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EVALUATION OF POTENTIAL OF HYDROGEN (PH) OF SODIUM HYPOCHLORITE AT THREE DIFFERENT MOMENTS BEFORE ITS EXPIRATION DATE

AVALIAÇÃO DO POTENCIAL HIDROGÊNIO (PH) DO HIPOCLORITO DE SÓDIO EM TRÊS DIFERENTES MOMENTOS ANTES DO SEU VENCIMENTO

EVALUACIÓN DEL POTENCIAL DE HIDRÓGENO (PH) DEL HIPOCLORITO DE SODIO EN TRES MOMENTOS DIFERENTES ANTES DE SU FECHA DE CADUCIDAD

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ABSTRACT
Sodium hypochlorite (NaOCl) is the most common used irrigant for root canal treatment, it was introduced into endodontics in the last half of XVIII century. the aim of this study was to evaluate the pH stability of sodium hypochlorite (of different concentrations) and saline solution at three different moments. The pH of the irrigants was measured in triplicate using a pH meter Mettler Toledo SevenEasy S20 (Mettler-Toledo AG. Analytical Instruments. Schwerzenbach, Switzerland) at three moments: S1: upon unpacking the solution bottle; S2: one month later; and S3: four months later. Data were submitted to normality tests. Then were analyzed by One-way Anova and Tukey tests by GraphPad Prism 6 (La Jolla, CA, USA) with a significance level of 5%. It was found that there was a statistically significant difference among the Sodium hypochlorite 1% and 2.5% in comparison with the saline solution and distilled water groups in S1, S2 and S3. Besides, it was found that sodium hypochlorite 1% loses its alkaline pH since the first month. However, sodium hypochlorite 2.5% was more stable after 4 months. It was concluded that The storage time has an effect on 1% sodium hypochlorite pH stability.

KEYWORDS: Sodium hypochlorite. Saline solution. Hydrogen-Ion Concentration.

RESUMO
O hipoclorito de sódio (NaOCl) é o irrigante mais utilizado no tratamento endodôntico, tendo sido introduzido na endodontia na última metade do século XVIII. O objetivo deste estudo foi avaliar a estabilidade do pH do hipoclorito de sódio (de diferentes concentrações) e solução salina em três momentos diferentes. O pH dos irrigantes foi medido em triplicata usando um medidor de pH Mettler Toledo SevenEasy S20 (Mettler-Toledo AG. Analytical Instruments. Schwerzenbach, Suíça) em três momentos: S1: ao desembalar o frasco da solução; S2: um mês depois; e S3: quatro meses depois. Os dados foram submetidos a testes de normalidade. Em seguida, foram analisados pelos testes One-way Anova e Tukey pelo GraphPad Prism 6 (La Jolla, CA, EUA) com nível de significância de 5%. Verificou-se que houve diferença estatisticamente significativa entre os grupos Hipoclorito de Sódio 1% e 2,5% em comparação com os grupos soro fisiológico e água destilada em S1, S2 e S3. Além disso, constatou-se que o hipoclorito de sódio 1% perde seu pH alcalino desde o primeiro mês. No entanto, o hipoclorito de sódio 2,5% foi mais estável após 4 meses. Concluiu-se que o tempo de armazenamento tem efeito na estabilidade do pH do hipoclorito de sódio 1%.

PALAVRAS-CHAVE Hipoclorito de sódio. Solução salina. Concentração de íons de hidrogênio.

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RESUMEN
El hipoclorito de sodio (NaOCl) es el irrigante más utilizado en el tratamiento de endodoncia, habiéndose introducido en la endodoncia en la última mitad del siglo XVIII. El objetivo de este estudio fue evaluar la estabilidad del pH del hipoclorito de sodio (de diferentes concentraciones) y solución salina en tres tiempos diferentes. El pH de los irrigantes se midió por triplicado utilizando un pHmetro Mettler Toledo SevenEasy S20 (Mettler-Toledo AG. Analytical Instruments. Schwerzenbach, Suiza) en tres momentos: S1: al desempacar el frasco de solución; S2: un mes después; y S3: cuatro meses después. Los datos fueron sometidos a pruebas de normalidad. Luego, se analizaron mediante pruebas One-way Anova y Tukey por GraphPad Prism 6 (La Jolla, CA, EE. UU.) con un nivel de significancia del 5%. Se encontró que hubo una diferencia estadísticamente significativa entre los grupos de Hipoclorito de Sodio 1% y 2,5% en comparación con los grupos de solución salina y agua destilada en S1, S2 y S3. Además, se encontró que el hipoclorito de sodio al 1% pierde su pH alcalino desde el primer mes. Sin embargo, el hipoclorito de sodio al 2,5% fue más estable después de 4 meses. Se concluyó que el tiempo de almacenamiento tiene efecto en la estabilidad del pH del hipoclorito de sodio al 1%.

