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Artigo

IN VITRO Cr(VI) SPECIATION IN SYNTHETIC SALIVA AFTER RELEASING FROM ORTHODONTIC BRACKETS USING SILICA-APTES SEPARATION AND GF AAS DETERMINATION

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A method for Cr(VI) speciation in synthetic saliva after releasing from orthodontic brackets, using silica nanoparticles organofunctionalized with (3-aminopropyl)triethoxysilane (APTES) for Cr(III)/Cr(VI) separation and GF AAS determination is proposed. Under the optimized conditions, Cr(VI) speciation was performed using 150 mg of silica organofunctionalized with 2.0% (v v^{-1}) of APTES at pH 8. It was observed different sensitivity when calibrations of GF AAS were performed using Cr(III) or Cr(VI) as standard solutions. Consequently, calibrations using stoichiometric mixtures (Cr(III) + Cr(VI)) were used for total Cr determination and calibration using Cr(VI) was used only for the determination of this specie. The reliability of the proposed silica-APTES separation procedure and GF AAS determination was checked by addition of both species in synthetic saliva. Recoveries ranging from 97 to 110% were obtained. The repeatability, based on the relative standard deviation (RSD) inter days was less than 6%. A corrosion test was carried out on 20 orthodontic brackets from two different models, after immersion in synthetic saliva (pH=6.0) at 37 °C with agitation (125 rpm) for 24 h. It was observed that about 40% of the total chromium released from the analyzed orthodontic brackets was Cr(VI).

Keywords: speciation, chromium, Cr(VI), saliva, brackets, GF AAS.

INTRODUCTION

Recent studies have demonstrated that some brands of orthodontic brackets can release large amounts of Cr, during contact with synthetic saliva.[1-4] The corrosion of the orthodontic brackets in the oral environment has concerned the clinicians for two main reasons: (i) the possibility of the body absorb the corrosion products suffering localized or systemic effects; or (ii) the clinical performance of the orthodontic appliances.[4,5] The former is concerned mainly to the toxic effect of Cr(VI), that can be present in the bracket composition or can be interconvert by Cr(III) oxidation in the saliva environment. In the oral environment, orthodontic appliances are potentially exposed to physical damage and chemical agents. Factors such as quantity and quality of saliva, salivary pH, board, amount of protein, chemical and physical properties of food and fluid intake and general health oral conditions can influence the orthodontic brackets corrosion in the oral cavity.[1,6]

Most metallic devices used during orthodontic treatment is made of stainless steel type austenitic (AISI type 316L stainless steel), which has 18% (m m^{-1}) chromium, 8% (m m^{-1}) nickel, 2 to 3% (m m^{-1}) molybdenum and low carbon content.[4,5] The corrosion process of metallic brackets has been linked to the deterioration of their mechanical properties and as mentioned above, to adverse biological effects.[6]

It is well known that the presence of chromium in an alloy can increase its corrosion resistant properties due to the ability to form a protective oxide film over the metallic surface. Consequently, the resistance to the corrosion is an essential criterion for its use as dentistry materials.[2]

Chromium coexists mainly in two oxidation states, Cr(III) that is essential to the metabolism of glucose, lipids and proteins and Cr(VI), which is highly toxic due to its allergic, carcinogenic, mutagenic and teratogenic effect for the humans.[7-9] Thus, the chemical speciation is important to identify species present in a sample and to access the real toxicity of chromium.

