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# **Interactions between the Tetrasodium Salts of EDTA and HEDP with Sodium Hypochlorite Irrigants**

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## Abstract

**Introduction:** A clinically useful all-in-one endodontic irrigant with combined proteolytic and decalcifying properties is still elusive. In this study, the chemical effects of dissolving the tetrasodium salts of 1-hydroxyethane 1,1-diphosphonic acid ( $\text{Na}_4\text{HEDP}$ ) or ethylenediaminetetraacetic acid ( $\text{Na}_4\text{EDTA}$ ) directly in sodium hypochlorite ( $\text{NaOCl}$ ) irrigants in polypropylene syringes were assessed over the course of 1 h. **Methods:** The solubility of the salts in water was determined. Their compatibility with 1% and 5%  $\text{NaOCl}$  was measured by iodometric titration, and in a calcium complexation experiment using a  $\text{Ca}^{2+}$ -selective electrode. **Results:** The salts dissolved within 1 min. The dissolution maximum of  $\text{Na}_4\text{HEDP}$  in water (wt/total wt) was  $44.6 \pm 1.6\%$ . The corresponding dissolution maximum of  $\text{Na}_4\text{EDTA}$  was  $38.2 \pm 0.8\%$ .  $\text{Na}_4\text{HEDP}$  at 18% in 5%  $\text{NaOCl}$  caused a mere loss of 16% of the initially available chlorine over 1 h. In contrast, a corresponding mixture between  $\text{NaOCl}$  and the  $\text{Na}_4\text{EDTA}$  salt caused 95% reduction in available chlorine after 1 min. Mixtures of 3%  $\text{Na}_4\text{EDTA}$  with 1%  $\text{NaOCl}$  were more stable, but only for 30 min. The  $\text{Na}_4\text{HEDP}$  lost 24% of its calcium complexation capacity after 60 min. The corresponding loss for  $\text{Na}_4\text{EDTA}$  was 34%. **Conclusions:** The compatibility and solubility of particulate  $\text{Na}_4\text{HEDP}$  with/in  $\text{NaOCl}$  solutions is such that these components can be mixed and used for up to 1 h. In contrast, short-term compatibility of the  $\text{Na}_4\text{EDTA}$  salt with  $\text{NaOCl}$  solutions was considerably lower, decreasing at higher concentrations of either compound. Especially for  $\text{Na}_4\text{HEDP}$ , but also for  $\text{Na}_4\text{EDTA}$ , the  $\text{NaOCl}$  had little effect on calcium complexation.

The term chemomechanical root canal preparation relates to the clinical concept of cleaning infected and/or inflamed root canal systems by means of instrumentation and irrigation with a chemically active substance. The goal is to clean the canal system and condition the canal wall for a subsequent filling procedure. Current approaches aim to remove the inflamed or necrotic soft tissue, smear layer, and biofilm, if present. Subsequently, the canal wall can be conditioned to improve the attachment of alloplastic filling materials (1), or to release growth factors to engineer a pulp-like tissue (2).

