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A spectroscopic assessment of interaction between 4% articaine hydrochloride with adrenaline and various endodontic irrigants

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ABSTRACT

Background: Interaction between 2% lidocaine HCl (with and without adrenaline) and sodium hypochlorite (NaOCl) resulted in a toxic precipitate formation. The aim of this *in vitro* study was to assess the interaction between 4% articaine hydrochloride with adrenaline (AHa) and commonly used endodontic irrigants 3% NaOCl, 2% chlorhexidine (CHX), and 17% ethylenediaminetetraacetic acid (EDTA) using spectroscopic analyses.

Materials and Methods: In this *in vitro* study, 3% NaOCl, 2% CHX, and 17% EDTA were mixed with 4% AHa individually. 1.7 ml of 4% AHa from the cartridge was mixed with 1.7 ml of each test irrigants. The solutions were subjected to a preliminary ultraviolet spectroscopic (UVS) analysis to assess forpotential interactions (if any). If the interaction was detected, the test solutions were further subjected to nuclear magnetic resonance (NMR) analysis for characterization. The precipitate formed (if any) was then subjected to NMR analysis.

Results: UVS analysis revealed a bathochromic shift when 3% NaOCl and 2% CHX were mixed with 4% AHa, respectively. This shift was not observed when EDTA was mixed with 4% AHa. 1H and 13C NMR spectra confirmed the interaction between 3% NaOCl with 4% AHa, which resulted in a precipitate formation, methyl 3-amino-4-methylthiophene-2-carboxylate (MAMC). The analysis of 1H NMR spectra showed peaks at 7.1 ppm, 2.21 ppm, and 9.93 ppm, respectively, which corresponds to aromatic ring protons. A peak at 3.8 ppm was assigned to methyl proton of methyl ester. The characteristic appearance of peaks at 14.82 and 51.16 ppm corresponds to aliphatic carbons. The five peaks occurring at 126, 134.5, 139.2, 156.5, and 162.9 ppm correspond to the aromatic carbon atoms present in the thiophene unit. NMR spectra revealed no interaction between 2% CHX and 4% AHa. 1H and 13C NMR spectra confirmed that 3% NaOCl interacted with 4% AHa, leading to the formation of a precipitate MAMC.

Conclusion: 3% NaOCl interacted with 4% AHa leading to the formation of a precipitate MAMC which is reported to exhibit the least toxicity. Until the precipitate is studied further, it would be advisable to avoid the immediate use of NaOCl following administration of intrapulpal anesthetic solution with articaine hydrochloride (with adrenaline).

Key Words: Anesthesia, chlorhexidine, lidocaine, magnetic resonance spectroscopy, sodium hypochlorite

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INTRODUCTION

Failure of primary conventional anesthetic techniques is not quite uncommon during endodontic therapy, particularly in cases of hot teeth. It has been reported that 4% articaine hydrochloride with adrenaline (AHa) is employed as supplemental injections, particularly, in cases of acutely inflamed pulps and is more effective when compared with that of 2% lidocaine hydrochloride with adrenaline (LHa).[1,2] A meta-analysis by Brandt *et al.* concluded that 4% AHa was 3.8 times more effective in achieving infiltration anesthesia than 2% LHa.[3] The chemical formula of AHa is 4-methyl-3-[2-(propyl amino)-prop ionamido]-2-thiophenec arboxylic acid, methyl ester hydrochloride, which attributes to its improved efficacy when compared to other anesthetic agents.[4] It is a unique amide local anesthetic agent which contains a thiophene ring instead of a benzene ring that allows for its greater lipid solubility and improved anesthetic potency. The thiophene ring may be activated to electrophilic intermediates by cytochrome P450-mediated oxidation resulting in toxic by-products.[5,6] In addition, some of the thiophene-containing therapeutic agents such as methapyrilene, tienilic acid, suprofen, tenidap, ticlopidine, and duloxetine exhibited various toxicities in humans and animals.[7,8] Schneider *et al.* compared the genotoxicity of various anesthetic agents and concluded that lidocaine and articaine did not produce any toxic metabolites.[9]

A previous study by Vidhya *et al.* reported that lidocaine hydrochloride (with or without adrenaline) interacts with sodium hypochlorite (NaOCl) to produce a toxic precipitate called 2,6-xylidine, a known metabolite of lidocaine.[10,11] Such potential interactions might arise in a clinical scenario, where intrapulpal anesthesia (IPA) is administered, followed by the immediate use of NaOCl for pulp tissue dissolution.