The determination of chromium in biological materials may be difficult due to the low concentration of this metal, the great variability of matrix from sample to sample and contamination. Among the instrumental techniques available, graphite furnace atomic absorption spectrometry (GF AAS) is still one of the most widely used for Cr determination in biological samples.[10,11] Particularly, AAS is a well-established analytical technique for chromium detection and speciation of Cr in biological materials using different approaches, such as selective volatilization as Cr(III)-thenoyltrifluoracetonate,[12] flow-injection on-line preconcentration on C18 mini-column, based on the selective formation of diethyldithiocarbanate complex of Cr(VI) in the 1-2 pH range and Cr(III) in the 4-9 pH range,[13] solid-phase extraction using ammonium pyrrolidinedithiocarbamate (APDC)-Cr(III) complex on Diaion HP-2MG resin,[14] Cr(III) extraction and preconcentration on silica gel chemically modified with Nb_2O_5[15] and glass beads surface modified with 3-aminopropyl triethoxysilane (APTES) and glutaraldehyde for Cr(III)/Cr(VI) speciation in seawater.[16] Among the solid-phase extraction, silica organofunctionalized with APTES[17-21] and 3-(2-aminoethylamino) propyltrimethoxysilane (AAPTMS)[22] has been successfully used for separation and preconcentration of chromium species in different samples. The APTES contains the amino group that can act as ligant for ions of transition metals (*e.g.* Cr) in solution.

Considering that chromium can be released from orthodontic brackets and the toxic effect of Cr(VI), the aim of this work is to propose an *in vitro* method for Cr(VI) speciation in synthetic saliva after releasing from orthodontic brackets, using silica nanoparticles organofunctionalized with (3-aminopropyl)triethoxysilane (APTES) for analyte separation prior to analyte detection using GF AAS.

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EXPERIMENTAL

Apparatus

The Cr determination was carried out using a GF AAS spectrometer, model SIMAA-6000®, equipped with Echelle grating, solid-state detector, longitudinal Zeeman-effect background correction system and standard THGA® tubes with pyrolytically coated integrated platform (PerkinElmer Life and Analytical Sciences, Shelton, CT, USA). A hollow cathode lamp of chromium (λ = 357.0 nm, current lamp = 25 mA, and spectral band pass = 0.7 nm) was used throughout in the work. An AS-72 autosampler (PerkinElmer) was used for transferring reference solutions and samples from polypropylene cups to the graphite tube. Argon 99.998% (v v^{-1}) (Air Liquid Brazil S/A, São Paulo, Brazil) was used as protective and purge gas. All absorbance signals were based on the integrated peak area.

An orbital shaker and a centrifuge (Quimis Scientific Equipments, São Paulo, Brazil) were used to shake and to separate silica from supernatant solution, respectively.

Reagents and materials

The glassware, polypropylene flasks (Falcon tubes) and autosampler cups were cleaned as follows: washed with detergent solution, soaked in 10% (v v^{-1}) HNO_3 for 24 h, rinsed with high-purity deionized water, dried and stored in a closed polypropylene container.

High-purity water with final resistivity of 18.2 MΩ cm was obtained by a Milli-Q® water purification system (Millipore, Bedford, MA, USA). Nitric acid 65% (m m^{-1}) (Merck, Darmstadt, Germany), hydrochloric acid 37% (m m^{-1}) (Merck) and sodium hydroxide (Merck) were used for silica pretreatment and pH adjustment. A high-purity 10 mg L^{-1} of $Mg(NO_3)_2$ (Merck) was used as chemical modifier. The calibration solutions were prepared by successive dilution of 1000 mg L^{-1} of Cr(III) ($CrCl_3$ in 4.2% v v^{-1} HCl) and Cr(VI) ($K_2Cr_2O_7$ in H_2O) Titrisol® from Merck.

Silica nanoparticles HDK® T40 (Wacker, Germany) with surface area of about 400 m^2 g^{-1} and 7 nm diameter were used throughout this work. The surface area of particle was calculated by means of BET (Brunauer, Emmet and Teller). The reagent (3-aminopropyl) triethoxysilane (APTES) 98% (v v^{-1}) (Sigma-Aldrich) was used for the silica nanoparticles organofuctionalization.

Three different solutions were used to prepare the synthetic saliva, with the following reagents/concentrations (mol L^{-1}): (Solution A) NaH_2PO_4/0.233 + KCl/1.164 + NaCl/0.123 + NH_4Cl/0.205 + sodium citrate/3.74x10^{-3} + lactic acid/0.039; (Solution B) urea/0.167 + uric acid/4.46x10^{-3} + NaOH/5x10^{-3}; (Solution C) KSCN/0.123.[23] The saliva solution was prepared daily by mixing the solutions A, B and C (1:1:1) and then diluted 50 times with high-purity deionized water. The pH of the synthetic saliva solution was adjusted with HCl up to the physiological values (pH~6).

