

# Influence of Radiopacifying Agents on the Solubility, pH and Antimicrobial Activity of Portland Cement

Paulo Henrique WECKWERTH<sup>1</sup>  
Adriano Cosme de Oliveira MACHADO<sup>1</sup>  
Milton Carlos KUGA<sup>2</sup>  
Rodrigo Ricci VIVAN<sup>1</sup>  
Raquel da Silva POLLETO<sup>3</sup>  
Marco Antonio Hungaro DUARTE<sup>3</sup>

<sup>1</sup>Center of Health Sciences, USC - Sagrado Coração University, Bauru, SP, Brazil

<sup>2</sup>Department of Restorative Dentistry, Araraquara Dental School,  
UNESP - Univ Estadual Paulista, Araraquara, SP, Brazil

<sup>3</sup>Department of Dentistry, Endodontics and Dental Materials, Bauru Dental School,  
USP - University of São Paulo, Bauru, SP, Brazil

The aim of this study was to evaluate the interference of the radiopacifiers bismuth oxide (BO), bismuth carbonate (BC), bismuth subnitrate (BS), and zirconium oxide (ZO) on the solubility, alkalinity and antimicrobial properties of white Portland cement (WPC). The substances were incorporated to PC, at a ratio of 1:4 (v/v) and subjected to a solubility test. To evaluate the pH, the cements were inserted into retrograde cavities prepared in simulated acrylic teeth and immediately immersed in deionized water. The pH of the solution was measured at 3, 24, 72 and 168 h. The antimicrobial activity was evaluated by a radial diffusion method against the microorganisms *S. aureus* (ATCC 25923), *P. aeruginosa* (ATCC 27853), *E. faecalis* (ATCC 29212) and *C. albicans* (ATCC 10231). The zone of microbial growth inhibition was measured after 24 h. The addition of BS and BC increased the solubility of the cement. The pH values demonstrated that all materials produced alkaline levels. At 3 h, BS showed lower pH than WPC ( $p < 0.05$ ). At 168 h, all materials showed similar pHs ( $p > 0.05$ ). The materials did not present antimicrobial activity for *S. aureus*, *P. aeruginosa* and *E. faecalis* ( $p > 0.05$ ). With regards to *C. albicans*, all materials formed an inhibition zone, mainly the mixture of WPC with ZO ( $p < 0.05$ ). The type of radiopacifier incorporated into WPC interfered with its physical and antimicrobial properties. ZO was found to be a viable radiopacifier that can be used with WPC.

Key words: antimicrobial activity, Portland cement, radiopacifier, pH.

## INTRODUCTION

The use of MTA, based on scientific evidence and clinical studies, is directed to the treatment of accidents and endodontic complications (1,2). The biocompatibility (3) and chemical composition (4,5) of Portland cement (PC) are similar to those of MTA, except for the absence of bismuth. It has been proposed as an alternative material to retrograde root canal filling (6,7), in substitution for MTA, since its physical properties are also comparable (8).

However, PC is less radiopaque than MTA (8), limiting its radiographic visualization after insertion

in the apical cavity. In order to solve this problem, radiopacifier agents that are usually used in dental materials have been proposed to be aggregated into PC, such as bismuth oxide (BO) (9), bismuth carbonate (BC) (10), bismuth subnitrate (BS) (10) and zirconium oxide (ZO) (11). All the combinations provide greater dentin radiopacity (11) in accordance with the minimum values required by the American National Standards Institute and American Dental Association (ANSI/ADA), specification No. 57 (12).

When evaluated by the radial diffusion method, PC did not present antimicrobial activity against *Enterococcus faecalis* (13,14), *Staphylococcus aureus*,

*Escherichia coli* and *Candida albicans* (13,15).

Independent of PC type, bacterial infiltration occurred after 15 days of exposure to microorganisms. Therefore, the mean period of infiltration is approximately 45 days when the PC is in its structural form (16). Meanwhile, in current studies (14,15) of antimicrobial activity, no additives or radiopacifiers were incorporated into white Portland cement (WPC).

The combination of 20% radiopacifier and 80% WPC (11) affects the final quantity of cement present in the mixture. Although the additions of BO, BC, BS and ZO provide better radiopacity to PC (9-11), their effects on the solubility, alkalization ability and antimicrobial activity in WPC is still unknown.

