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**Stability of Sodium Hypochlorite  
solution in different storage mediums: an in vitro study**

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# ABSTRACT

**Aim:** The aim of this study is to evaluate variations of NaOCl concentration when the solution exposed to different storage recipients found in dental practices, during 5 days.

**Materials and Methods:** This in vitro experience was performed using three different recipients: black (generic brand), white (generic brand) and frosted glass (generic brand). For each type of recipient there were two different capacities, 1L and 5L. Each of the recipients were filled with NaOCl (commercial generic brand bleach) with an expected concentration of 5%. As control it was used two other commercial generic bleaches, both capacities 1L and 5L, that remained in their original recipient. For one week, every hour from 9h to 17h it was poured 30ml of NaOCl in order to replicate a week's work at an endodontic clinic. Every hour it was measured the concentration of each sample of NaOCl using the iodometric titration method. At the end of each day all recipients were storage in a closed storage room at room temperature. The statistical analysis was performed using the software Excel Microsoft and IBM SPSS. Program. It was used the Spearman correlation test and p value of 0,001.

**Results:** There is a statistically significant correlation on the change of NaOCl concentration on the black recipient, on the white recipient and on the frosted glass recipient during the week. The black recipient was the one with the least variation of concentration.

**Conclusions:** The type of recipient for the storage of NaOCl influences its concentration during the week. The black recipient was considered the best one to use. Even though this variation on the concentration occurs, the NaOCl is still effective on the endodontic treatment.

**Keywords:** endodontics; sodium hypochlorite; irrigation; iodometric titration; storage.



## RESUMO

O principal objetivo do tratamento endodôntico é a remoção do tecido pulpar afetado de forma a criar um ambiente que promova o rejuvenescimento e previna o desenvolvimento de periodontite apical. Este tratamento endodôntico deve, portanto, eliminar a polpa dentária afetada, microrganismos, remover detritos e dar forma aos canais dentários de forma a facilitar uma limpeza e obturação eficaz.

O sucesso do tratamento endodôntico depende da eliminação de todo o tecido orgânico presente dentro dos canais, isto porque as bactérias e os seus subprodutos são o maior fator etiológico da periodontite apical. Como tal a desinfecção e dos canais interdentários deve ser criteriosa, através do uso de irrigantes e instrumentos eficazes que permitam atingir o sucesso clínico.

O objetivo da irrigação é eliminar microrganismos, eliminação da *smear layer*, remoção e dissolução de detritos e lubrificação dos instrumentos utilizados no canal dentário. Teoricamente, o irrigante perfeito tem as seguintes características: baixo custo, capacidade de lavagem, dissolução da matéria orgânica e inorgânica, boa penetração no sistema canalar, eliminação e separação de biofilme, não ser tóxico para os tecidos periapicais, não provocar alergia, não reagir com consequências negativas com outros materiais dentários e não enfraquecer a dentina.

O irrigante mais utilizado é o hipoclorito de sódio (NaOCl) devido às suas conhecidas propriedades e aos vários estudos que comprovam a sua capacidade anti-microbiana, bem como a sua capacidade de dissolução tecidual, no entanto existem outras substâncias que também podem ser utilizadas como irrigante, como a clorhexidina (CHX), ácido etilendiamino tetra-acético (EDTA), ácido cítrico e peróxido de hidrogénio (H<sub>2</sub>O<sub>2</sub>).

Atualmente, nenhuma das soluções irrigantes disponíveis para uso clínico abrange todas as características descritas anteriormente, e por este facto, em prática clínica utiliza-se uma combinação de soluções de forma a maximizar o sucesso do tratamento endodôntico.

O cloro (Cl<sup>-</sup>) presente no hipoclorito de sódio (NaOCl) é o responsável pelo efeito anti-microbiano e de dissolução, no entanto, o cloro é rapidamente consumido, o que demonstra que um regular reabastecimento e irrigação de NaOCl durante o tratamento endodôntico é indispensável para o sucesso desta terapia.

O segredo da eficácia da irrigação no tratamento endodôntico é adquirir soluções irrigantes com a concentração adequada. Atualmente existem duas formas de obtenção de hipoclorito de sódio: casas de venda de material dentário/farmácias e lixívia comercializadas para uso doméstico em supermercados. Apesar das farmácias parecer terem produtos com uma qualidade superior, as lixívia de uso comercial têm sido usadas desde há muitos anos para a realização de tratamento endodôntico.

Sabendo que o hipoclorito de sódio é uma solução instável por sua natureza, a sua atividade e efetividade é afetada pela quantidade de cloro disponível, cuja concentração diminui com o tempo, exposição à luz, contacto com o ar, metais e, também, uma temperatura de armazenamento aumentada leva a uma maior perda de quantidade de cloro.

A atividade e eficácia do hipoclorito de sódio é então determinada pela quantidade de cloro disponível. Existem vários métodos que podem ser utilizados para medir esta quantidade de cloro disponível, sendo que a titulação iodométrica, é o teste mais simples e mais utilizado. Este teste é uma titulação oxidação-redução, neste caso entre o NaOCl e o tiosulfato de sódio ( $\text{Na}_2\text{S}_2\text{O}_3$ ).

**Objetivo:** O objetivo deste trabalho é avaliar se a concentração de hipoclorito se mantém a mesma em três tipos de recipientes diferentes, um preto, um branco e outro de vidro fosco, durante um período de 5 dias. Para tal foram formuladas três hipóteses de estudo, uma para cada recipiente.

