted. Changes in available chlorine concentrations were investigated when the solutions were exposed to combinations of environmental and storage variables commonly found in dental practices.

MATERIALS AND METHODS

Sodium hypochlorite solutions

Milton (Proctor & Gamble Australia, Parramatta, NSW) (1 per cent w/v) and Sno-Wite (Kiwi Australia, Clayton South, Vic) (4 per cent w/v) were used as the sources of sodium hypochlorite for this study. Milton was used in the undiluted form only and Sno-Wite was also used undiluted or diluted to 1 per cent w/v.

Titrations

The level of available chlorine was assessed initially and at each test period, using Australian Standard 1087.[16] This test was developed for sodium hypochlorite solutions used in the dairy industry and involved addition of potassium iodide to a prepared sodium hypochlorite solution, with subsequent addition of acetic acid. Available chlorine oxidised the iodide ions to produce iodine which turned the solution brown. The resulting solution was then titrated against a standard sodium thiosulphate solution (0.1 mole/litre) until the colour just disappeared. The concentration of available chlorine was given by the following equation: per cent available chlorine equals $V \times C \times 3.546$, where V equals the volume of sodium thiosulphate used in millilitres and C equals the concentration of the standard sodium thiosulphate solution in moles/litre.

For all solutions except those in syringes, three samples were prepared and three titrations were performed on each sample at the end of each storage period. The solutions in syringes were treated differently – the contents of six syringes were combined and stirred to make a solution from which three titrations were carried out at each test period. Solutions in syringes exposed to sunlight were titrated by a single operator and all other titrations were carried out by teams of two operators, each making independent readings of the titration results. The 10 solutions were tested immediately upon preparation and at varying intervals depending on the storage conditions.

Table 1. Analysis of water from Kingaroy domestic supply used to dilute domestic bleach.*

Contents	Concentration mg/L
Cations	
Sodium	335
Potassium	12
Calcium	105
Magnesium	130
Hydrogen	0
Anions	
Bicarbonate	125
Carbonate	1.6
Hydroxide	0
Chloride	980
Fluoride	0.1
Nitrate	3.7
Sulphate	46
Other dissolved elements	
Iron	<0.02
Manganese	0.06
Zinc	<0.02
Aluminium	<0.05
Boron	0.1
Copper	<0.05

*Collected 28th August 1995 – tested April 1997 by Scientific Services section of Queensland Health.

Available chlorine

To test the effect of storage conditions on shelf-life, Sno-Wite solutions were diluted to one per cent w/v with both demineralised water and hard tapwater, then stored in the following manner:

(1) In one-litre bottles which were opened daily

Plastic one-litre bottles were half-filled with dilute Sno-Wite, then tightly capped, wrapped in aluminium foil to help exclude light and placed in a lightproof box. Each bottle was opened daily five days a week, agitated briefly then recapped after 10 minutes. Available chlorine levels were tested at varying intervals over eight months. The one per cent w/v solutions were stored in numbered containers. The titrating staff were not aware of the identity of solutions being tested.

(2) In open containers

In this segment of the study, an identical solution to that prepared in (1) above was prepared and placed in individual one-litre bottles which were wrapped in aluminium foil and stored in a lightproof, ventilated box in a photographic darkroom, with the bottles remaining uncapped.

Table 2. Sodium hypochlorite test solutions and details of conditions of storage and container type.

Parent solution	Diluent used	Nominal concentration of test solution*	Storage temperature	Container capped	Opened daily	Light exposure	Container type
Milton	Nil	1	Air conditioned	Yes	Yes	Dark	1L plastic
Sno-Wite	Nil	4	Air conditioned	Yes	Yes	Dark	1L plastic
Sno-Wite	Nil	4	50°C	No	Open	Ambient	100ml glass
Sno-Wite	Demin	1	Air conditioned	Yes	No	Sunlight	5ml syringe
Sno-Wite	Demin	1	Air conditioned	Yes	No	Dark	5ml syringe
Sno-Wite	Demin	1	Air conditioned	No	Open	Dark	1L plastic
Sno-Wite	Demin	1	Air conditioned	No	Open	Ambient	Stainless bowl
Sno-Wite	Demin	1	Air conditioned	Yes	No	Ambient	Stainless bowl
Sno-Wite	Hard	1	Air conditioned	Yes	Yes	Dark	1L plastic
Sno-Wite	Demin	1	Air conditioned	Yes	Yes	Dark	1L plastic

*Per cent available chlorine. Demin – demineralised water. Hard – hard water from Table 1. Sunlight – indirect sunlight. Ambient – sunlight from windows plus fluorescent lights.