The addition of BO affects the dimensional stability, fluid absorption and solubility of MTA (17). The high level of water absorption (18), compromises its physicochemical properties (17). This brings up the question whether this is the ideal radiopacifier to be added to WPC (11), requiring the evaluation of other radiopacifiers that might not cause alterations in the solubility of the cement, making it recommendable for periapical surgery.

The pH of WPC with 20% BO has been shown to be similar to that of white MTA Angelus<sup>®</sup>, maintaining the alkaline level after 168 h evaluation (19). However, the influence of other radiopacifiers in the alkalization capacity of WPC is unknown. ZO is a component of the epoxy-based cement AH Plus (Dentsply De Trey), which has a pH of approximately 7. However, the influence of this radiopacifier in the pH of WPC has not yet been established.

The antimicrobial activity of WPC is well known (13-16). However, the incorporation of 20% of a radiopacifier to WPC would result in a final mixture with significant alterations in its composition, possibly causing changes in the antimicrobial potential, mainly against bacteria, such as *E. faecalis*, and the fungus *C. albicans*.

Given the need of obtaining an ideal radiopacifier for WPC, the aim of the present study was to verify the interference of BO, BC, BS and ZO incorporated into WPC in a 1:4 (v/v) ratio on cement solubility, pH at different periods and antimicrobial activity against 3 species of bacteria and 1 species of fungus.

## MATERIAL AND METHODS

The following radiopacifiers were used:

bismuth oxide (BO) (Synth, Diadema, SP, Brazil), bismuth carbonate (BC) (Synth), bismuth subnitrate (BS) (Synth) and zirconium oxide (ZO) (JB Química, Suzano, SP, Brazil). The used cement was WPC (Irajazinho; Cimentos Rio Branco AS, Rio de Janeiro, RJ, Brazil). In all experiments, the proportion in volume of radiopacifier to WPC was in the ratio of 1 part (20%) of radiopacifier to 4 parts (80%) of WPC. To establish a workable material for dental applications, 1.0 g of powdered aggregate was incorporated to 0.3 mL of distilled water. As a control, the powdered WPC without radiopacifiers was also used with distilled water.

### Solubility Test

The cement specimens were molded using Teflon ring molds 20 mm in diameter and 1.5 mm high. Three specimens were fabricated for each material. The mould was supported by a larger glass plate and covered with a cellophane sheet. An impermeable nylon thread was placed inside the material and another glass plate, also covered with cellophane film, was positioned on the mold and pressed manually in such a way that the plates touched the entire mold in a uniform manner. The assembly was placed in an incubator (37°C, 95% relative humidity) for a period corresponding to three times the setting time. Immediately after being removed from the mold, the specimens were left in a desiccator for 6 h and were then weighed three times each with a degree of accuracy of 0.0001 g (Sartorius scale model 1662; Sartorius, Gottingen, Germany), the mean reading being recorded. The specimens were suspended by the nylon thread and placed two-by-two inside plastic containers with a wide opening containing 50 mL of deionized water, with care to avoid any contact between them and the inner surface of the containers. The sealed containers were stored for 24 h in an incubator (37°C, 95% relative humidity). After this period, the specimens were removed from the containers, rinsed with deionized distilled water, blotted dry with absorbent paper, placed in a desiccator for 24 h and then reweighed. The weight loss of each specimen (initial mass minus final mass) expressed as the percentage of the original weight, was taken as the solubility of the cement. According to the ANSI/ADA No. 57 specification (21) guideline, a root canal sealer should not have solubility greater than 3%.

Data resulting from the tests were analyzed statistically by ANOVA and Tukey's test at a significance level of 5%.

### pH Analysis

Fifty acrylic teeth with simulated root end cavities were used for the pH test. The teeth were divided into 5 groups (n=10), according to the tested material. The root-end cavities were filled with freshly prepared materials and the teeth were immediately sealed in glass flasks containing 15 mL of deionized water (Milli-Q water; Purelab Option DV25; Millipore Corp., Bellerica, MA, USA), and stored at 37°C. After 3 h of immersion, the teeth were carefully removed and placed into new flasks with an equal amount of new deionized water, and this procedure was repeated after all predetermined periods: 24, 72 and 168 h. After removal of the specimens, the glass flasks were vortexed for 5 s (Vortex Q-220A; Quimis Ap. Científicos Ltda., São Paulo, SP, Brazil) and the pH level of the solutions was analyzed using a pH meter (Quimis Ap. Científicos Ltda.) calibrated with a buffer solution (pH 4.0, 7.0 and 12.0) at a constant temperature (25°C). Data were compared by the ANOVA and Tukey's test at a significance level of 5%.

