

Original Article

Interaction between Irrigants commonly used in Endodontic Practice and their Characterization using ¹H NMR Spectra

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ABSTRACT:

Background: Primary Apical Periodontitis (AP) is caused by a microbial invasion of a root canal system which spread beyond the apex of the affected tooth. Most commonly used irrigant solution are NaOCl, CHX, EDTA, QMix™ and MTDA. Hence; we planned the present study to assess the interaction between the irrigants and characterize the by products formed in the associations between the most commonly used irrigants in endodontic practice through ¹H NMR analyses. **Materials & methods:** We planned the present study to evaluate interactions between the most commonly used irrigants in endodontic practice. A total of ten experimental groups were formed on mixing the irrigants with each other along with five control groups. Equal volumes (2ml) of the root canal irrigants to be assessed were mixed in round bottom polystyrene test tubes. The tubes were allowed to stand for 15 minutes to see if any interaction products are formed. These products were then centrifuged at 14000 rpm for 2.5 minutes. Spectra were taken with 400-MHz Varian NMR System at 25°C, in perpetuated (dimethyl sulfoxide) d6-DMSO solvent. **Results:** In this study, the dried precipitates were dissolved in DMSO (Dimethyl Sulfoxide) which also plays the role of internal reference in NMR was subjected to proton NMR data acquisition. The characteristic FID (Free Induction Decay) patterns were recorded using 400 MHz NMR Bruker spectrometer. **Conclusion:** Therefore within the limitations of this study it can be said that the use of chlorhexidine should be done judiciously, and its use should be avoided in combination with: NaOCl, MTAD, and Combination of MTAD and QMix should also be avoided

Key words: Irrigants, NMR spectra, Endodontic therapy

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INTRODUCTION

Primary Apical Periodontitis (AP) is caused by a microbial invasion of a root canal system which spread beyond the apex of the affected tooth. The goal of an endodontic treatment is to eliminate a cause of or to prevent a periapical disease. Addition of an irrigant such as sodium hypochlorite (NaOCl) or Ethylene diamine tetra acetic acid (EDTA) further increases the efficacy against microorganisms.¹⁻³ Most commonly used irrigant solution is Sodium Hypochlorite (NaOCl) at a concentration of 0.5% and 6%. Chlorhexidine gluconate (CHX) is also an effective antimicrobial agent and periodontal rinse agent. It is strongly basic and due to its ability to cationically bind to dentin, it seems to have substantivity effect in the roots.^{4, 5} EDTA is a chelating agent that dissolves inorganic components of the dentine but not the organic components⁶⁻⁸ and used to remove the smear layer. Most common concentrations range from 10 to 17%.^{9, 10} CHX cannot dissolve dentin effectively and

NaOCl can produce some surface erosion, therefore both of the components cannot effectively dissolve hard tissue components of a smear layer formed during the root canal instrumentation but EDTA a demineralizing agent can do so.^{11, 12} An alternative solution to EDTA for removing the smear layer is the use of BioPure™ MTAD™ (DENTSPLY Tulsa Dental Specialties, Tulsa, Okla.), a mixture of a tetracycline isomer, an acid (citric acid) and a detergent.¹¹ Recently, QMix™ (Dentsply Tulsa Dental, Tulsa, OK, USA), a novel irrigating solution for smear layer removal with added antimicrobial agents and disinfection was introduced. QMix™ contains EDTA, chlorhexidine and a detergent. Other interactions between EDTA and CHX as well as citric acid and CHX have also been evaluated, but the interaction between MTAD, QMix, EDTA and NaOCl or CHX has never been described and analysed by NMR spectra.¹² In this context, the aim of this study is to study the interaction between the irrigants and characterize the byproducts

formed in the associations between the most commonly used irrigants in endodontic practice through 1H NMR analyses.