Based on previous results, about the amounts of Cr released from commercially available brackets,[20] samples of two different models of stainless steel orthodontic brackets (MBT™), that presented high corrosion level, were used to perform the Cr(VI) speciation.

General procedure

The silica nanoparticles organofunctionalization and characterization were done as previously reported in the literature.[20] About 50 g of silica was washed with 200 mL of 0.2 mol L^{-1} HCl + 0.044 mol L^{-1} HNO_3 under agitation for 24 h, washed several times with high-purity deionized water and dried at 70 ºC for 12 h. About 1 g of washed silica was organofunctionalized with 0.2, 2.0 and 10% (v v^{-1}) APTES. The best conditions for Cr(VI) speciation was 150 mg of silica nanoparticles organofunctionalized with 2.0% (v v^{-1}) APTES.

The pH was measured using 150 mg of silica nanoparticles organofunctionalized with 2.0% (v v^{-1}) APTES suspended in 2.5 mL of 20 μg L^{-1} Cr(III) or 20 μg L^{-1} Cr(VI) in synthetic saliva.

The kinetic of adsorption of the Cr(III) and Cr(VI) species on organofunctionalized silica nanoparticles showed to be quite fast, although the amount of Cr(III) and Cr(VI) adsorbed were significantly different. For this reason, the time of stirring was not evaluated. About one minute was established for the adsorption. After pH adjustment with hydrochloric acid or sodium hydroxide, each suspension was centrifuged at 3000 rpm for 5 minutes and the supernatant was analyzed by GF AAS for chromium determination.

Solutions containing 20 μg L^{-1} Cr(III) or 20 μg L^{-1} Cr(VI) in water and synthetic saliva were used to optimize the heating program of the GF AAS. A volume of 10 μL reference solutions was used to obtain the pyrolysis and atomization temperatures without and with 10 μg of $Mg(NO_3)_2$ as chemical modifier.

Samples of 20 stainless steel orthodontic brackets from two different models were immersed in 5 mL of synthesized saliva (pH~6), at 37 ºC, stirred in an orbital shaker at 125 rpm for 24 h, simulating the oral environment. Subsequently, the brackets were separated and an aliquot of 1.0 mL of the resulting solutions was used for total Cr determination by GF AAS. Another aliquot of 2.5 mL was added to 150 mg of organofunctionalized silica modified with 2.0% v v^{-1} of APTES at pH 8, adjusted with NaOH. The suspension was manually shaken for 1 min, centrifuged at 3000 rpm for 5 min and the supernatant analyzed by GF AAS.

For GF AAS calibration, solutions containing equal mixtures of 1.0 to 10 μg L^{-1} of Cr(III) and 1.0 to 10 μg L^{-1} of Cr(VI), total Cr concentration of 2.0 to 20 μg L^{-1}; and solutions containing 2.0 to 20 μg L^{-1} of Cr(III) or 2.0 to 20 μg L^{-1} of Cr(VI) were used.

To check the Cr(III)/Cr(VI) interconversion and the reliability of the proposed procedure, two synthetic saliva aliquots were prepared with addition of 3 μg L^{-1} Cr(III) + 7 μg L^{-1} Cr(VI) or 7 μg L^{-1} Cr(III) + 3 μg L^{-1} Cr(VI) and keeping the solution for 1 hour at 37 ºC. Subsequently, these solutions were submitted to the speciation procedure.

RESULTS AND DISCUSSION

Effect of pH

The effect of synthesized saliva pH on the adsorption of Cr(III) and Cr(VI) on silica-APTES is shown in Figure 1.