### Antimicrobial Activity

The technique used was radial diffusion, by evaluating the zone of microbial growth inhibition on Petri dishes containing Brain Heart Infusion (BHI) agar (Merck KGaA, Darmstadt, Germany) for the bacteria and Sabouraud dextrose agar (Merck KGaA) for the fungi. The bacteria used in the study were *E. faecalis* (ATCC 29212), *S. aureus* (ATCC 25923) and *Pseudomonas aeruginosa* (ATCC 27853) and the fungus was *C. albicans* (ATCC 10231).

After reactivation of the microorganisms and adjusting the optical density to match 0.5 on the McFarland scale ( $1.5 \times 10^8$  CFU mL<sup>-1</sup>), 20 excavated (6 mm diameter x 3 mm depth) Petri dishes (5 per microorganism) were inoculated and incubated at 37°C for additional 30 min. WPC and the 4 experimental cements containing the radiopacifiers (BO, BC, BS and ZO) were taken individually to each excavation with a Luer syringe. After 2 h of pre-incubation at room temperature, the cultures were maintained in an oven at 37°C for 24 h.

After this period, the zone of microbial growth inhibition was measured with a digital caliper accurate to the nearest 0.01 mm (Mitutoyo Corporation, Tokyo, Japan). The experiment was performed five times for each species of microorganism. Cellulose disks

moistened with 2% chlorhexidine digluconate (CHX) or sterile saline solution (SS) were placed on the culture medium, being the positive and negative control, respectively. The inhibition zone measurement of the antimicrobial activity provided by the 2% CHX was 13.0 mm and 0.0 mm for the SS.

The results obtained with the cements were submitted to ANOVA and Tukey's testing at a significance level of 5%.

## RESULTS

Table 1 contains the mean and standard deviation of the solubility of WPC and the experimental cements. Only BS and BC increased significantly the solubility of WPC ( $p < 0.05$ ). The solubility of WPC may increase according to the type of radiopacifier used, with the exception of ZO.

After the immersion of the specimens and evaluation of the pH level of ultrapure water during the determined time periods, it was observed that the radiopacifiers interfered with the pH of WPC. Table 2 contains the mean and standard deviation of the pH level values shown in the cements. Within 3 h, only WPC/BS showed a lower pH, and significant differences ( $p < 0.05$ ) in WPC. Inversely, within 24 h, the WPC/BS showed a higher pH level, significantly different ( $p < 0.05$ ) from WPC/ZO. At the 72 h analysis period, the highest pH occurred for WPC/BS, followed by WPC/BO and WPC, showing significant differences ( $p < 0.05$ ) in WPC/ZO and WP/BC. Meanwhile, after 168 h of evaluation the cements displayed similar ( $p > 0.05$ ) pH levels.

Table 3 demonstrates the mean and standard deviation of the zone of microbial growth inhibition

Table 1. Means (in mg) and standard deviations of the solubility of the tested cements.

Cement	Solubility
WPC	0.91 (0.12) <sup>a</sup>
WPC/BO	1.16 (0.02) <sup>a</sup>
WPC/BC	5.29 (0.16) <sup>b</sup>
WPC/BS	8.30 (1.14) <sup>c</sup>
WPC/ZO	0.58 (0.33) <sup>a</sup>

WPC: White Portland cement. BO: Bismuth oxide. BC: Bismuth carbonate. BS: Bismuth subnitrate. ZO: Zirconium oxide. Different letters in columns indicate statistically significant difference ( $p < 0.05$ ).

produced by the cements. The means of the zone of microbial growth inhibition shown by the 2% CHX was 13.0 mm. There was no inhibition activity in the saline solution. In the evaluation of the antimicrobial activity by the radial diffusion method, none of the cements inhibited the microbial growth of *E. faecalis*, *S. aureus* and *P. aeruginosa* ( $p > 0.05$ ). On the other hand, *C. albicans* was inhibited by all cements, with WPC/ZO providing the largest zone of microbial inhibition ( $p < 0.05$ ).

## DISCUSSION

The addition of 20% radiopacifiers interfered with the physical and microbiological properties of WPC. BC and BS increased the solubility of WPC. The alkalization ability undergoes interference, depending on the analysis period and the type of radiopacifier. BO did not interfere with the alkalization ability of the WPC during all the analyzed periods. BS reduced the pH already in the first 3 h. BC and ZO reduced the pH of WPC within 72 h. However, the pH of all cements was similar to that of WPC within 168 h (7 days) of the evaluation. The cements did not demonstrate antimicrobial activity against the analyzed microorganisms, except for *C. albicans*, mainly WPC/ZO.