(3) Using hard tapwater for dilution

These solutions were prepared and stored as in (1) above, but the Sno-Wite was diluted with tapwater obtained from the Kingaroy reticulated supply during a period of prolonged drought. Detailed analysis of this water is provided in Table 1.

(4) In syringes exposed to sunlight

Diluted Sno-Wite was pre-loaded into 5mL disposable syringes (Becton Dickinson Medical, Singapore) with one inch 30 gauge needles attached. The syringes were stored on a window ledge in indirect sunlight. Solutions were tested after 6, 15, 23 and 30 days.

(5) In syringes stored in the dark

Syringes and solution identical to (4) above, were stored in a closed cardboard box, in air-conditioned premises, and tested after 42 and 98 days.

(6) In stainless steel bowls

In this part of the study, solutions of diluted Sno-Wite were placed into stainless steel bowls, both open to the atmosphere and covered with polythene cling-wrap to exclude air. The solutions were stirred prior to sampling and testing, which was carried out after six and five hours respectively.

Undiluted Milton and Sno-Wite were also tested in the following manner:

(7) In containers which were opened and agitated daily

The parent solutions of these two products were stored in identical one-litre plastic bottles, in the same lightproof box and subjected to the same regime of daily opening and agitation as in (1) above, and also tested over eight months.

(8) After storage at 50°C

Four per cent w/v Sno-Wite was placed in 100mL measuring cylinders immersed in water at a constant 50°C. The cylinders were loosely covered to prevent dilution by splash from the water bath and the contents evaluated after five hours.

The five test solutions in (1), (2), (3), and (7) above, were analysed at 35, 98 and 238 days. All solutions tested were tabulated according to the parent hypochlorite solution; type of water used for dilution (if any); storage temperature; whether the container was left open, covered or opened daily; the nature of exposure to light; and the type of container used (Table 2). Changes in chlorine concentration over time were graphed for all solutions, with chlorine content expressed as a percentage of the initial available chlorine concentration.

pH of solutions stored for eight months

The pH of all solutions tested over eight months was measured on the same day as the final titrations, using an appropriately calibrated Activon (Activon, Gladesville, NSW) model 109 pH meter, and tabulated to compare them statistically with the final concentrations of these solutions (Table 3).

Statistics

Statistical methods used to analyse the data varied for different segments of the study. These are specified in reporting the results. For clarity in detailing the preparation and storage of test solutions, textual divisions used in the results section vary slightly from those used in (1) to (8) above.

RESULTS

Solutions stored for eight months

Changes in mean concentration of the five solutions stored over approximately eight months are illustrated in Fig 1. Undiluted Sno-Wite was the most stable solution ($p<0.05$), losing just over 10 per cent of its original strength over the experimental period. However, the stability of Sno-Wite was not significantly different from the other solutions opened and agitated daily at the end of the first and third months ($p>0.05$). Milton was the least stable solution, deteriorating over the eight month period to just over 11 per cent of its initial concentration (mean concentration in nine samples 11.043 per cent with a standard error of 0.530). The other three solutions were not significantly different in mean concentration percentage after eight months ($p>0.05$), their mean values ranging between 41.8 (standard error 3.906) and 47.8 per cent (standard error 9.463). The Sno-Wite diluted with hard water

Table 3. pH and concentration of sodium hypochlorite solutions after storage for eight months while opened daily or left permanently open.

