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Metal Contamination and Stability of Household Bleach and Specially-Formulated Sodium Hypochlorite for Endodontic Use

(Pelumusan Logam dan Kestabilan Peluntur Isi Rumah dan Natrium Hipoklorit Dirumus Khas untuk Kegunaan Endodontik)

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ABSTRACT

The aims of this in vitro *study were to investigate metal contamination and short-term stability of two types of household bleach and a specially-formulated sodium hypochlorite (NaOCl) for endodontic use. The first part of the study was to compare traces of metal elements (Cu, Fe and Ni) between the two types of household bleach (Clorox and Milton) and a specially-formulated NaOCl (CanalPro^{TM} 3% NaOCl, Coltene, Whaledent)) using UV spectrophotometer. The second part of the study was to compare the available chlorine and pH of these different NaOCl formulations at different temperatures. Chemical stability of the NaOCl was assessed by measuring the amount of free available chlorine (FAC) using the iodometric titration assay at the temperature of 15, 30, 45, and 60 °C. The pH of the solutions was measured using calibrated pH meter. The results showed that Milton contained significantly higher concentration of Cu, Fe and Ni compared with the other formulations ($P < 0.001$). Concentrations of Fe detected in Clorox and CanalPro were higher than in the control, distilled water ($P < 0.05$). In all NaOCl samples, the concentration of available chlorine increased with temperature. Concomitantly, there was a significant decrease in pH with increasing temperature ($P < 0.001$) with all the NaOCl formulations. In conclusion, traces of metal remnants could be observed in all the NaOCl formulations, especially in Milton. Heating the NaOCl increases the FAC and decreases its pH.*

Keywords: Free available chlorine; metal element; pH; sodium hypochlorite

ABSTRAK

Kajian in vitro *ini adalah untuk mengkaji pelumusan logam dan kestabilan jangka pendek larutan sodium hipoklorit (NaOCl) pemutih isi rumah berbanding NaOCl yang dirumus khas untuk digunakan dalam rawatan endodontik. Bahagian pertama kajian ini bertujuan membandingkan unsur-unsur logam (Cu, Fe dan Ni) antara NaOCl pemutih isi rumah (Clorox dan Milton) dan NaOCl yang dirumus khas untuk rawatan endodontik (CanalPro) dengan menggunakan spektrofotometer UV. Bahagian kedua kajian ini adalah untuk membandingkan kepekatan klorin bebas tersisa dan nilai pH antara NaOCl pemutih isi rumah dan NaOCl yang dirumus khas pada suhu yang berbeza. Kestabilan larutan NaOCl dinilai dengan mengukur kepekatan klorin bebas tersisa menggunakan ujian pentitratan iodometrik. Kemudian, pH larutan diukur menggunakan pH meter pada suhu 15 °C, 30 °C, 45 °C dan 60 °C. Keputusan kajian menunjukkan larutan Milton mengandungi kepekatan Cu, Fe dan Ni yang ketara berbanding dengan larutan lain ($P < 0.001$). Kepekatan Fe yang didapati di dalam Clorox dan CanalPro adalah lebih daripada air suling ($P < 0.05$), tetapi kepekatan tersebut adalah kurang daripada yang terkandung dalam Milton ($P < 0.001$). Dalam semua sampel, kepekatan klorin bebas tersisa meningkat dengan pertambahan suhu. Pada masa yang sama, pH menurun dengan kenaikan suhu ($P < 0.001$). Kesimpulannya, kesan sisa logam dapat diperhatikan dalam larutan pemutih isi rumah dan larutan NaOCl yang dirumus khas untuk kegunaan endodontik, terutamanya dalam Milton. Pemanasan larutan NaOCl meningkatkan kepekatan klorin bebas tersisa dan mengurangkan nilai pH.*

Kata kunci: Klorin bebas tersisa; pH; sodium hipoklorit; unsur logam

Introduction

Sodium hypochlorite (NaOCl) is the most frequently used endodontic irrigant because of its excellent properties, such as broad antimicrobial activity and tissue-dissolving ability (Senia et al. 1971). NaOCl is a chlorine-containing oxidizer, which exists in solution as hypochlorous acid (HOCl) and hypochlorite ion (OCl^-). These active forms possess high oxidation activity and are proven to be one of the strongest disinfecting agents compare to the other endodontic irrigant solution. The level of free available chlorine (FAC) is the important factor affecting the stability and activity of NaOCl solutions. FAC is the sum of hypochlorous acid and hypochlorite ($HOCl + OCl^-$) in the solution. NaOCl, in concentrations ranging from 0.5% to 6%, is widely used as the root canal irrigant (Siqueira et al. 2000). All the unaltered NaOCl solutions that are available in household bleach and NaOCl specially-formulated for endodontic use have a pH ranging from 1.5 - 12.5 (Jungbluth et al. 2011).