NaOCl (in concentrations ranging from 0.5% to 5.25%), 2% chlorhexidine (CHX), and 17% ethylenediaminetetraacetic acid (EDTA) are the most commonly used endodontic irrigants. These irrigants are usually employed as a combination sequentially to achieve the desired antimicrobial and tissue dissolution effects. However, it has been reported that these irrigants interact with each other, which can lead to a reduction in the potency of the irrigants or formation of a toxic precipitate.[12,13] A careful review of the literature revealed that the interaction between 4% articaine hydrochloride and various endodontic irrigants has not been assessed yet. Hence, the aim of the present study was to assess the interaction between 4% AHa and various irrigants such as 3% NaOCl, 2% CHX, and 17% EDTA using spectroscopic analysis.

MATERIALS AND METHODS

The present *in vitro* study was conducted in a laboratory setup with the root canal irrigants (3% NaOCl, 2% CHX, and 17% EDTA) and the local anesthetic solution (4% articaine hydrochloride) which are to be assessed. The test solutions were subjected to a preliminary ultraviolet spectroscopy (UVS) evaluation before nuclear magnetic resonance (NMR) analysis.

Ultraviolet spectroscopy (Cary 5E, Japan)

The experimental groups analyzed for UVS are described in Table 1. The spectroscopic analysis of 4% AHa and the corresponding irrigant mixtures were compared to that of their respective original solutions. A shift in UV absorption in the spectrograph inferred that an interaction might have occurred between the mixed solutions. Those groups which showed a shift in the absorption of UV in comparison to that of original solutions were then subjected to further spectroscopic analyses.

Table 1: Control and experimental solutions assessed with ultraviolet spectroscopy for interaction of endodontic irrigants with 4% adrenaline

Groups	Description
Control solution	Cuvette-1: 2 ml DW (Aqua shine, SPARK, Chennai, India)
Test original solutions	Cuvette-2: 1.5 ml DW + 0.5 ml of 4% AHa (3M ESPE, Ubistes, Germany)
	Cuvette-3: 1.5 ml DW + 0.5ml of 3% NaOCl (Vensons India, Bengaluru, India)
	Cuvette-4: 1.5 ml DW + 0.5 ml of 2% CHX (Calypso, Septodent health care India Pvt. Ltd., Maharashtra, India)
	Cuvette-5: 1.5 ml DW + 0.5 ml of 17% EDTA (Dental avenue Pvt Ltd., Vasavi (E), Thane, India)
Test sample mixtures	Cuvette-6: 1.5 ml DW + 0.5 ml of 4% AHa mixed with 3% NaOCl
	Cuvette-7: 1.5 ml DW + 0.5ml of 4% AHa mixed with 2% CHX
	Cuvette-8: 1.5 ml DW + 0.5 ml of 4% AHa mixed with 17% EDTA

DW: Distilled water; AHa: Adrenaline; NaOCl: Sodium hypochlorite; CHX: Chlorhexidine; EDTA: Ethylenediaminetetraacetic acid

Experimental groups

Group I - Each cartridge of 4% AHa contains 1.7 ml of local anesthetic solution. This was dispensed into a glass test tube (BOROSIL, Gujarat, India) to which 1.7 ml of 3% NaOCl solution was micropipetted (ERBA, Mumbai, India) [Table 1].

Group II - In a glass test tube, 1.7 ml of 4% AHa from the cartridge was dispensed to which 1.7 ml of 2% CHX solution was micropipetted. The experimental solutions were observed for color change and precipitate formation for an hour.

Analysis of the by-products formed by mixing the irrigants with 4% AHa

The precipitate formed due to the interaction of an endodontic irrigant with 4% AHa was subjected to the following analyses:

1. Thin-layer chromatography (TLC) (Anna University, Chennai, India) was performed to assess the number of compounds formed on mixing of irrigants with 4% AHa
2. Column chromatography (Anna University, Chennai, India) was performed to obtain the pure form of the precipitate
3. NMR (Bruker Avance Iii 500 Mhz, Germany) analysis of the pure precipitate. If there was no precipitate formation, the solution was subjected directly to NMR analysis.