**Materiais e Métodos:** Este estudo *in vitro* foi realizado utilizando três tipos diferentes de recipientes: preto (marca branca), branco (marca branca) e vidro fosco (marca branca). Para cada tipo de recipiente foram utilizadas duas capacidades diferentes, 1L e 5L de B<sub>1</sub>/B<sub>5</sub>, W<sub>1</sub>/W<sub>5</sub> e FG<sub>1</sub>/FG<sub>5</sub>. Como controlo foram utilizados duas embalagens originais da marca branca do supermercado com ambas capacidades (1L e 5L) dos recipientes em estudo. A concentração esperada de NaOCl das lixívia adquiridas no supermercado era de 5%. Como controlo foram utilizados dois recipientes com a mesma capacidade (1L e 5L), que permaneceram na embalagem original. A cada dia e durante cinco dias, de hora a hora desde as 9h às 17h foram retirados 30mL de NaOCl de cada um dos recipientes de 1L de forma a replicar uma semana de trabalho numa clínica de endodontia. A cada hora foi tirada uma amostra de NaOCl e medida a sua concentração utilizando o método de titulação iodométrico. No final de cada dia todos os recipientes foram armazenados num armazém fechado à temperatura ambiente. No início do dia seguinte os recipientes de 1L

foram re-abastecidos de 1L com os seus correspondentes de 5L. A análise estatística foi feita utilizando os softwares *Excel Microsoft* e *IBM SPSS*. Foi utilizado o teste estatístico da correlação de *Spearman* e considerado um valor p de 0,001.

**Resultados:** Para cada tipo de recipiente, com uma concentração expectável inicial de 5%, a concentração medida na maioria das amostras foi mais baixa do que o esperado. A concentração de NaOCl sofreu variações durante o estudo. Comparando diariamente os recipientes de 1L todas amostras apresentaram uma decréscimo ao longo do dia, sendo que o que se manteve mais estável foi o recipiente preto, no entanto não com um comportamento muito semelhante ao de controlo, uma vez que o recipiente preto era agitado, aberto de hora em hora e mexido de forma a retirar os 30ml de hipoclorito de sódio, enquanto que o seu controlo apenas teve medições feitas 3 vezes por dia. Comparando os recipientes de 5L, o recipiente que sofreu a variação maior foi o branco com uma diminuição de 3,48% de concentração, seguido do vidro fusco com 2,09% e finalmente, o que revelou o melhor comportamento foi o recipiente preto, com uma diminuição de 1,77% ao longo da semana, valor semelhante ao recipiente de controlo que sofreu uma diminuição de 1,57%. Existe uma correlação estatisticamente significativa na variação da concentração de NaOCl ao longo do tempo no recipiente preto, branco e de vidro fusco, sendo que recipiente preto foi o que sofreu menor variação de concentração de NaOCl.

**Conclusão:** O tipo de recipiente para armazenamento de NaOCl é um fator influenciador da concentração do mesmo ao longo da semana. O recipiente preto é considerado o melhor para armazenamento e uso no dia-a-dia. Apesar das alterações que sofre, a concentração de NaOCl mantém-se eficaz para um tratamento endodontico com sucesso. Como recomendações para estudos futuros, poderia ser a realização por um período de tempo maior de forma a avaliar quanto tempo demora para que a % (w/v) de NaOCl baixe para valores que já não são recomendados para um tratamento endodontico eficaz.

**Palavras-chave:** endodontia; hipoclorito de sódio; irrigação; titulação iodométrica; armazenamento.



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# LIST OF ABBREVIATION

NaOCl – sodium hypochlorite

CHX - chlorhexidine

EDTA - ethylenediaminetetraacetic acid

H<sub>2</sub>O<sub>2</sub> - hydrogen peroxide

Na<sup>+</sup> - sodium ion

OCl<sup>-</sup> - hypochlorite ion

HOCl - hypochlorous acid

pH – potential of hydrogen

Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> - sodium thiosulfate

S<sub>2</sub>O<sub>3</sub><sup>2-</sup> - thiosulfate ion

I<sub>2</sub> - iodine

L – liter

ml - milliliter

S<sub>2</sub>O<sub>6</sub><sup>2-</sup> - tetrathionate ion

H<sup>+</sup> - hydrogen ion

Cl<sup>-</sup> - chlorine

H<sub>2</sub>O - water

HCl - Hydrochloric acid

B<sub>1</sub> - black recipient 1L

B<sub>5</sub> – black recipient 5L

W<sub>1</sub> – white recipient 1L

W<sub>5</sub> – white recipient 5L

FG<sub>1</sub> – frosted glass recipient 1L

FG<sub>5</sub> – frosted glass recipient 5L

C<sub>1</sub> – control recipient 1L

C<sub>5</sub> – control recipient 5L

L - liter

°C - degree celsius

M - molar concentration

g – gram

% - percentage

% (w/v) - percent of weight/volume

HCl - hydrochloric acid

KI - potassium iodide

$\bar{x}$  - mean

$\sigma$  – standard deviation

min – minimum

max - maximum

p – probability value

# **I. INTRODUCTION**

## **1. Endodontology – definition and objective**

According to the European Society of Endodontology (2006), endodontology it's the science that studies the shape, function, health and diseases of the dental pulp and periradicular region, both treatment and prevention. The main disease is caused by an infection, apical periodontitis. All the diseases, diagnosis of dental pain and their aetiology are all components of endodontic practice.(1,2)

The main objective of endodontic treatment is the removal of diseased pulpal tissue in order to form an environment which promote healing and prevent the development of apical periodontitis. Root canal treatment must eliminate diseased dental pulp, microorganisms, remove of debris and shape the root canals in order to facilitate an effective cleanse and filling. A proper disinfection and filling come a long way in favour of keeping the health and function and aesthetic of teeth.(1-3)

## **2. Irrigation**

Endodontic treatment success depends on the chemical and mechanical debridement of the root canals by using effective irrigants and instruments. (4)

The purpose of irrigation is to eliminate microorganisms, elimination of smear layer, remove and dissolve debris, and lubricate the root canal instruments. The irrigant solution should be delivered in large amounts alongside all the root canal system without passing the foramen. This can either be executed in a traditional way – with the help of a syringe and needle, or mechanical – ultrasonic systems. The syringe delivery system allows full control of the volume injected inside the root canal as well as how depth the needle would go. In contrast, when using a continuous flow of irrigant the volume is not precise.(2,3,5-8)

