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Evaluation of Time-Dependent Chemical Alterations in Sodium Hypochlorite Solution Kept on the Unit Tray During an Average Root Canal Treatment Period

Authors' Contribution:

Study Design A

Data Collection B

Statistical Analysis C

Data Interpretation D

Manuscript Preparation E

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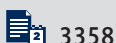
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Background: Sodium hypochlorite (NaOCl) is the most commonly used irrigant in root canal treatment because of its antimicrobial activity and tissue-dissolving capacity. Since these properties are related to its physicochemical characteristics, changes during chairside handling may be clinically relevant. This study aimed to evaluate time-dependent changes in pH, active chlorine content, and surface tension of NaOCl kept in transparent, open-lid containers during a typical chairside root canal treatment period.

Material/Methods: A commercially available 5% NaOCl solution was tested under 3 conditions: fresh (T_0), after 1 hour (T_1), and after 2 hours (T_2) of exposure in standardized transparent, open-lid containers under ambient clinic conditions ($\approx 22^\circ\text{C}$). For each outcome (pH, active chlorine content, and surface tension), 3 groups were evaluated ($n=10$ per group), yielding 90 measurements in total (3 outcomes \times 3 groups \times $n=10$). Data were analyzed using one-way analysis of variance followed by Duncan's multiple-comparison test ($P<0.05$).

Results: pH decreased significantly after 1 hour and further decreased after 2 hours, compared with fresh NaOCl ($P<0.05$). Surface tension did not differ significantly among groups ($P>0.05$). Active chlorine values did not differ between T_0 and T_1 ($P>0.05$), whereas T_2 showed significantly higher measured active chlorine than both T_0 and T_1 ($P<0.05$).

Conclusions: In this in vitro study, leaving NaOCl in transparent, open-lid containers for up to 2 hours was associated with decreased pH and increased measured active chlorine values, while surface tension remained unchanged. Further studies are needed to determine the clinical relevance of these physicochemical changes.

Keywords: Chlorine • pH • Sodium Hypochlorite • Surface Tension**Full-text PDF:** <https://www.medscimonit.com/abstract/index/idArt/952416>

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Introduction

Given the irregular and complex structure of the root canal system, effective irrigation using a suitable solution is necessary to remove pulp, microorganisms, and dentin residues in conjunction with mechanical instrumentation. An ideal irrigation agent should dissolve vital and necrotic pulp tissue while neutralizing microorganisms and their byproducts [1,2]. Sodium hypochlorite (NaOCl), with its exceptional tissue-dissolving properties, antimicrobial efficacy, and ability to neutralize toxic by-products, stands as the cornerstone of irrigation solutions in endodontics [3]. The effectiveness of this solution is influenced by various factors, including concentration, active chlorine content, pH, and surface tension [4-6].

NaOCl demonstrates its antibacterial and tissue-dissolving activity by engaging the active chlorine in its content with bacteria, biofilm, and the organic contents of pulp tissue [7]. It has been observed that the available chlorine level in the solution can decrease when exposed to air, light, certain temperatures, and organic and inorganic pollutants [8]. Similarly, a change in pH value can lead to significant changes in the chemical properties of the solution by altering the ratio of hypochlorite (OCl^-) and hypochlorous acid (HOCl^-) in the NaOCl solution [9]. A decrease in pH value enhances the antibacterial activity of the solution by increasing the ratio of HOCl^- , a potent antiseptic. However, it can also lead to a significant decrease in the solution's tissue-dissolving capacity, which can impact the thorough cleaning of the root canal system, thereby emphasizing the importance of maintaining a balanced pH value of the solution [10-12]. Conversely, an increase in the pH value can have the opposite effect [13]. Therefore, it is crucial to maintain the pH level of the solution at values that can provide this balance, to ensure both the antibacterial and tissue-dissolving effects.