Solutions	Average concentrations %	Average pH
Milton	0.125	8.533
Hard	0.415	9.363
Demin	0.474	9.507
Sno-Wite	3.554	12.517
Open	0.465	9.657

Hard – Sno-Wite diluted with hard water. Demin – Sno-Wite diluted with demineralised water. Open – Sno-Wite diluted with demineralised water and left permanently open.

produced a fine white precipitate in the bottom of the containers of all three samples.

The solution stored in open containers initially had a much greater loss of activity, with its concentration being significantly different from the other solutions at the first and third months ($p<0.05$), the loss of active chlorine concentration decreasing between the third and eighth month. Statistical comparisons of the solutions were based on an approximate test of equality of means when variances are heterogeneous, with the Games and Howell method[17] being used to make pair-wise comparisons. At three months, the data were transformed using the arcsin transformation to achieve approximate normality of the samples and comparisons made on this transformed scale.

Solutions stored in syringes exposed to sunlight

The one per cent w/v solutions stored in syringes exposed to indirect sunlight showed the most dramatic

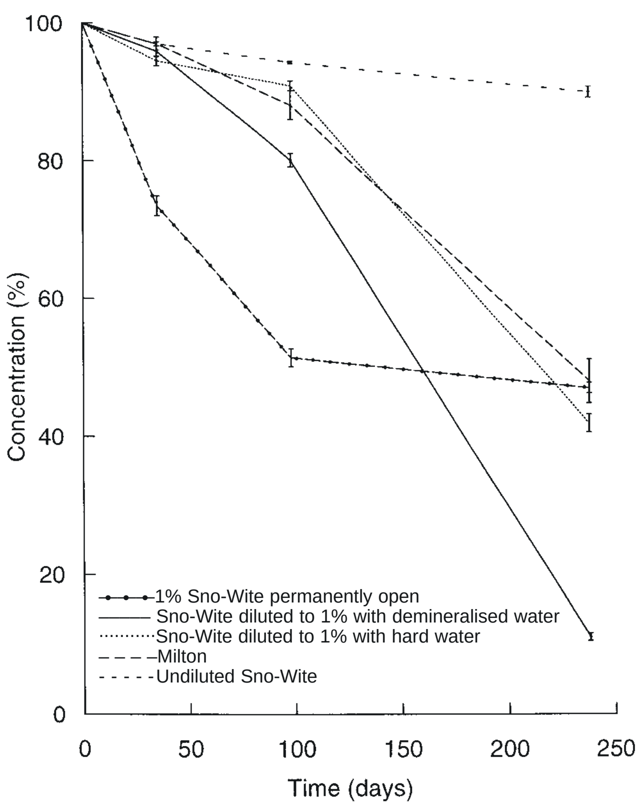

Fig 1. Change in percentage concentration of available chlorine in sodium hypochlorite solutions stored over eight months while opened and agitated daily or left permanently open.

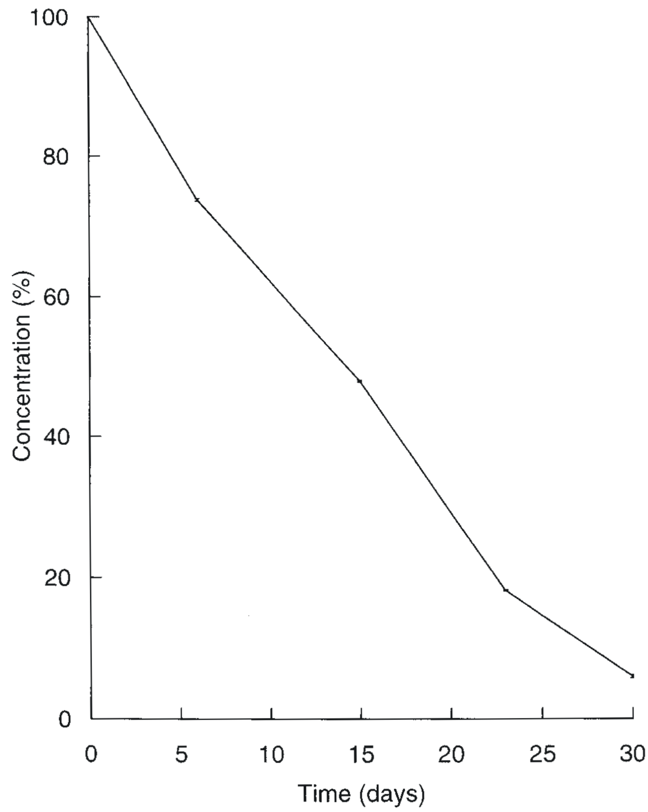

Fig 2. Change in percentage concentration with time of available chlorine in Sno-Wite diluted to 1 per cent when stored in translucent syringes exposed to indirect sunlight.

