

IRRIGANTS FREQUENTLY UTILIZED IN ENDODONTICS PRACTICE AND CALCIUM HYPOCHLORITE INTERACTIONS; A CHEMICAL EVALUATION

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Abstract

Introduction-A root canal treatment's primary goals are the complete elimination of connective tissue, the eradication of any remaining microorganisms discovered in infected root canals, and the creation of a strong seal that will avoid bacterial colonization of the root canal system in the future.

Methodology-In the current investigation, 0.16%, 1%, 2.5%, and 5.25% NaOCl solutions, 2% CHX solution and gel, 17% EDTA, 10% citric acid, 37% phosphoric acid, 0.9% saline solution, ethanol, and distilled water were examined. The very same company manufactured all of the chemicals, which were then used right away. Data were analysed using the statistical package SPSS 21 and the QTOF acquisition rate was set to 1.0 second with a 0.4-second inter-scan delay. The studies were performed using a syringe pump at a flow rate of 5.0 mL/min for direct infusion.

Results-The relationships' organoleptic qualities. When combined with 1%, 2.5%, and 5.25% NaOCl solutions, 2% CHX gel and solution instantly generated an orange-brown precipitate. When combined with 0.16% NaOCl, it created an orange-white precipitate. A white milky precipitate was noticed right away when CHX was mixed with 17% EDTA. After 5 seconds, a salt precipitation was formed by the associations CHX/saline solution and CHX/ethanol. **Conclusion:** NaOCl, an oxidising agent that chlorinates the guanidino nitrogens of CHX, is what causes the orange-brown precipitate that is seen in the connection amongst CHX and NaOCl.

Introduction

A root canal treatment's primary goals are the complete elimination of connective tissue, the eradication of any remaining microorganisms discovered in infected root canals, and the creation of a strong seal that will avoid bacterial colonisation of the root canal system in the future. [1]Therefore, ensuring root canal sanitation and avoiding reinfection must be the main endodontic therapy objectives. The root canals are thoroughly cleaned using irrigation treatments. The root canal system's complexity, the abundance of dentinal tubules in the roots, the invasion of the tubules by microorganisms, the creation of a smear layer while on instrumentation, and the existence of dentin as a tissue are the main barriers to completing the root canal cleaning and shaping processes[2]. Nevertheless, using irrigation solutions to clean and shape the root canals can lead to a number of accidents. Sodium hypochlorite (NaOCl), chlorhexidine, ethylene diaminetetra acetic acid (EDTA), and a combination of tetracycline, an acid, and a detergent (MTAD) are a few of the often employed irritants.

Keywords-

NaOCl, CHX,
10% Citric
Acid, 37%
Phosphoric
Acid, Saline
Solution

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Despite the fact that numerous correlations have already been researched, there is little literature on these interactions in general. While other writers looked at the connection between NaOCl and CHX, Baumgartner and Ibay³ explored the interactions between NaOCl/EDTA and NaOCl/citric acid.

The orange-brown precipitate observed's chemical make-up is not entirely clear, though. Parachloroaniline was found by Basrani et al⁴ and Krishnamurthy and Sudhakaran⁵. However, this chemical was not found by Thomas and Sem⁶ or Nowicki and Sem⁷. The chemical reactions among phosphoric acid, a powerful tool for smear layer elimination, and NaOCl or CHX have never been studied. Other interactions involving EDTA and CHX as well as citric acid and CHX have also been explored. The hybrid mass spectrometer known as electrospray ionisation quadrupole time-of-flight mass spectrometry (ESI-QTOF-MS) combines high-resolution tandem mass spectrometry (quadrupole plus time-of-flight) with a soft ionisation method in atmospheric pressure.

Due to the high mass resolution power and the mass accuracy of the TOF analyzer, ESI-QTOF-MS has been utilised as a significant fingerprint tool for complicated samples. Furthermore, unlike the chromatography approach, it allows for the direct entry of extremely small amounts of sample without the requirement for a pre-separation method. Applications of the approach in other complex matrices, including beer, vegetable oils, and snake venom, are particularly interesting.

In this situation, the purpose of this study was to use ESI-QTOF-MS studies to characterise the by-products created in associations among the most often used irrigants in endodontic practise.

Methodology

In the current investigation, 0.16%, 1%, 2.5%, and 5.25% NaOCl solutions, 2% CHX solution and gel, 17% EDTA, 10% citric acid, 37% phosphoric acid, 0.9% saline solution, ethanol, and distilled water were examined.

Results

The same company manufactured all of the chemicals, which were then used right away.