Alkaline sodium hypochlorite (NaOCl) solutions are the most widely used endodontic irrigants (3, 4). These NaOCl solutions are relatively cheap and readily available from various commercial sources (5). Furthermore, NaOCl solutions feature unique soft tissue and biofilm matrix dissolution properties (6, 7). However, irrigation with NaOCl solutions has no effect on the inorganic aspects of the smear layer (8), and does not prevent the accumulation of inorganic debris during instrumentation (9). Consequently, irrigation protocols using sodium hypochlorite in an alternating mode with an aqueous solution containing a decalcifying agent such as EDTA or citric acid have been proposed (10). However, these protocols are not necessarily user-friendly. Because of the chemical reactivity of OCl<sup>-</sup> (hypochlorite) with most decalcifying agents, especially those in acidic form, combined use is not possible (11, 12). The search for hypochlorite-compatible decalcifying agents or chelators in endodontic research has yielded two candidates thus far: tetrasodium HEDP (Na<sub>4</sub>HEDP)(12, 13) and tetrasodium EDTA (Na<sub>4</sub>EDTA)(14, 15). Both, 1-hydroxyethane 1,1-diphosphonic acid (HEDP or etidronate) and ethylenediaminetetraacetic acid (EDTA) are available as disodium and tetrasodium salts, depending on the desired pH. These biocompatible chelators are commonly used in the cosmetic, food and dish washing, and the laundry detergent industry (16). HEDP has a higher calcium binding capacity per gram chelator (16), while EDTA has a stronger affinity to calcium, i.e. a higher dissociation constant of the complex (17). Consequently, EDTA has a higher demineralizing effect than HEDP, and can erode dentin (18). EDTA solutions of a concentration lower than the usual 17% have thus been proposed. It has been shown that 3% EDTA can remove smear layer as effectively as 24% EDTA (19). It would appear that the reduction of the available chlorine that occurs in direct mixtures with Na<sub>4</sub>HEDP is less than that in counterparts containing Na<sub>4</sub>EDTA (12, 15). However, a direct comparison is elusive. Furthermore, it is not known how

the calcium binding capacity (complexation) of these two chelators is affected by the presence of NaOCl in aqueous mixtures.

It is known from previous research that no chelator investigated thus far has true long-term compatibility with NaOCl (12). Consequently, it was the goal of the present investigation to study short-term chemical interactions between the tetrasodium salts of HEDP and EDTA with NaOCl irrigants at different concentrations recommended by previous research. To simulate a clinical scenario, mixtures were made directly in 10-mL polypropylene irrigation syringes.

## **Materials and Methods**

### **Particles and Solutions**

The tetrasodium salts of 1-hydroxyethane 1,1-diphosphonic acid (HEDP) and ethylenediaminetetraacetic acid (EDTA) were obtained from commercial sources (HEDP: Cublen K8514GR, Zschimmer & Schwarz, Burgstädt, Germany, batch no. 92729; EDTA: Sigma Aldrich, Steinheim, Germany, batch no. 058K0136). The salts were sieved through a stack of precision sieves with defined screen openings (Test Sieve, Retsch GmbH, Haan, Germany). Particles from the 300 to 500- $\mu\text{m}$  fraction were used for all subsequent experiments.

The solutions used in these experiments were 5.0% and 1.0% NaOCl. They were diluted from a concentrated 14% stock solution using pure reagent-grade water. The stock solution was kept at 5°C in an amber glass bottle. Diluted solutions were prepared freshly before each experiment (see below) and warmed to ambient temperature. Available chlorine was determined and verified using iodometric titration (20).

### **Dissolution maximum**

The particles were dissolved in 25 mL of deionized water at room temperature. The powders were incrementally added under constant stirring until the particles stopped to dissolve. This procedure was repeated twice per chelator salt under investigation (triplicate experiments). Subsequently, the solutions were filtered using a .22- $\mu\text{m}$  syringe filter (TPP Techno Plastic Products AG, Trasadingen, Switzerland). To determine the weight of the dissolved salts per total weight of solution, 5 mL of the filtered solutions were pipetted into a pre-weighed glass vial. Weights were determined using a precision balance (Mettler AT261 DeltaRange, Mettler Toledo Int. Inc., Columbus, OH). Subsequently, the probes were desiccated in a drying oven (HORO

type 038A, Dr. Ing. A. Hoffmann, Ostfildern, Germany) at 120°C until all the water had evaporated and a steady weight was achieved.