deterioration in concentration. After six days, the solutions had lost more than 25 per cent of their available chlorine (mean concentration in the three samples 73.7 per cent with a standard error of 0.207) and after 23 days their concentration was less than 20 per cent of that at the start of the experiment (mean concentration in the three samples 18.2 per cent with a standard error of 0.095). Figure 2 displays mean concentrations of solutions exposed to sunlight. Standard error bars are included, though they are particularly small here because of the low standard error.

Solutions stored in syringes kept in the dark

One per cent bleach solutions stored in the dark in syringes identical to those used for solutions exposed to sunlight deteriorated at a much slower rate than the solutions described above (Fig 3). After three months, these solutions still retained more of the original available chlorine content than solutions in syringes exposed to sunlight after 30 days. After six days, mean concentration of the nine samples was 78.7 per cent with a standard error of 0.345, giving 95 per cent confidence that the mean loss in concentration would fall between 20.460 and 22.052 per cent. This and subsequent confidence intervals are constructed assuming normality of the nine samples, where standard statistical tests indicate this assumption is reasonable. The plungers of the syringes showed marked adhesion to the barrel wall and, by the end of

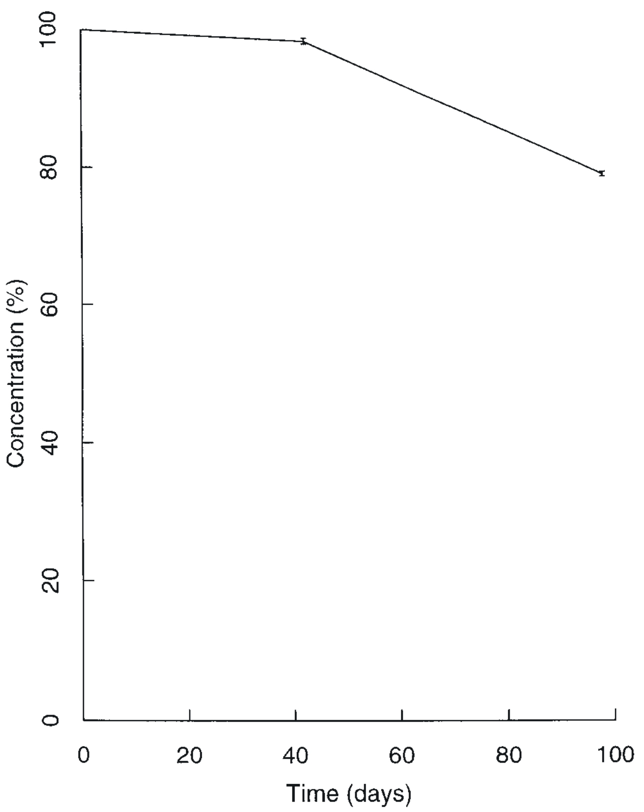

Fig 3. Change in percentage concentration with time of available chlorine in Sno-Wite diluted to 1 per cent when stored in translucent syringes stored in the dark.

the experiment, a black coloration of the hypochlorite solution was apparent adjacent to the plunger. This coloration disappeared rapidly when the solutions were pooled in a beaker ready for testing. When Milton was stored in syringes in the dark, as demonstrated in Fig 4, rust was also apparent by day seven. Obvious rust in droplets in the needle sheath is demonstrated in Fig 4a, and in Fig 4b the lumen of the needle at the bevel end appears totally occluded by rust. For this reason, this section of the study was abandoned.