Another factor that influences the effectiveness of irrigation solutions is the solution's surface tension and contact angle properties, which determine its penetration into the dentin surface and tubules [14]. Research has revealed that reducing the surface tension of NaOCl can increase its penetration into irregular areas of the root canal system and tubule depths [15]. This significant finding emphasizes the need for further research on the environmental factors that may affect the surface tension value of NaOCl. While there are numerous studies in the literature on the chemical stability and shelf life of NaOCl solutions [16-21], to the best of our knowledge, there is no study that evaluates the changes that can occur depending on the conditions under and duration for which the solution remains on the unit table. Chairside time for a typical non-surgical root canal appointment commonly falls within 60 to 120 minutes, with clinical studies reporting approximately 60 to 75 minutes for single-sitting procedures and up

to approximately 112.5 minutes total treatment time in randomized trials [22-24]. Therefore, in this study, we aimed to determine changes in pH, active chlorine amount, and surface tension of NaOCl solutions stored in transparent open-lid containers over 1- and 2-hour intervals.

Material and Methods

Preparation of NaOCl Samples

A commercially available 5% NaOCl solution (Mikrovem AF, Istanbul, Türkiye) for dentistry was used for this study. To minimize batch-related variability, all samples were prepared from a single bottle (same batch/lot) opened at the beginning of the experiment. Three experimental groups were established: fresh NaOCl analyzed immediately (T_0), NaOCl exposed to ambient conditions for 1 hour (T_1), and NaOCl exposed to ambient conditions for 2 hours (T_2). Each group consisted of 10 independent samples (20 mL per sample) placed in standardized, unused transparent polyethylene terephthalate (PET) cups with open lids.

Environmental conditions during exposure were those of a typical endodontic operator. Room temperature was maintained at approximately 22 °C (air-conditioned clinical room). Light intensity (lux), relative humidity, and airflow were not instrumentally recorded. No direct sunlight was allowed on the samples.

pH Measurements

The pH values of NaOCl samples were measured using a digital pH meter (HANNA Instruments, Romania) (Figure 1). To ensure homogeneity, each sample was placed on a magnetic stirrer in a beaker. The pH meter was calibrated for accuracy prior to measurement. While the stirrer was operating, the pH probe was immersed in the solution, and readings were recorded after 1 minute of stabilization. All measurements were performed by a single trained operator. Each sample was measured once. This process was systematically applied to all 3 sample groups: fresh NaOCl (n=10), NaOCl stored for 1 hour (n=10), and NaOCl stored for 2 hours (n=10).

Surface Tension Measurements

The density of NaOCl solutions was determined using a precision 10-mL pycnometer (Çalışkan LG025.31.0010, Ankara, Türkiye). After thorough cleaning with ethyl alcohol, the pycnometer was dried and weighed with its stopper on a Denver Instrument SI 234 balance. Then, the tare weight was recorded. The pycnometer was filled with freshly prepared distilled water, ensuring no air gaps, and the stopper was secured. Thereafter, the exterior was dried, and the combined mass

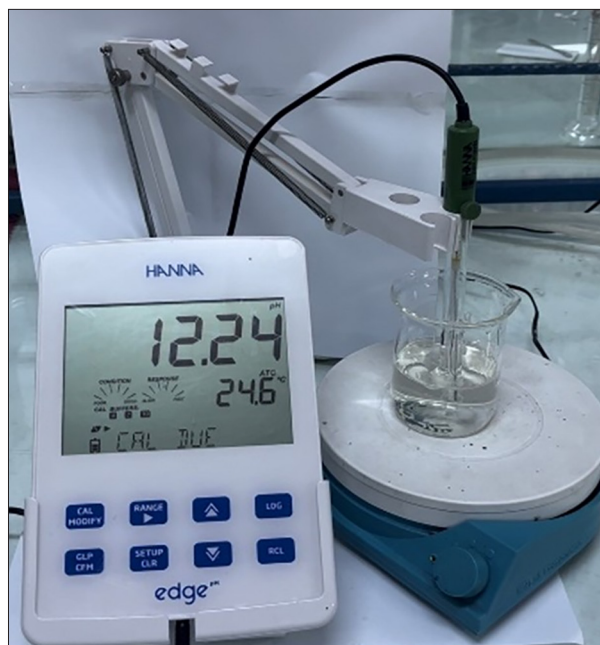


Figure 1. Digital pH meter.