### **Effect of chelator salts on available chlorine**

In this experiment, the chelator salts were added to and dissolved in pre-weighed amounts of 1% or 5% NaOCl. The mixtures were prepared directly in 10-mL polypropylene syringes (Omnifix REF4617100V, B.Braun, Melsungen, Germany). The plungers were removed and the syringe openings closed using paraffin film (Parafilm, Bemis, Neenah, WI). The NaOCl solution and the chelator salt were added and the syringe hubs were closed using Parafilm (Bemis). Experiments were performed in triplicates at the intervals and concentration measured, i.e. three separate syringes were prepared each (total of 150 syringes for these experiments, see Tables 1 and 2). Experiments were performed at ambient temperature. The triplicate samples per concentration/time were placed in a centrifuge tube rack and gently shaken by hand for 1 min. By that time, the chelator salts had dissolved completely. Aliquots of the mixed solutions, approximately 0.5 g, were weighed in the precision balance. They were removed after 1, 10, 20, 30, and 60 min and assessed for their amount of available chlorine using a titration apparatus (665 Dosimat, Deutsche Metrohm GmbH & Co. KG, Filderstadt, Germany). A 0.1 mol/L sodium thiosulfate solution (Merck KGaA Darmstadt, Darmstadt, Germany, Lot. no. HC378976) was used as titrator. In addition, the temperature and the pH of the reaction mixtures were recorded. Temperature measurements were performed using an electronic thermometer (Checktemp 1, Hanna Instruments, Leighton Buzzard, Great Britain) and pH measurements using a calibrated microelectrode (827 pH lab, Deutsche Metrohm GmbH & Co. KG).

### **Effect of hypochlorite on Ca<sup>2+</sup> complexing capacity**

To study the effect of the interactions between the hypochlorite and the chelators under investigation, the salts were added to a 5% NaOCl solution. (From the available chlorine experiment), it was expected that there was more interaction in this solution than in the 1% counterpart. Again, mixtures were prepared in individual syringes in triplicates for each time point and combination as described above (36 syringes in total, see Table 3). To stop the reaction, 0.6 g sodium thiosulfate was added to the mix immediately, after 30 or 60 min. To determine the Ca<sup>2+</sup> complexing capacity of the two chelators in the presence of NaOCl, a calcium chloride standard solution (CaCl<sub>2</sub> \* 2 H<sub>2</sub>O, 18.35g/5L, EMSURE, Merck) was used instead of the

commonly used calcium carbonate standard. The concentration of the standard solution (1000 ppm  $\text{Ca}^{2+}$ , pH 6.1) was verified using an atomic absorption spectrometer (contraAA 300, Analytik Jena AG, Jena, Germany) and compared to that of a commercially available 1000 ppm  $\text{Ca}^{2+}$  standard solution (CertiPUR calcium carbonate, Merck, no. 1.19778.0100). A pilot study revealed that the pH effects of NaOCl gave rise to false readings when using a calcium carbonate standard solution (pH 1), which needs to be acidic (data not shown). Thus, the experiments had to be performed in a high-pH environment. The  $\text{CaCl}_2$  solution was buffered at pH 10 using a commercially available boric acid/ potassium chloride/ sodium hydroxide buffer (2 mL per 100 mL standard solution, EMSURE, Merck). For the experiment the 1000 ppm  $\text{Ca}^{2+}$  standard solution was diluted to 100 ppm. The potential of 100 mL of this solution was determined using a calcium-selective electrode (ORION 93 series 9300 BN, Thermo Fisher Scientific, Waltham, MA). Subsequently, 0.1 mL increments of the reaction mixtures were added to 100 mL of the 100 ppm  $\text{Ca}^{2+}$  standard solution. Control measurements were performed using corresponding amounts of 6% sodium thiosulfate in 5% NaOCl or deionized water.

### **Data presentation**

All the percentage (%) values reported relate to mass, i.e. weight of chemicals per total weight of the resulting solution. The data presented are based on triplicate experiments. Mean values and standard deviations are shown. As these data represent results from chemical experiments with little variance, only descriptive statistics were applied.

### **Results**

Both the  $\text{Na}_4\text{HEDP}$  and  $\text{Na}_4\text{EDTA}$  salts dissolved in the NaOCl solutions within 1 min.. The dissolution maximum of  $\text{Na}_4\text{HEDP}$  in water (wt/ total wt) was  $44.6 \pm 1.6\%$ . The corresponding dissolution maximum of  $\text{Na}_4\text{EDTA}$  was  $38.2 \pm 0.8\%$ .