MATERIALS AND METHODS

We planned the present study in the department of conservative dentistry of Govt dental college, Shilma, and it included evaluation of interactions between the most commonly used irrigants in endodontic practice. After procurement of the materials required for the experiment, the study was conducted in laboratory setup at Sophisticated Analytical Instrumentation Facility, Punjab University, Chandigarh for the assessment of the various root canal irrigants used in different combinations. Standardized protocol was followed during the mixing of irrigants. A total of ten experimental groups were formed on mixing the irrigants with each other along with five control groups. Equal volumes (2ml) of the root canal irrigants to be assessed were mixed in round bottom polysterene test tubes. The tubes were allowed to stand for 15 minutes to see if any interaction products are formed. These products were then centrifuged at 14000 rpm for 2.5 minutes. On centrifugation precipitation was seen in Group II, IV, VII, VIII, IX and X. These precipitates were then collected in sterile eppendorf tube and dried. 0.7ml i.e. (700

microliter) of the dried precipitate was taken and dissolved in d-6 DMSO (dimethyl sulfoxide) solvent then subjected to 1 H NMR analysis. Spectra were taken with 400-MHz Varian NMR System at 25°C, in perpetuated (dimethyl sulfoxide) d6-DMSO solvent.

RESULTS

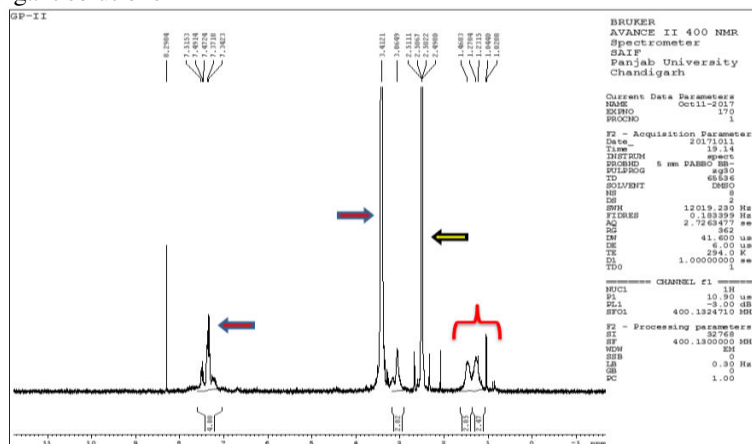
In the group II, IV, VII, VIII, IX and X precipitate formation was observed at the time of mixing of the solutions. Groups III, VI, XI, XII, XIII, XIV and XV have shown no precipitate formation or color change, until 2 hours. Similarly, no change was observed the control groups which included group XI, XII, XIII, XIV and XV.

In this study, the dried precipitates were dissolved in DMSO (Dimethyl Sulfoxide) which also plays the role of internal reference in NMR was subjected to proton NMR data acquisition. The characteristic FID (Free Induction Decay) patterns were recorded using 400 MHz NMR Bruker spectrometer. The FID file number was obtained for each sample of experimental groups of irrigants in which precipitates were evident. The FID patterns were then Fourier transformed from the time domain data to frequency domain data to get spectrum for each sample precipitate.

Table 1: Changes observed in different experimental and control groups of irrigant solutions during course of experiment

S. NO.	GROUPS	SOLUTION 1	SOLUTION 2	OBSERVATION
1.	Group I	3% NaOCl	17% EDTA	Bubble formation
2.	Group II	3% NaOCl	2% CHX	Orange-brown ppt
3.	Group III	3% NaOCl	Q MIX	No precipitates
4.	Group IV	3% NaOCl	MTAD	Mud Colourppt
5.	Group V	17% EDTA	2% CHX	Milky-White ppt
6.	Group VI	17% EDTA	Q MIX	No precipitates
7.	Group VII	17% EDTA	MTAD	Brown ppt
8.	Group VIII	2% CHX	Q MIX	Milky White ppt
9.	Group IX	2% CHX	MTAD	Dark brown ppt
10.	Group X	Q MIX	MTAD	Dark brown ppt
11.	Group XI	3% NaOCl	Distilled water	No precipitates
12.	Group XII	17% EDTA	Distilled water	No precipitates
13.	Group XIII	2% CHX	Distilled water	No precipitates
14.	Group XIV	Q MIX	Distilled water	No precipitates
15.	Group XV	MTAD	Distilled water	No precipitates

GRAPH 1 (a): Graph showing NMR spectra of the precipitate characteristic of group ii (2% chlorhexidine and 3% sodium hypochlorite) irrigant solutions



GROUP III- 3% NaOCl and QMix

In Group III in our study when we mixed 2% NaOCl and QMix no precipitation was seen. This finding was similar to a finding made earlier by Stojicic *et al.*²⁴ 2012 who also reported that mixing of NaOCl and QMix does not show precipitation or the solution does not turn orange or brown, even though QMix has chlorhexidine in it.