of the filled and empty pycnometer was recorded. The difference between these 2 weightings was the mass of distilled water. Subsequently, the volume of the pycnometer was computed by dividing the mass of the water by its density, taken as 0.997 g/cm^3 at approximately 25°C [25]. After rinsing and drying the pycnometer, it was filled with the NaOCl solution, and the mass was rerecorded. Density was calculated as the difference in mass between the full and empty pycnometer divided by the pycnometer's volume. This process was replicated for all NaOCl samples, including fresh samples ($n=10$) and those kept open for 1 hour ($n=10$) and 2 hours ($n=10$).

For measuring the surface tension of NaOCl samples, a 3.5-mL Traube stalagmometer (Çalışkan, LG028.20.00, Ankara, Türkiye) and the drop counting method were used. All stalagmometric measurements were performed by a single calibrated operator to minimize operator-dependent variability. Measurements were conducted at room temperature ($\approx 22^\circ\text{C}$). Each sample was measured once. The surface tension was calculated based on the number of drops falling from the stalagmometer, the density of the NaOCl samples measured with a pycnometer, and the surface tension of the reference liquid.

In addition, freshly prepared distilled water was used as the reference liquid for this study. Before beginning the experiment with the prepared NaOCl solutions, the number of drops formed by the distilled water with known surface tension in the determined volume V was counted and noted (Figure 2). The drop-counting process began when the liquid level reached point A. The last drop was taken at point B to determine the number of drops counted in the total volume, V . First, the number

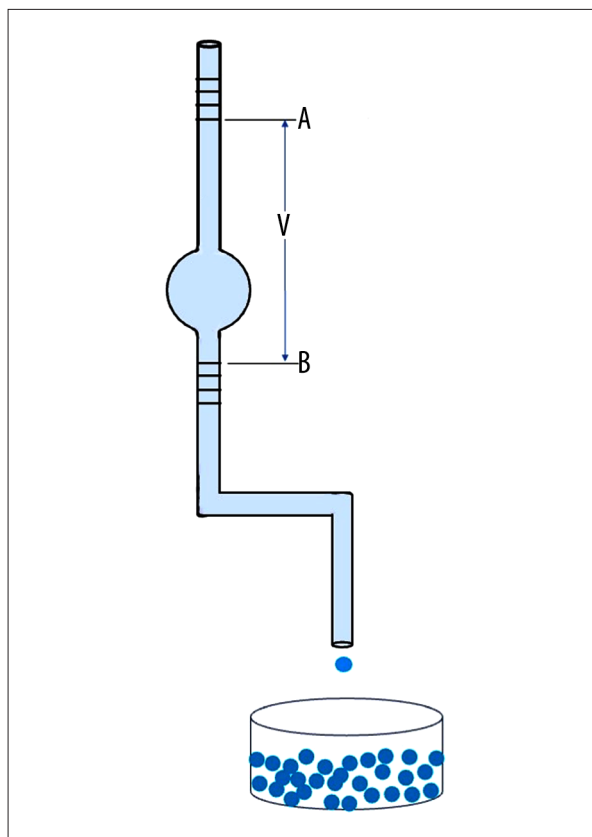


Figure 2. Traube stalagmometer drop counting method. **A:** The drop-counting process begin when the liquid level reached point A. **V:** Determined volume of the solution. **B:** The last drop is taken at point B to determine the number of drops counted in the total volume, V .

of drops of water was determined; thereafter, the number of drops of NaOCl solutions in fresh, 1-hour, and 2-hour samples were determined and noted, respectively.