PALABRAS CLAVE: Hipoclorito de sódio. Solución salina. Concentración de iones de hidrogeno.

INTRODUCTION

The root canal treatment is carried-out to disinfect/prevent the endodontic infection, in which the microorganisms' role is essential. For this, cleaning and shaping (1) aim to, mechanically and chemically, eliminate the microorganisms and their subproducts (2–4). However, despite all the technological evolution in the area of endodontics, it is clear that an adequate decontamination of root canals demands the use of auxiliary chemical substances, because of the anatomy complexity and the microorganisms resistance (5–7).

Sodium hypochlorite (NaOCl) is the most common used irrigant for root canal treatment, it was introduced into endodontics in the last half of XVIII century (8). It has a wide bactericidal effect against variety of Gram-positive and Gram-negative microorganisms, (9–11) and their byproducts. like Lipopolysaccharides (LPS) and lipoteichoic acid (LTA) (10,12). It has the ability to dissolve organic matter from the root canal (13). Its effectiveness is directly related to its concentration, becoming greater as its concentration increases, being found in concentrations at 0.5%, 1%, 2.5%, 5.25%. However, the higher it is, the greater its toxic effect on periapical tissues, and may also cause incidents during its use (14).

Its major disadvantage is the storage instability (15). Thus, its effectiveness can be reduced upon increased temperature, exposure to light, transport, and long period storage. Its chemical effectiveness depends on the active chlorine content, which is influenced by concentration, exposure time, pH and temperature in which the alkaline pH maintains the stability of NaOCl solutions, and have a greater bactericidal effect when pH is close to neutral or basic (16).

Therefore, the aim of this study was to evaluate the pH stability of sodium hypochlorite (of different concentrations) and saline solution at three different moments. The null hypothesis was that the storage time have no effect on the pH stability of the irrigant.

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MATERIAL AND METHODS

Ph measurement

Sodium hypochlorite of 1% and 2.5 % (Asfer, São Caetano do Sul, SP, Brazil), sterilized saline solution **(**Eurofarma laboratórios, São Paulo, SP, Brazil), and purified water (prepared at our laboratory) were used in this study as found in table 1.

Table 1 the evaluated endodontic irrigants

Irrigant	Manufacturer
Sodium hypochlorite 1%	Asfer, São Caetano do Sul, SP, Brazil
Sodium hypochlorite 2.5 %	Asfer, São Caetano do Sul, SP, Brazil
Sterilized saline solution	Eurofarma laboratórios, São Paulo, SP, Brazil
Distilled water	Institute of science and technology, São Paulo State University.

The pH of the irrigants was measured in triplicate using a pH meter Mettler Toledo SevenEasy S20 (Mettler-Toledo AG. Analytical Instruments. Schwerzenbach, Switzerland) at three moments:

1. S1: upon unpacking the solution bottle;
2. S2: one month later;
3. S3: four months later.

The pH meter was calibrated, then was immersed in a Falcon-tube containing the irrigant. All the irrigants were stored at refrigerator at 9 ºC between the intervals of measurement.

STATISTICAL ANALYSIS

Data were submitted to normality tests. Then were analyzed by One-way Anova and Tukey tests by GraphPad Prism 6 (La Jolla, CA, USA) with a significance level of 5%.

RESULTS

Inter-group comparisons

It was found that there was a statistically significant difference among the Sodium hypochlorite 1% and 2.5% in comparison with the saline solution and distilled water groups in S1, S2 and S3. (Table 1) and (Figure 1).