In theory, the perfect irrigating solution has the following characteristics: low cost, washing action, reduction of friction, improving cutting of dentine by the instruments, temperature control, dissolution of organic and inorganic matter, good penetration within the root canal system, killing of biofilm microbes, detachment of biofilm, non-toxic to periapical tissue, non-allergenic, does not react with negative consequences with other dental materials and does not weaken dentin. (3,9)

Although the most used irrigant is sodium hypochlorite (NaOCl), there are other substances that can be used as irrigants as well, such as chlorhexidine (CHX), ethylenediaminetetraacetic acid (EDTA), citric acid, hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>).<sup>(10)</sup>

In our present time, none of the available irrigating solutions matches the criteria described before, but in clinical practice a combination of solutions is used to maximize the success of root canal treatment.<sup>(3)</sup>

## **2.1 Sodium Hypochlorite**

The use of NaOCl was first recommended by Henry Dakin to disinfect open wounds in World War I at a concentration of 0.5% to 0.6%, and today it still remains as the gold-standard irrigant solution in root canal treatment based on its antibacterial effect, tissue-dissolving and lubricating properties, not to mention that they are cheap and easily found from many sources. <sup>(4,11–14)</sup>

Moorer & Wesselink 1982, established that chlorine is the one responsible for the dissolution and antimicrobial effect of NaOCl, nevertheless chlorine is rapidly consumed, which proves that regular replenishments of NaOCl is indispensable during endodontic therapy.<sup>(15)</sup>

The secret to effectiveness of the irrigation on the endodontic treatment is to acquire a solution of the appropriate concentration. There are two main sources of sodium hypochlorite nowadays: pharmaceutical supplies or commercially available household bleaches. Although pharmaceutical sources may seem to have a higher quality product, the commercial bleaches have been used from many years now. One may think that household bleaches are inferior because of the presence of other additives, thickening agents and surfactants. <sup>(11)</sup>

Through the years the choice of concentration of NaOCl has divided several opinions, with a range of usage extending from 0.5% to 5.25%. Estrela et al 2002, reported that a low concentrated NaOCl solution, as low as 1%, presents acceptable compatibility, and a higher concentration is more aggressive, more cytotoxic. So, although a higher concentration may mean more efficiency, it also may be not so worthwhile, which is also shown by Pashley et al 1985.<sup>(7,16,17)</sup>

According to Zehnder 2006, increasing the temperature of the NaOCl solution just before its use can increase its efficiency regarding its dissolution capacity of tissues. It is

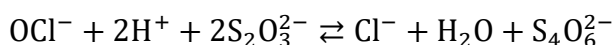
also proven that even heating the solution, as soon as it contacts the dental canal, it becomes at room temperature. (17,18)

Knowing that NaOCl is an unstable solution, its activity is affected by the level of available chlorine which its concentration declines with time, exposure to light, contact with air, metals and also, the higher the temperature of the place where the solutions are stored, the greater the loss of chlorine content.(19,20)

In water NaOCl ionizes into sodium ( $\text{Na}^+$ ) and the hypochlorite ( $\text{OCl}^-$ ) ions and establishes an equilibrium with hypochlorous acid (HOCl). Between pH four and seven, most of the chlorine exists as HOCl, whereas at pH of nine and above,  $\text{OCl}^-$  is most abundant. (3,17)

As reported by Estrela et al 2002, NaOCl is a strong base with a pH over 11, which is harmful on bacterial cells. This high pH alters the integrity of the cytoplasmic membrane causing damage in the cytoplasmic membrane integrity with an irreversible enzymatic inhibition, alterations in cellular metabolism and phospholipid degradation as observed in lipidic peroxidation. Also, it is referred that the higher initial concentration of the NaOCl solution, the minor was the reduction of its pH.(17)

As mentioned beforehand, the activity of NaOCl can be determined by the amount of available chlorine. There are several methods that can be used, including iodometric titration, it is the simplest and most used test for available chlorine. The iodometric titration it's a redox titration between, in this case, NaOCl and sodium thiosulfate ( $\text{Na}_2\text{S}_2\text{O}_3$ ). The thiosulfate ion ( $\text{S}_2\text{O}_3^{2-}$ ) it's a reducing agent widely used to determine oxidizing agents through an indirect process that involves iodine as an intermediate. When both thiosulfate ion and iodine ( $\text{I}_2$ ) come into contact, the first one is quantitatively oxidized forming tetrathionate ion ( $\text{S}_4\text{O}_6^{2-}$ ). All this translates in the final equation below. (15,17,21–24)





## **II. AIM**

The aim of this study is to evaluate variations of NaOCl concentration when the solution exposed to different storage recipients found in dental practices, during 5 days.

## **III. HYPOTHESIS**

### **1. The concentration of NaOCl modifies in the black recipient**

H1.0: The concentration does not change in the black recipient

H1.1: The concentration changes in the black recipient

### **2. The concentration of NaOCl modifies in the white recipient**

H2.0: The concentration does not change in the white recipient

H2.1: The concentration changes in the white recipient

### **3. The concentration of NaOCl modifies in the frosted glass recipient**

H3.0: The concentration does not change in the frosted glass recipient

H3.1: The concentration changes in the frosted glass recipient



## **IV. MATERIALS AND METHODS**

### **1. Study Type**

An in vitro experimental study in order to assess whether the concentration of NaOCl is maintained in different recipients at the same shelf in a closed storage at room temperature.

### **2. Study Design**

Three different types of recipients were used: black (generic brand), white (generic brand) and frosted glass (generic brand). For each type of recipient there were two different capacities, 1L and 5L denominated as B<sub>1</sub>/B<sub>5</sub> (black 1L/5L), W<sub>1</sub>/W<sub>5</sub> (white 1L/5L) and FG<sub>1</sub>/FG<sub>5</sub> (frosted glass 1L/5L). Each of the 5L recipients were filled with NaOCl (commercial generic brand bleach) with an expected concentration of 5%. As control it was used two other commercial generic bleaches, both capacities 1L and 5L denominated as C<sub>1</sub>/C<sub>5</sub> (control 1L/5L), that remained in their original recipient.

