The Impact of Iodoform on the Hydration, Bioactivity and Antimicrobial Properties of White Portland Cement

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Abstract. Iodoform (CHI_3) is a potential radiopacifying agent for use in Portland cement-based root-filling materials. During this study, the impact of 20 wt% iodoform on the hydration and setting of white Portland cement (WPC) was monitored by powder X-ray diffraction, ^{29}Si magic angle spinning nuclear magnetic resonance spectroscopy and Vicat apparatus. The presence of 20 wt% iodoform reduced the initial and final setting times of WPC from 150 to 121 min and 200 to 165 min, respectively. Iodoform had little impact on the products and extent of hydration after 7 days of curing; although, it did cause a reduction in the mean silicate chain length of the C-S-H gel (from 4.11 to 3.47 units). Both iodoform-blended and unblended cement pastes exhibited similar in vitro bioactivity, with the formation of crystalline hydroxyapatite on their surfaces within 1 day of exposure to simulated body fluid. An inhibition zone assay confirmed that WPC possesses intrinsic antimicrobial activity against *S. aureus*, *P. aeruginosa* and *E. coli*, which is significantly enhanced in the presence of iodoform. This study indicates that iodoform may be a suitable radiopacifying agent for Portland cement-based dental restoratives; although, further work is required to determine its long-term stability within the cement matrix.

1 Introduction

For the past two decades, Mineral trioxide aggregate (MTA), a commercial formulation comprising 80 wt% Portland cement and 20 wt% bismuth oxide (to confer radiopacity), has been used as a root filling material in dentistry [1, 2]. MTA is supplied as 1 g of powder which is manually mixed with 0.35 g of sterile water.

The major components of Portland cement are the impure phases; 'alite' (tricalcium silicate, Ca_3SiO_5), 'belite' (dicalcium silicate, Ca_2SiO_4), 'aluminate' (tricalcium aluminate, $Ca_3Al_2O_6$) and 'ferrite' (tetracalcium aluminoferrite, Ca_2 (Al/Fe) O5). Up to 5% of gypsum ($CaSO_4.2H_2O$) is also ground into the anhydrous cement during production to regulate the setting of the aluminate phase. 'Tooth-colored' MTA is formulated from white Portland cement (WPC) which contains low quantities of iron and manganese that impart the grey colour to ordinary Portland cement (OPC) from which 'Gray' MTA is produced [2].

The aluminate and ferrite phases react with water and gypsum to give rise to needle-like crystals of ettringite ($6CaO.Al_2O_3.3SO_3.32H_2O$) and its Fe-substituted analogue. Alite and belite react with water

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to form a poorly crystalline calcium silicate hydrate gel phase (C-S-H) of approximate formula $Ca_3Si_2O_7.3H_2O$ and portlandite (Ca (OH) $_2$). During hydration, the unpolymerised (Q^0) silicate tetrahedra of alite and belite become hydrated (Q^0 (H)) and then condense together to form dimers (Q^1). Ongoing condensation reactions produce short silicate chains comprising mid-chain (Q^2) species linked to two other silicate tetrahedra, and mid-chain Q^2(1Al) units linked to one silicate and one aluminate tetrahedron.

Iodoform (CHI_3) is among a range of candidate radiopacifying agents for use in Portland cement-based dental restoratives with potentially superior physicochemical, biological and antimicrobial properties to those of bismuth oxide [3-5]. Results of animal and human studies indicate that iodoform is sufficiently biocompatible and radiopaque at 20% addition to Portland cement [4, 5].

The objectives of this study were to investigate the impact of 20 wt% of iodoform on the hydration chemistry, in vitro bioactivity and antimicrobial properties of white Portland cement. Iodoform-blended (WPC-I) and unblended (WPC) cement paste samples were hydrated for 7 days prior to analysis by powder X-ray diffraction analysis (XRD), ^{29}Si magic angle spinning nuclear magnetic resonance spectroscopy (MAS NMR) and Fourier transform infrared spectroscopy (FTIR). The impact of iodoform on the initial and final setting times of the cement pastes was determined by penetration using a Vicat apparatus. The bioactivity of the cement samples was evaluated in vitro by monitoring the formation of hydroxyapatite on their surfaces, by FTIR, after immersion in simulated body fluid. And an inhibition zone assay was used to determine the antimicrobial activity of the cements against *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli*.

