Sodyum hipoklorit ve klorheksidin karışımından sonra oluşan çökeltinin titreşim spektroskopisi ile analizi: Potansiyel bir klinik problem

Vibrational spectroscopy analysis of the precipitate form after mixing sodium hypochlorite and chlorhexidine: A potential clinical problem

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ÖZ

Amaç Endodontik işlemlerde en yaygın yıkama ajanları olan sodyum hipoklorit ve klorheksidin ile karıştırılması reaksiyonuyla oluşan çözünmeyen kahverengi renkli maddenin moleküler yapısı henüz literatürde tam olarak açıklanmamıştır. Amaç sodyum hipoklorit ve klorheksidin karıştırma yoluyla oluşan çökeltiyi Fourier-transform infrared (FT-IR) spektroskopisi kullanarak analiz etmektir.

Yöntem Deneysel çökeltinin FT-IR spektrumu kaydedildi. Deneysel çökeltinin titreşimli FT-IR spektrumu analiz edildi ve mevcut veri tabanlarından elde edilen iletim modundaki klorheksidin spektrumu ile karşılaştırıldı (4000-400 cm-1).

Bulgular Deneysel çökelti spektrumda 3335, 2436, 2160, 2004, 1896, 1636, 579 ve 555 cm-1'de olmak üzere 8 pik kaydedildi. Bu pikler, klorheksidinin titreşimleriyle karşılaştırıldı.

Sonuç Sodyum hipoklorit ile etkileşimin ardından, klorheksidin yapısı kırılmış ve yeni bir çözünmeyen ürüne (1,6-Hekzandilbis{imino [({[(4-klorfenil)amonyo](imino)metil} amino) metiliden]})diamonyum)'a dönüşmüştür.

Anahtar Kelimeler: *Klorheksidin, Yıkama, Çökelti, Sodyum hipoklorit, Spektroskopi.*

ABSTRACT

Objective: The molecular structure of the insoluble brown-colored substance is produced through the reaction of mixing the most common irrigation agents in endodontic treatments as sodium hypochlorite and chlorhexidine has not been fully described in the literature yet. The aim was to analyze the precipitate is formed through the reaction of mixing sodium hypochlorite and chlorhexidine by using Fourier-transform infrared (FT-IR) spectroscopy.

Methods: FT-IR spectrum of the experimental precipitate was recorded. The vibrational FT-IR spectrum of the experimental precipitate was analyzed and compared with the spectrum of the chlorhexidine obtained from available databases in transmission mode (4000-400 cm-1).

Results: Eight-shift was recorded at 3335, 2436, 2160, 2004, 1896, 1636, 579 and 555 cm-1 in the spectrum. The experimental precipitate was analyzed and the results were compared with the vibrations of chlorhexidine.

Conclusion: After interaction with sodium hypochlorite, chlorhexidine structure was broken and was converted to a new insoluble product as 1,6-Hexandiylbis{imino[({[(4-chlorphenyl)ammonio](imino)methyl}amino)methylyliden]}) diammonium.

Keywords: *Chlorhexidine, Irrigant, Precipitation, Sodium hypochlorite, Spectroscopy.*

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INTRODUCTION

Sodium hypochlorite (NaOCl) is one of the most frequently used root canal irrigation solutions presenting organic tissue dissolving capabilities and antibacterial action in endodontic treatments.[1,2] To improve effectiveness of irrigation, different disinfectant solutions such as chlorhexidine (CHX) are used in combination with NaOCl due to their synergistic antimicrobial effects in endodontic infections.[3] However, this combination could result in an orange-brown precipitate, when CHX is introduced directly on NaOCl in the canal without interim flushing with distilled water.[4] This precipitate can adhere resolutely to the canal walls which is difficult to remove; and it may cause discoloration and sealing-related obturation failures.[3,4] Previous studies have conflicting results regarding the presence of para-chloroaniline (PCA) in this precipitate.[5-8] Although a recent study employing multiple analysis have proven that the precipitate does not contain PCA,[8] the molecular structure of the insoluble substance has not been fully described in the literature yet. Only a single study has identified the first time the molecular structure of the precipitate using a somewhat destructive analysis.[6] However, nondestructive molecular analysis could be more suitable to identify the chemical composition of the precipitate instead of any destructive methods such as mass spectrometry and its combinations.[8]

The infrared spectrum analysis of any compound means to investigate its unique "finger print".[9] Fourier-transform infrared (FT-IR) spectroscopy, is a nondestructive molecular spectroscopic technique for detection of molecular changes in reactions.[10] FT-IR spectroscopy has been used for screening the setting reactions of various dental materials[11-13] and for investigating possible interactions between the sealers used in root canal treatment.[14] FT-IR spectroscopy allows to analyze vibration amplitudes at various component frequencies of molecules; thus, is a useful tool in the characterization of molecular structure.[15] The purpose of this study was identification and discrimination of the precipitate formed by the reaction of mixing NaOCl and CHX by using FT-IR spectroscopy analysis.