The behavior of Cr(VI) in the synthetic saliva was very close to that observed for Cr(VI) in water, while that of Cr(III) was completely different.[20] At pH 2, the adsorption was about 60% of Cr(VI) and 70% of Cr(III) species (see Figure 1). In this pH, the protonation of $–CH_2NH_2$ group of the APTES can occur to form $–CH_2NH_3^+$ and, consequently, the electrostatic attraction of Cr(VI) and repulsion of Cr(III) on the silica-APTES surface is possible.[16,20] Opposite behaviors were observed for Cr(III) and Cr(VI) specie (Figure 1). Previous results for aqueous solutions, at pH 2, revealed almost none adsorption of Cr(III) on the silica-APTES.[20] Based on this results, it is possible to suppose some interaction between Cr(III) and the components of synthetic saliva to form anionic species, allowing the interaction with silica-ATPES.

The increase of the pH solution decreases the protonation of amino group and increases the electrostatic repulsion of Cr(VI) specie, as observed in Figure 1. Additionally, the interaction of Cr(III) with the $–CH_2NH_2$ group takes place, increasing the adsorption of this specie on the silica-APTES. At pH 8, the adsorption of Cr(III) over

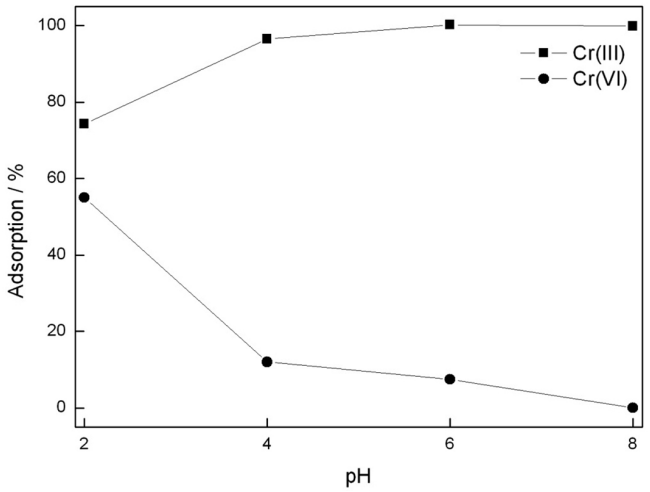

Figure 1. *Effect of the synthetic saliva pH on the adsorption of Cr species (20 µg L^{-1} each) on the organofunctionalized silica-APTES*

the silica-APTES was almost 100% while Cr(VI) was negligible.

At pH 8 it was possible to adsorb Cr(III) on the silica-APTES and determine Cr(VI) in the solution by GF AAS. The concentration of Cr(III) can also be calculated by the difference between Cr_{Total} and Cr(VI) concentrations.

The reusability of the silica-APTES was evaluated, whereas 1.0 mol L^{-1} of HCl was used to desorb Cr(III). Despite the efficient desorption of Cr(III), the subsequent procedure of conditioning silica-APTES was not so effective. Considering the small mass of silica-APTES used and the facility to modify a large mass of material, it was decided to not reuse the organic functionalized particles.

Heating program optimization for Cr(VI) speciation

The GF AAS heating program was evaluated using 20 µg L^{-1} Cr(III) and 20 µg L^{-1} Cr(VI) in synthetic saliva. The best results were observed at 1400 °C for pyrolysis and 2400 °C for atomization.

During the optimization of the heating program different sensitivity was observed for Cr(III) and Cr(VI) for the same concentration of each species. The absorbance of Cr(III) in both media studied (water and synthetic saliva solution) was always lower than Cr(VI). The same results were obtained for Cr(III) and Cr(VI) in synthetic saliva, Cr(III) in HCl solution or Cr(VI) in water.

To investigate the reason of the different sensitivities of Cr(III) and Cr(VI), calibration curves of Cr(III) (2.0 to 20 µg L^{-1}) or Cr(VI) (2.0 to 20 µg L^{-1}) were obtained, for calibration solutions prepared in water or synthetic saliva, in the absence or presence of 10 µg $Mg(NO_3)_2$, used as chemical modifier in the graphite tube. The results are depicted in Figure 2.