2 Materials and methods

2.1 Materials and sample preparation

The white Portland cement used in this study was supplied by Lafarge and is commercially available as 'Snowcrete' [6]. Iodoform, ex. Sigma Aldrich, UK, was used as received. Cement paste specimens (WPC) were prepared in duplicate by manually mixing the cement with distilled water at a water:cement ratio of 0.35 by mass. The samples were packed into polypropylene tubes, hermetically sealed and cured at 37 °C (i.e. body temperature). Samples blended with iodoform (WPC-I) were prepared similarly with partial replacement of the WPC by 20 wt% iodoform (at the same water:cement ratio).

2.2 Initial and final setting times

Initial and final setting times of the cement pastes were determined in triplicate in accordance with ASTM C191-08 using a manual Vicat apparatus [6]. Setting data were collected in triplicate and were subjected to a two-tailed *t*-test at P = 0.05.

2.3 Characterisation

For the purposes of characterisation, the specimens were cured for 7 hours before hydration was quenched by solvent exchange with propan-2-ol. Powder XRD was performed using a Philips D8 diffractometer with Cu Kα = 1.5406 Å, a step-size of 0.019° from 5° to 50° and a measuring time of 141.8 s per step.

MAS NMR spectra were collected on a JEOL JNM-ECX 300 MHz spectrometer. Single pulse ^{29}Si MAS NMR spectra were obtained with a pulse delay of 5 s, an acquisition time of 0.02048 s and 35000 scans. The ^{29}Si chemical shifts were referenced to tetramethylsilane (TMS) and the FIDs were processed by Delta software (provided by JEOL) to obtain spectra which were then analysed using Igor Pro software. The method used to deconvolute and analyse the ^{29}Si MAS NMR spectra is detailed elsewhere [6].

2.4 In vitro bioactivity analysis

For the analysis of bioactivity of WPC and WPC-I, cement pastes were cured for 7 days and immediately exposed to simulated body fluid (SBF) according to the method described by Kokubo and Takadama [7]. In each case, 0.15 g of cement sample were contacted with 150 cm^3 of SBF in hermetically sealed polypropylene containers at 37 °C for 3, 6, 24, 48, 72 and 168 hours. Each analysis was carried out in triplicate. The recovered cement samples were rinsed with deionised water, dried in air at 37 °C for 24 h and analysed by FTIR using a Perkin Elmer Paragon spectrometer.

2.5 Antimicrobial zone of inhibition assay

The antimicrobial properties of WPC and WPC-I were assessed using the inhibition zone method against *Staphylococcus aureus* NCIMB 9518, *Pseudomonas aeruginosa* NCIMB 8628 and *Escherichia coli* NCIMB 9132. Overnight cultures of each bacterium were spread on nutrient agar plates. Individual discs (1 cm diameter x 1 mm thickness) of cement were placed in the centre of each spread plate. Each assay was conducted in quadruplicate. The plates were examined for clear zones after incubation at 37 °C for 24 hours. The final population densities of the plates spread with *S. aureus*, *P. aeruginosa* and *E. coli* were approximately 2.4 x 10^8, 5.3 x 10^8, and 3.7 x 10^7 colony forming units per plate. The data were subjected to a one-tailed *t*-test at (n - 2) degrees of freedom and P = 0.05.

3 Results and discussion

3.1 Initial and final setting times

The initial and final setting times for samples WPC and WPC-I were obtained using a manual Vicat apparatus in accordance with ASTM C191-08, and are listed in Table 1. The presence of 20 wt% iodoform was found to reduce both the initial and final setting times of WPC by 29 min and 35 min, respectively.

Table 1. Initial and final setting times for WPC and WPC-I.

Cement	WPC	WPC-I
Initial set (min)	150 ± 15	121 ± 3
Final set (min)	200 ± 17	165 ± 5

3.2 Characterisation

Powder XRD data for WPC and WPC-I are presented in Fig. 1. The formation of ettringite and portlandite is noted in the diffraction patterns of both cement pastes, along with the residual unreacted parent cement phases, alite and belite [8]. The reflections of iodoform are seen to persist in the diffraction pattern of the bended paste up to 7 days, and there is no evidence for the formation of any other anomalous phases arising from the presence of iodoform within this cement system.