MATERIALS AND METHODS

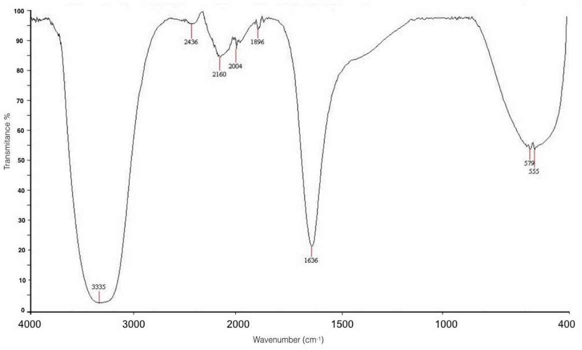
Commercially available CHX (Gluco-Chex solution 2%, Lot # 2909151, Cerkamed Medical Company, Stalowa Wola, Poland) and fresh NaOCl (Chlorax 5.25%, Lot # 2708151, Cerkamed Medical Co., Stalowa Wola, Poland) solutions were used. Experiments were conducted at 25°C. 10 mL CHX was added to 10 mL NaOCl in a glass beaker and mixed (Stirring Rod, Acrol Scientific Laboratory Systems, İstanbul, Turkey) continuously for a minute. An instant precipitate formation was observed.

A brown-colored solid substance was extracted from this mixture as described in the previous study.[8] Analytical grade ethyl acetate was used for extraction. The precipitate was extracted three times in 15 mL of ethyl acetate to transfer the precipitates into organic solvent. Organic layer was separated and dried over anhydrous sodium sulphate. Solvent was removed under reduced pressure and the solid brown precipitate was obtained. Triplicates of the solid brown precipitate was used for FT-IR analysis.

FT-IR spectrum of the solid brown precipitate was recorded with a FTIR spectrometer (Jasco FT-IR-300 E, Jasco Inc., MD, USA) using the KBr technique in the region 4000–400 cm^{-1}.[16] The spectrum resolution was ±1 cm^{-1}. All spectra were also electronically recorded on a double beam spectrophotometer (Unico UV-4802, Unico Co., NJ, USA).

RESULTS

FT-IR spectrum in transmission mode of the experimental precipitate was collected in the frequency range of 4000-400 cm^{-1} which is shown in (Figure 1). Eight-shifts were recorded at 3335, 2436, 2160, 2004, 1896, 1636, 579 and 555 cm^{-1} in the spectrum. Two strong stretching peaks were observed at 3335 and 1636 cm^{-1} whereas, weak stretching peaks were recorded at 2436, 2160, 2004, and 1896 cm^{-1}. Medium stretching with two



FT-IR spectra of experimental precipitate

Figure 1. The experimental FT-IR spectrum of the precipitate formed after 5.25% NaOCl and 2% CHX.

spikes were observed at 579 and 555 cm^{-1}. Spectral band assignments of CHX was obtained by FT-IR databases cited in the literature.[17]

DISCUSSION

CHX is a divalent biguanidine compound used as a broad spectrum antiseptic agent and its salt soluble form use in endodontic infection.[18] Strong oxidants, such as hypochlorite should be removed before introducing CHX into the root canal system due to chemical interaction resulting in formation of solid brown precipitate.[4,18] CHX has a positively charged (dicationic) acid molecule whereas, aqueous NaOCl solution is alkaline.[19] Formation of the solid brown precipitate after mixing NaOCl with CHX is the result of neutralization type of reaction.[19]

First time in the literature, we used FT-IR spectroscopy to analyze the vibrational spectra of the solid brown precipitate formed after interaction between CHX and NaOCl. Vibrational spectroscopy is an effective technique to examine molecular structure of organic compounds.[20,21] Development of FT-IR spectroscopic screening systems have been accelerated the application of vibrational spectroscopy in dentistry such as identification of mineral and organic phases of dental structures after acid and laser treatments, interface between the adhesive and dental hard tissues, and after bleaching of dental tissues.[21] In this study, FT-IR was used to compare the characteristic peaks of different functional groups in the solid brown precipitate and CHX. The FT-IR spectrum of CHX was obtained from the recent literature[17] and confirmed by open access databases (Figure 2).[22,23]

