

Original Article

Effect of storage temperature and heating on the concentration of available chlorine and pH of 2.5% sodium hypochlorite

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Abstract

Aim: This study investigates the amount of available chlorine and pH of sodium hypochlorite after dilution and the influence of storage temperature, heating, and aging.

Materials and Methods: A 5.25% sodium hypochlorite solution was diluted to 2.5%, and free available chlorine (FAC) and pH were measured. This was labeled as the control group. The diluted solution was divided into four groups. The first and second groups were stored at 4°C and room temperature, respectively, and FAC and pH were measured at room temperature at intervals of 30 min, 1, 3, and 6 h. The third and fourth groups were stored at 4°C and room temperature, respectively. Samples were collected at intervals of 30 min, 1, 3, and 6 h after heating to 60°C–70°C, and FAC and pH were measured. An iodometric titration method was used for FAC estimation. pH was measured using a digital pH meter. Data obtained were statistically analyzed.

Results: All groups showed depletion in FAC and decrease in pH over time. Available chlorine was utmost in Group III and least in Group II. No statistically significant difference in pH was recorded.

Conclusions: Sodium hypochlorite at room temperature should be used within 3 h of dilution. Its shelf life can be enhanced by storing at 4°C. Heating the solution to 60°C–70°C before irrigation is more efficacious.

Key Words: Free chlorine, pH, shelf life, sodium hypochlorite, storage temperature

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INTRODUCTION

Sodium hypochlorite is the most preferred irrigant in modern endodontic practice owing to its antibacterial, pulp dissolution, and lubricating qualities.^[1] It causes biosynthetic alterations in cellular metabolism and phospholipid destruction. Formation of chloramines cause interference in cellular metabolism and

degrade lipids and fatty acids in bacteria. Chlorine, a strong antioxidant, is released when in contact with organic tissue, resulting in inhibition of bacterial enzymes by irreversible oxidation of the sulfhydryl group.^[2] Therefore, the presence of “available” chlorine is critical for efficacious disinfection.

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In aqueous solution, the available chlorine is the sum of hypochlorous acid (HOCl) and hypochlorite ion (OCl^-). The available chlorine is measured by the oxidizing capacity of the solution and expressed in terms of the amount of elemental chlorine.^[3]

Sodium hypochlorite solutions ranging from 0.5% to 5.25% are recommended as endodontic irrigants.^[4] Certain studies have concluded that the antimicrobial and tissue-dissolving properties occur faster in higher concentrations, but they also pose the risk of toxicity to vital tissues.^[4] 5.25% sodium hypochlorite was efficient in eliminating spores and viruses in addition to the bacteria commonly prevailing in the root canal but posed the risk of toxicity and irritation when forced beyond the root apex.^[5,6] At lower concentrations, the antimicrobial and pulp dissolution properties are lowered. Concentrations of 1% or more are recommended for tissue dissolution and for the purpose of disinfection; the concentration of sodium hypochlorite should be at least 0.5%.^[7] However, it has been verified that 0.5% sodium hypochlorite was less irritant but required more time for the dissolution of organic tissues. 5.25% sodium hypochlorite was more potent in dissolving organic tissue, had better antibacterial action, and faster reaction time. In contrast, it was excoriating to the vital tissues, favoring the use of 2.5% concentration of sodium hypochlorite which showed better antimicrobial action with good tissue dissolution time and lesser cytotoxicity.^[7] Studies have shown that there was a marked decrease in the microhardness of root dentin after irrigation with 6% sodium hypochlorite than a 2.5% solution.^[8] Sodium hypochlorite has deteriorative effects on the biomechanical properties of dentine as it causes degradation of the organic dentine constituents.^[8,9] Thus, 2.5% sodium hypochlorite is recommended for routine root canal treatment procedures.^[7]

Pure hypochlorite solutions used in endodontics have a pH of 12. Investigations have shown that solutions are least prone to decomposition when the pH is above 11. Below pH 11, the rate of decomposition is dependent on the pH, where it rapidly increases from pH 11 to 7, with the peak rate at pH 7.^[9] Hence, the pH of the diluted sodium hypochlorite solution also plays an important role in the stability of the solution.

There is a paucity of literature that assesses the free available chlorine (FAC) and pH in sodium hypochlorite postdilution with distilled water, stored at different temperatures, and its efficacy when heated. The aim of the current study was to investigate the variables of storage temperature, the temperature of the solution while working and time on the FAC and pH of 5.25% sodium hypochlorite diluted to 2.5%.