The subtracted, deconvoluted and fitted ^{29}Si MAS NMR spectra, together with the residue of the subtracted and fitted spectra, for the WPC and WPC-I pastes are shown in Fig. 2. These spectra comprise a sharp peak at -72 ppm which is assigned to the residual unreacted Q^0 species in belite, and hydrated Q^0(H), Q^1, Q^2(1Al) and Q^2 species of C-S-H gel in relative abundancies that are typical of white Portland cement that has hydrated for 7 days under similar conditions [6, 9]. The position and number of signals in the spectra of WPC are the same as those of WPC-I, and the extents of hydration of both cements (~60%) do not differ significantly (Fig. 2). This suggests that iodoform does not directly participate in the hydration reactions of white Portland cement; however, the decrease in

mean silicate chain length indicates that it may exert an influence on the assembly of the C-S-H gel phase.

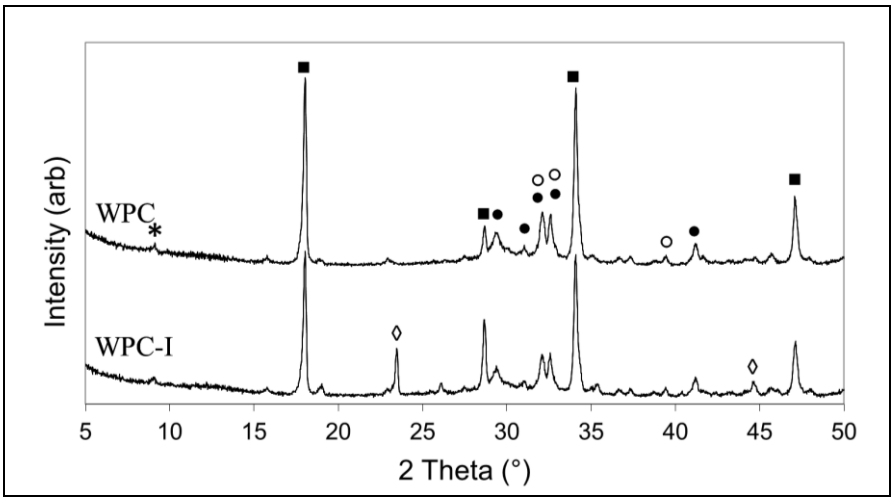
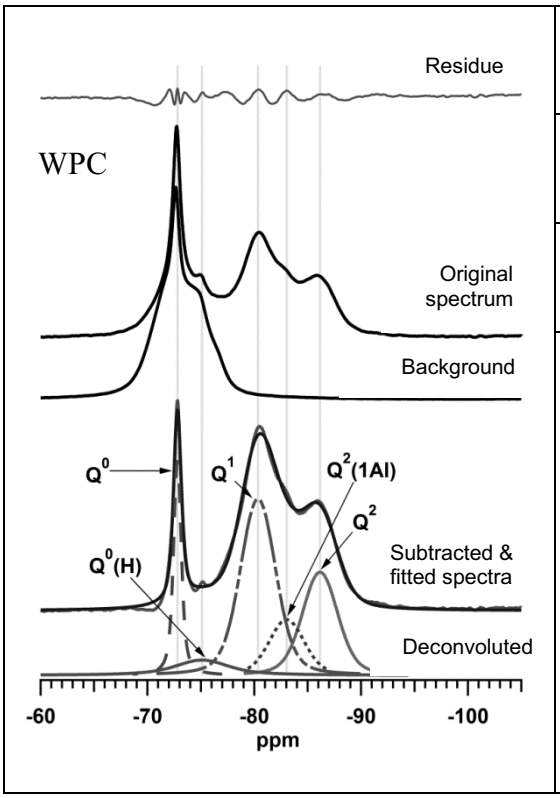


Figure 1. XRD data for WPC and WPC-I. (Key: ● alite; ○ belite; * ettringite; ■ portlandite; ◊ iodoform).



Sample	WPC	WPC-I
Extent of hydration	60%	61%
Mean silicate chain length	4.11	3.47

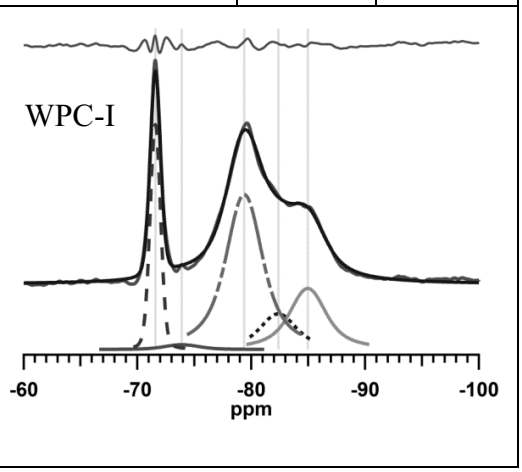


Figure 2. ^{29}Si MAS NMR data for WPC and WPC-I.

