IOSR Journal of Dental and Medical Sciences (IOSR-JDMS)
e-ISSN: 2279-0853, p-ISSN: 2279-0861.Volume 14, Issue 4 Ver. I (Apr. 2015), PP 69-74
www.iosrjournals.org

Chemical Interactions between Different Irrigating Solutions: A Spectrometric Study

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Abstract: *The aim of this study was to characterize the by-products formed on interacting different irrigating solution used in Endodontic practice through Hybrid Qudrupole-Orbital Mass Spectrometer. Method: Sodium hypochlorite (NaOCl) (0.16%, 1%, 2.5% and 5.25%) was interacted with Chlorhexidine (CHX) solution, 10% citric acid, 37% phosphoric acid, 7% maleic acid, distilled water, saline solution and ethanol The solutions were mixed in a ratio of 1:1and Hybrid Qudrupole-Orbital Mass Spectrometer were used to characterize the by-products. Results: An orange-brown precipitate is formed on interacting CHX with NaOCl (1%, 2.5% and 5.25%). No precipitate was observed on CHX was associated with 0.16% NaOCl, citric acid, phosphoric acid, maleic acid and distilled water. When associated with ethanol and saline solution, salt precipitaion was formed. Conclusion: The interaction of NaOCl and CHX forms orange-brown precipitate because of oxidizing agent NaOCl. The precipitate formed in association with ethanol and saline solution was due to lower solubility and salting-out process respectively. NaOCl solution on reacting with citric acid and phosphoric acid mainly lead to chlorine formation. Intermediate flushes with distilled water helps to prevent or decrease the formation of precipitate thus preventing the detrimental effect of precipitate.*

Keywords – *Chlorhexidine, sodium hypochlorite, Mass spectrometry, citric acid, phosphoric acid*

I. Introduction

Bacteria in the root canal system can initiate and cause periapical inflammatory lesions. The aim of root canal treatment is to eliminate bacteria from the infected root canal and to prevent reinfection. Biomechanical cleaning and shaping of the root canal greatly reduces the number of bacteria [1], but the anatomical complexity of the root canal system, organic and inorganic residues and bacteria cannot be completely removed and often persist [2]. Mechanical instrumentation forms a smear layer on the canal surface [3]. Thus, chemical debridement in the form of various irrigants is performed to aid in removal of residual debris, necrotic tissue, microbes and smear layer. Various irrigants have been used for canal disinfections like sodium hypochlorite, chlorhexidine, citric acid, phosphoric acid [4].

During the course of endodontic therapy, multiple irrigants are often used. Typically, an irrigant is not thoroughly flushed from the root canal before the next irrigant is applied. As a result, endodontic irrigants routinely come into contact with each other inside the root canal [5]. Although such combination of irrigants may enhance their antimicrobial properties, possible chemical interactions among the irrigants may lead to by-product formation [6]. This precipitate acts as a chemical smear layer and could compromise dentin permeability, the intracanal medication diffusion, and the obturation sealing. This raises a potential concern with respect to leaching of the precipitate into the surrounding tissues and the seal of the root canal [7-9].

II. Materials And Methods

Solutions:

The solutions used in these study were 2% CHX solution, 5.25%, 2.5%, 1%, 0.16% NaOCl solution, 10% Citric acid, 37% phosphoric acid, 7 % Maleic acid, distilled water, saline solution and ethanol. All chemical solution was prepared at Central Research Laboratory, Sawangi, Wardha. Solutions were used immediately after preparation.

Color Changes And Precipitate Formation:

Chlorhexidine solution was mixed with NaOCl, citric acid, phosphoric acid, Maleic acid, distilled water, saline solution and ethanol. Whereas, NaOCl was reacted with Citric acid, Phosphoric acid, Maleic acid, distilled water, saline solution and ethanol. To determine the change of color and formation of precipitate, 0.5 ml of each solution were mixed in a ratio 1:1 [11, 12, and 19] on flat 1.5 ml of polypropylene microtubes. The test was repeated for three times and the color change and formation of precipitate was recorded.