Both chelator salts showed some negative effects on free available chlorine when dissolved in NaOCl solutions (Tables 1 and 2). When  $\text{Na}_4\text{HEDP}$  powder was mixed with 1% or 5% NaOCl, between 72% and 87% of the initially available chlorine remained in solution after 60 min (Table 1 and 2). The higher concentration of HEDP (18%) caused a slightly higher loss of available chlorine than the lower concentration (9%), while the concentration of the NaOCl solution had little impact (Fig. 1). In contrast,  $\text{Na}_4\text{EDTA}$  showed some strong and dose-dependent effects,

especially in 5% NaOCl (Table 2). When 18% Na<sub>4</sub>EDTA was present in 5% NaOCl, the reaction was immediate (Fig. 1), with a concurrent drop in pH and rise in temperature (Table 2). In the presence of 3% Na<sub>4</sub>EDTA, 5% NaOCl reacted in the first 10 min, while the 1% solution retained 87% of the available chlorine in the first 30 min (Table 1).

The tetrasodium salt of HEDP in 5% NaOCl initially lost 9% of its calcium chelating effect compared to a pure solution in water (Table 3), and 24% after 60 min. These losses correlated to the time course of the interaction with the available chlorine (Table 2). The corresponding immediate loss for Na<sub>4</sub>EDTA was 13%, reaching 34% after 60 min. Consequently, despite its apparent interaction with the hypochlorite (OCl<sup>-</sup>), the Na<sub>4</sub>EDTA retained its ability to chelate Ca<sup>2+</sup> over the course of this experiment.

## Discussion

The current study investigated the potential of adding particulate chelators to NaOCl solutions in order to generate an *ad hoc* all-in-one irrigant. The NaOCl should maintain its pH and available hypochlorite (OCl<sup>-</sup>) with an additional decalcifying effect over the approximate course of an endodontic treatment. This approach would circumvent the technical problems of storing and/or mixing solutions. The results showed that the tetrasodium salt of HEDP, but not necessarily that of EDTA, is suitable for this purpose, even if the EDTA concentration is kept low.

Although the current experiments were performed in a simulated clinical environment, this is a strictly chemical investigation, and no clinical measures were obtained. The chelator salts were mixed with sodium hypochlorite solutions at room temperature in polypropylene syringes. For the experiments on the effect of hypochlorite on the chelators, a 100-ppm Ca<sup>2+</sup> solution was selected, because this amount of calcium corresponds to that removed from root canals during the irrigation with chelating solutions (13). It is clear from the available literature that all the effects of NaOCl solutions desired by the endodontist, i.e. biofilm and necrotic tissue dissolution, are related to available chlorine and pH in that solution (21). HEDP in aqueous solution, when freshly mixed with a NaOCl solution, neither impedes the antimicrobial properties of the NaOCl (22), nor its ability to dissolve necrotic tissue (23). A recent study also showed that this may be the case with an alkaline solution of Na<sub>4</sub>EDTA (15). The EDTA solution normally used by dentists has a pH between 7 and 8.5, and is prepared by adding sodium hydroxide (NaOH) to the disodium salt of EDTA in water. Such a solution strongly interferes with available chlorine