Thin-layer chromatography analysis

One milligram of precipitate was removed from the glass tube using spatula and mixed with 2 ml of CDCL3 (chloroform) to make a solution. Four drops of this solution were placed in TLC plate coated with silica gel (Trade-Link Pvt. Limited Khadalpur, Mehsana, Gujarat, India). This TLC plate was then placed in the TLC chamber-containing hexane solvent. The CDCL3 TLC plate was removed from the chamber when the hexane solvent reached the full length. The solvent was allowed to evaporate, following which the number and nature of compounds were analyzed using UV light.

Column chromatography analysis

The pure form of precipitate was then isolated by removing the impurities (formed, if any) using column chromatography. One hundred milligrams of the precipitate was collected by mixing 4% AHa and 3% NaOCl, respectively, in a beaker. This was further mixed with 500 mg of silica powder and 500 ml of hexane solvent. The solvent was then evaporated

to achieve a homogeneous mixture by heating the solution up to 47°C. A vertical glass column (24 mm diameters × 15″ height) with a control knob in the lower end was taken. A cotton ball was placed as a stationary phase in the lower end of the column, to which the homogeneous mixture was added. Hexane was added into the vertical column as a solvent and another cotton ball was placed on top of the interactive precipitate in the column. The knob in the lower end of the glass column was opened, and the pure form of interactive by-products was collected. Isolation of purified compound was later confirmed with TLC.

Nuclear magnetic resonance spectroscopy analysis

The purified compound (obtained, if any) was allowed to dry for 24 h, and the resultant powder was mixed with 3 ml of CDCL3. If no precipitate was formed, the experimental solutions were mixed with CDCL3 solution and subjected to NMR analysis directly. This solution was then micropipetted to an NMR tube (5 mm diameter × 18 cm height dimension), which was further subjected to NMR analysis, and the samples were analyzed thrice. One-dimension (H1 C13) NMR spectra were then obtained for each sample at 21°C, and the resulting spectra were fully assigned in terms of chemical shifts of all proton and carbon atoms with the corresponding irrigants and 4% AHa.

RESULTS

Ultraviolet spectroscopic analysis

The control solutions of 3% NaOCl, 17% EDTA, 2% CHX, and 4% AHa exhibited a UV max at 295 nm, 0 nm, 240 nm, and 270 nm respectively. The mixture of the test sample of 4% AHa and 3% NaOCl mixture showed a bathochromic shift (redshift) to 285 nm. Similarly, a bathochromic shift was observed on mixing 2% CHX and 4% AHa, in which UV max was shifted to 260 nm. These bathochromic shifts confirmed that 3% NaOCl and 2% CHX interacted with 4% AHa. The test sample of 4% AHa mixed with 17% EDTA revealed a UV max at 270 nm which corresponds to 4% AHa original wavelength.

Color change and precipitate formation

A white precipitate was formed immediately on mixing 3% NaOCl with 4% AHa. This precipitate showed a gradual color change to light yellow within

30 min and the yellow precipitate sedimented in the bottom of the test tube within an hour [Figure 1]. No color change or precipitate formation was observed up to 1 h on mixing 2% CHX with 4% AHa.

Thin-layer chromatography analysis
The precipitate formed by mixing 3% NaOCl with 4% AHa was subjected to TLC. The spotting pattern observed in TLC plate showed the presence of two distinct spots with an Rf value of 0.4 and 0.3, respectively. Both the spots were observed in a single lane and the spot with Rf value of 0.4 was observed to be darker in shade under UV light which indicated that the compound was impure. The spotting pattern observed in TLC plate on cross-verification following column chromatography showed only one distinct compound at an Rf value of 0.4.

Nuclear magnetic resonance analysis
The spectra obtained with 1H NMR and 13C NMR of the precipitate on mixing 3% NaOCl with 4% AHa is represented in Figure 2.

1H nuclear magnetic resonance spectral analysis of precipitate formed on mixing 3% sodium hypochlorite and 4% AHa
1H NMR spectra were assigned for the aromatic ring protons with peaks at 7.1 ppm, 2.21 ppm, and 9.93 ppm, respectively, in which each peak exhibited a characteristic singlet pattern corresponding to protons in the 5th, 4th, and 3rd position of the thiophene ring of the 4% AHa. A peak at 3.8 ppm was assigned to methyl proton of methyl ester.