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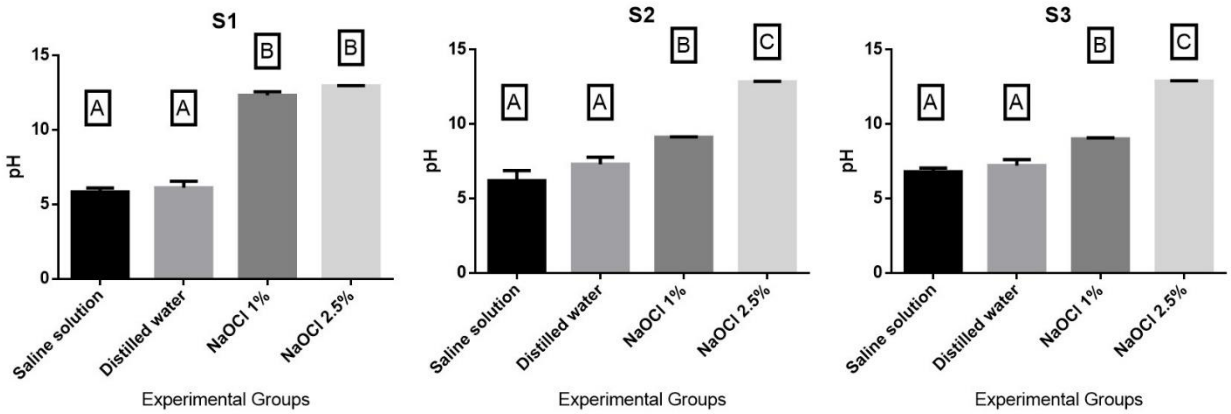
Table 1. Mean values and statistical difference among the experimental groups at three moments of pH measurement (S1, S2 and S3) (inter-groups comparisons).

Samples	Saline solution	Distilled water	NaOCl 1%	NaOCl 2.5%
S1	5.84 (A)	6.12 (A)	12.18 (B)	12.96 (B)
S2	6.20 (A)	7.30 (A)	9.11 (B)	12.84 (C)
S3	6.80 (A)	7.21 (A)	9.00 (B)	12.89 (C)

Figure 1. pH values and statistical difference among the experimental groups at three moments of pH measurement (S1, S2 and S3). Different uppercase letters mean statistically significant difference.



INTRA-GROUPS COMPARISONS

It was found that sodium hypochlorite 1% loses its alkaline pH since the first month. However, sodium hypochlorite 2.5% was more stable after 4 months (Table 2) and (Figure 2).

Table 2. Mean values and statistical difference among the three moments of pH measurement (S1, S2 and S3) of experimental groups (intra-groups comparisons).

	S1	S2	S3
Saline Solution	5.84 (A)	6.20 (A)	6.80 (A)
Distilled water	6.12 (A)	7.30 (B)	7.21 (B)
NaOCl 1%	12.18 (A)	9.11 (B)	9.00 (B)
NaOCl 2.5%	12.96 (A)	12.84 (A)	12.89 (A)

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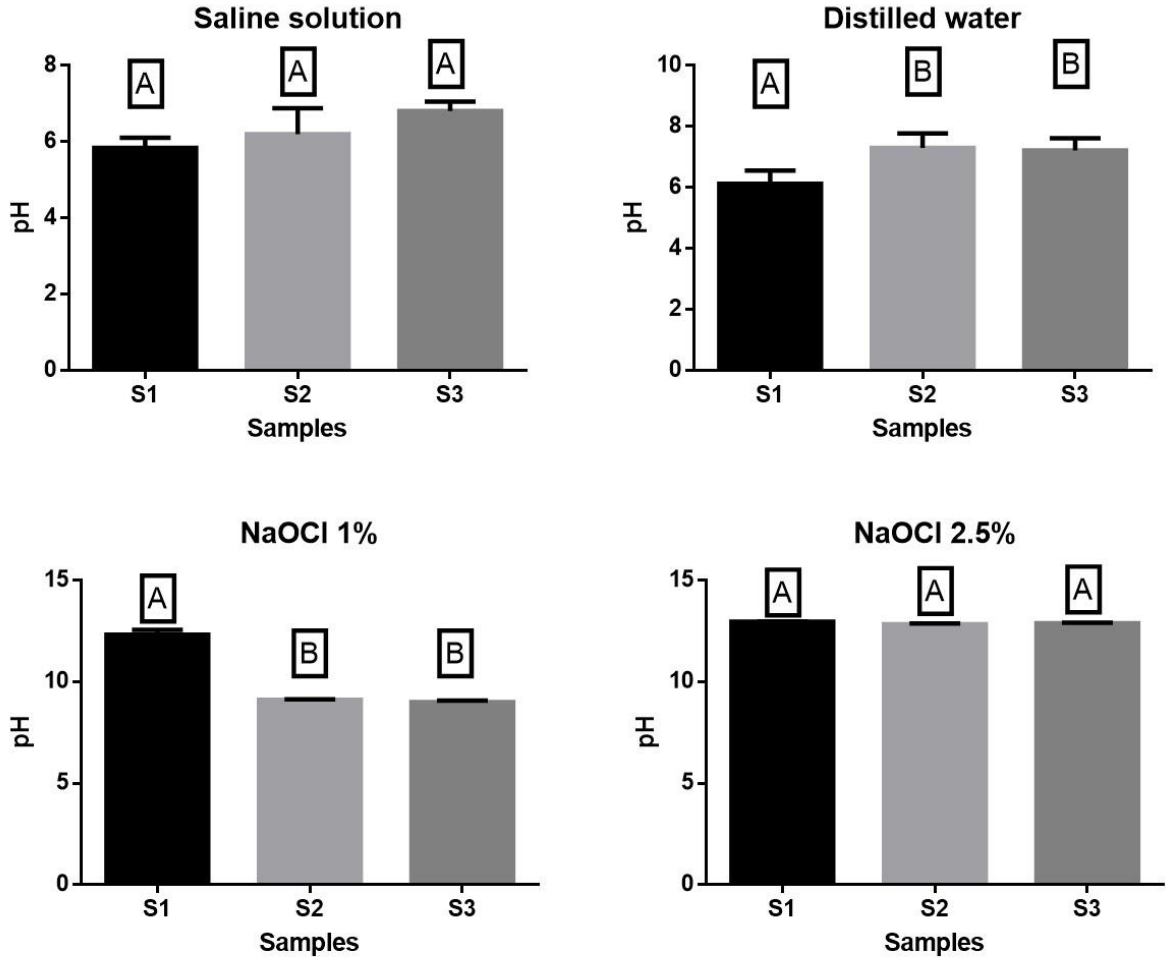
Figure 2. pH values and statistical difference among three moments of pH measurement (S1, S2 and S3) of the experimental groups. Different uppercase letters mean statistically significant difference.