(hypochlorite) in NaOCl solutions (24). Tartari and co-workers reported that Na<sub>4</sub>EDTA, when mixed with NaOCl, continues to remove smear layer and has little effect on tissue dissolution in the short-term. This is in seeming contrast to the current results. However, these authors mixed 5% NaOCl with solutions of 10% and 20% Na<sub>4</sub>EDTA (resulting in 2.5% NaOCl solutions), and then assessed the smear layer (5 min) and soft tissue dissolution (15 min). The focus of the current study was different: we investigated the possibility to directly mix chelator salts with NaOCl solutions (resulting in higher available concentrations of chelator and hypochlorite), store these mixtures for different time intervals, and then assess their efficacy. The results reported here on the loss of available chlorine in NaOCl solutions are comparable to those reported by other authors; their solution initially containing 2.5% NaOCl and 10% Na<sub>4</sub>EDTA lost 51% of the available chlorine in the first hour (15). The current results show that the more available chlorine and Na<sub>4</sub>EDTA was in the system, the stronger was the chemical interaction between these two compounds (Tables 1 and 2). This can be explained due to kinetics, as the reaction rate is increased for higher concentrations of substrates. Consequently, the tetrasodium salt of EDTA does have some compatibility with NaOCl, but only at low concentrations and in the short term. As reported earlier, this may be useful when two solutions are freshly mixed (15). For the application intended in this study and in the clinical situation, however, the tetrasodium salt of HEDP appears much more suitable than the EDTA counterpart.

The limited interference of the NaOCl solutions with the Ca<sup>2+</sup> complexation of the chelators under investigation has also been reported in earlier studies assessing mixtures of the respective solutions (12, 13, 23). Although NaOCl is a strong oxidizing agent, it had little effect on calcium chelating ability. Comparing the mixture HEDP/H<sub>2</sub>O to HEDP/NaOCl, the calcium chelation was slightly reduced due to the oxidized chelator, which decomposes to acetic acid. Although there is also an oxidation of EDTA, the decomposition is rather slow (25) and can yield unknown by-products and iminoacetic acid, which can still chelate Ca<sup>2+</sup>. Hence, EDTA could possibly chelate Ca<sup>2+</sup> but NaOCl is also decomposed and there is not enough available chlorine present if used in a combined solution (Tables 1 and 2).

Anytime new materials or combinations (irrigants) are used in different combinations or with different techniques, there are many questions regarding biocompatibility and efficacy that need to be addressed. This manuscript provides a first step to at least provide the feasibility for this irrigant combination. However, apart from the self-evident advantage for clinicians to use just one irrigant, it remains unclear whether the effective concentrations of chlorine and the capacity

of a chelating agent maintained better when 1) undiluted stable individual components are used and frequently refreshed, or 2) a single component is refreshed but constantly degrading over the hour of use. Future studies should also address the compatibility of Na<sub>4</sub>HEDP with chlorhexidine, Ca(OH)<sub>2</sub>, as well as etching and bonding agents. The effects of temperature and ultrasonic activation on the combined irrigant should also be assessed. Furthermore, dentin conditioning using a NaOCl/HEDP combination should be scrutinized regarding its effects on pluripotent cells/ regenerative endodontic procedures. Last but not least, clinical studies should be performed. Indeed, it is not entirely clear whether a decalcifying agent is truly necessary to improve the outcome of root canal treatment, although follow-up studies gave a hint in that direction, at least in the case of re-treatments (26).

## **Conclusion**

The compatibility and solubility of particulate Na<sub>4</sub>HEDP with/in NaOCl solutions is such that these components can be mixed and used for up to 1 h. In contrast, short-term compatibility of the Na<sub>4</sub>EDTA salt with NaOCl solutions was considerably lower, decreasing at higher concentrations of either compound. Especially for Na<sub>4</sub>HEDP, but also for Na<sub>4</sub>EDTA, the NaOCl had little effect on calcium complexation. However, it needs to be cautioned that these statements are based entirely on a laboratory study model and not on actual clinical conditions.

