

Negligible Expression of Arsenic in Some Commercially Available Brands of Portland Cement and Mineral Trioxide Aggregate

Gustavo De-Deus, MD, MSc, PhD,^{*†} Maria Claudia Brandão de Souza, MS,^{*}
Rivail Antonio Sergio Fidel, PhD,^{*} Sandra Rivera Fidel, PhD,^{*}
Reinaldo Calixto de Campos, PhD,[‡] and Aderval S. Luna, PhD[§]

Abstract

Introduction: This study was designed aiming to determine and compare the amount of arsenic in some brands of mineral trioxide aggregate (MTA) and Portland cement. **Methods:** In the present study, arsenic species (As[III], As[V], and dimethylarsinic acid) were separated by high-performance liquid chromatography (HPLC) using a strong anion exchange column and converted into arsines by online HG. The instrumental coupling, HPLC-HG-AFS, was applied to 0.2 g of each cement that was prior digested in a solution of HCl, HNO₃, and HBF₄. Data were expressed as a part per million, and the preliminary analysis of the raw pooled data revealed a bell-shaped distribution. Statistical analysis was performed using one-way analysis of variance for multiple comparisons. **Results:** In all chromatograms obtained, only type III arsenic could be detected. The minimum amount of arsenic was detected in samples of white MTA ProRoot (3.3×10^{-4}) and the maximum in the samples MTA Bio Angelus (Angelus, Londrina, PR, Brazil) (8.6×10^{-4}). In the Gray MTA (Angelus), gray ProRoot MTA (Tulsa/Dentsply, Tulsa, OK) and CP Juntalider (Brasilatex Ltda, Diadema, SP, Brazil) did not detect any trace of arsenic. The values of arsenic found in CP Irajazinho (Votorantim Cimentos, Rio Branco, SP, Brazil) and white MTA Angelus were intermediaries to minimum and maximum values. The nonparametric test Kruskal-Wallis showed statistically similar results among all cements tested ($p > 0.5$). **Conclusions:** Overall, the present study showed that all cements showed insignificant amounts of type III arsenic as well as no trace of arsenic DMA and type V could be detected. (*J Endod* 2009;35:887–890)

Key Words

Arsenic, MTA, biomaterials, Portland cement

Mineral trioxide aggregate (MTA) has an almost 15-year history of clinical and experimental successes with an ample variety of applications (1, 2). The idea that MTA is Portland cement plus bismuth oxide (3, 4) has been generating a significant body of research in order to evaluate Portland cement as a low-cost alternative to MTA (5–9). Spångberg (10) was wise in summarizing this background: “The fact responsible to exacerbate this issue is the unreasonably high price of ProRoot MTA in relationship to the inexpensive raw material for manufacturing Portland cement...ProRoot MTA (Tulsa/DENTSPLY, Tulsa, OK) is a new material but for practical purposes not much different from Portland cement. The factors responsible for the beneficial effects in ProRoot are also found in Portland cement.” This statement is supported by several studies that have shown comparable physical and biological properties (11–14).

One important point in the development of a low-cost Portland cement for clinical usage is the concern of the amount of arsenic present in the material (15). High concentrations of arsenic have been found in the cement dust, an alkaline byproduct of cement manufacturing. With the aim of avoiding the presence of arsenic and lead, an experimental, water-based cement, labeled as MTA Bio, has been developed by a Brazilian dental company (Angelus, Londrina, PR, Brazil). MTA Bio is fully synthesized in a laboratory under controlled, clean, and segregated conditions to ensure that the final product is free of any undesirable contamination (16).

Therefore, the present study was designed to determine and compare the release of arsenic in some brands of commercially available Portland and MTA cements. The tested hypotheses were (1) that there is a difference in the arsenic release among the Portland cements and MTAs and (2) that the level of arsenic release from all hydraulic cements tested is below those considered to be hazardous.