In water medium and without chemical modifier (Figure 2A) the sensitivity of Cr(VI) (slope=0.008580 L μg^{-1} s^{-1}) was twice of that observed for Cr(III) (slope=0.004201 L μg^{-1} s^{-1}). When 10 µg $Mg(NO_3)_2$ was used as chemical modifier the sensitivity was almost the same for Cr(VI) (slope=0.006836 L μg^{-1} s^{-1}) and Cr(III) (slope=0.006671 L μg^{-1} s^{-1}). In saliva medium and without chemical modifier (Figure 2B) the sensitivity for Cr(VI) (slope=0.007885 L μg^{-1} s^{-1}) and Cr(III) (slope=0.006529 L μg^{-1} s^{-1}) was also different, but the difference was lower than that observed for water medium. When the chemical modifier was used the sensitivity for both species in saliva medium was drastically reduced and the difference between Cr(VI) (slope=0.002800 L μg^{-1} s^{-1}) and Cr(III) (slope=0.002174 L μg^{-1} s^{-1}) still persisted. In this case, the total chromium determination in synthetic saliva using only one of the chromium species to prepare the calibration curve is not recommended. So, to avoid systematic errors the use of calibration solutions resulting from the mixture of Cr(III) and Cr(VI) species revealed to be more adequate.

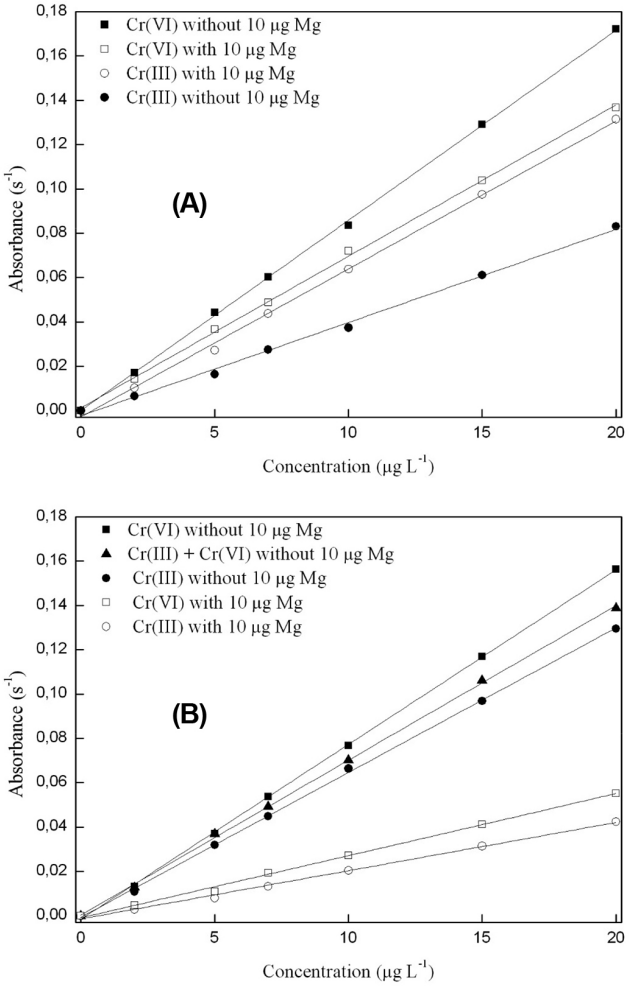

Figure 2. *Analytical curves for Cr(III) and Cr(VI) in water (A) and synthetic saliva (B) with and without 10 µg of $Mg(NO_3)_2$ as chemical modifier*

The explanation for the obtained results can be related to the different interaction of Cr(III) and Cr(VI) with the graphite surface, resulting in different atomization mechanism. Thermal decomposition of the carbide[24] or vaporization of the free metal[25] has been proposed as atomization mechanisms for chromium in GF AAS. However, these proposed mechanisms do not explain the behavior observed in the present work. It could be supposed that Cr(III) sensitivity for water medium is strongly affected due to the more effective formation of chromium carbide that is retained on the graphite tube. On the other hand, in presence of synthetic saliva this interaction is reduced due to the formation of Cr(III) complex with synthetic saliva components and, consequently, the carbide formation is less favored.