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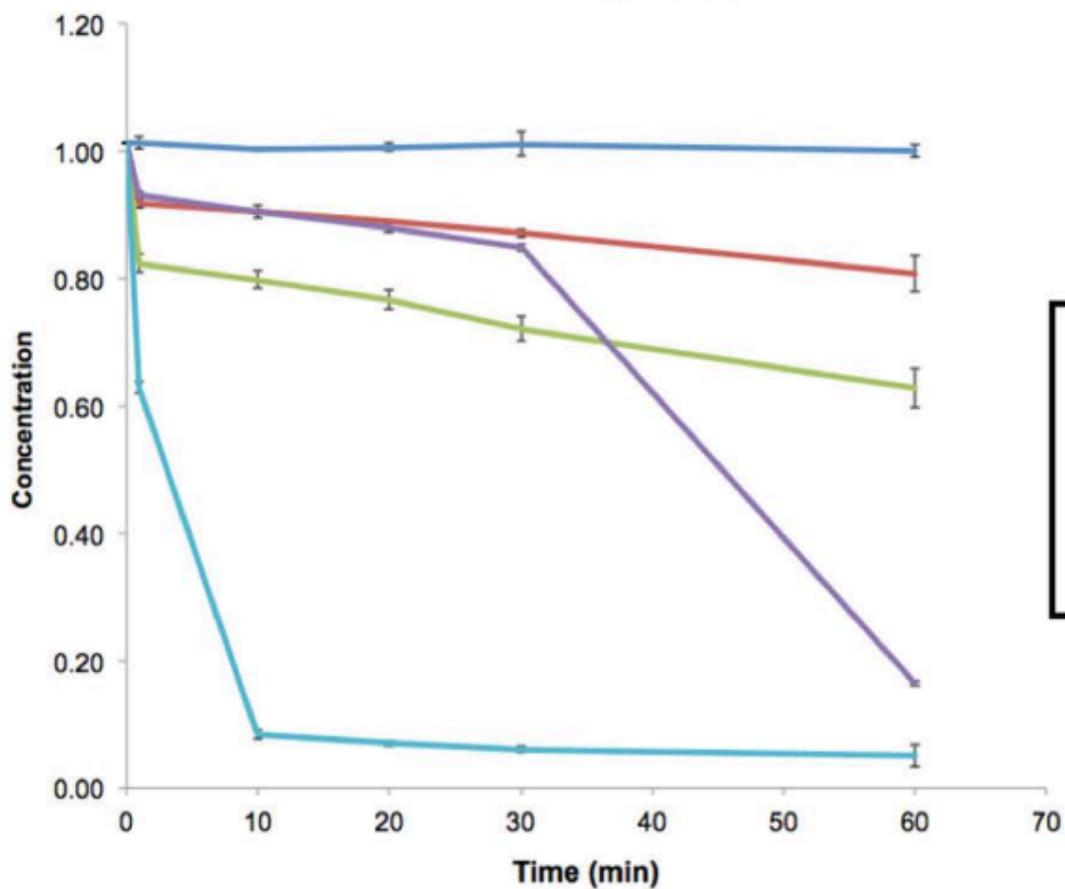
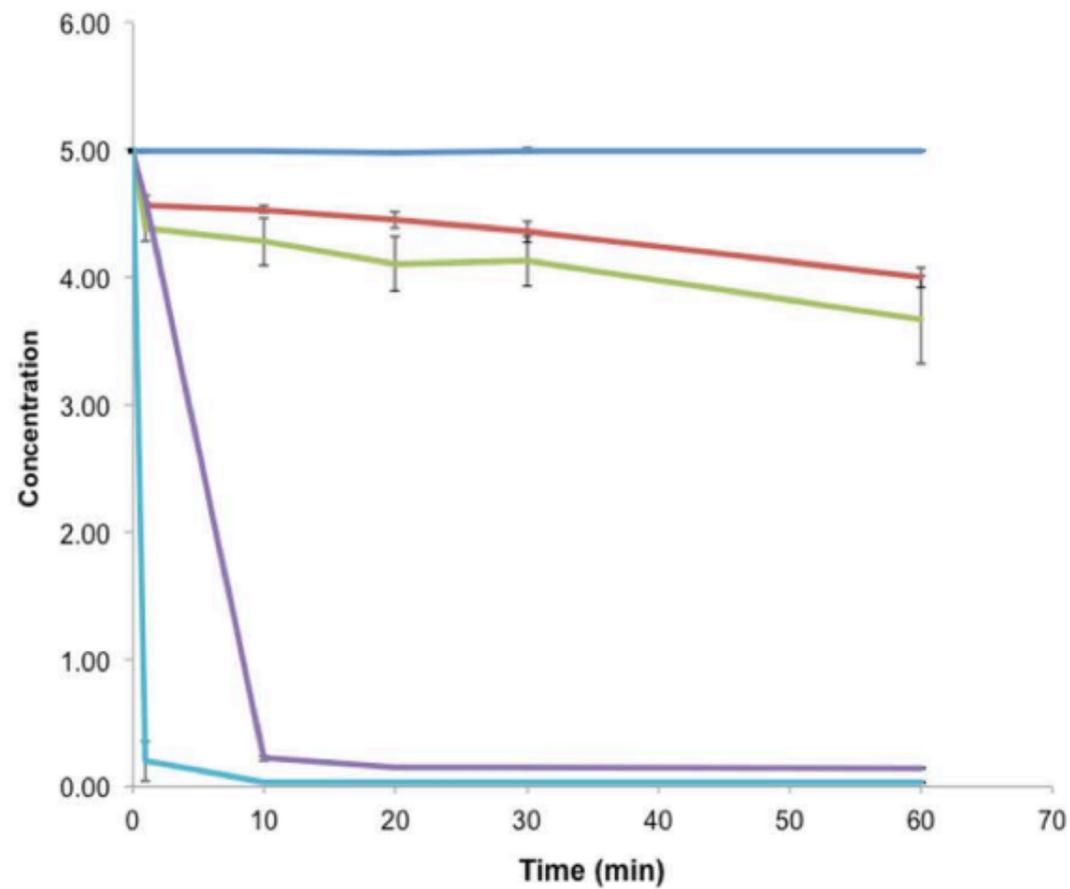
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Caption:

**Figure 1.** NaOCl concentrations in the 2 solutions used in this study over time, as assessed by iodometric titration. Note that Na<sub>4</sub>EDTA salt caused a faster decline in available chlorine in the 5% compared to the 1% NaOCl solution. This was not the case with the Na<sub>4</sub>HEDP salt.

**1% NaOCl****5% NaOCl**

**TABLE 1.** Available chlorine (% of theoretical maximum), pH and temperature (°C) in 10 mL of a 1.0% NaOCl solution in a polypropylene syringe after addition of the chelator salt

Salt (wt/wt)	1 min	10 min	20 min	30 min	60 min
<b>18% Na<sub>4</sub>HEDP</b>					
Available chlorine	95 ± 2%	92 ± 2%	88 ± 2%	83 ± 2%	72 ± 3%
pH range	11.6	11.5 – 11.7	11.4 – 11.6	11.3 – 11.5	11.1 – 11.2
Temperature	24 ± 0°C	24 ± 0°C	24 ± 0°C	23 ± 0°C	23 ± 0°C
<b>9% Na<sub>4</sub>HEDP</b>					
Available chlorine	99 ± 1%	97 ± 0%	95 ± 0%	93 ± 1%	87 ± 3%
pH range	11.0 – 11.2	11.1 – 11.2	11.1	11.0 – 11.1	10.9 – 11.0
Temperature	22 ± 0°C				
<b>18% Na<sub>4</sub>EDTA</b>					
Available chlorine	73 ± 1%	10 ± 1%	8 ± 0%	7 ± 1%	6 ± 2%
pH range	11.2 – 11.5	9.8	9.7 – 9.8	9.7 – 9.8	9.7
Temperature	21 ± 1°C	25 ± 0°C	23 ± 0°C	22 ± 0°C	21 ± 1°C
<b>3% Na<sub>4</sub>EDTA</b>					
Available chlorine	95 ± 1%	93 ± 1%	90 ± 1%	87 ± 1%	17 ± 0%
pH range	12.1 – 12.6	12.1 – 12.2	11.5 – 12.0	11.5 – 11.9	9.7 – 9.8
Temperature	20 ± 1°C	21 ± 0°C	21 ± 0°C	21 ± 0°C	23 ± 1°C
<b>none (control)</b>					
Available chlorine	100 ± 1%	99 ± 0%	100 ± 1%	100 ± 2%	99 ± 1%
pH range	11.7	11.6 – 11.8	11.7	11.7 – 11.8	11.6 – 11.8
Temperature	22 ± 1°C	22 ± 0°C	22 ± 0°C	22 ± 0°C	22 ± 0°C

Triplicate experiments, means and standard deviations except pH: total ranges.