For one week, every hour from 9h to 17h it was poured 30ml of NaOCl in order to replicate a week's work at an endodontic clinic. Every hour it was measured the concentration of each sample of NaOCl using the iodometric titration method. It is worth mentioning that were titrated two samples for each recipient and the mean obtained. At the end of each day all recipients were storage in a closed storage at room temperature. Although they were different recipients, the same laboratorial procedure was implemented:

1. Take initial concentration measurement of all 5L recipients, including controls.
2. Fill each one of the 1L recipients with NaOCl from their 5L correspondent. Storage the 5L until the end of the day;
3. After one hour, shake the recipient and pour 30ml of NaOCl from all 1L recipients, and then measure the concentration (repeat this step 3x);
4. Measure the concentration of every 1L recipient, including control;
5. 2 hours after step 4, shake the recipient and pour 30ml of NaOCl from all 1L recipients and then measure the concentration (repeat this step 3x);
6. Measure the concentration of every recipient, including control;
7. Storage all recipients in a closed storage at room temperature;

8. Repeat all steps in the next 4 days.

## **2.1 Iodometric titration**

1. Prepare the sodium thiosulfate solution to a concentration of 0,1M;
2. Prepare the burette with 50ml with sodium thiosulfate;
3. Pipette 20ml of NaOCl to an Erlenmeyer flask, to which were added 50ml of distilled water;
4. Then add 3g of potassium iodide and 15ml of 6% acetic acid with a pipette;
5. With this the titration of potassium iodide has started until the solution changed from a brownish colour to a light yellow;
6. Once reached this colour add 3ml of starch solution (0,1%) (w/v) with the help of a pipette, which will change the colour of the solution to blue;
7. Continue the titration with sodium thiosulfate until the solution becomes colourless;
8. As soon as the solution becomes colourless, the titration was stopped and the volume was recorded for the calculation of NaOCl content.

## **3. Materials used**

During this study the materials used were commercial bleach NaOCl, black plastic recipient, white plastic recipient, frosted glass recipient, graduated syringe, starch solution 2%, hydrochloric acid (HCl) 3M, sodium thiosulfate solution ( $\text{Na}_2\text{S}_2\text{O}_3$ ) 0,1M, potassium iodide (KI), balance, ring stand, burette assembly, burette clamp, 500ml Erlenmeyer flask, pipette 25ml, pipette pump and magnetic stirrer bar.

## **4. Statistical Analysis**

The statistical analysis was performed using the software Excel® Microsoft Corporation (Seattle, WA, E.U.A.) and IBM SPSS® program (Statistical Package for the Social Sciences v.26, SPSS Inc., Chicago, United states of America).

## V. RESULTS

Table 1 – Concentration of NaOCl on the beginning of the study on the recipients B<sub>5</sub>, W<sub>5</sub>, FG<sub>5</sub>, C<sub>5</sub> and C<sub>1</sub>

	% of NaOCl (w/v) at the beginning
B <sub>5</sub>	5.08
W <sub>5</sub>	4.88
FG <sub>5</sub>	4.79
C <sub>5</sub>	5.09
C <sub>1</sub>	4.06

From each recipient with an expected concentration of NaOCl of 5%, the measured concentration for the most samples were lower than expected. The W<sub>5</sub> recipient had minus 2,4%, the FG<sub>5</sub> recipient 4,2% and the C<sub>1</sub> recipient 2,8%. On the other hand, two recipients had a slightly higher concentration than expected, the B<sub>5</sub> had more 1,6%, and the C<sub>5</sub> recipient 1,8%.

Table 2- N, Range, Minimum, Maximum, Mean ( $\bar{x}$ ) and Standard Deviation ( $\sigma$ ) of the concentration values of NaOCl (expressed in percentage of weight per volume) for each type of recipient for each day.

Recipient / Day / Concentration		N	Range	Min	Max	$\bar{x}$	$\sigma$	
B <sub>1</sub>	1	% of NaOCl (w/v)	8	.03	5.04	5.07	5.05	.01309
	2	% of NaOCl (w/v)	8	.02	5.05	5.07	5.06	.01035
	3	% of NaOCl (w/v)	8	.01	5.05	5.06	5.06	.00518
	4	% of NaOCl (w/v)	8	.01	5.00	5.01	5.01	.00518
	5	% of NaOCl (w/v)	8	.01	4.98	4.99	4.99	.00463
W <sub>1</sub>	1	% of NaOCl (w/v)	8	.12	4.76	4.88	4.82	.04496
	2	% of NaOCl (w/v)	8	.07	4.81	4.88	4.85	.02449
	3	% of NaOCl (w/v)	8	.03	4.81	4.84	4.82	.01389
	4	% of NaOCl (w/v)	8	.06	4.67	4.73	4.71	.02295
	5	% of NaOCl (w/v)	8	.09	4.62	4.71	4.68	.03625
FG <sub>1</sub>	1	% of NaOCl (w/v)	8	.05	4.75	4.80	4.78	.01690
	2	% of NaOCl (w/v)	8	.04	4.75	4.79	4.78	.01389
	3	% of NaOCl (w/v)	8	.06	4.68	4.74	4.72	.02390
	4	% of NaOCl (w/v)	8	.05	4.68	4.73	4.71	.01642
	5	% of NaOCl (w/v)	8	.05	4.64	4.69	4.67	.01669
C <sub>1</sub>	1-5	% of NaOCl (w/v)	15	.05	4.81	4.86	4.84	.02264
B <sub>5</sub>	1-5	% of NaOCl (w/v)	15	.09	4.99	5.08	5.04	.03645
W <sub>5</sub>	1-5	% of NaOCl (w/v)	15	.17	4.71	4.88	4.81	.07554
FG <sub>5</sub>	1-5	% of NaOCl (w/v)	15	.10	4.69	4.79	4.75	.03950
C <sub>5</sub>	1-5	% of NaOCl (w/v)	15	.08	5.01	5.09	5.06	.03195

The concentration of NaOCl suffered variations during the study. B<sub>1</sub> was the recipient with the least variation by day with a mean decreasing of 0,32%, then FG<sub>1</sub> with a 1,05% and last W<sub>1</sub> with a 1,60%. From the maximum value measured to the minimum, there were a decreased of the concentration of NaOCl of 1,77% for B<sub>5</sub>, 3,48% for W<sub>5</sub>, and 2,09% for FG<sub>5</sub>, where the control only diminished 1,57%.