Sno Wite heated to 50°C

Undiluted Sno-Wite stored at 50°C deteriorated to a mean of only 94.7 per cent of its original concentration after five hours, with a standard error of 0.135, giving 95 per cent confidence that the mean loss in percentage concentration would fall between 4.935 and 5.560 per cent. These changes are graphed in Fig 5 along with those for all solutions stored for less than a day, comparing average percentage concentrations and showing standard error bars for these means. One of the 100mL measuring cylinders used for this section of the study had previously suffered noticeable etching of its inner surface and the solution it contained showed continuous 'gassing' during the entire test period while the other solutions did not. The results reported earlier therefore exclude this cylinder, with its final available mean chlorine concentration being 92 per cent, compared to 97.8 per cent for the other cylinders.

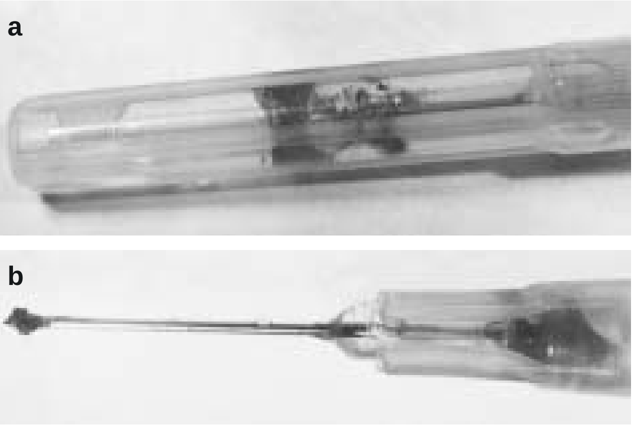

Fig 4. Rust formation after seven days in syringes loaded with Milton and stored in the dark. Fig 4a shows demonstrable rust formation in drops of Milton trapped in needle sheath of syringe. In Fig 4b, the lumen of the 30 gauge needle appears completely occluded by rust.

Solutions stored in stainless steel bowls

The only solutions to show a significant increase in concentration during the study were those stored in open stainless steel bowls. The mean increase in concentration over a period of six hours was 3.5 per cent, with a standard error of 0.150 per cent, giving 95 per cent confidence that the value of the mean increase in concentration percentage would fall between 3.154 and 3.846 per cent. Similar solutions stored in identical bowls tightly covered with clingwrap showed a reduction in available chlorine of the original concentration over a slightly shorter period (five

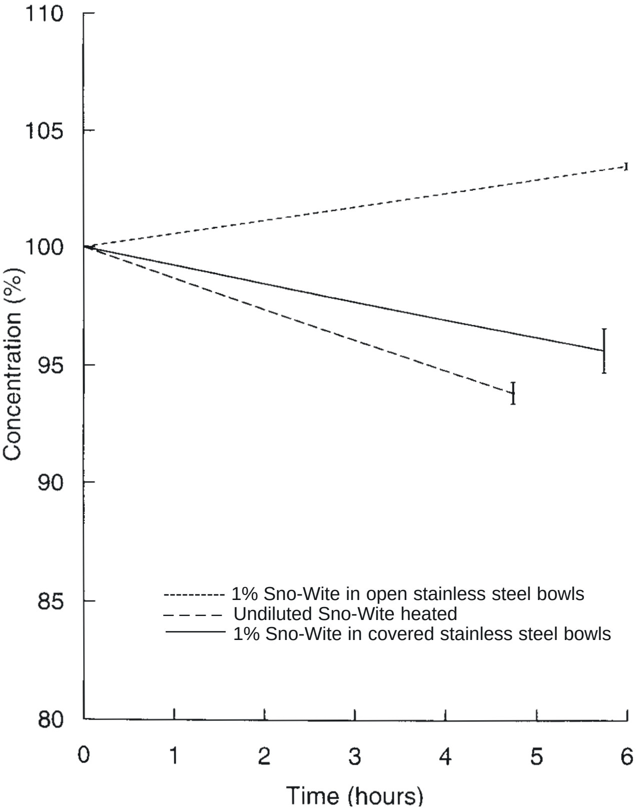

Fig 5. Change in percentage concentration with time of available chlorine of undiluted Sno-Wite heated to 50°C and diluted Sno-Wite stored in open and closed stainless steel bowls for periods up to six hours.