**TABLE 2.** Available chlorine (% of theoretical maximum), pH and temperature (°C) in 10 mL of a 5.0% NaOCl solution in a polypropylene syringe after addition of the chelator salt

Salt (wt/wt)	1 min	10 min	20 min	30 min	60 min
<b>18% Na<sub>4</sub>HEDP</b>					
Available chlorine	100 ± 0%	99 ± 4%	95 ± 5%	95 ± 5%	84 ± 8%
pH range	12.3 – 12.7	11.9 – 12.6	12.1 – 12.3	11.9 – 12.4	10.5 – 11.6
Temperature	23 ± 2°C	24 ± 2°C	24 ± 2°C	24 ± 1°C	25 ± 2°C
<b>9% Na<sub>4</sub>HEDP</b>					
Available chlorine	98 ± 1%	98 ± 1%	96 ± 1%	94 ± 2%	86 ± 2%
pH range	12.3	11.9 – 12.3	11.5 – 12.1	10.8 – 11.9	9.9 – 10.4
Temperature	22 ± 1°C	22 ± 1°C	22 ± 1°C	23 ± 1°C	24 ± 1°C
<b>18% Na<sub>4</sub>EDTA</b>					
Available chlorine	5 ± 4%	1 ± 0%	1 ± 0%	1 ± 0%	1 ± 0%
pH range	8.8 – 11.8	8.6 – 8.7	8.6 – 8.7	8.6 – 8.7	8.6 – 8.7
Temperature	38 ± 10°C	33 ± 2°C	28 ± 1°C	26 ± 0°C	22 ± 0°C
<b>3% Na<sub>4</sub>EDTA</b>					
Available chlorine	96 ± 1%	5 ± 0%	3 ± 0%	3 ± 0%	3 ± 0%
pH range	10.3 – 10.9	7.0 – 7.5	6.9 – 7.0	6.9 – 7.0	6.9 – 7.0
Temperature	22 ± 1°C	49 ± 3°C	37 ± 1°C	30 ± 1°C	26 ± 1°C
<b>none (control)</b>					
Available chlorine	100 ± 0%	100 ± 0%	100 ± 0%	100 ± 0%	100 ± 0%
pH range	12.4 – 12.5	12.4 – 12.6	12.3 – 12.5	12.5 – 12.7	12.5 – 12.6
Temperature	22 ± 0°C				

Triplicate experiments, means and standard deviations except pH: total ranges.

**TABLE 3.**  $\text{Ca}^{2+}$  that was chelated (% reduction) when 0.2 mL of mixtures of the tetrasodium salts of HEDP or EDTA with deionized water or sodium hypochlorite solutions pre-incubated in 10-mL polypropylene syringes for different time intervals were added to 100 mL of a 100 ppm calcium chloride standard solution

<b>Mixture (wt/wt)</b>	<b>Immediately</b>	<b>30 min</b>	<b>60 min</b>
18% HEDP in water	$82 \pm 1\%$	$83 \pm 1\%$	$82 \pm 2\%$
18% HEDP in 5% NaOCl	$75 \pm 1\%$	$68 \pm 2\%$	$62 \pm 3\%$
18% EDTA in water	$39 \pm 1\%$	$40 \pm 3\%$	$44 \pm 1\%$
18% EDTA in 5% NaOCl	$34 \pm 3\%$	$30 \pm 4\%$	$29 \pm 3\%$

Triplicate experiments, means and standard deviations.