Material and Methods

Experimental Design

In the present study, arsenic species (As[III], As[V], and dimethylarsinic acid [DM]) were separated by high-performance liquid chromatography (HPLC) using a strong anion exchange column and converted into arsines by online HG. The instrumental coupling, HPLC–HG–atomic fluorescence spectrometry (AFS), was applied to the following cements: white Portland cement (Irajazinho; Votorantim Cimentos, Rio Branco, SP, Brazil; and Juntalider; Brasilatex Ltda, Diadema, SP, Brazil); white, gray, and MTA Bio (Angelus); and white and gray MTA ProRoot (Tulsa/Dentsply).

From the ^{*}Department of Endodontics, Rio de Janeiro State University, Rio de Janeiro, Brazil; [†]Veiga de Almeida University, Rio de Janeiro, Brazil; [‡]Department of Chemistry, Pontifical Catholic University, Rio de Janeiro, Brazil; and [§]Department of Analytical Chemistry, Rio de Janeiro State University, Brazil.

Address requests for reprints to Dr Gustavo André de Deus, Carneiro Vianna, Rua Desembargador Renato Tavares, 11 ap. 102, Ipanema, Rio de Janeiro, Brazil 22411-060. E-mail address: endogus@gmail.com.

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Sample Preparation

To ensure that homogeneous samples were used, standard sampling techniques were applied. Eight samples of each material were triturated, homogenized, and dried overnight at 110°C and then left to cool in desiccators. An aliquot of approximately 200 mg of the sample was weighed directly in the PTFE flask of the microwave oven. The sample aliquot was digested with 1.0 mL of a mixture 1:1 of hydrochloric and nitric acids plus 5.0 mL of tetrafluoroboric acid in the microwave flask. The samples were then submitted to a 3-step microwave oven temperature program (8 minutes at 400 W, 15 minutes at 790 W, and 10 minutes at 950 W). After the digestion was finished, all solutions were colorless, indicating an effective digestion. After cooling the solution, the final volume of 50 mL was made up with deionized water.

Control of Contamination

To avoid possible contamination while handling the samples, disposable plastic gloves were worn at all times. Digestion tubes and pipette tips were always cleaned by immersion in 20% v/v nitric acid for at least 24 hours. Before use, instruments were thoroughly washed with Milli-Q water. All sample manipulations and analyte solution preparations were performed in a laminar flow hood (Karl Bleyemehl, Rosenheim, Germany). The efficiency of the cleaning procedures was confirmed by the low blank values.

Reagents

A total of 1,000 mg/L of stock standard solutions of arsenic species (as As) were prepared from arsenic trioxide (Merck, Darmstadt, Germany), sodium arsenate (Merck), and dimethylarsinic acid (Sigma, St Louis, MO) in purified water. Deionized water (resistance ≥ 18.2 M Ω) was obtained with a three stage ion-exchange filter system (Gehaka, São Paulo, SP, Brazil) and was used as a chromatographic eluant and for preparing and diluting all reagents. All reagents used in the experiments were of analytical grade (Merck S.A., São Paulo, SP, Brazil).

Instrumentation

The HPLC system consisted of a Varian 9012 ternary pump (Varian, San Fernando, CA) equipped with a Rheodyne 7125 injector (IDEX Health & Science, Oak Harbor, WA) and a 200- μ L loop for sample introduction. The separation of the arsenic species occurred in a 25 cm \times 4.1 mm Hamilton PRP X-100 column (Hamilton, Reno, NV). Hydride generation of volatile arsines before the detection was performed adding online solutions of HCl and NaBH₄ by means of a Gilson Minipuls 3 peristaltic pump (Gilson International VP, Middleton, WI). AFS detection was achieved with a Millennium System (PS Analytical, Orpington, Kent, UK) using a boosted-discharge hollow cathode lamp (Photron, Sydney, Australia). The separation of the gaseous arsines from the liquid stream was performed in a PS Analytical Type A gas-liquid separator using argon as a carrier gas. The analog signal output was connected to a computer equipped with chromatographic software (Agilent, Santa Clara, CA).

Chromatographic Conditions

Separation of the arsenic compounds was performed in approximately 10 minutes in the anion-exchange column using 10 mmol/L of phosphate buffer (pH = 5.8) as the mobile phase at an 0.8 mL/min flow rate, the elution order being As(III), DMA, and As(V). Figure 1 represents a typical chromatogram obtained with this instrument coupling.