Historically, household NaOCl solutions are produced by passing chlorine gas through a solution of sodium hydroxide (Frais et al. 2001). In recent times, electrosynthesis of NaOCl has become the standard manufacturing practice. Jungbluth et al. (2012) has questioned whether there are any differences in the content of heavy metal remnants such as cobalt (Co), copper (Cu), chromium (Cr), and nickel (Ni) in the electrolytic production process of NaOCl, thereby resulting in a higher potential for toxicity; this question needs to be investigated. It is also unclear if household bleach may be contaminated as their production for domestic use is much less stringent than that for healthcare use. Whether there are any other differences between these formulations in metal element content, and the extent these remnants may result in a higher allergenic or toxicity potential remains unanswered.

To reduce the risks of toxicity associated with high concentration NaOCl while still enjoying its beneficial effects, it has been advocated to use a lower concentration but to increase its temperature (Frais et al. 2001). Cunningham et al. (1980) showed that raising the temperature of NaOCl solutions to 35 °C increased its effectiveness in dissolving necrotic tissue. Another study reported that heating to 60 °C renders it superior in effectiveness, regardless of the concentration (2.6% or 5.25%) of the solution (Abou-Rass et al. 1981). In addition, there were recommendations to improve the effectiveness, increasing its FAC, by heating the NaOCl solution (Frais et al. 2001; Gambarini et al. 1998). However, increasing the temperature of NaOCl can also lead to an increase in ionization activity, thereby increases H+ and altering the $HOCl/OCl^-$ balance. It is uncertain if household bleach and NaOCl specially-formulated for endodontic use will react differently towards temperature changes.

Therefore, the aims of this study were to compare the presence of trace metal elements between the two types of household bleach and a specially-formulated NaOCl solution. The effect of various temperatures on the available chlorine and the pH were also investigated.

MATERIALS AND METHODS

SAMPLE SIZE

Sample size was calculated by using G Power Software (E Erdfelder 2007; Edgar Erdfelder & Buchner 1996) with the effect size of 0.25 and $\alpha = 0.05$. A total of 160 samples was required, 40 per group, to achieve the power of 80%.

SAMPLES AND GROUPING

Two different brands of household bleach (Clorox, Clorox Company, Kuala Lumpur, Malaysia and Milton, Proctor & Gamble Australia, Parramatta, NSW, Australia) were purchased from a supermarket in Kuala Lumpur, Malaysia. A specially-formulated 3% NaOCl solution (CanalPro™, Coltène/Whaledent AG, Altstätten, Switzerland) for endodontic use was purchased from a dental supplier. For each brand, ten bottles were acquired. Distilled water was used as negative control. Immediately after purchase, the solutions were transferred into amber glass bottles and stored at room temperature. Each bottle was fitted with an air-tight screw cap. There were four experimental groups:

1. Group 1: Clorox Regular
2. Group 2: Milton
3. Group 3: CanalPro
4. Group 4: Distilled water (Negative control)

METAL ELEMENT ANALYSIS

Metal element analysis of the samples as performed using a spectrophotometer (DR1900 Spectrophotometer, Hach, CO, USA). Three metal ions, namely iron (Fe), copper (Cu) and nickel (Ni) had been selected for analysis. The spectrophotometer was able to detect Fe in the range of 0.009 to 6.0 mg/L, Cu in the range of 0.001 mg/L to 8.0 mg/L and Ni in the range of 0.006 to 6.0 mg/L. Calibration of the spectrophotometer was carried out according to manufacturer's instructions and a pre-programmed calibration curve was permanently installed in its memory.

MEASUREMENT OF FAC AND pH

For each group, the solutions were randomly subdivided into 10 random samples for testing at four different temperatures (15 °C, 30 °C, 45 °C, and 60 °C). For each, a volume of 15 mL of solution was placed in a glass test tube and heated to 15 °C, 30 °C, 45 °C and 60 °C in an uncovered beaker using a water bath (Memmert, Schwabach, Germany) for 60 min. A mercury thermometer was used to monitor the temperature. 10 mL of the solution was used to calculate the available chlorine solution through iodometric titration and 5 mL was used to measure the pH with a calibrated a pH meter (FiveEasy pH meter, Mettler Toledo, Zürich, Switzerland).