hours). The mean decrease of the percentage concentration was 4.4, with a standard error of 0.948, giving 95 per cent confidence the mean loss would be between 2.168 and 6.542 per cent. When Milton was stored in stainless steel bowls for six hours, there was visible rust formation with marked pitting of the metal surface. For this reason, the result was not reported.

pH of solutions stored for eight months

The pH of each long term solution is listed in Table 3, along with the final mean concentration of available chlorine. Solutions which deteriorated more had lower pH readings – Milton, at 8.6 (standard error 0.052), having the lowest mean pH of the three samples tested. At 12.6 (standard error 0.034), undiluted Sno-Wite showed the highest mean pH in the three samples tested.

To establish and compare time frames within which the loss of concentration did not exceed 10 per cent, some further comparisons were made and confidence intervals constructed. Capped syringes stored in the dark and the 1 per cent w/v solutions opened and agitated daily were compared after three months. The best performing solution after this time was the solution diluted using hard water, with 95 per cent confidence that the mean loss in percentage concentration would give a value between 7.513 and 10.817. This solution was significantly different from Milton ($p<0.05$), where there was 95 per cent confidence that the value would fall between 17.738 and 22.131 for the mean loss in percentage concentration, but the loss was not significantly different from the solution diluted with demineralised water, where there was 95 per cent confidence that the loss of available chlorine concentration lay between 7.287 and 16.462. Solutions in capped syringes stored in the dark had the greatest loss in mean concentration after a period of three months, with 95 per cent confidence limits for these values of 20.572 and 22.052 per cent loss of original concentration, but was only significantly different from the best performing solution diluted with hard water. The comparisons were made on the arcsin scale, using the approximate test of equality of means. The confidence intervals reported have been back-transformed to the original scale. After the shorter one-month period, none of the solutions had measurements with a concentration loss exceeding 10 per cent.

DISCUSSION

In this study, loss of available chlorine concentration due to exposure of bleach solutions to light was much greater than previously reported. One per cent w/v solutions examined by Rutala et al[5] deteriorated to 83 per cent at 30 days when exposed to direct and indirect sunlight, while Aparecida et al[12] reported 2.6 per cent w/v solutions deteriorated to a similar level in continuous artificial light over the same period. This contrasts with less than 10 per cent available chlorine remaining after 30 days for the syringes exposed to sunlight in this study.

In comparing the results of this study to previous studies, consideration should be given to the different storage conditions – syringes rather than translucent bottles. Difficulties in quantifying exposure to light, variations in container volume and variations in transparency of containers make cross-study comparisons unreliable. From a chlorine concentration point of view, when the syringes are stored away from ambient light, preloading of bleach into capped syringes appears to be an acceptable practice.

Use of an open container, such as a stainless steel or plastic bowl from which syringes can be regularly refilled during the course of an appointment, would not appear to result in a noticeably weakened solution over the course of a few hours. Similarly, the results of this study suggest heating four per cent w/v solutions to 50°C for use during a single endodontic appointment would not appear to cause significant loss of available chlorine content. Gambarini et al[13] also reported minimal loss of available chlorine concentration when five per cent solutions were heated intermittently. However, it must be appreciated that accidental spillage from an open container may present a hazard to the clothing and eyes of operator, staff and patients, so care should be taken if this method of delivery is to be used clinically. The increase in concentration of the solution in an open (uncovered) bowl was surprising, and was presumably due to evaporation of water as a result of the large surface area of the solution in relation to its volume and the low relative humidity of the air-conditioned environment. A similar experiment with a covered bowl predictably showed loss of available chlorine due to air exposure and contact with metal.