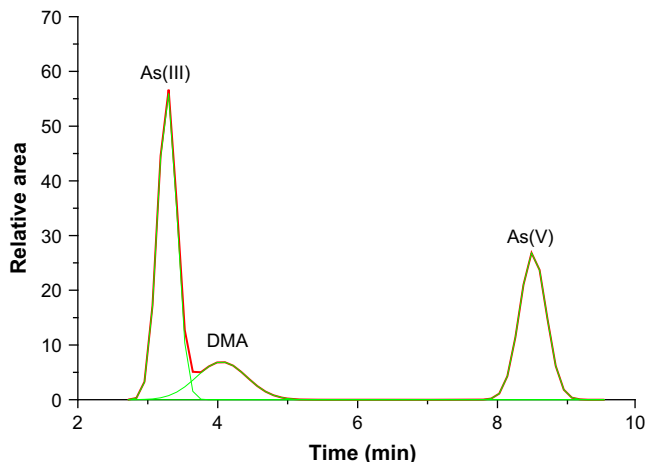


Figure 1. Typical chromatogram obtained with this coupling.

Arsenic Speciation by Coupled HPLC-HG-AFS

Two hundred microliters of the sample was injected in the HPLC system following the previously described chromatographic conditions. Before detection, online hydride generation was achieved adding first 3 mol/L of HCl and then 1.4% m/v of NaBH₄ solution (stabilized with 0.1 mol/L of NaOH), each at a flow rate of 1 mL/min, delivered with a peristaltic pump. After the hydride generation, the arsines were transported to the gas-liquid separator, where an argon flow of 200 mL/min carried them to the detector. Before the detection, the argon stream was dried with a hygroscopic membrane drier tube. The retention times were 3.3, 4.0, and 8.5 minutes for As(III), DMA, and As(V), respectively.

Determination of Total Arsenic

A total of 0.2 g of each material was placed in a Teflon vessel and 0.5 mL of 37% m/v HCl solution and 5.0 mL of a mixture in equal parts of 65% m/v HNO₃ plus 50% m/v HBF₄ solutions. The system was heated in a microwave digester (Provecto Analítica, São Paulo, Brazil). The final solution was obtained and transferred to a 50-mL volumetric flask after dilution with purified water. Arsenic content was evaluated by using HPLC-HG-AFS, with a manual valve for sample introduction (Fig. 2).

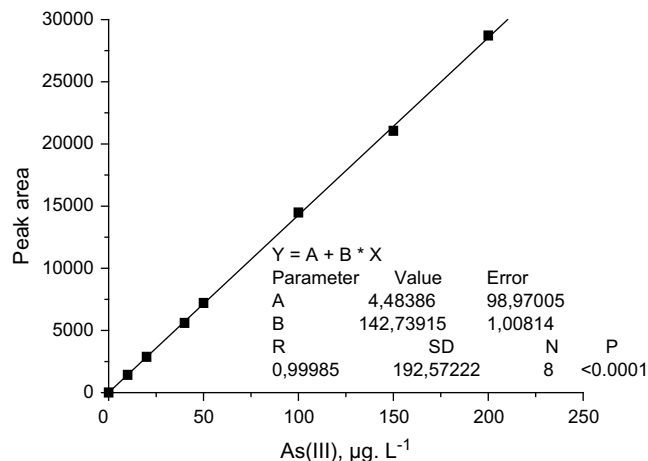


Figure 2. Calibration curve of As(III) using HPLC-HG-AFS.

TABLE 1. Determination of As(III) in Real Samples

Cements	As (ppm) \pm SD
White Portland Cement (Kajazinho, Brazil)	4.7 \pm 0.36
White Portland Cement (Juntalider, Brazil)	< LOD
MTA White (Angelus)	6.5 \pm 0.53
MTA Bio (Angelus)	8.6 \pm 0.85
White ProRoot MTA (Tulsa/Dentsply)	3.3 \pm 0.46
Gray ProRoot MTA (Tulsa/Dentsply)	< LOD
Gray MTA (Angelus)	< LOD

LOD, outside the limit of detection; MTA, mineral trioxide aggregate.

