Interaction between EDTA and Sodium Hypochlorite: A Nuclear Magnetic Resonance Analysis

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Abstract
Recent studies detected erosion of the dentinal walls following the use of EDTA as a final flush. Several authors have studied degradation of EDTA and it appears to be caused by an oxidation reaction. The objective of this paper was to verify through nuclear magnetic resonance (NMR) analysis if the oxidizing property of sodium hypochlorite inactivates EDTA. Solutions of sodium hypochlorite and EDTA were analyzed. EDTA tracing and the appearance of new signals indicative of by-products of the reaction, were studied at different time intervals with a NMR analysis. The tracings of NMR analysis confirmed that the reaction between sodium hypochlorite and EDTA lead to a very slow but progressive degradation of this compound. Mindful of the limitations of an in vitro study, the results of this study nevertheless demonstrated that a final flush with sodium hypochlorite cannot limit the chelating effects of EDTA in a clinically realistic time period. (J Endod 2006;32:460–464)

Key Words
Cleaning, EDTA, nuclear magnetic resonance (NMR), sodium hypochlorite
dizing properties of sodium hypochlorite, in addition to rinsing out the chelating solution, it could also reduce its chemical activity. Inactivation of EDTA would appear to be achieved through an oxidation reaction (29), which limits the progressive demineralization and could therefore prevent a further weakening of the inorganic structure of the tooth. The degradation of the EDTA molecule has been studied by several authors (29–32). This process brings about the formation of intermediate by-products such as gluoxalic acid and ethylenediaminotriacetic acid, both of which are biocompatible and not very aggressive towards the dentinal components. Neither one seems able to damage the peritubular cells in the event of their extrusion from the apex that should nonetheless be studiously avoided (12).

The reducing action of NaOCl on EDTA could influence the combined use of these irrigating solutions during root canal therapy. If degradation of EDTA would be immediate, its effect should be limited sooner.

Nuclear magnetic resonance (NMR) spectroscopy is based on the disturbance of the energy levels of the nuclei influenced by a strong magnetic field. Some nuclei have a certain spin that can be described as the rotation of the nuclei around an axis. A particular nuclear magnetic momentum can be associated to each nucleus. When one of these nuclei is inserted in a homogenous external magnetic field, there is a magnetic interaction between its magnetic momentum and the magnetic field itself. NMR utilizes this interaction to allow the sample to absorb a known wavelength of electromagnetic radiation. The use of NMR facilitates the recording of signals that indicate the progress of the oxidation of the original sample.

The aim of this study was to elucidate the chemical interactions between EDTA and sodium hypochlorite and to analyze the oxidation reaction utilizing NMR.

Materials and Methods

EDTA powder (SIGMA, St. Louis, MO) and 5% sodium hypochlorite solution (OGNA Laboratori Farm, Milan, Italy) were analyzed. All substances used in this study were analytically pure. The test solutions were kept at room temperature and away from direct light at all times. During the experiment the chemical reaction was monitored with reagents mixed inside a NMR tube. Solutions of EDTA (without the addition of NaOCl in the test tube) remained stable for many days under the same experimental conditions of temperature and partial oxygen pressure.

The oxidation process of EDTA was monitored by following the loss of nuclear magnetic signals relative to both the protons (\(^1\)H) and the \(^13\)C isotopes of EDTA. Optimal conditions to record the NMR signal was obtained utilizing 40 mg of EDTA dissolved in 0.7 ml deuterated water (D\(_2\)O). The spectrum of the \(^1\)H- 300 MHz proton and the spectrum of \(^13\)C- 75.5 MHz carbon were obtained using a Gemini spectrometer (Varian SpA, Leini, Torino, Italy).

The calculation of the number of moles (\(n\)) in a certain substance was derived using the following formula:

\[
 n = \frac{g}{Mr}
\]

in which \(g\) represents the grams and \(Mr\) the relative mass of a compound. The number of moles (\(n\)) of EDTA in 40 mg is equal to 0.096 mmoles.

This data was important because it allowed the calculation of the quantity of sodium hypochlorite that needed to be added to the EDTA for oxidation to take place. The interaction between sodium hypochlorite and EDTA involves the transfer of electrons (\(e^-\)) from one atom to another. The complete oxidation of a molecular group \(-\text{CH}_2-\text{COO}^-\) requires 1.5 equivalent molecules of sodium hypochlorite. Because the EDTA molecule is made up of four \(-\text{CH}_2-\text{COO}^-\) groups, the relationship would be 1:6 and therefore, to completely inactivate the 0.096 mmoles of EDTA, a quantity of sodium hypochlorite containing a number of mmoles six times greater (0.576 mmoles) needed to be added. Utilizing the same formula as before, it was determined that the number of moles (\(n\)) in 0.1 ml of 5% NaOCl would have to be 0.067 mmoles. Therefore, 0.86 ml of 5% NaOCl was added to the 0.7 ml of EDTA to guarantee its complete deactivation, at least on a theoretical basis.