Precipitate Characterization:

The precipitate formed was analyzed by Hybrid Qudrupole- Orbital Mass Spectrometer (Q- Exactive) for determining the chemical composition. All the microtubes were centrifuged at 3000 rpm for 10 min and supernatant was discarded. The precipitate was so thick that it was stuck inside the microtube. The precipitate was washed with deionized water, acetonitrile, methanol or combination of these. It was then acidified with 0.1% formic acid (for protonation in positive ion mode). Then LC-MS/ MS were performed on Hybrid Qudrupole- Orbital Mass Spectrometer. The mass was described as mass-to-charge ratio (m/z) and it was recorded between m/z 70 to 1000 in positive ion mode [ESI (+)- MS]. The parameters for mass spectrometer were set as: Source temperature was set at 320^o C and Spray voltage set to 3.6 kV. The acquisition rate of was set to 1 second with inter-scan delay of 0.4 second. Xcalibur data analysis software was used for data processing. Syringe pump at flow ratio of 5 μL/ min by direct infusion was carried out for analysis.

III. Results

Sr. No	Solution 1	Solution 2	Observation
1	2% CHX solution	5.25 % NaOCl	Orange- brown precipitate
2	2% CHX solution	2.5 % NaOCl	Orange- brown precipitate
	2% CHX solution	1% NaOCl	Orange- brown precipitate
4	2% CHX solution	0.16% NaOCl	Unchanged
5	2% CHX solution	10% Citric Acid	Unchanged
6	2% CHX solution	37% Phosphoric Acid	Unchanged
7	2% CHX solution	Distilled Water	Unchanged
8	2% CHX solution	Saline solution	Salt Precipitation
9	2% CHX solution	Ethanol	Salt Precipitation
10	2% CHX solution	7% Maleic Acid	Unchanged
11	5.25 % NaOCl	10% Citric Acid	Bubble formation
12	5.25 % NaOCl	37% Phosphoric Acid	Yellow solution with Bubble formation
13	5.25 % NaOCl	Distilled Water	Unchanged
14	5.25 % NaOCl	Saline solution	Unchanged
15	5.25 % NaOCl	Ethanol	Unchanged
16	5.25 % NaOCl	7% Maleic Acid	Unchanged
17	2.5 % NaOCl	10% Citric Acid	Bubble formation
18	2.5 % NaOCl	37% Phosphoric Acid	Yellow solution with Bubble formation
19	2.5 % NaOCl	Distilled Water	Unchanged
20	2.5 % NaOCl	Saline solution	Unchanged
21	2.5 % NaOCl	Ethanol	Unchanged
22	2.5 % NaOCl	7% Maleic Acid	Unchanged
23	1% NaOCl	10% Citric Acid	Bubble formation
24	1% NaOCl	37% Phosphoric Acid	Yellow solution with Bubble formation
25	1% NaOCl	Distilled Water	Unchanged
26	1% NaOCl	Saline solution	Unchanged
27	1% NaOCl	Ethanol	Unchanged
28	1% NaOCl	7% Maleic Acid	Unchanged
29	0.16 % NaOCl	10% Citric Acid	Unchanged
30	0.16 % NaOCl	37% Phosphoric Acid	Unchanged
31	0.16 % NaOCl	Distilled Water	Unchanged
32	0.16 % NaOCl	Saline solution	Unchanged
33	0.16 % NaOCl	Ethanol	Unchanged
34	0.16 % NaOCl	7% Maleic Acid	Unchanged

5.25%, 2.5% and 1% NaOCl solution when reacted with 2% Chlorhexidine solution produced an orange-brown precipitate. Whereas, on interacting NaOCl at different concentration with 0.16% NaOCl, 10% citric Acid, 37% phosphoric acid, 7% maleic acid, distilled water, saline solution and ethanol, no precipitate was formed. NaOCl solution when associated with 10% citric acid led to the bubble formation. NaOCl solution showed yellow solution when reacted with 37% phosphoric acid.

Regarding Chlorhexidine solution, it produces orange brown precipitate when reacted with 5.25%, 2.5%, 1% NaOCl solution. Salt precipitation was seen when Chlorhexidine solution was interacted with saline solution and ethanol. With 7% maleic acid, 10% citric acid, phosphoric acid and distilled water no precipitate was formed in association with Chlorhexidine solution.