The surface tension of water was taken as approximately σ_W : 72 dyn/cm at around 25°C [26]. Since the surface tension is directly proportional to the drop mass and inversely proportional to the number of drops, the surface tension of the NaOCl solution in the samples was calculated using the following formula:

$$\sigma_L = (\sigma_W \times N_W \times \rho_L) / (N_L \times \rho_W),$$

where σ_L =surface tension of the liquid, σ_W =surface tension of water, ρ_L =density of the liquid, ρ_W =density of water, N_W =number of drops in water, and N_L =number of drops in liquid.

Active Chlorine Measurements

Purified water (1 L) was boiled for 5 minutes and then allowed to cool. Thereafter, 12 g of sodium thiosulfate pentahydrate ($\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$; molar mass: 248 g/mol) was dissolved in the

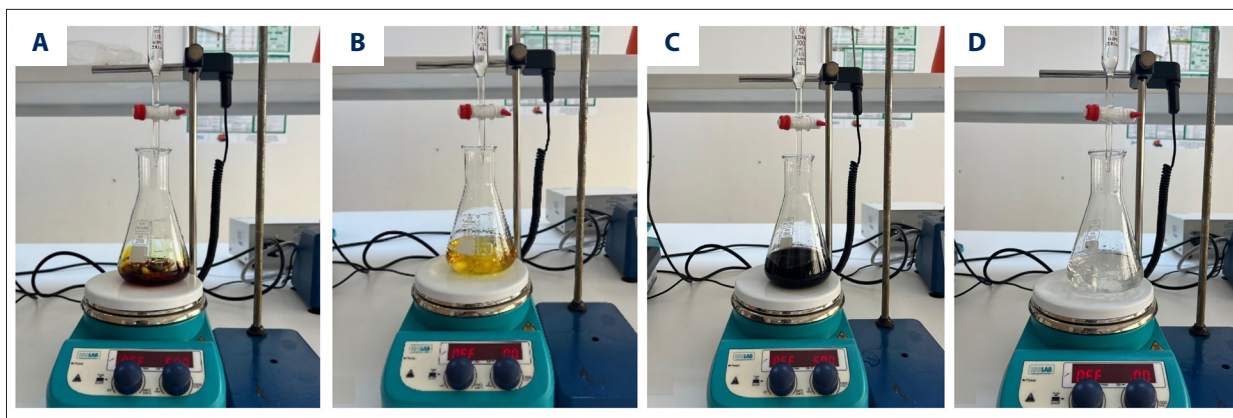
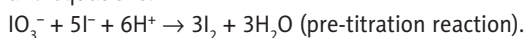


Figure 3. (A) Brown-colored solution before titration. (B) Light-yellow-colored solution after titration. (C) Blue-colored solution after adding 3 mL of starch solution. (D) Colorless solution after titration.

water, followed by the addition of 0.01 g of sodium carbonate (Na_2CO_3). The mixture was stirred until fully dissolved and then transferred to an opaque bottle for storage.

Thereafter, a 2% starch solution was prepared. Approximately 0.32 g of dried potassium iodate (KIO_3) was dissolved in a 250 mL graduated flask. To 50 mL of this solution, 2 g of potassium iodide (KI) and 10 mL of hydrochloric acid (HCl) were added. This mixture was titrated with the standardized sodium thiosulfate ($\text{Na}_2\text{S}_2\text{O}_3$) solution until a light-yellow endpoint was reached. Following this, 3 mL to 5 mL of starch solution was added, and titration was continued until the blue color disappeared (**Figure 3**). The concentration of the $\text{Na}_2\text{S}_2\text{O}_3$ solution was calculated using the following relevant reactions and equations:



At the equivalence point, 6*moles of iodate=mole $\text{Na}_2\text{S}_2\text{O}_3$.