Data Presentation and Statistical Analysis

Data were expressed as a part per million and the preliminary analysis of the raw pooled data revealed a bell-shaped distribution (D'Agostino and Pearson omnibus normality test). Further statistical analysis was performed by using one-way analysis of variance for multiple comparisons. The alpha-type error was set at 0.05.

Results

In all sample chromatograms, only As(III) could be detected. Therefore, Table 1 showed the results of As(III) in Portland cement and MTAs using HPLC-HG-AFS. The limit of detection is defined as the concentration in which the signal-to-noise ratio of 3:1 was found to be $1.0 \times 10^{-4}\%$ m/m for As(III). The signal-to-noise ratio was calculated by using software provided by the instrument manufacturer.

Discussion

The first null hypothesis was validated by the present results because a no difference in the arsenic release among the Portland cements and MTAs was achieved. In the same way, the second hypothesis was also confirmed because the total amount of arsenic released from all cements tested is far below those considered to be hazardous. For the human body, the poisoning by inorganic arsenic can be lethal for oral doses above 60 mg for each kilogram of corporal mass or 60 part per million. Thus, the lower values of arsenic present in the hydraulic cements tested cannot be seen as a contraindication for the clinical employment of these materials.

An interesting aspect of the present results is the detectable amount of arsenic found in the MTA Bio samples. This finding does not support the manufacturer's purpose that was to produce a cement totally free of undesirable contaminant substances, in particular, arsenic (16).

The lower amount of arsenic release found in the present study is consistent with the good cytotoxicity (6, 12) and subcutaneous tissue results (9) related to both MTA and Portland cements. Therefore, it can be expected that the leachable arsenic must be close to the ultra-trace typical quantity otherwise; the biological results should not be so favorable because of the fact that arsenic compounds can bind to sulfhydryl groups acting as an enzyme inhibitor and interfering, in a critical way, with cell metabolism (15).

One of the major obstacles for the clinical use of Portland cement is the concern around the amount of arsenic release. Curiously, to the best of the current authors' knowledge, just one single study was designed in order to determine the arsenic release of MTA and Portland cements so far. A study by Duarte et al (15) concluded that MTA and Portland cements displayed very low amounts of arsenic release. Although there is a substantial difference in the methodological models used, the present results are fully in agreement with the conclusions made by Duarte et al.

In a subsequent report, Primus (17) makes a serious criticism of the experimental model employed by Duarte et al (15). In the present study, the experimental model was improved in comparison to the study done by Duarte et al. First of all, eight samples were used in duplicate instead of the five single samples by Duarte et al. Moreover, Duarte et al made an extraction using a HCL 6 mol/L solution; this method is unable to guarantee that the total amount of arsenic is measured because the available arsenic concentration is only part of the total arsenic concentration in the sample (18). The main advantage of the present experimental model is related to the solid digestion process dilution with purified water in which the cements were submitted that is able to assure a reliable determination of the total amount of arsenic. The total concentration analysis is occasionally required for environmental and toxicologic investigations. Extreme conditions have to be used in this case to dissolve the sample such as hydrochloric/nitric acids mixture plus tetra-fluoroboric acid. Once in solution, the arsenic concentration can be determined by the standard technique described aforementioned (18).

The MTA cements would be classified as a permanent-contact implant device. Therefore, as Primus (17) stressed, "In this category, the biocompatibility tests that are needed include cytotoxicity, genotoxicity, sensitization, implantation effects, and endodontic usage tests." However, Portland cement has already been passed for all of these requirements. Moreover, a recently published single case report showed a very favorable clinical outcome when Portland cement was used as an apical plug in a tooth with a periapical radiolucency and a wide-open apex (8).

Historically, arsenic is the poison of choice for many murders in fiction and in reality. Although it has been used as a poison, arsenic has many chemical applications and is quite an important element. For instance, arsenic is an essential ultratrace element for human life. It is also necessary for red algae, chickens, rats, goats, and pigs. A deficiency of arsenic results in inhibited growth. In conclusion, the negligible amount of arsenic present in the Portland cements cannot be seen as a real obstacle for its clinical usage. The present results add one further point to support the use of the Portland-based cements.

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