It does not appear that other researchers have studied the effect of daily opening and agitation of bleach solutions for the purpose of decanting small quantities into syringes. Such procedures result in only modest loss of available chlorine. If a loss of 10 per cent of available chlorine is regarded as acceptable, then domestic bleach diluted to one per cent w/v should have a shelf-life well in excess of one month and perhaps as much as three months. Capped syringes of diluted bleach stored in the dark should have a similar storage life. Undiluted Sno-Wite would appear to have a shelf-life approaching eight months. This latter result is surprising, as more dilute solutions normally have a longer shelf-life, but it is likely the greater content of sodium hydroxide in the undiluted samples may counter the drop in pH associated with exposure of these solutions to carbon dioxide in air. Reduction in pH has a dramatic effect on storage life for sodium hypochlorite solutions.[11] As would be expected, in this study solutions with the lowest end concentrations had the lowest pH (Table 3). In this study, the poor performance of Milton may be explained by differences in the relative content of sodium hydroxide compared to diluted bleach, although Milton does have sodium chloride added as a buffer to reduce its rate of decomposition.

Solutions diluted with hard tapwater might have been expected to deteriorate more rapidly given the presence of metallic ions in such water. It should be stressed that the water used here was very high in salt content and failed to meet National Health and Medical Research Council standards for drinking water. The high sodium content of this water sample may also have had a buffering effect, counteracting the potential reduction in available chlorine content in the solution. Reticulated water from other supplies may not behave as this sample did. The nature of the precipitate in the hard water solutions is not known but may be calcium or magnesium salts precipitated by the high pH of the bleach solutions. Despite its good performance in these tests, water from reticulated supplies cannot be recommended for dilution of bleach because variations in dissolved salts could vary the results and the unknown nature of precipitates may present a hazard.

Initially, diluted bleach solutions stored in open containers showed rapid deterioration but after eight months had similar concentrations to those of identical solutions opened daily. This result is at variance with the findings of Aparecida et al,[12] who reported that 2.6 per cent solutions stored in open containers, while also giving a 40 per cent residual chlorine concentration at eight months, showed less initial deterioration. This result is perplexing but may be explained by the fact that solutions in the current study were stored in a photographic darkroom. Vapour from acetic acid stop baths used for photography could theoretically result in a drop in pH, causing accelerated reduction in chlorine concentration in the early stages.

The results of this study have caused the authors to review their previous endorsement of Milton for endodontic irrigation,[1] due to its relatively poor shelf-life and corrosive properties. Milton is more corrosive of stainless steel than dilute bleach, therefore all Milton/metals contact should be avoided where possible. Milton should not be stored in syringes with needles attached, because of rapid corrosion of the needle, and should not be dispensed from stainless steel bowls. Disposal of Milton into stainless steel sinks and metal drains should be accompanied by flushing with copious quantities of water. A cautious recommendation for the use of domestic bleach, after appropriate dilution, could be made where it is stored undiluted initially then kept in plastic bottles or syringes away from light.

CONCLUSIONS

Undiluted Sno-Wite was the most stable solution of those opened daily and Milton was the least stable solution of those opened daily.

Undiluted bleach should maintain 90 per cent of its original strength for at least six months. Heating of undiluted bleach causes only modest loss of available chlorine content. Initially, diluted bleach left open deteriorated rapidly, but this rate slowed over time.

The contents of syringes exposed to sunlight showed the most rapid loss of chlorine content. If a 10 per cent loss of available chlorine concentration is regarded as the maximum acceptable, then domestic bleach diluted to one per cent w/v with demineralised water should not be stored for more than three months.

Heated bleach lost nearly five per cent of its strength in six hours. Diluted bleach increased its chlorine concentration in open bowls by more than three per cent over five hours.

Containers of Milton or bleach solutions should be tightly closed at all times and stored away from light. The results of this study suggest that, from the point of view of available chlorine concentration, preloading of one per cent bleach solutions into capped, disposable plastic syringes which are prominently labelled is acceptable provided they are stored away from light and discarded after about two months. Under no circumstances should local anaesthetic cartridges be refilled for dispensing sodium hypochlorite. Practitioners should discard any batch of syringes which shows deterioration of the rubber seal, due to risk of the seal sticking to the syringe barrel with forceful injection into the canal and because of the unknown nature of breakdown products.

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