

Reduction of Microbial-Derived Components in Dental Unit Water Lines by Ozonated Water

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Introduction

Interest in the contamination of dental unit water lines (DUWLs) is growing amongst dental researchers, and previous studies have shown that these water supplies are highly populated by planktonic bacteria. Fortunately, the reported cases of infection by bacteria from DUWL are few. However, dental surgeons should attempt to ensure optimum hygiene standards in the dental office, particularly when treating immuno-compromised patients. A wide range of measures and methods to control the problem have been suggested, although to date they have proved not to be completely effective. In this investigation, we have conducted a multicomponent evaluation of the molecular nature and levels of bacterial catabolites present in DUWL specimens by high-resolution proton nuclear magnetic resonance (NMR) spectroscopy, and also assessed the efficacy of a novel ozonation process to diminish the concentrations of these 'markers' of microbial activity.

Objectives

This experiment aimed at examining the ability of high resolution, high field ^1H NMR analysis to provide useful molecular information regarding the nature and level of microbial catabolites present in DUWLs and to assess the effect of the use of ozonated water on these catabolites after 7 days.

Materials and Methods

Sample Collection

Water from the dental unit water lines (DUWLs) of 8 individual dental units was collected. A series of further DUWL samples were collected 7 days following the once daily ozonation of the water using ozonated water from the TherOzone Unit, CA, USA.

Each specimen was then centrifuged at 16,000 g for 30 minutes (40 °C) and the supernatant removed for ^1H NMR analysis. Proton NMR measurements: Proton (^1H) NMR measurements on the above samples were conducted on a Bruker AMX-600 spectrometer (Queen Mary, University of London facility), operating at a frequency of 600.13 MHz and a probe temperature of 298 K. Typically, 0.60 ml of sample was placed in a 5-mm diameter NMR tube, and 0.07 ml of $2\text{H}_2\text{O}$ was added to provide a field frequency lock. The intense water signal ($\delta=4.80$ ppm) was suppressed by pre-saturation via gated decoupling during the delay between pulses. Pulsing condition for the one-dimensional (1-D) spectra acquired on the samples examined were: 64 or 128 free induction decays (FIDs); 16,384 data points; 3-7 μs pulses; and 1.0 s pulse repetition rate. Line-broadening functions of 0.30 Hz were routinely employed for the purpose of processing experimental NMR data. Chemical shifts were referenced to external sodium 3-trimethylsilyl [2,2,3,3- $^2\text{H}_4$] propionate (TSP, $\delta=0.00$ ppm). Where present, the methyl group resonance of acetate ($\delta=1.920$ ppm) served as a secondary internal reference. The identities of biomolecules present in the ^1H NMR spectra of the DUWL samples acquired were routinely assigned by a consideration of chemical shift values, coupling patterns and coupling constants.

Results

A 600 MHz single pulse ^1H NMR spectrum of a typical DUWL specimen is shown in Fig 1. This spectrum contains many prominent, sharp resonances ascribable to a range of low-molecular-mass biomolecules. Indeed, the most intense signals present are those ascribable to microbial-derived organic acid anions, notably acetate, formate, lactate and propionate. Further biomolecules detectable included the amino acid glycine, a number of aromatic compounds and occasionally ethanol. Treatment of DUWLs with the powerful microbicidal agent ozone gave rise to a substantial reduction in many of the microbial catabolites products detectable in samples ($p < 0.01$). Treatment of these samples with ozonated water gave rise to:

Discussion

Multicomponent ^1H NMR investigations of DUWLs provide much valuable information regarding their chemical composition. Indeed, since many of the components detectable in these samples represent chemotaxonomic 'markers' of microorganisms (e.g. acetate, formate and propionate), the technique employed here is likely to serve as a valuable means of determining microbial colonisation of DUWLs, and also as a method for determining the effectiveness of microbicidal agents such as O_3 .

Conclusion

High resolution, high field ^1H NMR spectroscopy is a technique which offers many advantages over alternative time-consuming and labour-intensive analytical methods since (1) it allows for the rapid, non-invasive and simultaneous study of chemical compounds present in complex and multicomponent samples and (2) it generally requires little or no knowledge of sample composition prior to analysis. Furthermore, chemical shift values, coupling pattern and coupling constants of resonances present in ^1H NMR spectra of such multicomponent systems such as DUWL provide much valuable molecular information regarding the molecular nature of chemical species detectable. Treatment of these DUWL samples with O_3 in ozonated water from the TherOzone unit for seven days reduced microbial catabolite levels in DUWLs.