The peaks in the EDTA graphs as well as the emergence of new signals related to the by-products of its interaction with NaOCl were analyzed with NMR at different time intervals, 1.5 min, 4.5 min, 7 min, 11 min, 16 min, 25 min, 50 min, 80 min, 120 min.

Results

The graphs obtained from the NMR analysis confirmed that the reactions between NaOCl and EDTA caused the oxidation of the latter which lead to its progressive deactivation, however, they also showed that this reaction was extremely slow (Fig. 1). The peaks of EDTA and its by-products are detailed in Fig. 2. The kinetics of this oxidation was not immediate, rather under the conditions of this in vitro experiment they were observed at the different time intervals at which the peaks were analyzed. It was incomplete, even after 120 min. Table 1 presents both the frequency in Hz [frequency/(300 MHz) = chemical shift = part per million (ppm)] and the intensity (in arbitrary units). It demonstrates that the oxidation of EDTA by sodium hypochlorite is complex and requires time. In addition, the kinetic oxidation of both peak 1 (\(=\text{N-CH}_2-\text{C}(=\text{O})\text{OR}\)) and peak 2 (\(=\text{N-CH}_2-\text{CO-N}\)) progresses at a different rate. This indicates the progressive formation of by-products of the reaction between the two chemicals. The continuous evolution of the signals relative to the EDTA protons indicate that during the first 7 min of the reaction between the two compounds no significant changes in the peaks were recorded, between 7 and 11 min some as a result of the formation of the by-products of the reaction were noted. This increased during the following time intervals 11 to 16, 16 to 25, 25 to 50, and 50 to 80 min (Fig. 1). These signals indicate the progression of the oxidation reaction of the chelating agent (EDTA). At the end of the experiment, the spectra indicated that the reaction between NaOCl and EDTA caused the formation of unknown by-products. Therefore the possible action of the EDTA by-products on both Ca and Mg ions is also unknown.

Discussion

EDTA can potentially modify dentin and therefore its physical and mechanical properties through excessively demineralization of the inorganic portion. This has been demonstrated in various studies by means of images of dentinal walls, which showed noticeable signs of erosion (7, 10, 11, 19–23). Radicular dentinal structures represent the substrate for a root canal filling. The frequency with which different authors have found these alterations as well as the need to ensure a hermetic seal, has lead to the search for solutions, which preserve tooth structure.

In a recent study, Cobankara et al. (33) showed that the presence of the smear layer could negatively influence both coronal and apical leakage of root canal treated teeth obturated with different methods and materials. This could be explained by the fact that complete removal of the smear layer allows sealers to penetrate into the dentinal tubules as recently was demonstrated by Kokkas et al. (16). In this study the authors showed, that when the smear layer was not removed, no penetration of the sealers into the dentinal tubules could be achieved (14, 15). However, it has been hypothesized that excessive demineralization of dentin could create more difficulties in the adaptation of the root-
filling materials to the canal walls. Park et al. (34) suggested that an increased coronal leakage in samples treated with EDTA compared with those treated with MTAD might be caused by the erosive property of EDTA and the length of dentin exposure to this solution. It has been hypothesized that the effects of EDTA within the canal are a function of contact time (23, 35) because its self-limiting action is known to be completed within 7 h (24). Crumpton et al. (35) have demonstrated that the volume of irrigation does not influence the quality of smear layer removal, since this is a function of contact time. In this study 1 ml of EDTA with a contact time of 1 min was just as effective as 10 ml.

The results obtained in the present study lead to certain questions regarding the combined use of NaOCl and EDTA. The experiment determined that the oxidation of the EDTA molecule is not immediate, but progressive over time. In particular, the use of NMR accurately demonstrated the slow oxidation of the chelating agent. The frequency of the radiation necessary for the absorption of energy depends on both the type of nucleus (e.g. \(^{1}H\) or \(^{13}C\)) as well as its chemical surrounding. Identical nuclei in different molecules absorb energy differently. This is why the oxidation of EDTA, which involves a change in the molecular structure, can easily be identified with NMR.

The fact that the quantity of NaOCl utilized did not completely oxidize all of the EDTA, even though theoretically it would have been sufficient, may be explained by the fact that in a solution the oxidation follows a statistical criteria. Therefore, it is reasonable to expect to find a certain number of molecules completely oxidized, another not oxidized at all, as well as other possible intermediate combinations. Furthermore, if the oxidation process does not exclusively involve EDTA, but also some of its by-products, then the quantity of molecules actually oxidized reduces even more. Table 1, though indicative of the oxidation process, gives an idea of the gradual change in the oxidation rate of EDTA over time.