The iodometric titration used in this study was in accordance with the standards of the American Society for Testing Material International (ASTM D 2022-89). The chemical assessment for the percentage of concentration of FAC (weight/volume) of the samples was performed at laboratory room temperature. This was determined with an iodine titration procedure with a standardized 0.1N sodium thiosulphate (Na_2S_2O) solution, after acidification of the NaOCl solution with acetic acid. Each sample was analysed three times. The titration was carried out with pipettes with an accuracy of 0.05 mL.

The content of FAC in sodium hypochlorite was calculated based on the following equation;

$$\%Cl_2 = \frac{V1N\ meqCl2 * 100}{Valiq\ S}$$

where V_1 is the $Na_2S_2O_3$ volume required in the titration; N is the normality of the solution of $Na_2S_2O_3$; $meqCl_2$ is the milliequivalents-gram = 35.5×10^{-3}; V_{aliq} is the volume of the aliquot for dilution used in titration; and S is the specific gravity = 1.1.

STATISTICAL ANALYSIS

The data collected was transferred and analysed using the SPSS Version 22.0 (IBM, Armonk, NY, USA). Distribution of data was examined using Shapiro-Wilk test. The test indicated that the data was normally distributed (p>0.05). Statistical analysis was performed using One-way analysis of variance (ANOVA) test was used to compare metal ion release between each brand of sodium hypochlorite. Mixed ANOVA was used to compare the available chlorine concentration and pH between the two types of household bleach and the NaOCl specially-formulated for endodontic use at various temperatures.

RESULTS AND DISCUSSION

COPPER (Cu), IRON (Fe) & NICKEL (Ni)

Milton had the highest Cu, Fe, and Ni concentration detected compared to the other solutions. The mean Ni concentration in Clorox, CanalPro, and distilled water was below the detection limit (<0.006 mg/L). Comparison using one-way ANOVA test indicated that there is statistically significant difference of Cu, Fe and Ni concentration between different solutions (Table 1).

TABLE 1. The mean concentration of copper, iron and nickel (mg/L) in different solution

	Mean ± Standard deviation				P value
	Clorox	Milton	CanalPro	Distilled water	
Copper (Cu)	0.018 ± 0.01	0.582 ± 0.09	0.023 ± 0.01	0.010 ± 0.00	<0.001*
Iron (Fe)	0.029 ± 0.01	0.070 ± 0.02	0.042 ± 0.02	0.010 ± 0.00	<0.001*
Nickel (Ni)	0†	0.048 ± 0.01	0†	0†	<0.001*

*Statistical significance in one-way ANOVA, † below detection limit

FAC CONCENTRATION

The present study showed that there was a statistically significant difference in the available chlorine between the solutions at 15 °C, 30 °C, 45 °C, and 60 °C. The results showed a significant influence of temperature ($P < 0.001$) on available chlorine (Table 2). Available chlorine increased with increasing temperatures as shown in Figure 1. Thus, amongst the tested solutions, Milton at 15 °C has the least available chlorine (1.378) while Clorox at 60 °C has the most FAC (4.644). There were significantly higher levels of FAC for all NaOCl solutions at 60 °C compared to 15 °C, but the distilled water remained constant at zero despite temperature increments.

TABLE 2. The mean of available chlorine in different solution at different temperature

Group	Mean & Standard deviation				P value
	15 °C	30 °C	45 °C	60 °C	
Clorox	4.349 ± 0.07	4.456 ± 0.03	4.565 ± 0.03	4.644 ± 0.06	
Milton	1.378 ± 0.01	1.394 ± 0.01	1.419 ± 0.01	1.442 ± 0.01	<0.001*
CanalPro	3.201 ± 0.02	3.230 ± 0.02	3.295 ± 0.02	3.413 ± 0.02	
Distilled water	0	0	0	0	