A graduated burette was filled with $\text{Na}_2\text{S}_2\text{O}_3$ solution, and the initial volume was recorded. Approximately 10 mL of NaOCl was measured into a tared 50 mL beaker, weighed to the nearest 0.1 mg, and then transferred to a 250-mL conical flask. After adding 5 mL of glacial acetic acid, approximately 2 g of KI was introduced and mixed. The solution was allowed to stand for approximately 5 minutes in the dark to ensure reaction completion. Once a brown color developed, titration with the $\text{Na}_2\text{S}_2\text{O}_3$ solution was conducted using a magnetic stirrer until the color transitioned to light yellow. Upon adding 3 mL of starch solution to this solution, a blue-colored complex was formed. This solution was then titrated with sodium thiosulfate ($\text{Na}_2\text{S}_2\text{O}_3$) until it became colorless, thus marking the endpoint. The final volume of thiosulfate utilized was recorded, and the active chlorine (AC) percentage was calculated using the specified formula $[\text{AC}\% = (\text{V}_1 \times \text{M}_1 \times 3.546^*)/\text{m}]$, where V_1 : volume of standardized sodium thiosulphate solution; M_1 :

molar concentration of standardized sodium thiosulphate solution; and m : weight of the sample analyzed (*1 mL of 0.1 mol/L sodium thiosulfate pentahydrate equals 3.546 mg active chlorine)]. The data obtained were documented for statistical analysis. All titrations were performed by a single trained operator. Evaporation during the 1- to 2-hour exposure period was not directly quantified.

Statistical Analysis

Descriptive statistics for continuous variables, which are among the characteristics under consideration, were expressed as median, mean, standard deviation (SD), minimum (min), and maximum (max) values. For continuous variables, the Kolmogorov-Smirnov test was used to assess normality, and the Levene test was used to assess homogeneity of variances. Following these tests, a one-way analysis of variance (ANOVA) was performed to compare group means for normally distributed characteristics. Duncan's multiple-comparison test was used to identify differences among groups after the ANOVA. The statistical significance level was set at 5%, and the SPSS (version 21) statistical package program was used for the calculations.

Results

The mean, SD, min, max, and P values of the pH levels of fresh, 1-hour, and 2-hour NaOCl samples are presented in **Table 1**. A statistically significant difference was determined between the pH values of the groups ($P < 0.05$). The pH value of the T_1 samples was significantly lower than that of the T_0 samples ($P < 0.05$), while the pH value of the T_2 samples was significantly lower than that of the T_0 and T_1 samples ($P < 0.05$).

Table 2 presents the mean, SD, min, max, and P values of the surface tension values determined by the Traube stalagmometer for the 3 NaOCl groups. No statistically significant difference

Table 1. Mean, standard deviation (SD), minimum (Min), maximum (Max), and *P* values of NaOCl solutions; pH measurements at 3 different time points.

Group	Mean±SD	Min	Max
Fresh (T ₀)	12.266±0.139 ^a	12.00	12.41
1. hour (T ₁)	10.320±0.215 ^b	9.96	10.63
2. hour (T ₂)	10.087±0.307 ^c	9.75	10.79

$p_{T_0-T_1}=0.001$; $p_{T_0-T_2}=0.001$ $p_{T_1-T_2}=0.043$. T₀: Immediate measurement after unboxing; T₁: measurement at 1 hour; T₂: measurement at 2 hours. Different lowercase letters indicate statistically significant difference between the time points (Duncan post hoc test; $P<0.05$).

Table 2. Mean, standard deviation (SD), minimum (Min), maximum (Max), and *P* values of NaOCl solutions; surface tension (dyn/cm) measurements at 3 different time points.

Group	Mean±SD	Min	Max
Fresh (T ₀)	74.009±1.119	73.09	75.32
1. hour (T ₁)	75.185±1.631	73.21	77.66
2. hour (T ₂)	74.322±1.559	73.18	77.68

$p=0.192$. T₀: Immediate measurement after unboxing; T₁: measurement at 1 hour; T₂: measurement at 2 hours. (Duncan post hoc test; $P<0.05$).

Table 3. Mean, standard deviation (SD), minimum (Min), maximum (Max), and *P* values of NaOCl solutions; active chlorine measurements at 3 different time points.