DISCUSSION

It is known that the purpose of endodontic treatment is to remove organic tissues, microorganisms and their by-products presents in the root canal system (2,6,13,17). Among the irrigation protocols, the use of sodium hypochlorite (NaOCl) is clinically predominant (2,4), however its chemical instability puts the effectiveness of disinfection and tissue dissolution to the test. The present study consisted of a longitudinal analysis to evaluate the pH stability of NaOCl at different concentrations (1% and 2.5%) during the storage period, the null hypothesis being rejected.

The pH of the NaOCl solution has a direct influence on the biological effect, whether for tissue dissolution capacity or antimicrobial effect. In alkaline solutions, the hypochlorite ion (OCl−) prevails, which exhibits greater capacity for tissue dissolution. In acidic solutions, hypochlorous acid (HOCl) is more abundant, presenting an antimicrobial effect (18,19). In the study by Nicoletti et al. (2005), it was verified that factors such as sun exposure and temperature promote the chemical instability of

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chlorinated solutions. Care during storage of the solutions influences the maintenance of properties and stability (15). Even more, it was reported that there is a relation between the pH, antimicrobial effect, detergent and dissolving capacity of these solutions when exposed to sunlight or high temperatures. According to Siqueira (2000) sodium hypochlorite solutions have a greater bactericidal effect when at a pH close to neutral or basic, and even if kept under refrigeration they can remain stable for 120 days (20).

According to Camargo et. Al (2008), it was found that the NaOCl solutions showed pH stability for 30 days (21). In the present study, it was found that the 1% NaOCl solution underwent a significant change in pH, with an initial value of 12.18 (when opening the package) and 9.00 at the end of 4 months. Such results raise questions regarding the effectiveness of these solutions used for cleaning and disinfecting root canals, since, at first, the pH conditions of the solution were more favorable to the dissolution of the organic tissue and, after 4 months, its conditions of pH were no longer the same, which could influence their biological actions and consequently their therapeutic efficacy. Regarding the highest concentration evaluated, NaOCl 2.5%, it was observed that there were no significant changes in the pH of the solution. This remained stable until the fourth month of analysis. Such results are in line with findings in the literature, as it is known that NaOCl instability occurs due to the decomposition of hypochlorite ions into chlorate and chlorine ions and this process directly depends on the concentration of the solution (16).

By associating physicochemical factors in the search for greater elimination of microorganisms, 2.5% NaOCl at pH 12 was able to significantly decrease the cell viability of the mixed bacterial biofilm (formed in situ), but when this solution (NaOCl 2, 5%) had an acidic pH, a more significant reduction was observed than that found at basic pH (19).

Finally, NaOCl has a great effect on the success of the root canal treatment (2,4,5,13). However, due to the fact that the present study has a longitudinal character, in which the same sample was analyzed at different times, with the objective of evaluating the influence of time, it was possible to verify the instability of the 1% NaOCl solution, and this fact is of great relevance clinic, since the biological properties of this solution may be compromised.

CONCLUSION

The storage time has an effect on 1% sodium hypochlorite pH stability.

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