*statistical significance in one-way ANOVA test

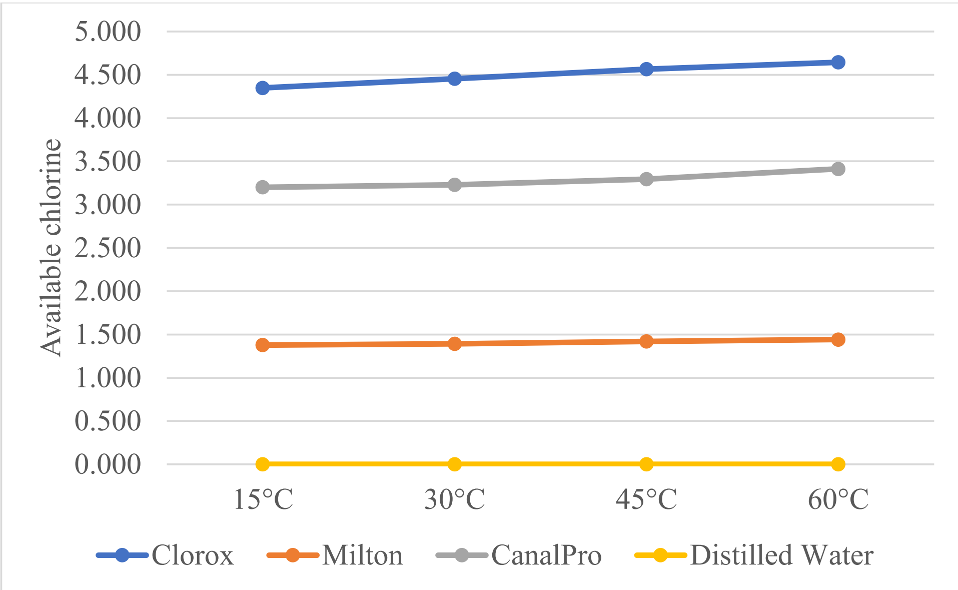

FIGURE 1. Mean available chlorine of various solutions at different temperature

pH

In all samples, the pH decrease with increased temperature as shown in Figure 2. All our test and control group; which included Clorox, Milton, CanalPro, and distilled water showed decreasing pH with the increase in temperature. Comparison using two-way repeated measure ANOVA test indicated statistically significant difference of pH between the solutions at different temperatures. Statistically significant difference was observed for all the solutions at the tested temperatures. Thus, among the tested solutions, CanalPro at 15 °C has the highest pH (13.1) while Milton at 60 °C has the lowest pH (11.2).

TABLE 3. The mean of pH in different solution at different temperature

Group	Mean & Standard deviation				P value
	15 °C	30 °C	45 °C	60 °C	
Clorox	12.317 ± 0.02	12.233 ± 0.07	11.847 ± 0.04	11.772 ± 0.04	
Milton	11.609 ± 0.05	11.510 ± 0.03	11.398 ± 0.05	11.238 ± 0.05	<0.001*
CanalPro	13.101 ± 0.02	13.059 ± 0.01	13.000 ± 0.02	12.900 ± 0.05	
Distilled water	6.976 ± 0.01	6.956 ± 0.01	6.884 ± 0.01	6.809 ± 0.01	