13C nuclear magnetic resonance spectral analysis of precipitate formed on mixing 3% sodium hypochlorite and 4% AHa
The characteristic appearance of peaks at 14.82 and 51.16 ppm corresponds to aliphatic carbons. The five peaks occurring at 126, 134.5, 139.2, 156.5, and 162.9 ppm correspond to the aromatic carbon atoms present in the thiophene unit. Both 1H and 13C NMR spectra confirmed the presence of methyl-3-amino4 methylthiophene-2-carboxylate (MAMC) compound in the resultant precipitate.
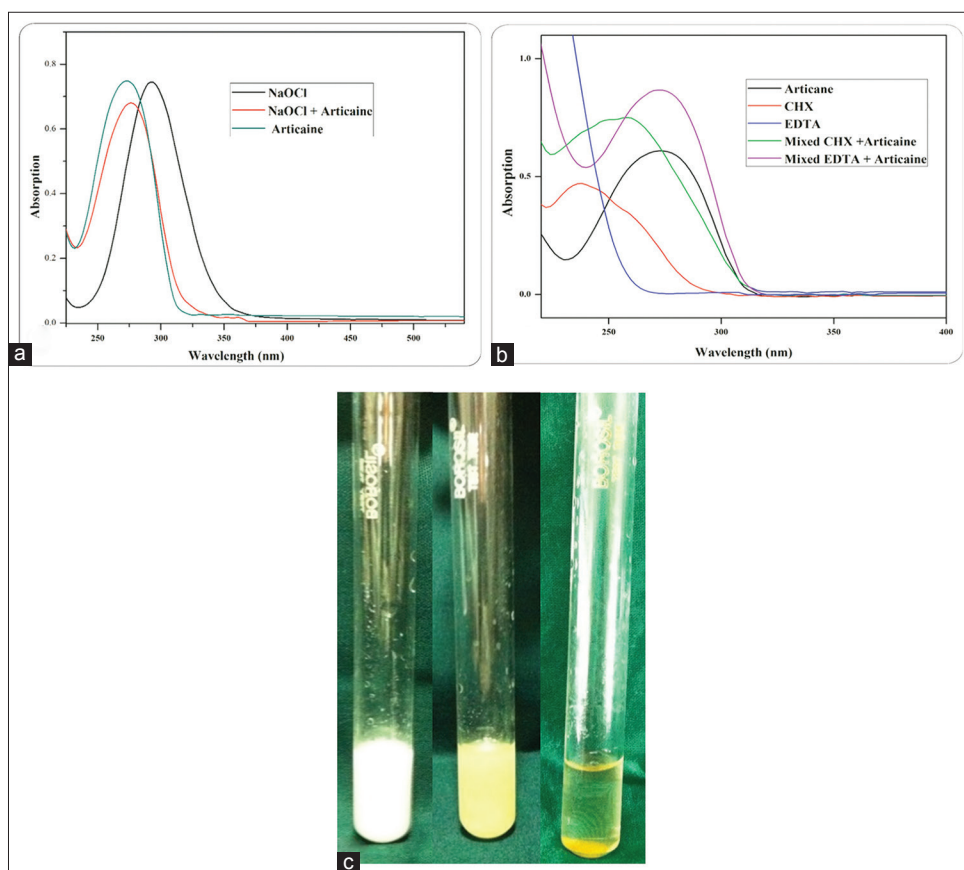