Fig 1: (From right), CHX reaction with 5.25% NaOCl, 2.5% NaOCl, 1% NaOCl, 0.16% NaOCl, 7% maleic acid, 10% citric acid, 37% phosphoric acid, saline solution, ethanol and distilled water

NaOCl being an oxidizing agent, redox-type of reaction were observed with precipitate formed with NaOCl and CHX, as set by mass spectrometry analysis. Fig 2 shows ESI (+) – MS for different experimental group of CHX. The first spectrum (Fig 2 A, B and C) and second spectrum (Fig 3 A, B and C) shows the association between NaOCl and CHX. The main signals attributed to these groups were the following: Fig 2A: m/z 327 $[M+H]^+$ $C_{15}H_7N_5Cl_2^+$, Fig 2B m/z 742 $[M+H]^+$ $C_{10}H_{14}N_{11}Cl_{13}^+$, Fig 2C m/z 866 $[M+H]^+$ $C_{16}H_3N_3Cl_{18}^+$; Fig 3A m/z 361 $[M+H]^+$ $C_{14}H_{38}N_{10}Cl^+$, m/z 739 $[M+H]^+$ $C_{16}H_4NCl_{13}^+$, m/z 826 $[M+H]^+$ $C_{33}H_{18}N_2Cl_{11}^+$. The profile of the observed m/z signals was evaluated because of presence of 2 chlorine atoms in molecule of CHX and chlorine obtained from the oxidation of NaOCl. It was found to be in accordance with Xcalibur software.