*statistical significance in one-way ANOVA test

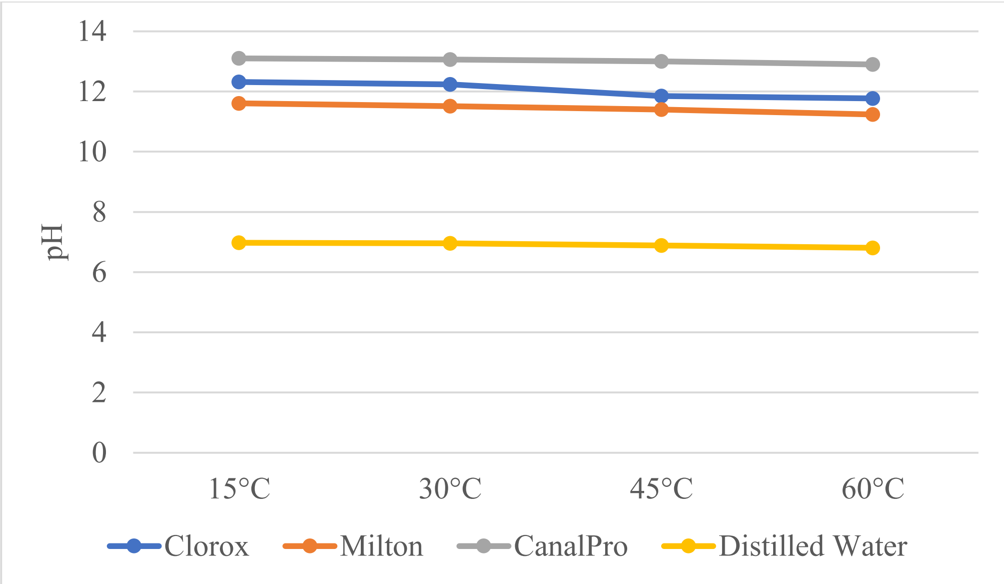

FIGURE 2. pH of various solutions at different temperature

In this study, the results showed that Cu was detected in Milton, CanalPro, Clorox and distilled water at concentrations of 0.582, 0.023, 0.018, and 0.010 mg/L, respectively. Since the Cu concentration of CanalPro and Clorox were below 0.5 mg/L, it was considered to meet the minimum standard. In contrast, Milton showed slightly higher concentration of Cu than the 0.5 mg/L benchmark for NaOCl processing. However, it is less than the maximum allowable level of Cu as regulated by USEPA (United States Environmental Protection Agency 2018). The major factor influencing the elevated Cu ion levels in Milton could be attributed to the copper water lines involved in the manufacturing process (Powell Fabrication & Manufacturing, Inc. 2015). Another source of Cu could be from metal containers. According to Clarkson et al. (1998), depending on the roof, tank and pipe material, some metallic ions may come from storage tanks. Cu is known to be a catalyst of NaOCl decomposition, causing NaOCl to break down more rapidly (Clarkson & Moule 1998).

The Fe content in Milton, CanalPro, Clorox, and distilled water were 0.070, 0.042, 0.029 and 0.010 mg/L, respectively. In the present study, CanalPro, Clorox and distilled water contained a much smaller amount of iron compared with Milton solution. Nevertheless, considering that the amount of Fe detected in all the NaOCl solutions tested is much less than the typical specifications for Fe, 0.5 mg/L in NaOCl (The Chlorine Institute 2011) and 0.3 mg/L in water (USEPA 2018), Milton, CanalPro and Clorox could be regarded as acceptable for endodontic use. Nevertheless, the presence of Fe may cause discolouration of teeth because Fe^{+3} is one of the strongest chromophores. However, NaOCl only starts to turn a slightly reddish-brown colour when the Fe levels exceed 1 mg/L (Odyssey Manufacturing Company 2007). Since the Fe concentrations found in Milton, CanalPro and Clorox were all below 1 mg/L, concerns about tooth discolouration would not be an issue.

In the present study, the highest total amount of nickel was detected in Milton (0.048 mg/L). The level of Ni in Clorox, CanalPro and distilled water was below the detectable limit. Typical specifications of Ni is < 0.05 mg/L for the manufacturing of domestic bleach (Powell Fabrication & Manufacturing, Inc. 2015) and 0.1 mg/L in water (USEPA 2018). Considering the value of Ni concentrations in all NaOCl solutions tested were below the standards, it can be concluded that the Ni concentrations in Milton, Clorox and CanalPro were within safety limits.

In the present study, the lowest available chlorine of both household and specially-formulated NaOCl solutions was obtained at a temperature of 15 °C, while the highest available chlorine was achieved when the solutions were heated to 60 °C. Post-hoc Tukey test showed significant differences between the mean of available chlorine of all NaOCl solutions as temperature increased (Table 3). However, the findings are inconsistent with the findings by Sirtes et al. (2005), who showed that NaOCl solutions of 1%, 2.62%, and 5.25% maintained constant availability of chlorine in spite of temperature changes and variable heating periods. The increase in concentration noted in the present study might be due to the fact that the solutions, in the uncovered glass test tubes, suffered evaporation of water, thereby shifting the equilibrium towards dissociation of ions.

According to Le Chatelier's principle, increasing the temperature of the reaction increases the internal energy of the system. When a liquid is heated, the molecule kinetic energy increases. The energy of this movement is enough to overcome the forces that bind the molecules together, allowing the liquid to become more fluidity and decreasing its viscosity (Giardino et al. 2016). This phenomenon could also explain the increasing amount of HOCL and OCl^- (Wright et al. 2017).