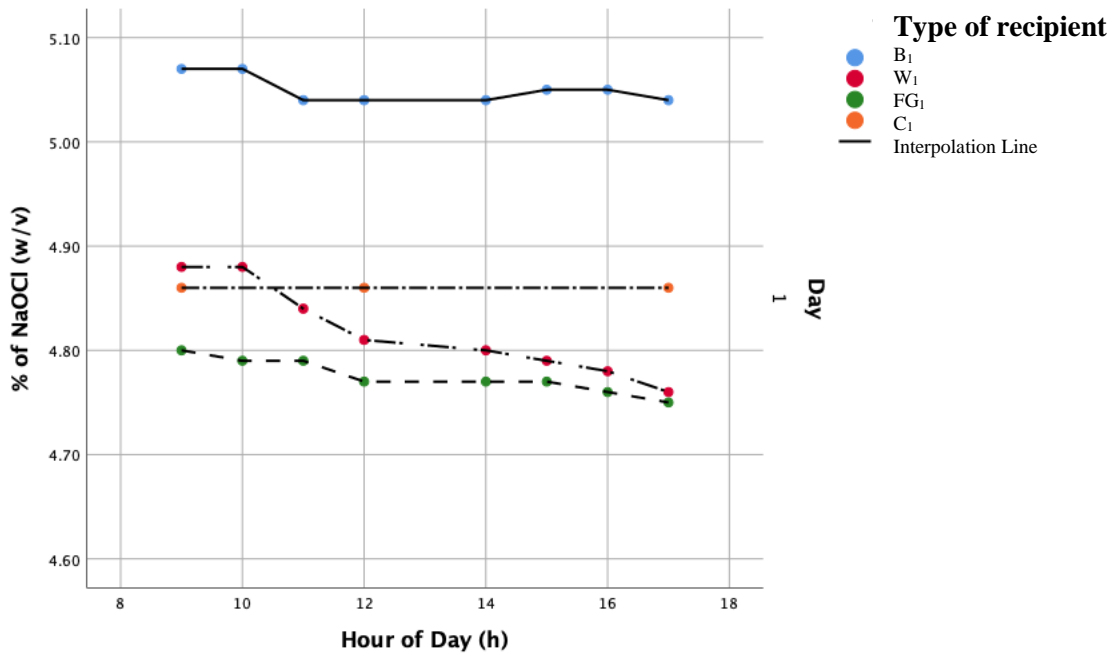


Figure 1 - Variation of the concentration of NaOCl through day 1, on the IL recipients

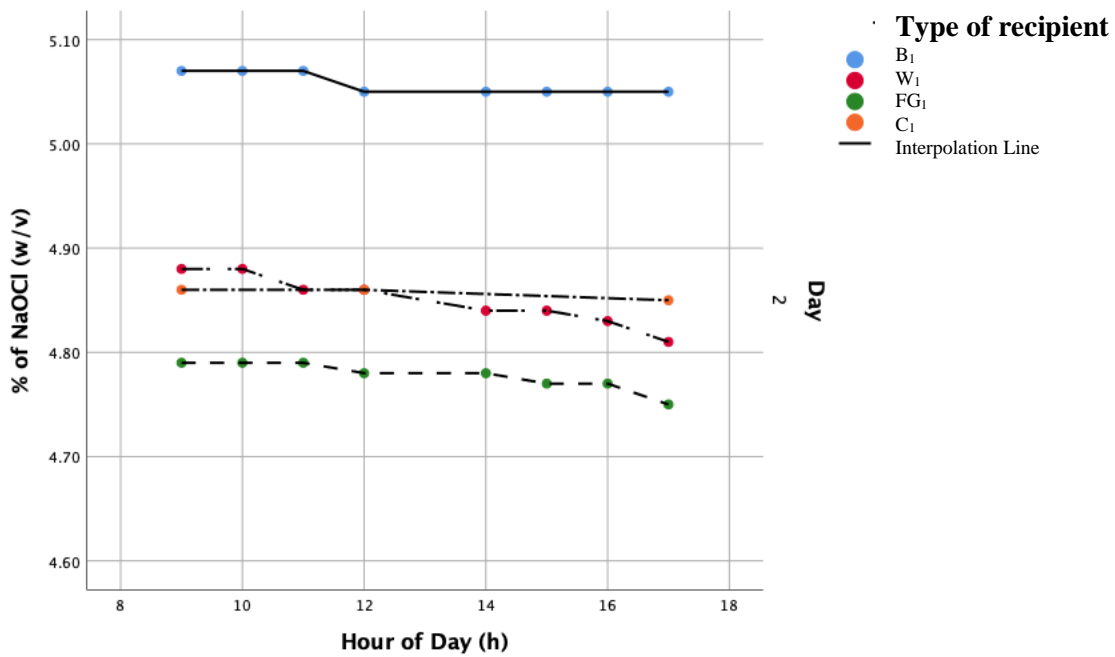


Figure 2 - Variation of the concentration of NaOCl through day 2, on the IL recipients