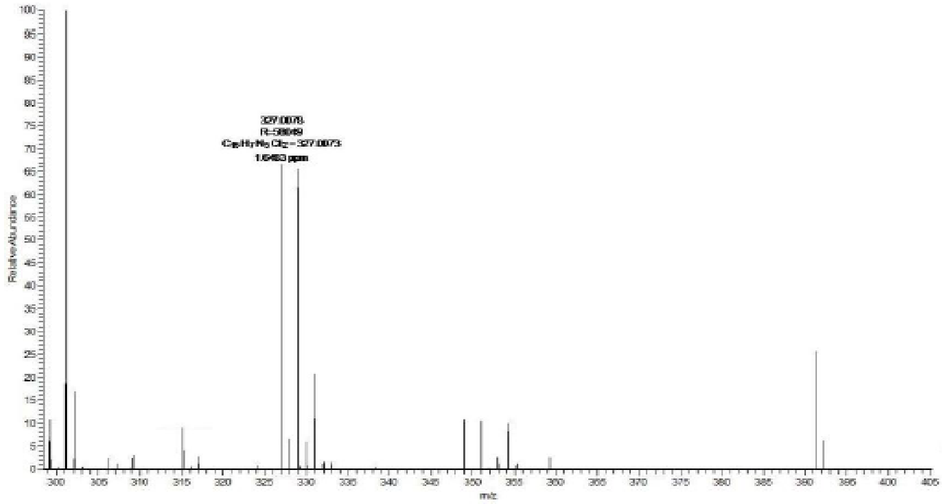

Fig 2A: 5.25% NaOCl + 2% CHX

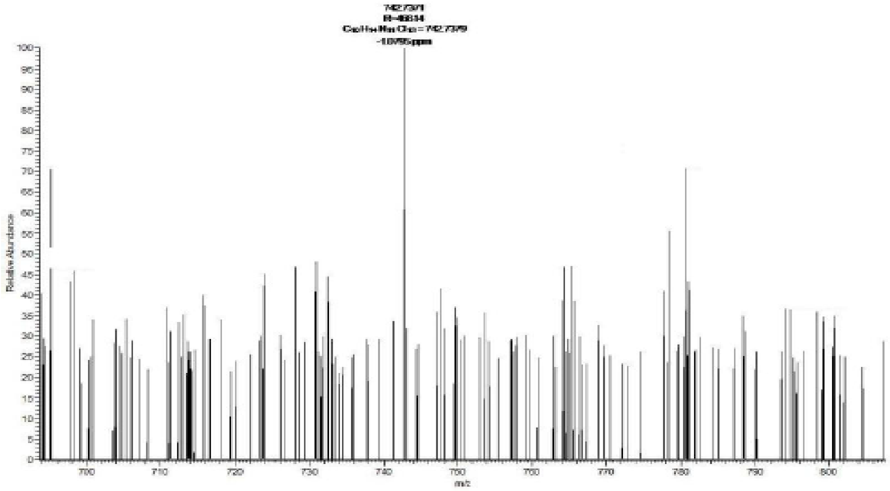

Fig 2B: 5.25% NaOCl + 2% CHX

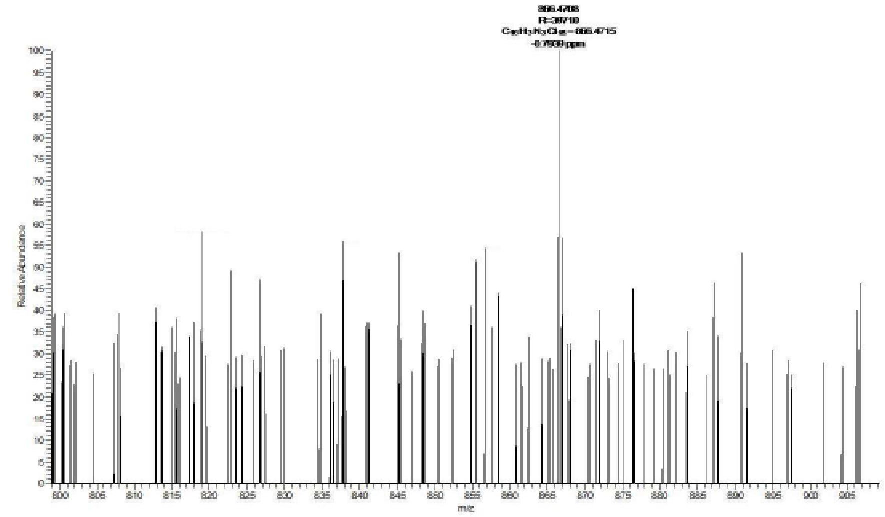

Fig 2C: 5.25% NaOCl + 2% CHX

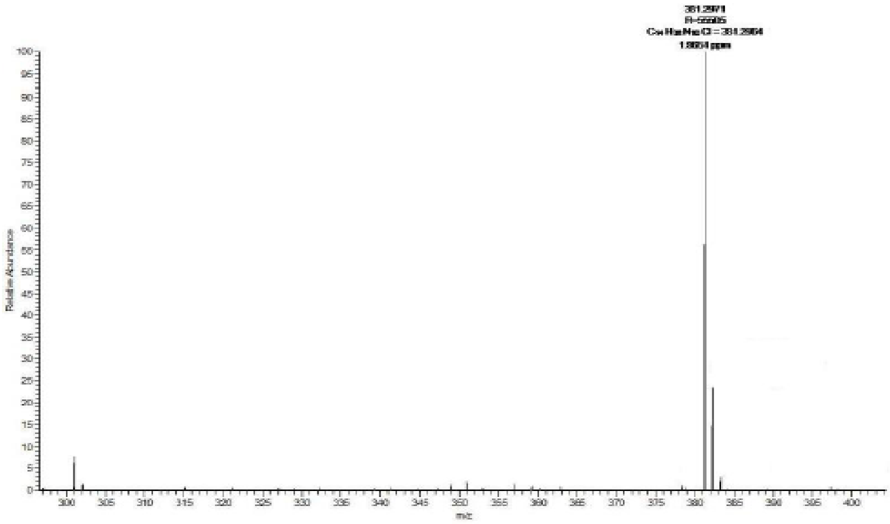

Fig 3A: 2.5% NaOCl + 2% CHX

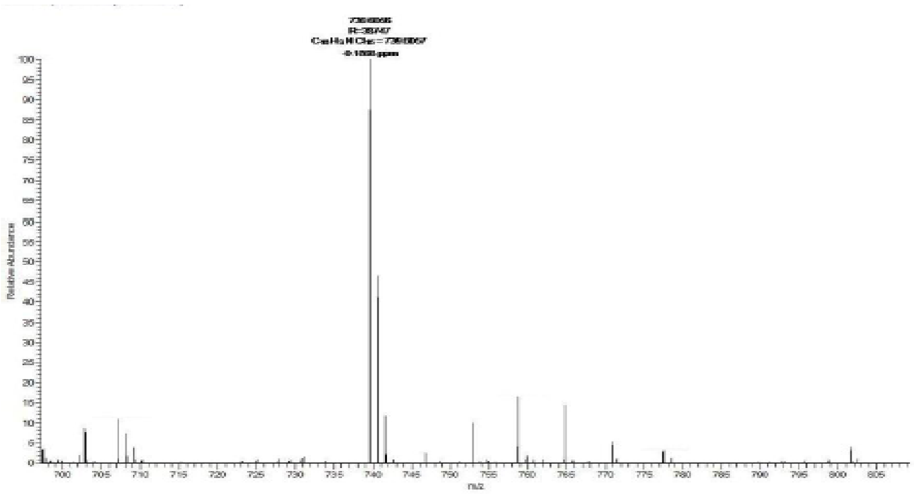

Fig 3B: 2.5% NaOCl + 2% CHX

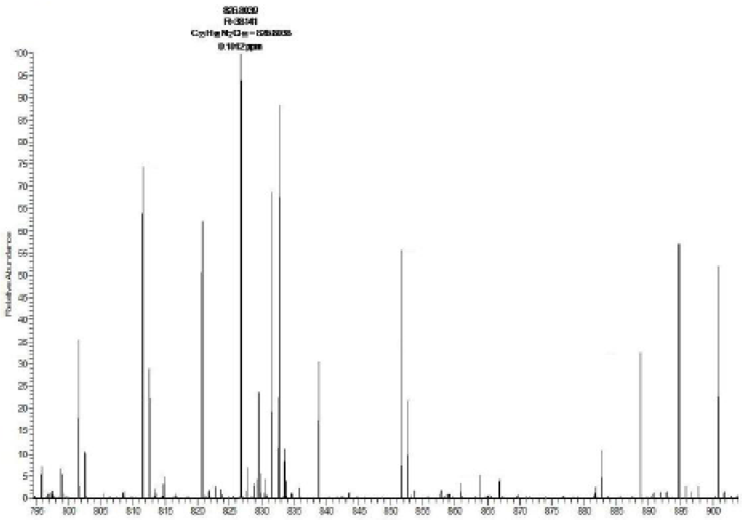

Fig 3C: 2.5% NaOCl + 2% CHX

IV. Discussion

This study analyzed the by-products formed by interaction of routinely used irrigants for endodontic treatment. Biomechanical cleaning and shaping reduces the bacterial count but does not completely eliminate the bacteria from the canals and, therefore requires the use of various irrigants in a sequential manner or in combination to enhance the antimicrobial effect [4]. The interaction of this irrigants could be detrimental to the outcome of root canal therapy. A small amount of precipitate left behind raises a potential concern regarding leaching of the precipitate in surrounding tissues and seal of the root canal as reaction. The insoluble product is difficult to remove from the canal preventing the penetration of intracanal medicaments and thus compromising the seal of obturated canal [8, 13].

In this study, the interaction of NaOCl and CHX solution resulted in orange-brown precipitate. Several products of chlorination were confirmed by mass spectrometry from oxidizing agent NaOCl [20-22]. The orange brown color may be associated with guanidine oxidation [23]. Basrani et al [6] in their study using X-ray photon spectroscopy (XPS) and time-of-flight secondary ion mass spectrometry (TOF-SIMS) found the presence of para-chloroaniline on combining NaOCl and CHX. Basrani et al [19] using Gas chromatography mass-spectrometer found the presence of para-chloroaniline. However, on using Nuclear Magnetic Resonance Thomas and Sem [11] and Nowicki and Sem [12)] could not detect the presence of para-chloroaniline. The difference of the result may be attributed to different techniques used.

Baumgartner and Ibay [10] studied the chemical reaction by mixing other irrigating solutions like hydrogen peroxide, citric acid and EDTA. Also, Krishnamurthy et al [13] in their study detected the presence of para-chloroaniline.