The present study confirms that the FAC differed by the type of NaOCl solutions. Clorox showed the highest FAC concentration followed by CanalPro and Milton at all temperatures. Clorox is 5.25% NaOCl, whereas Milton is 1% NaOCl. Hence, the FAC of Clorox is higher than that of Milton. The lower NaOCl concentration is because Milton is marketed primarily as a sterilizing solution for breastfeeding equipment, milk bottles and baby feeding accessories. CanalPro is available in both 3% and 6% NaOCl concentrations; the 3% NaOCl formulation was used in this study. Interestingly, although the NaOCl concentration (and FAC) is lower, the manufacturer reported in their marketing brochure that the tissue dissolution capability of CanalPro is superior than conventional NaOCl because surface active agent was added to CanalPro (Coltene 2018; Stojicic et al. 2010).

In the present study, the test solutions reacted differently with increasing temperature. The available chlorine remained at zero at the different temperature, while both household bleach and specially-formulated NaOCl display variable increment rate of FAC with increasing temperature. The increment rate of FAC was the lowest for Milton, probably because of the lower existing FAC (1%) to start with.

The results of this study suggest heating NaOCl solutions to 60 °C for endodontic use in a single visit appointment will increase the FAC and it would not result in existing chlorine loss. In contrast, Gambarini et al. (1998) reported a slight loss of FAC when 5% solutions were heated intermittently; however, measurement of FAC were only taken after the samples were allowed to cool at room temperature. Conversely, in this study, the measurements were taken immediately after completing the cooling or heating without any delay, to avoid further decomposition.

In the present study, the pH of all NaOCl solutions decreased with increasing temperature. The mean pH for all type NaOCl solutions after heating to 60 °C were in the range of 11.2 - 12.9. Even though there was a reduction of pH when temperature increased, both types of household bleach and specially-formulated NaOCl solution maintained high pH (> 11). This finding is consistent with a recent study by de Wright et al. (2018) who investigated the influence of heating of NaOCl admixed with etidronate and ethylenediaminetetraacetic acid (EDTA); they found that there was reduction in the pH when the solution was heated to 35 °C.

Ionization usually increases with temperature. The reduction in pH is because hypochlorous acid (HOCl) rapidly dissociates into hydrogen ions (H^+) and hypochlorite ions (OCl^-) when the temperature is increased. Theoretically, as the pH of NaOCl decreases, more HOCL is formed and the antibacterial activity should increase. It is known that the bactericidal activity is greater at acidic pH (<6). Hence, tracking the changes in pH over time could be a useful means to assess the antibacterial efficacy of NaOCl. Assessing pH can be performed in clinical situation and it is less complex than measuring FAC.

When other types of acid are added in NaOCl, this will decrease the pH and increase antibacterial effect (Rossi-Fedele et al. 2011). Therefore, apart from heating the NaOCl, antibacterial effects could also be improved by formulating more acidic solutions to drive the equilibrium towards formation of HOCl. Nevertheless, the reduction of pH through heating NaOCl is minimal, but addition of acid could drastically reduce the pH. The additional H^+ depletes the OCl^- rapidly, thereby, reducing the tissue dissolving capability and compromising the shelf-life of NaOCl.

The findings of this study indicate that different types of NaOCl solution had different overall pH across temperature changes. Milton, the lowest NaOCl concentration (1%), had the lowest pH; hence, it is more easily decomposed. All NaOCl solutions contain sodium hydroxide with a resulting high pH, but Milton also contains additional sodium chloride as a buffer to reduce its rate of decomposition (Clarkson et al. 2001).

The findings of this study confirm that temperature has an effect on the pH of NaOCl. Although the overall pH drop could be observed with all NaOCl solutions, the rates of pH drop were different. The pH drop was noticeable for Clorox, particularly between 30 °C - 45 °C. The reason for this distinctive pattern remains unclear.

Conclusion

From the results of this study, it may be concluded that trace metals were found in the two types of household bleach and the specially-formulated NaOCl tested. Milton had the highest Cu, Fe and Ni contamination compared to Clorox and CanalPro. However, none of the NaOCl solutions tested should raise health concerns if used as endodontic irrigant as the concentrations of Cu, Fe and Ni are within the recommended safety limits. Heating of NaOCl solutions resulted in a significant increase of the available chlorine while inversely reducing its pH. The results were particularly marked when NaOCl was heated to 60 °C. Heating may enhance the chemical activity of NaOCl, thereby improving the ability to disinfect the root canal system more effectively but it will also alter its pH.

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