With CHX solution and gel, EDTA, citric acid, phosphoric acid, saline solution, ethanol, and distilled water, NaOCl formulations were connected. Furthermore, EDTA, citric acid, phosphoric acid, saline solution, ethanol, and distilled water were combined with the CHX solution and gel.

As shown in Table 1, the solutions were combined in a 1:1 ratio using 0.5 mL of each solution at room temperature in flat-top 1.5-mL polypropylene microtubes. The investigation was carried out three times. Following the solutions had been combined, the microtubes were qualitatively assessed for bubble presence, precipitate production, and colour changes in the solutions.

The chemical composition of the solutions was ascertained using ESI-QTOF-MS after mixing the formulations and watching the precipitate development (entry 1-5, 9-15, 19, and 20 in Table 1).

The specimens were centrifuged, the supernatant was eliminated, and 0.5 mg of the precipitate became soluble in 1.0 mL of deionized water (Type I, 18 mU.cm), aceto- nitrile, methanol (HPLC/Spectro), or mixtures of these, before being acidified with 0.1% formic acid (to aid protonation in the positive ion mode; ACS, Tedia). ESI-QTOF-MS investigations were carried out on a QTOF Micro mass spectrometer (Waters, Wythen-shawe, Manchester, UK) following the solubilization of the precipitate. The analyses between m/z 90 and 1000 in positive ion mode [ESI(+)-MS] were taken into account. The mass was explained as mass-to-charge ratio (m/z). The nebulization gas was adjusted to 500 L/h at 120 C, the cone gas to 50 L/h, and the source temperature to 100 C for the mass spectrometer. The voltages for the capillary and cone were set at 3000 V and 30 V, respectively. Data processing was done using the MassLynx 4.0 program, and the QTOF collection rate was adjusted to 1.0 second with a 0.4-second inter-scan delay. The studies were performed using a syringe pump at a flow rate of 5.0 mL/min for direct infusion.

TABLE 1. Association of Irrigants and Visual Characteristics of the Products

Entry	Solution 1	Solution 2	Appearance of the resultant association*
1	2% CHX gel	5.25% NaOCl	Orange-brown precipitate
2	2% CHX gel	2.5% NaOCl	Orange-brown precipitate
3	2% CHX gel	1% NaOCl	Orange-brown precipitate
4	2% CHX gel	0.16% NaOCl	Orange-white precipitate
5	2% CHX gel	17% EDTA	White milky precipitate
6	2% CHX gel	10% Citric acid	Unchanged
7	2% CHX gel	37% Phosphoric acid	Unchanged
8	2% CHX gel	Distilled water	Unchanged
9	2% CHX gel	Saline solution	Salt precipitation
10	2% CHX gel	Ethanol	Salt precipitation
11	2% CHX solution	5.25% NaOCl	Orange-brown precipitate
12	2% CHX solution	2.5% NaOCl	Orange-brown precipitate
13	2% CHX solution	1% NaOCl	Orange-brown precipitate
14	2% CHX solution	0.16% NaOCl	Orange-white precipitate
15	2% CHX solution	17% EDTA	White milky precipitate
16	2% CHX solution	10% Citric acid	Unchanged

17	2%CHXsolution	37%Phosphoricacid	Unchanged
18	2%CHXsolution	Distilledwater	Unchanged
19	2%CHXsolution	Salinesolution	Saltprecipitation
20	2%CHXsolution	Ethanol	Saltprecipitation
21	5.25%NaOCl	17%EDTA	Bubbleformation
22	5.25%NaOCl	10%Citricacid	Bubbleformation
23	5.25%NaOCl	37%Phosphoricacid	Yellowsolutionwithbubbleformation
24	5.25%NaOCl	Distilledwater	Unchanged
25	5.25%NaOCl	Salinesolution	Unchanged
26	5.25%NaOCl	Ethanol	Unchanged
27	2.5%NaOCl	17%EDTA	Bubbleformation
28	2.5%NaOCl	10%Citricacid	Bubbleformation
29	2.5%NaOCl	37%Phosphoricacid	Yellowsolutionwithbubbleformation
30	2.5%NaOCl	Distilledwater	Unchanged
31	2.5%NaOCl	Salinesolution	Unchanged
32	2.5%NaOCl	Ethanol	Unchanged
33	1%NaOCl	17%EDTA	Bubbleformation
34	1%NaOCl	10%Citricacid	Bubbleformation
35	1%NaOCl	37%Phosphoricacid	Slightyellowsolutionwithbubbleformation
36	1%NaOCl	Distilledwater	Unchanged
37	1%NaOCl	Salinesolution	Unchanged
38	1%NaOCl	Ethanol	Unchanged
39	0.16%NaOCl	17%EDTA	Unchanged
40	0.16%NaOCl	10%Citricacid	Unchanged
41	0.16%NaOCl	37%Phosphoricacid	Unchanged
42	0.16%NaOCl	Distilledwater	Unchanged
43	0.16%NaOCl	Salinesolution	Unchanged
44	0.16%NaOCl	Ethanol	Unchanged