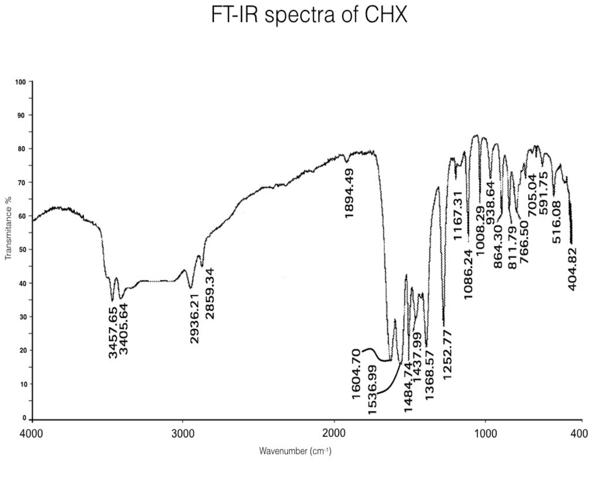


Figure 2. FT-IR spectrum of CHX molecule was obtained by FT-IR databases.[24]

CHX ($C_{22}H_{30}Cl_2N_{10}$) or (1E)-2-[6-[[amino-[(E)-[amino-(4-chloroanilino) methylidene] amino] methylidene] amino] hexyl]-1-[amino-(4-chloroanilino) methylidene] guanidine molecule consists of C-Cl, C= C, C-C, C= C, C-C, C= C, C-C, C-NH, HN-C, C= N, C-NH, HN-C, C= N, C-NH, HN-C, C-C, C-C, C-C, C-C, C-C, C-NH, HN-C, C=N, C-NH, HN-C, C= N, C-NH, NH-C, C-C, C= C, C-Cl, C= C, C-C, C= C bonds are illustrated in (Figure 3).[24] The typical FT-IR spectrum of a CHX is made up from C=C, C-C, C-NH, C=NH, and C-Cl stretching vibrations modes.[22,23] The characteristic N-H stretching of amine is expected to appear in 3500-3250 cm^{-1} frequency ranges.[17,22,23,25] The N-H stretching of secondary amine salts is characterized by major peaks at 2542, 2956 cm^{-1}, and the N-H bending of secondary aromatic amine is 1519.80 cm^{-1}.[17] The characteristic bands of C=N stretching vibrations modes are expected to appear in 1690 - 1640 cm^{-1} frequency ranges in aromatic guanidine (ArNHC(=N-H)NHAr) and ((CH3)2NC(=N-H)C(CH3)2). [17] Generally, stretching vibrations of C=C group is expected to appear at 1670 - 1626 cm^{-1} for CHX.[25,26] As for the C-C alkaline stretching are expected to appear in 1400 - 600 cm^{-1} in fingerprint region of the FT-IR spectrum.[24,25] The C-N secondary aromatic amine stretching are expected to appear in 1300 - 1000 cm^{-1} frequency ranges.[17,22,23] The characteristic C-Cl stretching vibrational modes of structure are expected to appear in 800 - 600 cm^{-1} wave numbers.[22,23] The FT-IR spectrum of the experimental precipitate was assigned with the corresponding values in literature.[17, 22, 23, 25-27] The recorded peaks were defined as follows: The strong and varying stretching peak was observed at 3335 cm^{-1}. The characteristic N-H stretching vibrations of amide structure are expected to appear in 3335 cm^{-1} frequency ranges. A very strong C-NH2 vibration mode was observed at 2436 cm^{-1}. A very strong C=NH vibration mode with two spikes were observed at 2160 and 2004 cm^{-1}. An overtone C-H bending was observed at 1896 cm^{-1}. The medium, sharp C=C peak was stretched at 1636 cm^{-1}. Solid brown precipitate also had the aromatic guanidine peak at this wavelength (typically appearing between 1690 and 1595 cm^{-1}). The decreased frequency was attributable to hydrogen bonding. A medium N-Cl stretching with two spikes was observed at 579 and 555 cm^{-1}. Similarly, a previous study has been reported that the probable sites of Cl addition to CHX occur at the N atoms when interact with NaOCl.[9]

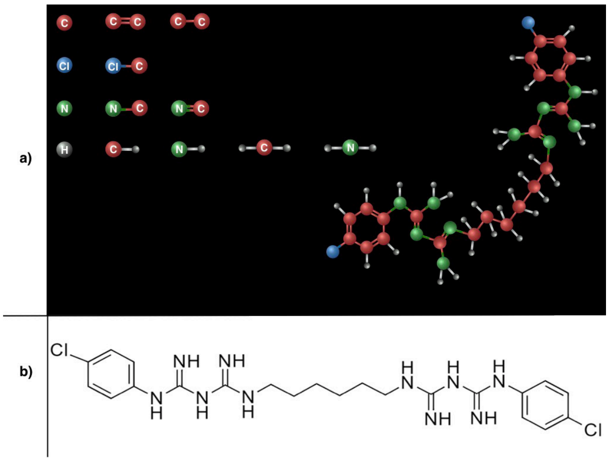


Figure 3. a; The chemical bonds of CHX (C22H30Cl2N10) or (1E)-2-[6-[[amino-[(E)-[amino-(4-chloroanilino) methylidene] amino] methylidene] amino] hexyl]-1-[amino-(4-chloroanilino) methylidene] guanidine, b; The molecular structure of CHX was drawn according to the U.S. National Library of Medicine Database (24) with ChemDraw 16 (PerkinElmer, Inc., Waltham, MA).