In the solubility test, the combination of WPC with BC and BS did not comply with the ADA's standards (12), which stipulate a maximum of 3% mass difference. BO is the radiopacifier used in MTA (1). When incorporated into WPC, an alteration occurs in the

hydration process (18) and causes structural failure (11).

During the setting time of PC, an expansion of the material occurs due to the hydration process (8,18). There are space formations within the cement matrix, which originate from the absorption of water (1,18). With the evolution of the process, initially these particles tend to leave empty spaces in the structure and eventually form components. In the next phase, the hydration process causes an increase of solid volume, reducing the porosity and the final mass when compared initially (2,18). In this study, the solubility test of cements was recorded only after the final setting, discounting the interference of this process the along the way.

BC and BS are excipients used in oral medications. Only the cements incorporated with these radiopacifiers displayed greater solubility than WPC, similar to bismuth increasing the absorption of water in WPC (18) and in the solubility test, dehydration occurred with the specimens to obtain the final weight (12), and the mass reduction was always evident.

Probably because of the natural reaction of hydration, the presence of BC and BS interfered with the structure and final weight of the WPC. ZO is one of the components of the endodontic cement AHPlus (Dentsply DeTrey GmbH, Konstanz, Germany), and displayed the least interference with the solubility of WPC.

Differing from the solubility test, the evaluation of the alkalization capacity (pH) of the cements started immediately after mixing, which made impossible establishing a correlation between the tests. In the pH

Table 2. Means and standard deviations of the pH of the cements at the different evaluation times.

Cement	3 h	24 h	72 h	168 h
WPC	13.54 (0.98) <sup>a</sup>	8.85 (0.93) <sup>ab</sup>	8.63 (0.84) <sup>a</sup>	8.14 (0.88) <sup>a</sup>
WPC/BO	11.45 (1.13) <sup>ab</sup>	8.99 (0.94) <sup>ab</sup>	8.78 (0.69) <sup>a</sup>	7.93 (0.67) <sup>a</sup>
WPC/BC	11.32 (1.39) <sup>ab</sup>	8.98 (0.78) <sup>ab</sup>	7.68 (0.26) <sup>b</sup>	7.94 (0.66) <sup>a</sup>
WPC/BS	10.59 (1.34) <sup>b</sup>	9.26 (1.10) <sup>b</sup>	9.26 (0.77) <sup>a</sup>	8.32 (0.76) <sup>a</sup>
WPC/ZO	11.24 (1.48) <sup>ab</sup>	7.91 (0.69) <sup>a</sup>	7.61 (0.18) <sup>b</sup>	7.62 (0.31) <sup>a</sup>

WPC: White Portland cement. BO: Bismuth oxide. BC: Bismuth carbonate. BS: Bismuth subnitrate. ZO: zirconium oxide. Different letters in columns indicate statistically significant difference ( $p < 0.05$ ).

Table 3. Means and standard deviation (in mm) of the zones of microbial growth inhibition for the tested cements.

Cement	<i>S. aureus</i>	<i>E. faecalis</i>	<i>P. aeruginosa</i>	<i>C. albicans</i>
WPC	0 <sup>a</sup>	0 <sup>a</sup>	0 <sup>a</sup>	6.75 (0.50) <sup>a</sup>
WPC/BO	0 <sup>a</sup>	0 <sup>a</sup>	0 <sup>a</sup>	7.50 (0.57) <sup>a</sup>
WPC/BC	0 <sup>a</sup>	0 <sup>a</sup>	0 <sup>a</sup>	7.50 (0.57) <sup>a</sup>
WPC/BS	0 <sup>a</sup>	0 <sup>a</sup>	0 <sup>a</sup>	7.00 (0.00) <sup>a</sup>
WPC/ZO	0 <sup>a</sup>	0 <sup>a</sup>	0 <sup>a</sup>	9.00 (0.81) <sup>b</sup>

WPC: White Portland cement. BO: Bismuth oxide. BC: Bismuth carbonate. BS: Bismuth subnitrate. ZO: zirconium oxide. Different letters in columns indicate statistically significant difference ( $p < 0.05$ ).

analysis, for all hydration processes, expansion and eventual migration of byproducts to the surface of the cements should be considered (2). Inversely, the solubility test showed that the specimens had already concluded the setting.