The groups that were tested and the associations' organoleptic properties are shown in Table 1. In terms of the CHX associations, 2% CHX gel and solution instantly formed orange-brown precipitates with 1%, 2.5%, and 5.25% NaOCl solution (Fig. 1A) and orange-white precipitates with 0.16% NaOCl (Fig. 1B). An immediate white milky precipitate (Fig. 1C) was seen when CHX was combined with 17% EDTA. After 5 seconds, both the CHX/saline solution and CHX/ethanol interactions resulted in a salt precipitate (Fig. 1D and E). Whenever CHX was combined with 10% citric acid, phosphoric acid, or distilled water, no precipitate was seen. Because NaOCl is known to be an oxidising agent, redox-type interactions were seen for the precipitate generated in the reaction between NaOCl solutions and CHX, which was allocated by mass spectrometry analyses. The ESI(+)-MS for the various experimental groups of CHX is illustrated in Figure 2. The first spectrum for 2% CHX (Fig. 2A) serves as a control; the second (Fig. 2B) and third

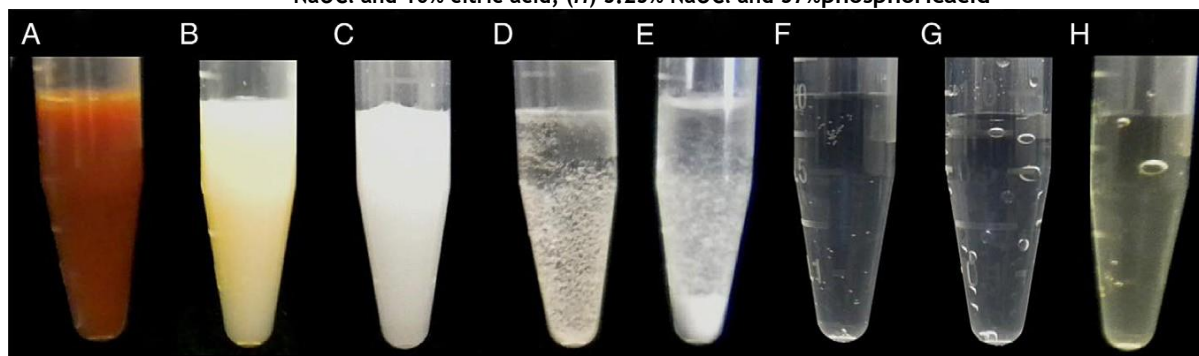
(Fig. 2C) spectra (relative to entries 1, 4, 11, and 14 in Table 1) demonstrate the connection amongst NaOCl and CHX, leading to the creation of orange-brown and orange-white precipitates, correspondingly.

The main signals attributed to these groups were the following:

Figure 2A: m/z 253 [M+2H]²⁺/ C₂₂H₃₂Cl₂N₁₀²⁺, m/z 505 [M+H]⁺+C₂₂H₃₁Cl₂N₁₀⁺, m/z 701 [M+H]⁺+C₂₈H₄₃Cl₂N₁₀⁷⁺ (CHX gluconate);

Figure 2B and C: m/z 270 [M+2H]²⁺ C₂₂H₃₁Cl₃N₁₀²⁺/ m/z 539 [M+H]⁺+C₂₂H₃₀Cl₃N₁₀⁺, m/z 287 [M+2H]²⁺+C₂₂H₃₀Cl₄N₁₀²⁺/m/z 573 [M+H]⁺+C₂₂H₂₉Cl₄N₁₀⁺, m/z 304 [M+2H]²⁺+C₂₂H₂₉Cl₅N₁₀²⁺/ m/z 607 [M+H]⁺+C₂₂H₂₈Cl₅N₁₀⁺, m/z 321 [M+2H]²⁺+C₂₂H₂₈Cl₆N₁₀²⁺/ m/z 641 [M+H]⁺+C₂₂H₂₇Cl₆N₁₀⁺, m/z 338 [M+2H]²⁺+C₂₂H₂₇Cl₇N₁₀²⁺/ m/z 675 [M+H]⁺+C₂₂H₂₆Cl₇N₁₀⁺, m/z 355 [M+2H]²⁺+C₂₂H₂₆Cl₈N₁₀²⁺/ m/z 709 [M+H]⁺+C₂₂H₂₅Cl₈N₁₀⁺.