In order to verify the influence of saliva components on the chromium species sensitivity, calibration curves of Cr(III) or Cr(VI) were prepared in each solution (A, B and C) used to prepare the synthetic saliva. The sensitivity for Cr(VI) in all solutions was similar, maybe due to the low complexing capability of this species. The presence of oxygen in the Cr(VI) species (CrO_4^{2-} or $Cr_2O_7^{2-}$) can reduce the chromium interaction with graphite, reducing carbide formation and, consequently, increasing the sensitivity. In this way, vaporization of chromium oxide would be one probable atomization route. With

respect to Cr(III), the sensitivity was higher for solution A, which contained complexing agents, such as PO_4^{-3}, $(C_3H_5O(COO)_3)^{3-}$ (citrate) and $C_3H_5O_3^-$ (lactate) that decrease the interaction of Cr(III) with the graphite tube.

Considering all results, it is possible to conclude that calibration curves with only one of the chromium species (III or VI) in synthetic saliva medium is not recommended for total chromium determination. In this way, stoichiometric mixtures of Cr(III) and Cr(VI) were tested to obtain the calibration curve. Indeed, this is not the best way for GF AAS calibration, but this strategy decreases systematic errors associated to the Cr(III) and Cr(VI) determination (Figure 2B). It is important to emphasize that this is the first time that the different behavior on the atomization of Cr(III) and Cr(VI) was observed and reported in the literature for GF AAS.

An additional study was conducted in order to investigate the necessity to apply the separation procedure for the calibration solutions. Using the silica-APTES separation procedure for each stoichiometric calibration solution of Cr(III) + Cr(VI) (2.0 to 20 μg L^{-1}), the slopes of Cr(VI) (0.007648 L μg^{-1} s^{-1}) after Cr(III) separation was very close to that obtained for Cr(VI) in solution prepared without addition of Cr(III) (0.007885 L μg^{-1} s^{-1}). This study demonstrated that the determination of Cr(VI) remaining in the synthetic saliva after Cr(III) separation can be performed without previous separation of Cr(III).

The heating program adopted for chromium determination without chemical modifier is depicted in Table 1.

Table 1. Heating program for Cr determination by GF AAS

Step	Temp. (°C)	Ramp (s)	Hold (s)	Ar flow (mL min^{-1})	Read
Drying I	110	10	25	250	No
Drying II	130	5	15	250	No
Pyrolysis	1400	10	20	250	No
Atomization	2400	0	5	0	Yes
Cleaning	2600	1	3	250	No

Injection temperature: 30 °C; Pipette speed: 100%.

Figures of merit

The calibration curves for total chromium determination and Cr(VI) speciation in synthetic saliva after the corrosion assay were prepared using stoichiometric mixtures of Cr(III) + Cr(VI) (2.0 to 20 μg L^{-1}) and Cr(VI) (2.0 to 20 μg L^{-1}), at pH 8.0, respectively.

The limit of detection (LOD) for total Cr determination was calculated considering the variability of 10 consecutive measurements of synthesized saliva solution (blank), according to 3 s_{blk}/b (s_{blk} = standard deviation of the blank and b = calibration curve slope). To estimate the LOD of Cr(VI), 2.5 mL of synthesized saliva was shaken manually with 150 mg of silica organofunctionalized with 2.0% (v v^{-1}) APTES, centrifuged at 3000 rpm for 5 min and the supernatant measured (n=10) by GF AAS. Considering the adopted conditions, the LOD for total Cr and Cr(VI) determination was 0.1 μg L^{-1}.