Group	Mean±SD	Min	Max
Fresh (T ₀)	3.775±0.065 ^b	3.64	3.86
1. hour (T ₁)	3.791±0.051 ^b	3.72	3.89
2. hour (T ₂)	3.889±0.076 ^a	3.76	3.97

$p_{T_0-T_1}=0.496$; $p_{T_0-T_2}=0.001$ $p_{T_1-T_2}=0.001$. T₀: Immediate measurement after unboxing; T₁: measurement at 1 hour; T₂: measurement at 2 hours. Different lowercase letters indicate statistically significant difference between the time points (Duncan post hoc test; $P<0.05$).

was observed among the surface tensions of the T₀, T₁ and T₂ solution groups ($P>0.05$).

The mean, SD, min, max, and *P* values of the active chlorine percentages of NaOCl samples determined by iodometric titration are presented in **Table 3**. The mean active chlorine percentages of the NaOCl solution groups revealed a statistically significant difference. According to the paired comparisons, no statistically significant difference was found between the active chlorine contents of the T₀ and the T₁ samples ($P>0.05$). In contrast, the active chlorine percentage in the T₂ group was significantly higher than that in the T₀ and T₁ groups ($P<0.05$).

Discussion

NaOCl, the most commonly used solution in endodontics, is easily affected by heat, light, pH, heavy metals, storage conditions, and time due to its unstable structure [27]. Various studies have examined the chemical changes of NaOCl solution

under different temperatures and concentrations, with varying waiting times and in diverse environments [9,28-32]. A related in vitro study evaluated NaOCl stability in different storage media over longer periods (days) [33]; however, it did not simulate chairside open-tray exposure during a typical appointment. To the best of our knowledge, no previous study has evaluated time-dependent changes occurring while NaOCl is kept on the unit tray during an average endodontic treatment period. Accordingly, this study compared pH, active chlorine content, and surface tension among fresh NaOCl samples taken directly from a closed bottle and those left for 1 and 2 hours in transparent open-lid PET cups under common clinical conditions.

In this study, 20 mL of NaOCl solution was used for each sample, thus representing the approximate amount of NaOCl solution that is placed on the unit table in a transparent PET glass during preparation for root canal treatment. Environmental conditions were kept constant by considering an average treatment room's temperature (22°C) and brightness.

The active chloride content of NaOCl solutions used in endodontics has been determined via iodometric titration in most extant studies [8,27,28,30,34-37]. Therefore, iodometric titration was used to determine the active chloride content of NaOCl solutions in this study. Although the British Pharmacopoeia Commission has defined iodometry as the most efficient and reliable method for determining active chloride concentration [27], this method requires many dilutions to reach the titration itself; moreover, since it is a completely manual and colorimetric method, it requires precise work. For this reason, a drop added or removed during application may cause a difference in the final result [30]. Although it was conducted meticulously, there is a possibility that this method's sensitive nature may have created a slight limitation in our study.

Van der Waal et al (2014) [38] examined NaOCl solutions taken from dental offices. They showed that most of the active chlorine concentrations stated on the packaging did not match the measured active chlorine concentration, and 27% of the samples had less active chlorine content than the stated values. Similar to the findings of these researchers, in our study, an average of 3.7% active chlorine amount was detected. In contrast, the label stated the NaOCl percentage as being 5% (which equals 4.77% of active chlorine).

Further, in this study, comparisons between freshly opened samples and those exposed to air for 1 and 2 hours revealed a gradual increase in active chlorine concentration. While no significant difference was observed between the fresh and 1-hour samples, the solution exposed for 2 hours exhibited a significantly higher active chlorine percentage than the fresh and 1-hour samples. This finding is compatible with a concentration effect in open containers, in which preferential water loss over time may increase the measured active chlorine percentage even if the total amount of chlorine does not increase. Similarly, Clarkson et al (2001) [8] reported time-dependent changes in available chlorine in NaOCl solutions stored in open containers. However, because evaporation-related mass and volume loss was not quantified in the present study, the proposed evaporation-driven concentration effect should be interpreted as a plausible hypothesis rather than a confirmed mechanism.