Figure 1. Visual aspect of the interactions between the following: (A) 5.25% NaOCl and 2% CHX; (B) 0.16% NaOCl and 2% CHX; (C) 17% EDTA and 2% CHX; (D) saline solution and 2% CHX; (E) ethanol and 2% CHX; (F) 5.25% NaOCl and 17% EDTA; (G) 5.25% NaOCl and 10% citric acid; (H) 5.25% NaOCl and 37% phosphoric acid



It was possible to link the production of the milky precipitate with acid-base reactions since the signals in the ESI(+)-MS spectra of the interactions amongst CHX and EDTA precipitate were solely related to the isolated chemicals. The precipitates that resulted from mixing saline solution-containing CHX with ethanol had something to

do with the salting-out process and the reduced solubility, respectively. No precipitate was observed when NaOCl at the various concentrations was combined with 17% EDTA, 10% citric acid, 37% phosphoric acid, distilled water, saline solution, and ethanol.

The association of NaOCl with 17% EDTA (Fig.1F) and 10%citricacid (Fig.1G)led to the formation of bubbles. NaOCl associated with 37% phosphoric acid (Fig.1H)exhibited a yellow coloring plus formation of bubbles.

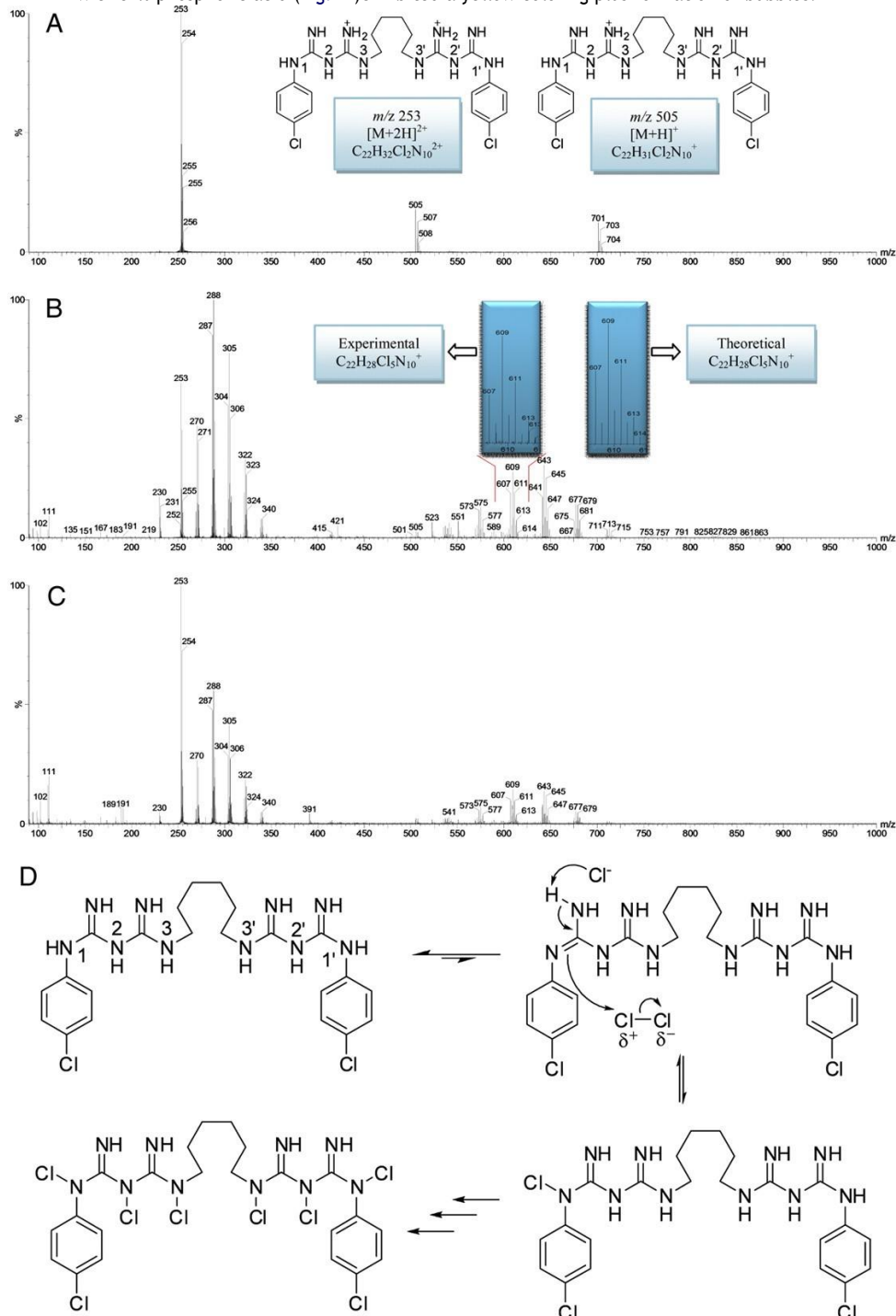


Figure 2. Electrospray ionization mass spectrum in positive ion mode [ESI(+)-MS] for the different experimental groups of chlorhexidine digluconate [(A) 2% CHX; (B) 5.25% NaOCl + 2% CHX; (C) 0.16% NaOCl + 2% CHX]; and (D) the proposed mechanism of chlorination of the guanidine nitrogens (1-3 and 1'0-3'0).

Discussion

In the current investigation, the by-products created by the relationship of the irrigants frequently

employed in endodontic therapy were assessed. Knowing this is crucial since such byproducts can precipitate into a chemical smear layer that obstructs

the dentinal tubules and may affect the root filling's seal and are potentially harmful to the periapical tissues. As a result, every effort should be made to eliminate these byproducts. Orange brown precipitates were produced in the current investigation when NaOCl at concentrations of 1%-5.25% was combined with 2% CHX solution and gel. Para-chloroaniline's signal at m/z 128 ($[M+H]^+$ C₆H₇ClN⁺) could not be found (Fig. 2B and C).

The existence of numerous products of chlorination from the oxidising agent NaOCl, which arises at 1 to 6 guanidino nitrogens of CHX, was verified by the mass spectrometry studies. The guanidine oxidation may be responsible for the orange-brown hue. Our results are consistent with those of Thomas and Sem⁶ and Nowicki and Sem⁷, who used nuclear magnetic resonance but were unable to detect the presence of para-chloroaniline. They differ from Basrani et al⁴, who discovered it by utilising time-of-flight secondary ion