3.3 In vitro bioactivity analysis

The in vitro formation of a layer of bone-like substituted hydroxyapatite (HA), $Ca_{10}(PO_4)_6(OH)_2$, on the surface of a material placed in SBF solution provides an indication of its bioactivity (i.e. the ability of the material to bond with living bone tissue and to stimulate its regeneration) [7]. The FTIR spectra of WPC and WPC-I, as functions of residence time in SBF, are presented in Fig. 3. Si–O stretching modes of the C-S-H gel give rise to the broad band at ~970 cm^{-1} with contributions at 870 and 1120 cm^{-1} from carbonate and sulphate groups, respectively [8]. The unresolved doublet at 1420 and 1490 cm^{-1} also arises from carbonate species, and the signals at 1660 and 3470 cm^{-1} are attributed to O–H vibrations of water and hydroxyl groups. Stretching modes of O–H in portlandite are assigned to the sharp signal at 3670 cm^{-1}. Weak sharp bands arising from iodoform are noted to contribute to the spectrum of WPC-I at 2975, 1263 and 868 cm^{-1}.

The broad signal at 600 cm^{-1} in the FTIR spectra of the cements exposed to SBF for 1 hour, is indicative of the rapid deposition of a layer of amorphous hydroxyapatite on their surfaces (Fig. 3) [8]. The increase in relative intensity and resolution of this band into a doublet signify the ongoing development and crystallisation of the hydroxyapatite layer as a function of residence time in SBF. The concurrent disappearance of the sharp O–H signal at 3670 cm^{-1} in the FTIR spectra of both cements after 1 hour in SBF, is attributed to the dissolution of portlandite. These results confirm that the in vitro bioactivities of WPC and WPC-I are similar and that the incorporation of iodoform into the cement mixture does not adversely affect this clinically important property of the material.

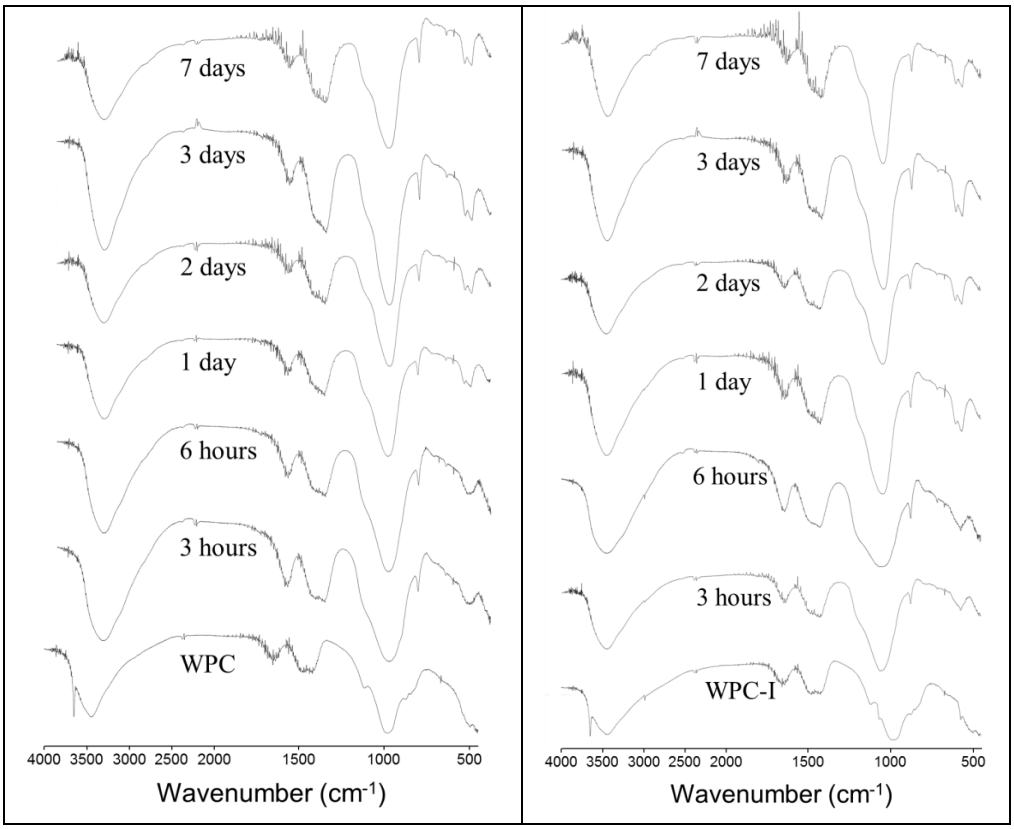


Figure 3. FTIR spectra of WPC and WPC-I as functions of residence time in SBF.