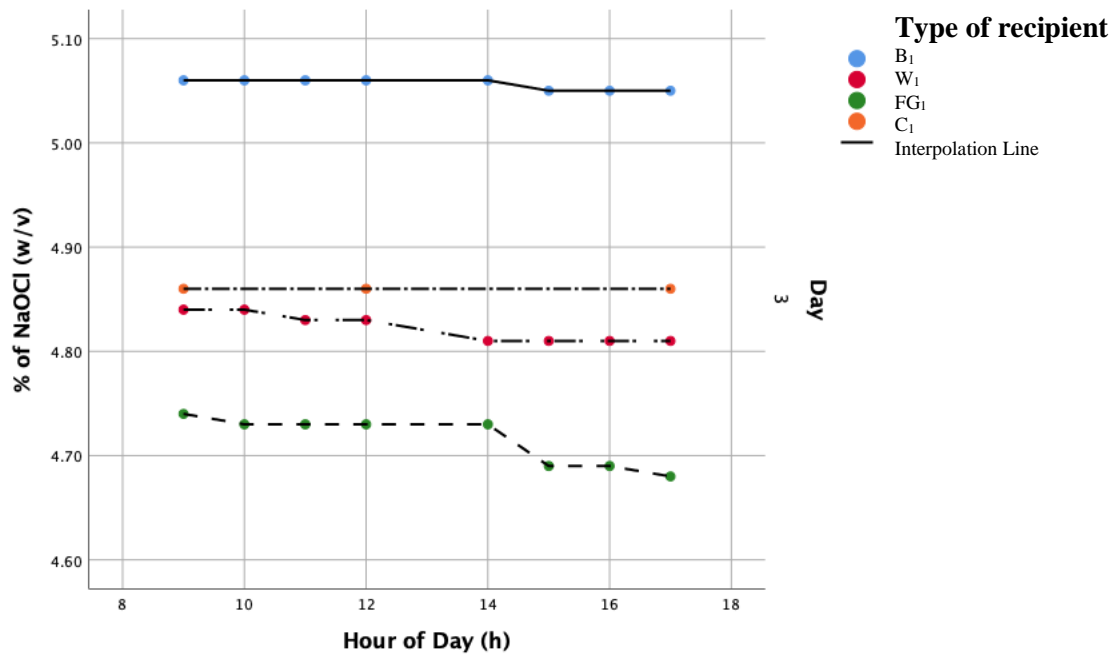


Figure 3 - Variation of the concentration of NaOCl through day 3, on the IL recipients

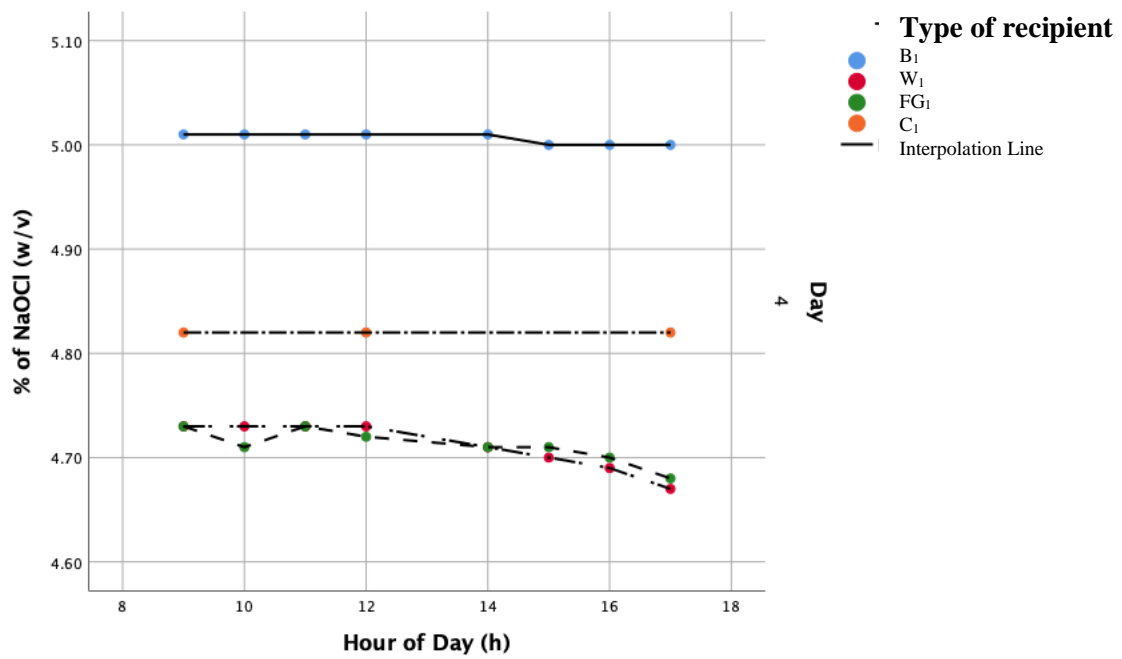


Figure 4 - Variation of the concentration of NaOCl through day 4, on the IL recipients