MATERIALS AND METHODS

Samples

A 5.25% sodium hypochlorite unbuffered solution (Medilise Chemicals, Kannur, India) was used. The bottle was obtained within 3 months of manufacture. It was full at the beginning of the study and was opened only during sampling. As the purpose was to obtain a final concentration of 2.5% sodium hypochlorite, 1000 ml of undiluted sodium hypochlorite was mixed with 1100 ml of distilled water.

Grouping

Control group

A 25 ml sample of the 2.5% sodium hypochlorite was taken, and the FAC was measured by titration with sodium thiosulfate. The pH was determined using a digital pH meter (Labtronics, Panchkula, Haryana, India). This sample was labeled as the control group. The control group, i.e., 2.5% sodium hypochlorite at 0 h aided comparison of percentage loss of available chlorine among various groups over time.

Experimental group

Half of the 2.5% sodium hypochlorite solution was stored at 4°C, and the other half at room temperature in opaque, airtight, nonreactive polyethylene bottles protected from light, mimicking clinical conditions. To evaluate the FAC at different storage temperatures, time, and temperature of the solution, four separate groups were prepared as follows:

- Group I: Sample stored at 4°C, available chlorine calculated at room temperature after 30 min, 1, 3, and 6 h
- Group II: Sample kept at room temperature and FAC calculated at the end of 30 min, 1, 3, and 6 h
- Group III: Sample stored at 4°C, FAC calculated immediately after the solution was heated to 60°C–70°C at intervals of 30 min, 1, 3, and 6 h
- Group IV: 2.5% sodium hypochlorite kept at room temperature, FAC calculated immediately after the solution was heated to 60°C–70°C at intervals of 30 min, 1, 3, and 6 h.

The room temperature was noted to be in the range of 28°C–30°C when the study was conducted. The sodium hypochlorite was heated to 60°C–70°C in a covered beaker using a Bunsen burner, and temperature was determined by a thermometer. A temperature range (60°C–70°C) was used instead of a particular temperature as accurate temperature control devices are unlikely to be available in a clinical scenario.

Determination of free available chlorine

The percentage of available chlorine was measured by iodometric (redox) titration, which involved the conversion of the hypochlorite ion (ClO^-) to iodine (I_2) by the addition of

KI and then titration of the iodine with standardized sodium thiosulfate solution. The measurements were carried out in triplicate for each sample, and the mean value determined.

Measuring the pH

The pH of the samples was recorded with a digital pH meter at the end of 30 min, 1, 3, and 6 h from the time of dilution. Two readings were obtained, and the mean values were calculated.

Statistical analyses

The data were entered into IBM Statistics for Windows (Version 20; IBM Corp, Armonk, NY, USA). The variations in FAC among the groups over time were compared using one-way analysis of variance (ANOVA) with *post hoc* Tukey test. Differences in pH among the groups were compared using independent *t*-test.

RESULTS

The FAC concentration of the control group was adjusted to 2.5%, and the pH was 11.68.

Free available chlorine concentration

All samples showed a reduction in FAC when compared to the control group over time, irrespective of the storage temperature. The loss of chlorine content in Group I when compared to the control group after 30 min of dilution was 7%, 10% after 1 h, and 22% and 35% at 3 and 6 h, respectively. Group II recorded the maximum reduction in chlorine titer. Available chlorine decreased by 14% after 30 min and further by 18%, 42%, and 53% after 1, 3, and 6 h, respectively. Group III showed minimal loss of available chlorine. There was a 6% fall in chlorine titer at 30 min of dilution, 8% at 1 h, and 17% and 28% at 3 and 6 h, respectively. Group IV showed 9% depletion of the initial value at 30 min of dilution, 10% at 1 h, 33% at 3 h, and 46% at 6 h. Comparison using one-way ANOVA test showed that FAC is highest in Group III followed by Group I, Group IV, and least in Group II [Table 1 and Figure 1]. *Post hoc* Tukey test showed that there was no significant statistical difference between any of the groups at 30 min and 1 h of dilution. At 3 h, there was a statistically significant difference between all the groups, except between Group I and III. Comparison of available chlorine at

6 h showed that there was a statistically significant difference between Groups I and II, II and III, and III and IV [Table 2].

pH variations

Although there was no statistically significant difference in pH among all the groups, at all times ($P > 0.05$), it could be observed that the pH was slightly higher in samples stored at 4°C than at room temperature at any given time [Table 3 and Figure 2].