Hybrid Qudrupole- Orbital Mass Spectrometer combines quadrupole precursor ion selection with high resolution, accurate mass (HRAM) orbital detection to deliver exceptional performance and versatility. Orbitrap-based mass spectrometers are used in proteomics [14,15] and are also used in life science mass spectrometry such metabolism, metabolomics, environmental [16], food and safety analysis[17]. Most of them are interfaced to liquid chromatography separations, though they are also used with gas chromatography [18].

The precipitate between CHX solution and saline solution is caused by the concentration of salt of saline solution and precipitated CHX salts i.e. salting-out process [24]. Due to reduced solubility of CHX salt in ethanol, precipitate is formed between ethanol and CHX solution.

An exothermic reaction with bubble formation was observed when NaOCl was associated with citric acid and phosphoric acid. The bubbles are mainly chlorine gas [25], a toxic product. The bubble formation result from increase in proton (H^+) concentration in presence of chloride ions (Cl^-).

The yellow color on mixing phosphoric acid was associated with high formation of Cl_2 caused by more acidic nature of phosphoric acid. Thus it is important to use intermediate flushes while smear layer removal to remove or at least to reduce the concentration of NaOCl present in the root canal.

NaOCl at different concentration and 2% CHX solution do not form any precipitate when reacted with distilled water.

V. Conclusion

This study shows that the interaction of NaOCl and CHX forms orange-brown precipitate because of oxidizing agent NaOCl. The precipitate formed in association with ethanol and saline solution was due to lower solubility and salting-out process respectively. NaOCl solution on reacting with citric acid and phosphoric acid mainly lead to chlorine formation. Intermediate flushes with distilled water helps to prevent or decrease the formation of precipitate thus preventing the detrimental effect of precipitate.

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