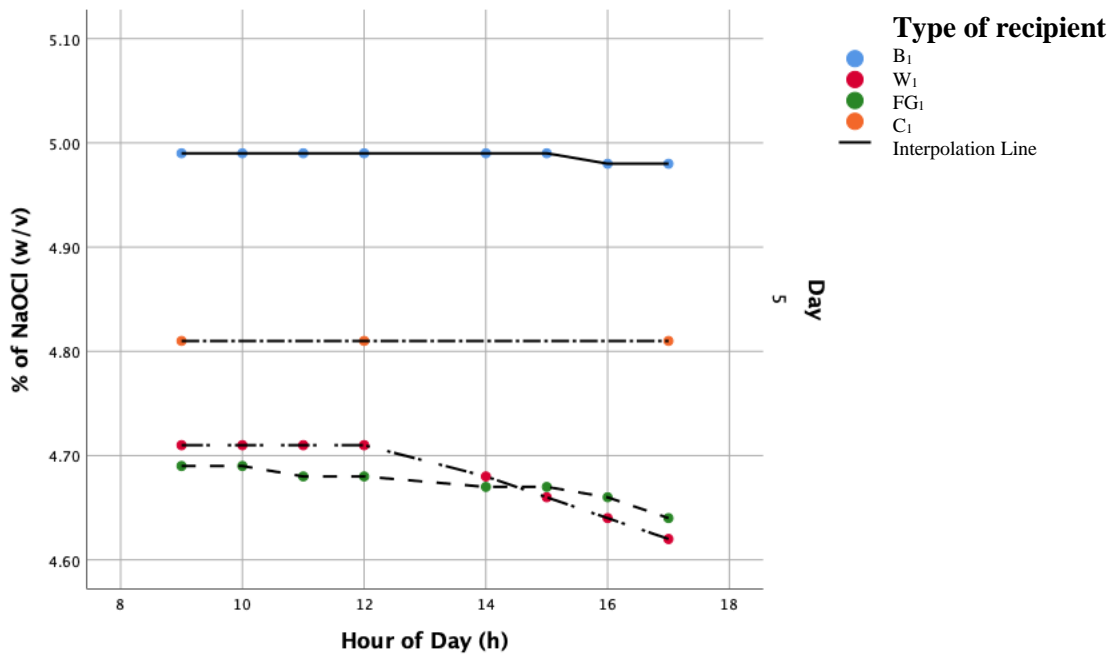
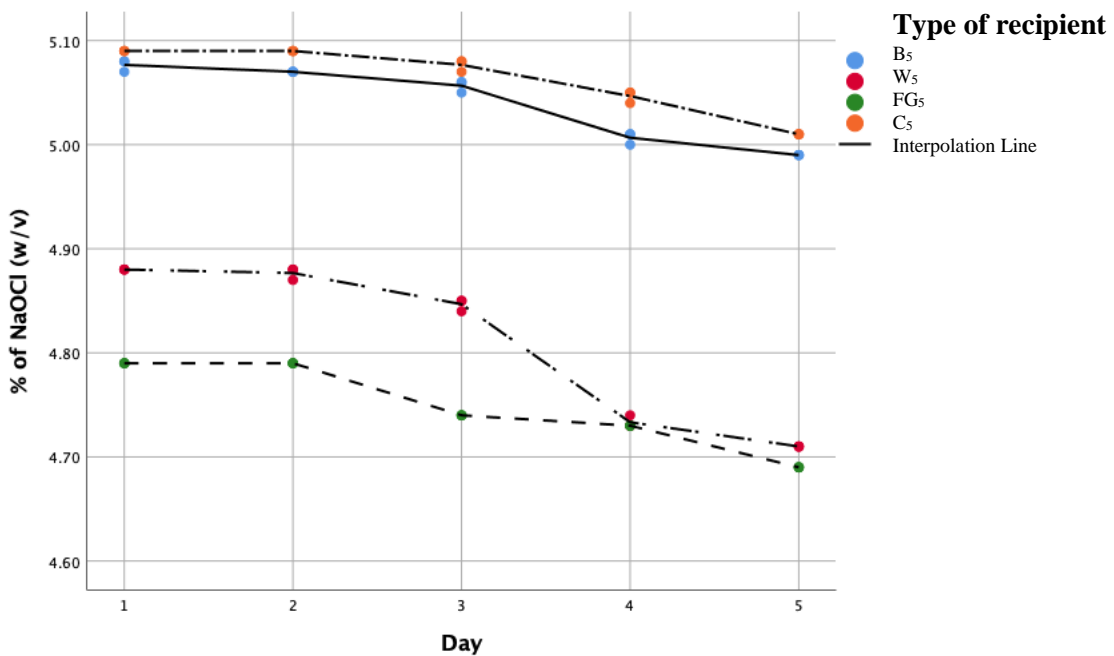


Figure 5 - Variation of the concentration of NaOCl through day 5, on the 1L recipients

From day 1 to day 5 (figures 1-5), all the 1L recipients suffered variations, with a slight decreased throughout the day. The control recipient was the one that had the least changes.



All the 5L recipients suffered variations during the week as well. The concentration of W<sub>5</sub> was the one that decreased the most, followed by FG<sub>5</sub>. The B<sub>5</sub> had a similar behaviour as the control recipient.

Table 3 - Spearman statistic test that correlates the variation of percentage of NaOCl during the week in the B5. \*\* Correlation is significant at the 0.001 level (2-tailed).

			Day	% of NaOCl
Spearman's rho	Day	Correlation Coefficient	1.000	-.981**
		Sig. (2-tailed)	.	.000
		N	15	15
	% of NaOCl	Correlation Coefficient	-.981**	1.000
		Sig. (2-tailed)	.000	.
		N	15	15

There is a statistically significant correlation on the modification of NaOCl on the white recipient during the week. The null hypostasis (H1.0) is rejected and the alternative hypostasis (H1.1) is accepted.

Table 4 - Spearman statistic test that correlates the variation of percentage of NaOCl during the week in the W5. \*\* Correlation is significant at the 0.001 level (2-tailed).

			Day	% of NaOCl
Spearman's rho	Day	Correlation Coefficient	1.000	-.972**
		Sig. (2-tailed)	.	.000
		N	15	15
	% of NaOCl	Correlation Coefficient	-.972**	1.000
		Sig. (2-tailed)	.000	.
		N	15	15

There is a statistically significant correlation on the modification of NaOCl on the white recipient during the week. The null hypostasis (H2.0) is rejected and the alternative hypostasis (H2.1) is accepted.

Table 5 - Spearman statistic test that correlates the variation of percentage of NaOCl during the week in the FG5. \*\* Correlation is significant at the 0.001 level (2-tailed).

		Day	% of NaOCl
Spearman's rho	Day	Correlation Coefficient	1.000
		Sig. (2-tailed)	.
		N	15
	% of NaOCl	Correlation Coefficient	-.975**
		Sig. (2-tailed)	.000
		N	15

There is a statistically significant correlation on the modification of NaOCl on the white recipient during the week. The null hypothesis (H3.0) is rejected and the alternative hypothesis (H3.1) is accepted.