DISCUSSION

The results of the current study support the common impression that sodium hypochlorite solutions are inherently unstable after dilution and are to be used within 3 h of opening the sealed bottle and diluting when intended for endodontic irrigation purposes. Fraiss *et al.*^[9] noted that pH and concentration of the solution, temperature, ultraviolet light exposure, and presence of trace ions play an important role in the decomposition of the hypochlorite ions to chlorate (ClO_3^-) and chlorine (Cl_2) ions. In the current study, a gradual decrease in the pH of all solutions irrespective of the storage temperature was seen over time which was proportional to the decomposition rate of the solutions. However, the decomposition rate in this study can

Table 1: Comparison of free available chlorine depending on storage temperature, heating of solution, and time interval

Time interval	Group	n	Mean	SD	Mean squares	F	P
30 min	Group I	3	2.33	0.07	0.023	2.281	0.156
	Group II	3	2.15	0.17			
	Group III	3	2.35	0.07			
	Group IV	3	2.28	0.06			
1 h	Group I	3	2.25	0.09	0.037	1.925	0.204
	Group II	3	2.04	0.25			
	Group III	3	2.28	0.07			
	Group IV	3	2.26	0.06			
3 h	Group I	3	1.94	0.06	0.242	39.71	<0.001
	Group II	3	1.44	0.07			
	Group III	3	2.08	0.09			
	Group IV	3	1.67	0.09			
6 h	Group I	3	1.63	0.14	0.231	9.403	0.005
	Group II	3	1.17	0.12			
	Group III	3	1.79	0.24			
	Group IV	3	1.34	0.09			

n: Number of samples, F: Test value for ANOVA, SD: Standard deviation, ANOVA: Analysis of variance

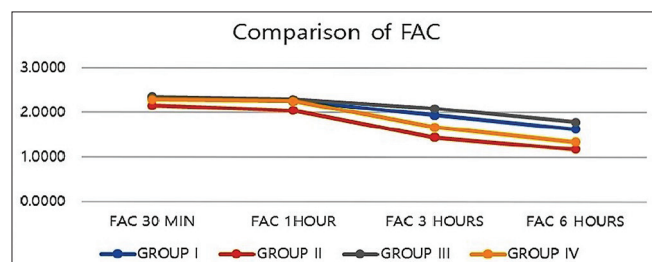


Figure 1: Graphical representation of free available chlorine in various groups

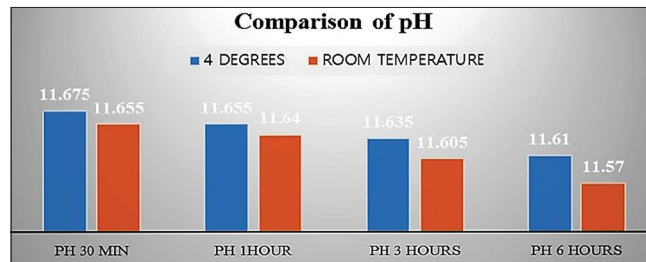


Figure 2: Graphical representation of the pH of sodium hypochlorite in different storage temperatures at various time intervals

Table 2: Intergroup comparison of the free available chlorine

Storage time	Comparison group	Compared with	Mean difference	SE	P
30 min	Group I	Group II	0.18	0.082597	0.209
		Group III	-0.0133333	0.082597	0.998
		Group IV	0.05	0.082597	0.928
	Group II	Group III	-0.1933333	0.082597	0.168
		Group IV	-0.13	0.082597	0.443
		Group IV	0.0633333	0.082597	0.867
1 h	Group I	Group II	0.21	0.113529	0.319
		Group III	-0.03	0.113529	0.993
		Group IV	-0.0033333	0.113529	1
	Group II	Group III	-0.24	0.113529	0.227
		Group IV	-0.2133333	0.113529	0.308
		Group IV	0.0266667	0.113529	0.995
3 h	Group I	Group II	0.49667*	0.06377	<0.001
		Group III	-0.14333	0.06377	0.19
		Group IV	0.26667*	0.06377	0.013
	Group II	Group III	-0.64000*	0.06377	<0.001
		Group IV	-0.23000*	0.06377	0.028
		Group IV	0.41000*	0.06377	0.001
6 h	Group I	Group II	0.45333*	0.12804	0.031
		Group III	-0.16333	0.12804	0.601
		Group IV	0.28667	0.12804	0.192
	Group II	Group III	-0.61667*	0.12804	0.006
		Group IV	-0.16667	0.12804	0.587
		Group IV	0.45000*	0.12804	0.032

*Statistically significant difference ($P < 0.05$). SE: Standard error**Table 3: Comparison of pH of sodium hypochlorite in different storage temperatures at various time intervals**