In this study, as in certain existing studies in the literature [14,39], surface tension was measured using the Traube Stalagmometer and the drop method. It has been reported that the use of solutions with low surface tension in root canal irrigation can achieve more effective and thorough cleaning [31] and that higher concentrations of NaOCl can provide deeper penetration into dentin by reducing surface tension [40]. At the same time, Palazzi et al (2016) [40] stated that the difference in surface tension between solutions with very similar concentrations was not statistically significant; the only significant difference was detected between 1% and 6%

NaOCl. In our study, no statistically significant difference was observed among the surface tensions of the fresh, 1-hour, and 2-hour solution groups. The average active chlorine content of our solution was 3.7% when fresh and 3.8% after 2 hours. This increase by 0.1% might not have resulted in a significant difference in surface tension, which is consistent with the results of the study by Palazzi et al [40].

Further, in this study, the pH of NaOCl solution was measured using a calibrated digital pH meter, which has also been used in previous studies [13,30,35,38] due to its practicality and high reliability in obtaining accurate pH readings. In our study, the average initial pH was 12.2, while it was measured as 10.3 at the end of the first hour and 10 at the end of the second hour. The observed pH reduction from approximately 12.2 to 10 is potentially clinically relevant because NaOCl chemistry is pH-dependent: lowering pH shifts the equilibrium toward hypochlorous acid (HOCl), which may increase antimicrobial activity, but may also reduce tissue-dissolving capacity and alter solution reactivity during irrigation [9-12]. Therefore, if NaOCl is left exposed on the unit tray for prolonged periods, the altered pH could change the balance between antibacterial efficacy and organic tissue dissolution, underscoring the importance of limiting unnecessary exposure time and using dispensing practices that reduce air and light contact.

To the best of our knowledge, there are no studies in the literature that examine changes in the pH of NaOCl solutions exposed to air and light for short periods of time, such as 1 to 2 hours. However, in a study by Van der Waal et al (2014) [32], in which they examined the pH changes of NaOCl solutions from different brands when fresh, at 2 weeks, and at 22 weeks, the initial pH average of the solutions was 11.84. By week 22, it had decreased to an average of 11.3. In contrast, Johnson and Remeikis (1993) [19] examined the storage conditions and time variables of NaOCl solutions with different concentrations (5.25%, 2.62%, and 1.0%) and concluded that the pH value decreased with increased storage time. The results of these 2 studies are parallel to our results. We assume that the much more rapid decrease in the pH value of our solutions compared with those in other studies is related to the storage of the samples in transparent open-lid containers.

Limitations

This in vitro study evaluated physicochemical parameters (pH, active chlorine, and surface tension) and did not directly assess antimicrobial efficacy or pulp tissue dissolution under clinical conditions. Organic load (eg, dentin or pulp tissue), canal anatomy, irrigation dynamics, and clinically relevant interactions with other irrigants were not simulated. Environmental parameters that can affect evaporation and degradation (light intensity, relative humidity, and airflow) were not instrumentally

quantified. In addition, evaporation (mass/volume loss) during the exposure of 1 and 2 hours was not measured, so the mechanism underlying the observed change in active chlorine percentage cannot be confirmed. Moreover, stalagmometry and iodometric titration are manual, operator-dependent methods; therefore, although measurements were performed by a single trained operator using a standardized protocol, some degree of operator-related variability cannot be excluded. Finally, measurements were performed without technical replication per sample and only 1 commercial NaOCl product was tested, which may limit generalizability. The relatively small sample size (n=10 per group) may have limited statistical power to detect small between-group differences.

Conclusions

Within the limitations of this in vitro study, keeping NaOCl in transparent open-lid containers for 1 to 2 hours was associated with a decrease in pH and a statistically significant increase in the measured active chlorine percentage at 2 hours (compared with fresh and 1-hour samples), while no statistically significant change was observed in surface tension. Because the present study did not compare container opacity or different dispensing bottles, conclusions should be limited to the tested condition (transparent open-lid cups). From

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