Figure 1: (a) Ultraviolet spectra of 3% sodium hypochlorite, 4% AHa and the bathochromic shift of sodium hypochlorite/AHa mixture. (b) Ultraviolet spectra of 17% ethylenediaminetetraacetic acid, 2% chlorhexidine, 4% AHa and the bathochromic shift of chlorhexidine/AHa mixture. (c) Test tubes depicting the formation of white precipitate (immediately), and yellow precipitate (after 30 min) followed by sedimentation of the yellow precipitate (after 1 h) on mixing 3% sodium hypochlorite and 4% AHa.
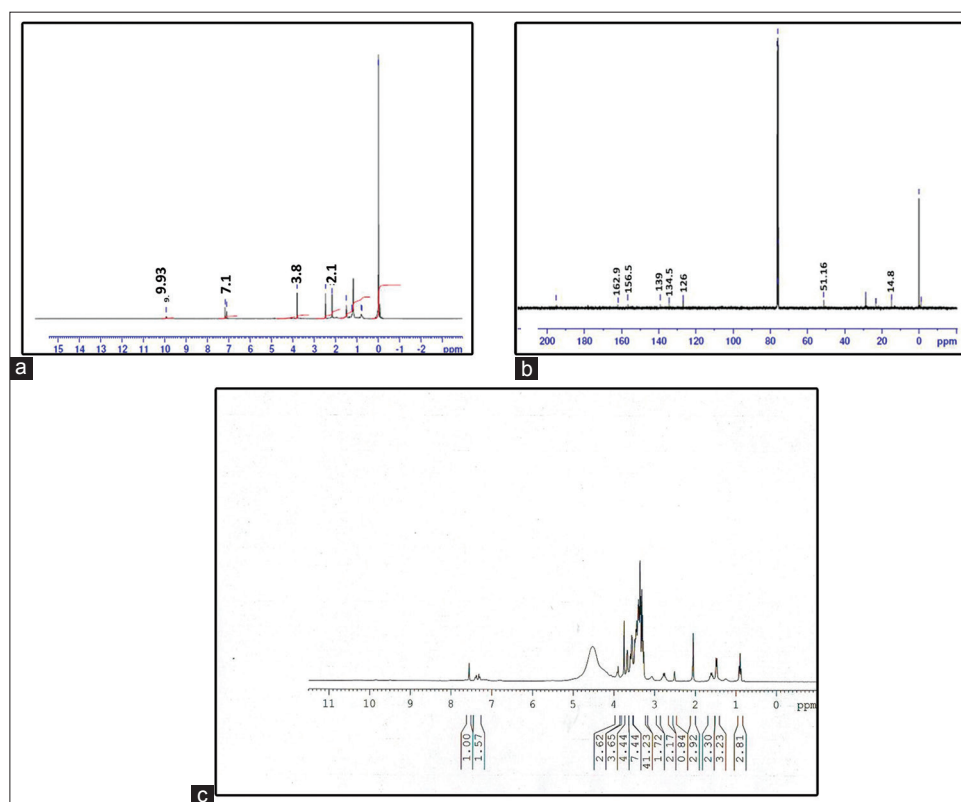
Figure 2: (a) 1H nuclear magnetic resonance spectra of the precipitate formed by mixing 3% sodium hypochlorite and 4% AHa. (b) 13C nuclear magnetic resonance spectra of the precipitate formed by mixing 3% sodium hypochlorite and 4% AHa. (c) 1H nuclear magnetic resonance spectra of the precipitate formed by mixing 2% chlorhexidine and 4% AHa.

1H nuclear magnetic resonance spectral analysis of resultant solution on mixing 2% chlorhexidine and 4% AHa

The singlet peak at 7.3 ppm was assigned to the aromatic ring protons representing the hydrogen at 5th position of thiophene unit. A peak at 2.51 ppm corresponds to the methylene protons present in the 4th position of thiophene unit. The peak at 3.89 ppm was assigned for methyl proton of methyl ester. The peaks around 0.87–3.80 ppm corresponds to methylene protons present in AHa and CHX. The peak at around 4.5 ppm corresponds to the aliphatic amide protons observed in both the solutions. The peaks around 6.8 ppm to 7.55 ppm correspond to the aromatic proton present in CHX.

13C nuclear magnetic resonance spectral analysis of the resultant solution on mixing 2% chlorhexidine and 4% AHa

The characteristic appearance of peaks at 42.10–27.43 ppm corresponds to hexamethylene alkyl chain and the amino (-C = NH) carbons appeared in the range of 161.94 and 157.07 ppm. The peaks occurring at 139.99–121.25 ppm correspond to the aromatic carbon atom present in the thiophene unit. Both 1H

and 13C NMR peaks obtained on mixing 2% CHX and 4% AHa correspond to the individual peaks of 2% CHX and 4% AHa which proved that there was no interaction between these two solutions.

DISCUSSION

Various methods have been employed to assess the chemical interactions between two or more compounds, namely time-of-flight-secondary ion mass spectrometry gas chromatography-mass spectrometry, X-ray photon spectroscopy, and NMR spectroscopy.[12-14] Mass spectrometry may not be a reliable method for determining the presence of degradation products because it relies on gas phase ionization, which can fragment molecules.[15] On the contrary, gas chromatography can analyze the compounds which possess a boiling point of <400°C, whereas the boiling point of AHa is 440°C. NMR spectroscopy is a noninvasive and nondestructive method to analyze the samples and determines the chemical composition of the breakdown products.[15] NMR has also been used in previous studies to analyze the interaction of various endodontic irrigants.