Storage time	Temperature	n	Mean	SD	t	df	P
pH 30 min	4°C	2	11.68	0.00707	2.828	2	0.106
	Room temperature	2	11.66	0.00707			
pH 1 h	4°C	2	11.66	0.00707	3	2	0.095
	Room temperature	2	11.64	0			
pH 3 h	4°C	2	11.64	0.00707	4.243	2	0.051
	Room temperature	2	11.61	0.00707			
pH 6 h	4°C	2	11.61	0.01414	2.828	2	0.106
	Room temperature	2	11.57	0.01414			

n: Number of samples, t: t value of the independent Student's t-test, df: Degrees of freedom, SD: Standard deviation

be said to be independent of pH value as it was noted to be above 11 at all times, and the drop in pH was not statistically significant in any of the groups.^[9] A similar study by Piskin and Türkün^[1] compared various brands of commercial bleach as a source of sodium hypochlorite as a source of sodium hypochlorite. In their study, they concluded that the pH of the solutions used in their study did not affect the stability; however, storage temperature and concentration of the solutions were said to have significant effects on the degradation rate.

The FAC is the total of HOCl^- and OCl^- concentration in the solution. These molecules are responsible for the chlorinating and oxidizing action on pulp tissue and microorganisms.^[3] The FAC was measured using an iodometric titration method according to Piskin and Türkün^[1] and Fraiss *et al.*^[9] All groups illustrated a negative correlation between the FAC and time. The dilution of sodium hypochlorite resulted in the loss of

available chlorine that was not statistically significant up to 3 h. Solutions kept at room temperature showed maximum loss of available chlorine. This finding is consistent with research done previously by investigators that reported a gradual decline in available chlorine over time.^[1] Based on the report of our study, storage temperature is a crucial factor in the stability of sodium hypochlorite solutions. Solutions stored at 4°C showed a remarkably higher concentration of available chlorine than those kept at room temperature. The fall in FAC is almost 1.5–2 times more in solutions stored at room temperature than those stored at 4°C at the end of 6 h. This is in accordance with studies conducted by Piskin and Türkün^[1] and Hoffman *et al.*^[10] which reported storage of sodium hypochlorite at 4°C kept the solutions stable for a longer period of time.

The content of FAC in sodium hypochlorite solution after heating for endodontic irrigation has not been researched thoroughly. Certain studies have reported that heating was more efficacious in tissue debridement.^[11,12] Other studies demonstrated that the capability of 1% sodium hypochlorite at 45°C to dissolve human pulps was similar to 5.25% solution at 20°C, and a temperature rise of 25°C increased sodium hypochlorite efficacy on steady-state *Enterococcus faecalis* cells by a factor 100.^[13] The bactericidal activity is more than doubled for every 5° increase in temperature in the range of 5°C–60°C.^[14]

In the present study, results show that there was no statistically significant difference in available chlorine versus time between the heated and nonheated solutions at 30 min and 1 h. This is in consistence with experiments carried out by Gambarini *et al.*^[14] which demonstrated both heated and nonheated solutions maintained high available chlorine content and pH values consistent with excellent tissue-dissolving and antibacterial properties. On the other hand, this is in opposition to the study done by Cunningham and Balekjian^[15] which reported that there was degradation of heated samples at 24 h when tested at 37°C. In the current study, a statistically significant difference was seen between Groups I and IV at III h, wherein the chlorine titer was higher in the former. It was also noted that the available chlorine was higher in Group I than in Group IV although the difference was not statistically relevant. This may have occurred due to a difference in storage temperatures; Group I samples were stored at 4°C and Group IV samples at room temperature. Fraiss *et al.*^[9] conducted an experiment to evaluate various factors affecting the FAC concentration. They heated 5.25% samples of sodium hypochlorite in covered and uncovered beakers at 70°C–75°C for up to 4 h. The available chlorine titer was measured at hourly intervals. Results obviously showed a higher concentration of available chlorine in samples that were heated for a longer period of time in uncovered beakers due to evaporation. In the current

study, the use of heated sodium hypochlorite solution that was stored at 4°C shows good stability even at 6 h. Therefore, the use of warm sodium hypochlorite is safer than using nonheated, higher concentrated ones. However, the effect of warm sodium hypochlorite on periapical tissues remains controversial, and further investigation on this has to be documented.

CONCLUSIONS

The results of this study conclude the following:

- Sodium hypochlorite should be used within 3 h of dilution, especially when stored at room temperature. As endodontic procedures seldom exceed 3 h, this problem can be solved by diluting small amounts when required
- Storage temperature plays a pivotal role in the degradation rate of sodium hypochlorite
- Storing sodium hypochlorite in 4°C is optimal for longer shelf life
- The use of warm sodium hypochlorite demonstrated more available chlorine than the nonheated counterparts
- pH of the samples did not affect the stability of sodium hypochlorite in this study.

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Conflicts of interest

There are no conflicts of interest.

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