GROUP IV- 3% NaOCl and MTAD

In group IV, when we mixed 3% NaOCl and MTAD, mud coloured precipitates were observed in the test tube. In our study in 1H NMR spectroscopy, peaks were recorded at around 2.5 ppm, 3.5ppm, 6.85-6.95 ppm, 7 ppm and 7.5ppm and on both sides of 2.5 ppm. The peak obtained at 2.5 ppm is characteristic of protons of DMSO solvent. Peaks recorded around 7 ppm and 7.5ppm represents the occurrence of doxycycline which is precipitated due to the addition of sodium hypochloride. This reaction is called as salting-out reaction. So far no study has been conducted on analysis of precipitates formed by mixing sodium hypochloride and MTAD by 1H NMR spectroscopy.

GROUP V- 17% EDTA and 2% CHX

In our study in Group V, when 17% EDTA and 2% CHX were mixed together milky white precipitate was observed in the test tube. Similar type of finding was found in another study conducted by Clarkson *et al.*¹⁵ 2011 in which they observed precipitate were white or white and milky when CHX was mixed with EDTA.

GROUP VII- 17% EDTA and MTAD

Group VII showed formation of brown precipitate when 17% EDTA and MTAD were mixed together. The precipitate was then subjected to 1H NMR spectroscopy and graphs were generated. Peaks were observed at 0.78 ppm, 2.5 ppm, 3.3-3.4 ppm, 6.85-6.95 and 8ppm. At 2.5 ppm the peak recorded is due to protons of DMSO and around 2.5 ppm the peaks are recorded due to detergent present in MTAD. At 0.78 ppm peak is because of outer hydrogen of EDTA. Peak at 3.3-3.4 ppm is observed due to unbroken MTAD in the solution.

GROUP VIII- 2% CHX and QMix

In our study in Group VIII when 2% CHX and QMix were mixed together milky white precipitates were observed. This could be due to interaction between EDTA present in QMix and chlorhexidine. Arslan *et al.*¹⁹ 2015 also observed precipitate formation when 2% CHX and QMix were used separately after 2.5% NaOCl irrigation and percentage of specimen free of precipitates were 0% for distilled water, 5.2% for chlorhexidine and 49.1% for QMixTM.

GROUP IX- 2% CHX and MTAD

In Group IX, 2% CHX and MTAD is mixed which led to formation of dark brown precipitates. These precipitates are formed due to reaction of chlorhexidine and citric acid present in the MTAD. In NMR spectra

peaks were observed at 2.5ppm, 3.5ppm, 3.25-4ppm, 6.85-6.95ppm, 7.30ppm, 7.55ppm. The peaks evident around 2.5 and 3.25-4 ppm is characteristic to the protons of DMSO and chlorhexidine.

GROUP X- QMix and MTAD

In Group X, when we mixed QMix and MTAD it led to the formation of dark brown precipitates. These precipitates were formed due to reaction of chlorhexidine present in the QMix and citric acid present in the MTAD. The characterization of the precipitate was confirmed with 1H NMR spectroscopy and various graphs were generated.

In our study when various irrigants were mixed precipitation was seen in the following groups II (3% NaOCl and 2% CHX), IV (3% NaOCl and MTAD), VII (17% EDTA and MTAD), VIII (2% CHX and QMix), IX (2% CHX and MTAD) and X (QMix and MTAD). This precipitation may act as chemical smear layer, prevent the diffusion of intracanal medicaments and interfere with the sealing ability of obturation materials. However when these precipitates were analysed using 1H NMR spectroscopy chloroaniline was found in Group no. II, VIII, IX and X. This Chloroaniline may be present at ortho, meta or para position. In all these groups where chloroaniline was formed chlorhexidine was present in one or the other form. The International Agency for Research on Cancer (IARC, 1993) has classified 4-chloroaniline in Group 2B (possibly carcinogenic to humans) based on inadequate evidence in humans and sufficient evidence in experimental animals for the carcinogenicity of 4-chloroaniline.

CONCLUSION

Therefore within the limitations of this study it can be said that the use of chlorhexidine should be done judiciously, and its use should be avoided in combination with

- NaOCl
- MTAD
- Combination of MTAD and QMix should also be avoided

As QMix contains chlorhexidine as one of its components and chlorhexidine is highly reactive and has a tendency to breakdown giving aromatic derivatives i.e. chloroaniline which are proven toxic compound.

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