In the current study, UV spectral analyses revealed a bathochromic shift when 3% NaOCl and 2% CHX solutions were mixed with 4% AHa. On the contrary, 17% EDTA did not show any shift in UV absorption. This proved that 17% of EDTA did not interact with 4% AHa, and hence, it was not subjected to further analyses. The potential chemical reaction expected with the interaction of 3% NaOCl and 4% AHa is the liberation of hypochlorous acid. This might combine with carbon atoms present in 4% Aha, resulting in disruption of the molecule with subsequent cleavage of the double bond. On further hydrolysis, MAMC precipitate might be formed, which is an aromatic amine consisting of a thiophene unit. The potential chemical reaction expected with the interaction of 2% CHX and 4% AHa is a base catalyst reaction with a cleavage at the guanidine carbon atom.[16] This breakdown product was expected to react with NH group of 4% AHa producing 1,4-chlorophenyl, 3-methyl 1H thieno 2,3-pyrazole-5-carboxylate (CMTPC) compound. Both MAMC and CMTPC compounds can be chemically determined using NMR analysis, and hence, this method was employed in the present study.

In the present study, on mixing 3% NaOCl with 4% AHa, the precipitate formed was identified as MAMC using NMR analysis. Alhough the precipitate (MAMC) is formed with a lesser volume of AHa, 1.7 ml of AHa was mixed to 1.7 ml of NaOCl in an attempt to produce the precipitate in sufficient quantities, required for NMR analysis. MAMC is a known precursor of AHa which contains a thiophene unit.[17] It has been reported that a thiophene compound, methapyrilene, was withdrawn from clinical use owing to periportal hepatic necrosis and hepatocarcinogenicity in rats.[18] Thiophene compounds have been largely reported to get bioactivated to form electrophilic intermediates by the oxidation process. These thiophenes can produce toxic by-products (toxicophores) on oxidation of hepatocytes.[8] Lepailleur *et al.* have assessed the genotoxicity and carcinogenic potential of MAMC and two other compounds using the Syrian hamster embryo cell-transmission assay.[17] The authors had reported that 3-amino 4-methylthiophene displayed a carcinogenic potential with a profile of a nongenotoxic carcinogen.[17] However, the presence of an ester in MAMC compound modifies its reactivity and has been proven to be a very stable derivative with an intramolecular H+ bond. Thus, its metabolic activation to its potential toxicophores was proven to be minimal. The presence of the carboxylate group in the alpha position of the sulfur atom in the thiophene ring also makes MAMC less carcinogenic.[18]

On the contrary, lidocaine hydrochloride (with or without adrenaline) has been shown to produce a toxic precipitate (2,6, xylidine) when it comes in contact with NaOCl.[10] Although the use of 4% AHa might be an appropriate alternative for achieving adequate intrapulpal supplemental anesthesia during endodontic therapy, especially in cases of hot pulp, the resultant precipitate might get attached to the root canal surface and hinder the penetration of intracanal irrigants, medicaments, and root canal sealers into the dentinal tubules. Further research on the dentin tubule occlusion by this precipitate is needed, and the amount of precipitate extruded during cleaning and shaping and its cytotoxicity on human periodontal fibroblast requires to be assessed. Since the exact consequences of this precipitate are not known currently, immediate use of NaOCl following the administration of intrapulpal/intracanal anesthetic solution should be avoided.

The peaks obtained on mixing 2% CHX and 4% AHa do not correspond to the CMTPC compound, which proved that there was no significant interaction occurred between CHX and AHa.

This study is not without its limitations. It can be possibly argued that IPA is usually employed in the pulp chamber and that the probability of the anesthetic solution entering into the root canal following IPA is very minimal. Practitioners can also neglect this significant interaction attributing to the reason that only a small amount of local anesthesia (0.2–0.5 ml) is employed for supplemental IPA. However, it is an unacceptable fact that acutely inflamed pulp remnants might necessitate such frequent IPA administrations into the pulpal space to ensure the patient's maximum comfort during the endodontic procedure.[19]

CONCLUSION

Within the limitations of this *in vitro* study, it could be concluded that:
1. UV spectra analysis confirmed that 3% NaOCl and 2% CHX interacted with 4% AHa
2. 17% EDTA showed no interaction on mixing with 4% AHa with adrenaline
3. 1H and 13C NMR spectra confirmed that only 3% NaOCl interacted with 4% AHa, resulting in the formation of a precipitate MAMC.

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Conflicts of interest

The authors of this manuscript declare that they have no conflicts of interest, real or perceived, financial or nonfinancial in this article.

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