The higher solubility observed for BS and BC can be related to the negative interference of these substances with the hydration of WPC, increasing the porosity of the material and consequently its solubility.

Although BC and BS showed a higher solubility, this property did not implicate in an increase of the pH level after 72 h. Perhaps the formation of calcium hydroxide and the release of hydroxyl ions does not occur during the solubility stage, but a release of other components such as bismuth does.

Independent of the evaluation period, the cements promoted alkalization of the ultrapure water, reducing the pH during the experiment. The pH of the ultrapure water where WPC and WPC/BO were immersed always maintained alkaline levels, showing a marked reduction after 24 h of analysis. Similar results were also observed in other studies (18,19). Camilleri (18) found lower pH values for WPC/BO in the first 24 h, whereby the evaluation was done directly on cement surface. This possibly resulted when the calcium hydroxide formed during the process of hydration reacted with the carbon dioxide in the air, forming calcium carbonate.

Some differences of pH were observed among the experimental cements during the evaluation times, but after 168 h (7 days) all values were similar, agreeing with the results when compared with WMTA Angelus® and WPC/BO (19). In this study, the ultrapure water was replaced for each experimental period, avoiding saturation in the middle. Due to the hydration reaction in the setting of WPC, a portion of the formed calcium hydroxide could have dissolved in the liquids. This replacement of new ultrapure water was favored exclusively in the evaluation of the alkalization ability of the cement within the determined analyzed period. This justified the similar values of pH after 168 h, probably because the formation of calcium hydroxide is reduced after the final setting of the cement (19).

The evaluation method of antimicrobial activity was the radial diffusion in agar. This methodology is used in the literature as one of the initial tests to verify antimicrobial activity of the material against certain microorganisms (13,15). *E. faecalis* and *C. albicans* are frequently associated with endodontic failures and used as a parameter for material comparisons (13,16).

The antimicrobial activity of WPC and the experimental cements did not occur in relation to all types of bacteria used in the present study, agreeing with the results of other authors that tested PC against *S. aureus*, *E. faecalis* and *E. coli* (13). On the other hand, the cements displayed antimicrobial activity against *C. albicans*, providing zones of microbial growth inhibition varying from 6.75 mm to 9.0 mm, smaller than the zone produced by the positive control (2% CHX). ZO provided a greater microbial inhibition zone when compared with the other cements ( $p < 0.05$ ).

Estrela et al. (15) did not observe inhibition zones of microbial growth when testing gray PC. However, considering other studies on the antifungal activity of PC (13,15,16), only the present study evaluated the effectiveness of WPC added with diverse radiopacifiers. The difference of the results of this study and other studies (13,16) in relation to the efficacy of WPC against *C. albicans*, is probably due to the microorganism used in the study. While this study used *C. albicans* ATCC 10231, the other studies (13,16) used *C. albicans* ICB/ USP-562, which can be more resistant to WPC.

The use of MTA in Endodontics is well established (2), with favorable results and prognostics (1). The chemical similarity of MTA with WPC is indisputable (4), presenting similar biological results (3). Meanwhile, its radiopacity is lower (11) than that recommended by the American Dental Association (ADA) No. 57 specification (12). The addition of a radiopacifier, such as BO, is recommended (18), regardless of occasional negative interferences in the cement's physical properties (17,18). In this proposal, BS, BC and ZO, in spite of being poorly studied radiopacifiers, can be considered as an alternative (11).

According to its low solubility, final alkalization potential and antimicrobial activity similar to WPC, makes ZO a promising radiopacifier agent to be used as an aggregate for WPC, at the proportion of 20% volume in WPC. The reduction of the pH of WPC with ZO after 24 h is related to the lower solubility of this association. Possibly the zirconium oxide participates effectively in the hydration of PC, favoring a more homogeneous cement with less porosities. Other studies must be conducted with this combination to finally verify cement expansion, intensity of water absorption, material resistance and biological compatibility.

In summary, WPC represents an alternative material to be used as a retrograde filling material. The addition of 20% ZO in volume to WPC resulted in less

solubility when compared with BC and BS, similar to pure WPC. The alkalization capacity was similar to that of WPC, and therefore the antimicrobial activity against *C. albicans* was superior to that of the other cements. However, with regard to the methodologies used in the present study and according to the interpretation of the obtained results, it may be concluded that the radiopacifiers BS and BC had a negative influence on WPC, while ZO did not affect the results of WPC, appearing as a promising radiopacifier to be investigated.

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Received May 22, 2012  
Accepted October 31, 2012