## VI. DISCUSSION

The present study investigated whether the NaOCl concentration of available chlorine to be used in endodontic treatments stays the same in different types of storage recipients. Samples were collected from every recipient and its concentration measured, every 8 hours, every day, during a week. To our knowledge, there was no other study that replicate the clinical usage and manipulation of sodium hypochlorite during weekly practice.

Initially only two samples matched the expected concentration of 5% (w/v), the other three, on average, were 3,13% off the expected concentration as seen on table 1. This could be explained by some factors that may influence such as age of solution, in the current study the time span between manufacturing and purchase is unknown, or less restrict quality directives for the manufacturing, packaging or handling as well.(25) Based on this results, we should always check the concentration of NaOCl in order to be assured that we are irrigating at an desired value.

They were expected variations of % NaOCl across all recipients (table - 2), but from all the 1L recipients, the B<sub>1</sub> was the recipient which had less variation each day of the study. Also the B<sub>5</sub> recipient was the one who was less affected (1,77%) during the week with a similar behaviour with his similar capacity control recipient (C<sub>5</sub> - 1,56%), suggesting that this type of recipient should be considered as the main way to storage.(11,25)

As it was the objective of the present study to replicate a week's work at a endodontic clinic, everyday usage with NaOCl for root canal treatment purposes is shown in figures 1-5. In all days studied, each type of 1L recipient suffered changes of its % of hypochlorite throughout the day, with ending the day with a slightly less compared to the beginning of the day. This result is explained by some factors: constantly open and closed the recipient, mechanical agitation can also influence and most important the exposure to daylight.(15,19,25) To remind that at the beginning of each day the 1L recipients were filled with new samples from their correspondent 5L recipient, that's why there are variations between measurements in the beginning of the day and the day before.

As reported by Clarkson et al 2001, the chemical instability of NaOCl increases when its exposed to air or light, having said that, its recommended that amber glass or opaque plastic recipients should be considered as the NaOCl container.(19) The results from this

study are supported by this, as B<sub>1</sub> and FG<sub>1</sub> were the recipients with less variations each day during the week.

As shown before, the results were statistically significant (table 3-5), which means that there is a correlation, in this case negative, between the variation of the concentration of NaOCl and the storage time for the three recipients (B<sub>5</sub>, W<sub>5</sub>, FG<sub>5</sub>). Despite this significance the variation that occurred throughout the week was minor and the levels of concentration of NaOCl at the end of the week were still considered optimal as for endodontic treatment studies shows that we can have a successful root canal treatment with concentrations from 1% to 5%.(7,16,17)

Despite storage room conditions weren't the main focus of the present study, ideal conditions were chosen for this study. According to Frai el al 2001, with a guaranteed non-exposure of light and with a temperature of storage below 30°C, NaOCl samples wouldn't decay to much their % (w/v) over time, although the clinical factor of everyday use, with constant open and close of recipients, is an unknown factor.(11) With this study we filled that gap of information, and despite the variations of NaOCl concentration in different recipients were statistically significant, the results still shows optimal values for successful treatments. According to some investigators, storing NaOCl at lower temperatures helps preventing possible instabilities that would lower the concentration from the irrigant.

For possible future studies, we suggest longer periods of study time, so that we can evaluate how much time it would take to decreased the % (w/v) NaOCl to values that no longer are recommended for endodontic treatments.

## VII. CONCLUSION

From this study it was possible to confirm that the concentration of NaOCl in different storage recipients found in dental practices reduces throughout the day, during a week's work at a dental clinic.

The type of recipient is an influential factor in order to minimize the diminished of concentration of NaOCl.

The black type recipient in both capacities (1L and 5L) had the best results with the least variation of % NaOCl (w/v).

As confirmed before, the variation of NaOCl concentration in the samples used in this study is statistically significant ( $p = 0,001$ ), the values measured at the end of each day and at the end of the study were in range of optimal for endodontic treatment success.

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## IX. APPENDIX

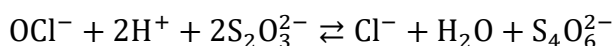
Calculations performed to determine the concentration of sodium hypochlorite from the volume of sodium thiosulfate spent in the titration:

$$V(\text{Na}_2\text{S}_2\text{O}_3)_{\text{spent}} = 272,7 \text{ ml}$$

$$[\text{Na}_2\text{S}_2\text{O}_3] = 0,1 \text{ M}$$

$$V(\text{NaOCl})_{\text{sample}} = 20 \text{ ml}$$

$$\text{Molar mass NaOCl} = 74,44 \text{ g/mol}$$



$$n^\circ \text{ mols of OCl}^- = \frac{V(\text{Na}_2\text{S}_2\text{O}_3)_{\text{spent}}(\text{L}) \times [\text{Na}_2\text{S}_2\text{O}_3]}{2}, \text{ by the stoichiometry of the reaction}$$

above

$$n^\circ \text{ moles of OCl}^- = \frac{V(\text{Na}_2\text{S}_2\text{O}_3)_{\text{gasto}}(\text{L}) \times [\text{Na}_2\text{S}_2\text{O}_3]}{2}$$

$$n^\circ \text{ mols of OCl}^- = \frac{272,7 \times 10^{-3} \times 0,1}{2}$$

$$n^\circ \text{ mole of OCl}^- = \frac{272,7 \times 10^{-3} \times 0,1}{2}$$

$$n^\circ \text{ moles of OCl}^- = 0,014 \text{ mols}$$

$$[\text{OCl}^-] = \frac{0,014}{20 \times 10^{-3}}$$

$$[\text{OCl}^-] = 0,68 \text{ M}$$

$$\text{mass of OCl}^- = 0,68 \times 74,44$$

$$\text{mass of OCl}^- = 50,75 \text{ g}$$

$$\%(\text{w/v}) = \frac{50,75}{1000} \times 100 = 5,07\%$$