3.4 Antimicrobial zone of inhibition assay

The results of the antibacterial inhibition zone assays using *S. aureus*, *P. aeruginosa* and *E. coli* are listed in Table 2. Clear zones around each of the WPC discs were noted in all cases indicating that this

material is intrinsically antimicrobial against all bacterial strains. There were found to be significant increases in the size of the inhibition zones around the WCP-I discs for all bacteria (P = 0.05), indicating that the incorporation of iodoform in the cement paste enhances its antimicrobial properties with respect to both Gram-positive (*S. aureus*) and Gram-negative (*P. aeruginosa*, *E. coli*) bacteria.

Table 2. Inhibition zone data for WPC and WPC-I.

Bacterium	Diameter of zone of inhibition (cm)	
	WPC	**WPC-I**
S. aureus	16.8 ± 1.5	18.0 ± 0.1
P. aeruginosa	14.5± 0.9	16.8 ± 1.5
E. coli	18.8 ± 1.0	20 ± 0.1

3.5 The role of iodoform as a radiopacifier in Portland cements

Iodoform is among the candidate radiopacifying agents that are currently considered for incorporation in Portland cements for dental applications [3-5]. The present study has demonstrated that 20 wt% replacement of iodoform for white Portland cement reduces both initial and final setting times. The ability of a radiopacifying agent to reduce setting time is a distinct benefit, as a common complaint regarding MTA is that its setting time is inconveniently long [10]. Bismuth oxide, the proprietary radiopacifier in MTA, is known to prolong both the initial and final setting times [11], so in this respect, iodoform would be an advantageous alternative.

This study has also confirmed that iodoform does not directly participate in or divert the normal hydration reaction of the cement; however, the mean silicate chain length of the C-S-H gel was reduced in the presence of iodoform, indicating that it may play a structure-directing role in the cement system. Further work to explore this phenomenon, and also to investigate the impact of iodoform on the evolution of the calcium aluminate hydrate phases is now warranted. Since the long-term chemical stability of iodoform in the highly alkaline environment of the cement matrix has not yet been established, further investigations of this nature are also recommended.

Additionally, the incorporation of iodoform into the cement system was not found to have an adverse impact on bioactivity in vitro. A layer of bone-like HA precipitates on the surface of an implanted bioactive material in vivo (from the components of human plasma) which provides a focus for bone-forming cells to adhere and proliferate. This is an acknowledged advantage of Portland-cement based biomaterials that exhibit the ability to stimulate the formation of new cementum, pulp and bone [3]. Animal and human studies have indicated that 20:80 mixtures of iodoform and Portland cement exhibit similar biocompatibility to that of commercial MTA [4, 5]. Hence, from a biological perspective, iodoform has the potential to enhance the antimicrobial properties of the cement without compromising its bioactivity and biocompatibility.

4 Conclusions

This study considers the impact of 20 wt% iodoform, a radiopacifying agent, on the hydration and setting of white Portland cement (WPC) by powder X-ray diffraction analysis, ^{29}Si magic angle spinning nuclear magnetic resonance spectroscopy, Fourier transform infrared spectroscopy and penetration by Vicat apparatus. The presence of iodoform was found to reduce the initial and final setting times of WPC by 29 min and 35 min, respectively. Iodoform had no significant impact on the products and extent of hydration after 7 days; although, it did cause a reduction in the mean silicate chain length of the C-S-H gel (from 4.11 to 3.47 units). Both iodoform-blended and unblended cement pastes exhibited similar in vitro bioactivity, with the formation of crystalline hydroxyapatite on their surfaces within 1 day of exposure to simulated body fluid. An inhibition zone assay confirmed that WPC possesses intrinsic antimicrobial activity against *S. aureus*, *P. aeruginosa* and *E. coli*, which is

significantly enhanced in the presence of iodoform. This study confirms that iodoform may be a suitable radiopacifying agent for Portland cement-based dental restoratives; although, further work is required to determine its long-term stability within the cement matrix.

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