The reliability of the proposed silica-APTES separation procedure was checked by addition and recovery of Cr(III) and Cr(VI) species in synthetic saliva and the results are presented in Table 2. For both additions, the recoveries were 97 and 110%. The repeatability of method was calculated based on all steps of the method, such as silica modification, separation and determination by GF AAS. The relative standard deviation (RSD) for inter days measurements was less than 6%.

Table 2. The Cr(III) and Cr(VI) interconversion study and species recovery

Species	Addition		Determination		Recovery	
	Cr(III) (μg L^{-1})	Cr(VI) (μg L^{-1})	Cr(III) (μg L^{-1})	Cr(VI) (μg L^{-1})	Cr_{Total} (μg L^{-1})	Cr(VI) (%)
Mixture 1	3	7	3.2**	6.8±0.2*	10.0±0.1	97
Mixture 2	7	3	6.4**	3.3±0.2*	9.7±0.2	110

* After separation using the proposed method (silica-APTES). ** Calculated by difference between Cr_{Total} and Cr(VI) determined.

Speciation of Cr(VI) released from brackets

The results for the 20 brackets corrosion assay analysis are given in Table 3. For both assays, it was detected the presence of Cr(VI) in the solution (about 39% of total Cr released). The high release of Cr from the brackets can be related to the poor quality of the metal alloys used for manufacturing and, consequently, the corrosion process of metallic brackets takes place.

Additional experiments were conducted to get information about Cr(VI) origin in saliva: (a) by interconversion in the saliva solution, or (b) directly released from the orthodontic brackets.

Table 3. Total Cr and Cr(VI) found in synthetic saliva after brackets corrosion assay and Cr(III) species separation on silica-APTES

Bracket Sample*	Cr_{Total} (μg L^{-1})	Cr(VI) (μg L^{-1})	Cr(III)** (μg L^{-1})
1	14.1 ± 0.3	5.6 ± 0.9	8.5
2	20.3 ± 0.7	7.2 ± 0.3	13

*n=20 brackets. **Calculated by difference between Cr_{Total} and Cr(VI) determined.

To know if the Cr(VI) present in synthesized saliva was released from brackets or originated from Cr(III) oxidation, more experiments were conducted. For this, about 40 mg of metallic chromium powder was submitted to the same procedure used in the brackets corrosion assay and separation using silica-APTES. The analysis of the supernatant revealed 108.0±0.6 μg L^{-1} of total Cr and according to the proposed method, 100% of released chromium was Cr(III), demonstrating that the saliva medium is not able to convert metallic Cr in Cr(VI). Additionally, results of the addition and recovery test (Table 2) demonstrate that there is not interconversion of Cr(III) to Cr(VI) or *vice-versa* in the synthesized saliva environment. Therefore, the Cr(VI) concentration in saliva after contact with orthodontic brackets is due to the direct release of Cr(VI) from the material.

In order to investigate the influence of a typical enzyme (mucin) found in the natural saliva on the Cr(VI) speciation, a mixture of 5 μg L^{-1} of Cr(III) and 5 μg L^{-1} of Cr(VI) was prepared with and without 0.1% (m v^{-1}) of mucin. The Cr(VI) concentration found was 5.2±0.1 μg L^{-1} and 5.3±0.1 μg L^{-1} in the presence and absence of mucin, respectively. Using Student's *t*-test, it was not observed statistic difference of the results for Cr(VI) in presence and absence of mucin, at 95% confidence level.

CONCLUSIONS

Silica nanoparticle organofunctionalized with APTES was very efficient for Cr(III)/Cr(VI) separation, allowing the Cr(VI) speciation analysis in synthetic saliva after releasing from orthodontic brackets. The proposed method is selective, leading to precise results. The different sensitivity observed for Cr(III) and Cr(VI) in GF AAS encouraged the investigation of different approach for total Cr determination in

saliva where Cr(III) and Cr(VI) coexists. The results indicated that the Cr(VI) is released from the orthodontic brackets of low quality. Considering the duration of the orthodontic treatment (2 years), the possibility of corrosion of the brackets in the oral environment deserves attention to avoid ingestion of highly toxic specie of chromium.

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