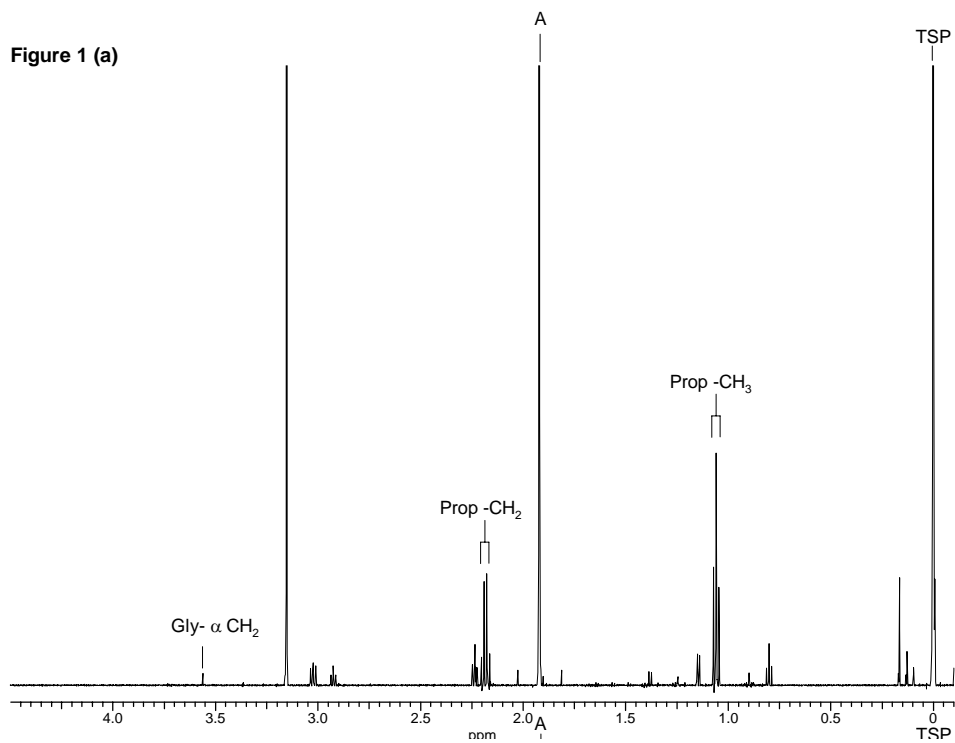


Figure 1 (b)



Figure 1 (a) and (b), High-low-field regions respectively of a typical and (c): Figure displaying a typical partial expansion (a) -0.5 to 4.5 ppm, (b) 5.0 to 8.5 ppm of a high- and low-field regions of a typical 600 MHz single-pulse ^1H NMR spectrum of a typical control (untreated) DUWL sample and (c) specimens respectively. Abbreviations: A, acetate- CH_3 ; Ar, resonances arising from aromatic microbial catabolites (including those ascribable to phenylalanine and tyrosine); Form, formate $-\text{H}$; Gly, glycine- CH_2 ; Prop - CH_2 and $-\text{CH}_3$, propionate- CH_3 and $-\text{CH}_2$.

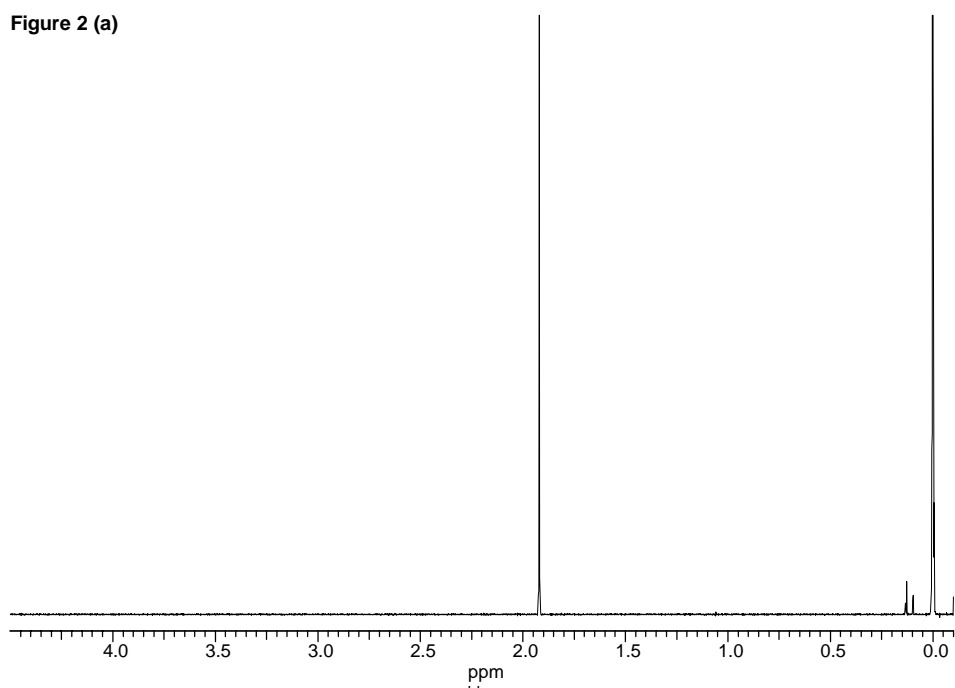


Figure 2 (b)

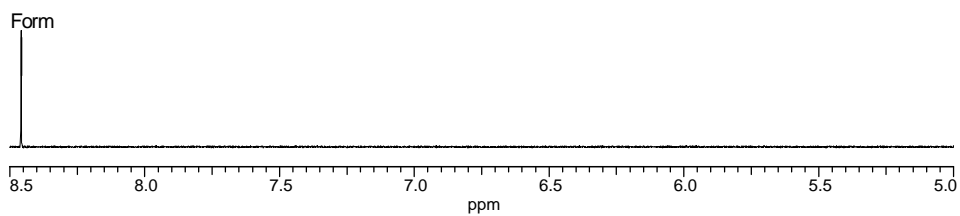


Figure 2 (a) and (b), corresponding high- and low-field regions of a typical DUWL sample collected 7 days following ozonation. Abbreviations: A, acetate-CH₃; Ar, resonances arising from aromatic microbial catabolites (including those ascribable to phenylalanine and tyrosine); Form, formate -H; Gly, glycine-CH₂; Prop -CH₂ and -CH₃, propionate-CH₃ and -CH₂.

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