mass spectrometry and x-ray photon spectroscopy. The various findings could be a result of different approaches. Comparable to x-ray photon spectroscopy, TOF secondary ion mass spectrometry studies surfaces and uses a different technique to introduce molecules or aggregates of them into the mass spectrometer. On the other hand, the precipitate is completely dissolved in a solvent and then the solution is analysed using nuclear magnetic resonance or ESI-QTOF-MS. In addition to the differences between these approaches, it is not possible to compare the outcomes of the mass spectrometry produced by multiple study teams because the MS spectra have not yet been thoroughly documented in the literature.

By using ESI(+)-MS to analyse the white milky precipitate formed when EDTA was combined with CHX solution and gel, it was discovered that the acid-base reactions were responsible. Our results are consistent with those of Rasimick et al.⁸, who used reversed-phase high-performance liquid chromatography to analyse the precipitate created by mixing 17% EDTA with 2% or 20% CHX and found that more than 90% of the precipitate mass was either EDTA or CHX salt. While the precipitate formed between CHX and saline solution was attributed to the salting-out process, which raised the concentration of salt and precipitated the CHX salts, the precipitate built between CHX and ethanol was credited to the CHX salt's diminished dissolution in ethanol. In the presence of EDTA, citric acid, or phosphoric acid, NaOCl produced an exothermic reaction that resulted in the creation of bubbles. The intensity of the bubbles decreased with increasing concentrations of phosphoric acid, citric acid, and EDTA. When combined

with distilled water, 9 NaOCl at various concentrations and 2% CHX gel and solution did not precipitate. As a result, distilled water appears to be the irrigant that is most recommended for use in intermediate rinses to get rid of leftovers of the chemical auxiliary component that was previously utilised.

Conclusion

In conclusion, NaOCl, an oxidising agent that chlorinates the guanidino nitrogens of CHX, is the cause of the orange-brown precipitate seen in the connection between CHX and NaOCl. When CHX reacts with EDTA, saline solution, or ethanol, precipitates are produced that are related to acid-base reactions, the salting-out process, or decreased solubility, respectively. NaOCl in combination with EDTA, citric acid, and phosphoric acid mostly causes the creation of chlorine gas. To minimise or at least minimise the production of byproducts, intermediate flushes with distilled water seem to be adequate.

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