![Figure 1](image1.png)
Figure 1. NMR analysis graph of the oxidation reaction occurring between NaOCl and EDTA—at the different time intervals (Time 0, 1.5 min, 4.5 min, 7 min, 11 min, 16 min, 25 min, 50 min, 80 min, 120 min)—in the test tube. The spectrum shows how the signals relative to the EDTA protons change at different times.

![Figure 2](image2.png)
Figure 2. Detail of Fig. 1. The peaks relative to the different chemical products are specified. The smaller peak between peak 1 and peak 2 is because of oxidation of EDTA. The presence in the spectrum of both the decreasing and the widening EDTA peaks, as well as the emergence of new signals, demonstrate the oxidation progress of the chelating agent.

<table>
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<tr>
<th>Time (min)</th>
<th>Frequency (Hz)</th>
<th>Intensity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Peak 1</td>
<td>Peak 2</td>
</tr>
<tr>
<td>0</td>
<td>1161.72</td>
<td>1093.04</td>
</tr>
<tr>
<td>1</td>
<td>1162.92</td>
<td>1094.41</td>
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<td>4.5–7.0</td>
<td>1162.54</td>
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</tr>
<tr>
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<td>80.0–120.0</td>
<td>1147.71</td>
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\*\(\text{Hz; frequency/(300 MHz) = chemical shift}\)
\(1\) (in arbitrary units)
\(\text{§}(\text{= N-CH2-C(-O)OR})\)
\(\text{I}(\text{= N-CH2-C(-O)OR})\)

*Oxidation started at about 2.5 min (25° C).*
kinetics, does not allow any characterization of the oxidation by-products, and therefore the effect of their action on both the inorganic (Ca and Mg ions) and organic matrix is still unknown.

Of importance to consider is not that deactivation occurs, but that it is extremely slow. This implies that active EDTA is present inside the endodontic space, which action is time dependent and not influenced by volume (35). This may also provide an explanation for the SEM images that show erosion of the dentinal walls and the presence of micro fractures of the intertubular septa.

The results of this study are in agreement with previous investigations in which the effect of the presence of sodium hypochlorite on the chelating ability of EDTA was tested with a calcium titration method, using murexide as an indicator (26) or a Ni-dimethylglyoxime titration method, flame spectrometry and measurements of dentin microhardness (36).

The self-limiting properties of EDTA demonstrate that its action goes on until all of its cationic receptors are saturated with Ca$^{2+}$ ions and therefore demineralization terminates when this equilibrium has been reached (24, 25). Perez et al. (37) observed how the self-limiting effect of EDTA is determined by changes in pH that occur during the demineralization process. Because the pH decreases as the process progresses, both the quantity of the demineralized dentin as well as the dissolved one decrease with time. However, not all authors agree on the self-limiting property of EDTA. For instance Patterson (21) does not come agree with this characteristic finding.

A final flush with NaOCl has been questioned in several studies that have shown that dentinal erosion is more evident when irrigation with EDTA is followed by a final flush with NaOCl (7, 20, 38). This finding suggests that using NaOCl after a flush with EDTA could further increase dentinal erosion. It was hypothesized that this could occur because the factor that limits the dissolution of dentin by EDTA appears to be the organic matrix of the dentin itself as it accumulates on the surface of the canal wall while it gets progressively demineralized, thereby avoiding further dissolution. Morphological findings suggest that EDTA may de-calcify peritubular dentin during the early stages of the final irrigation and that the subsequent use of NaOCl dissolves the exposed organic matrix (20, 38). These complementary effects may result in a deeper erosion of the dentinal wall (20). To limit this phenomenon, new irrigating solutions and protocols have been proposed (MTAD) and have been reported in the literature (34, 39). Other authors proposed to reduce the EDTA concentration and the duration of exposure. Nakashima and Terata (40) reported that an alkaline 3% EDTA solution with adjusted pH 9.0 allowed smear layer removal without excessive demineralization and therefore demineralization terminates when this equilibrium has been reached (24, 25). Perez et al. (37) observed how the self-limiting effect of EDTA is determined by changes in pH that occur during the demineralization process. Because the pH decreases as the process progresses, both the quantity of the demineralized dentin as well as the dissolved one decrease with time. However, not all authors agree on the self-limiting property of EDTA. For instance Patterson (21) does not come agree with this characteristic finding.

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Conclusions

Recognizing the inherent limitations of an in vitro experiment, the results of this study suggest that there appears to be no indication for a final flush with NaOCl for the purpose of utilizing its oxidation properties to deactivate the action of EDTA, at least not for time periods that are clinically realistic and acceptable.

Thus, irrigation with NaOCl for the purpose of mechanically rinsing out the EDTA solution does not seem sufficient. It demonstrated an aggressive action with root canal dentin and may accelerate erosion of the radicular substrate, which is needed to facilitate a matrix for the best possible three-dimensional root canal filling.

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