In the current study, chemical bonds of C-NH2, C=N and N-Cl were formed after mixing NaOCl and CHX; whereas, N-H, C-N, C-C and C-Cl bonds were missing in the solid brown precipitate. These bonds are present in FTIR spectrum of CHX at 2800-1800 cm^{-1} and at 1520-700 cm^{-1}.[22] Theoretically, molecules with low bond disassociation energies are more prone to breaking.[28] The C-N bonds of the CHX molecule could be broken because of the low bond dissociation energy between the two atoms when interacted with NaOCl.[19] The NH2-C=N-C band might be broken; thus, it is possible that four-hydrogen atoms originating from NaOCl or water molecule might have been included in the newly formed brown precipitate. Consequently, the E notation of the molecule geometry of CHX might be converted to Z rearrangement after interaction with NaOCl. We speculate that the new product occurred after mixing of 5.25% NaOCl and 2% CHX is the result of the following equation which is as follows.

“NaOCl + $C_{22}H_{30}Cl_2N_{10}$+ $2H_2O \rightarrow$ NaOCl + $C_{22}H_{34}Cl_2N_{10}$+ O_2”

This product, $C_{22}H_{34}Cl_2N_{10}$ as 1,6-Hexandiylbis {imino [({ [(4-chlorphenyl) ammonio] (imino) methyl} amino) methylyliden] }) diammonium after interaction with sodium hypochlorite also forms after a similar reaction of mixing perchloric acid or perchlorate with CHX.[29] This reaction is classified as a double replacement reaction where two compounds react, and the cations and the anions of the two reactants change their places. Furthermore, water is an excellent solvent for these polarized molecules transferring.[30]

Only single study has been reported the molecular structure of the precipitate using electrospray ionization quadrupole time-of- flight mass spectrometry (ESI-MS).[9] Authors emphasized that the chlorine addition to CHX structure occurred at the guanidino nitrogens in the presence of NaOCl, an oxidizing agent causing chlorination and this molecular change was resulted $C_{22}H_{28}Cl_5N_{10}$ production without presence of PCA.[9] However, another study reported controversial composition the precipitate by using ESI-MS method.[31] CHX is macromolecule and it has 505.45Da molecular weight. Instead of macromolecules, the ESI-MS method uses solute species with molecular weights small enough. Therefore, it could be analyzed the mass of ions with an available quadrupole mass filter that had a maximum mass range of about 450Da.[32] So that, the ESI-MS method may a disadvantage to focus mostly on pseudo molecular ions created by the discharging. [32] Thus, controversial results might be due to high voltage electric discharge in ESI-MS method. Contrary, the vibrational spectroscopy solely focuses on aerosol molecules and provides less information on the mass spectrum.[20] This might be the reason for inconsistent results with the previous study.

A recent study reported that the absence of free PCA in the precipitate.[33] Free or pure PCA has been two asymmetric -NH2 stretches at 3472 cm-1 and at 3381 cm-1, respectively.[33] Moreover, a scissoring signal of -NH2 group at 1617 cm-1 has also presented in the recent report.[33] None of these -NH2 signals have been recorded in the precipitate (NaOCl + CHX).[33] In agreement with the recent report, IR spectra of the precipitate yielded single stretch at 3335 cm-1 instead of –NH2 signals in the present study, confirming the absence of free PCA. Although the multiple analyses have proven that the precipitate does not contain PCA,[8] the precipitate can adhere resolutely to the canal walls which is difficult to remove; and it may cause discoloration and sealing-related obturation failures.[3,4] Further studies on this subject may clarify the molecular sequence, toxicity, solubility, and behavior of this precipitate in clinical conditions.

CONCLUSIONS

The vibrational frequencies of the essential modes in experimental precipitate have been accurately assigned, analyzed and the results were compared with the vibrations of chlorhexidine. Thus, we showed that chlorhexidine structure was broken and was converted into a new insoluble compound as 1,6-Hexandiylbis {imino [({ [(4-chlorphenyl) ammonio] (imino) methyl} amino) methylyliden] }) diammonium after interaction with sodium hypochlorite.

CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported

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