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Determination of liberated mercury from amalgam submerged in artificial saliva by cold vapor atomic absorption spectrometry

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Abstract

Mercury might be liberated from dental amalgam in toxic amounts for humans, which represent a real health risk. This work presents the direct determination *in vitro* liberated mercury from amalgam submerged in artificial saliva by cold vapor atomic absorption spectrometry (CVAAS). Artificial saliva was prepared with KCl, KSCN, $NaHCO_3$, $NaH_2PO_4.H_2O$ and lactic acid. The amalgams prepared with different types of trituration: few (F), normal (N) and excessive (E), were submerged in 10 mL of artificial saliva in tubes of 2.5 x 9.0 cm with cap and put into thermostated bath at 37°C. The total portions of test of saliva were took every 24 h the first three days and then every three days until completing 30 days; moving away the carved tooth of the saliva and storing this to 4°C until their spectrometric analysis. The precision were evaluated by mean of the Hg determination in 4 real samples, the results show average RSDs less than 5%. The accuracy was verified by analyzing two certified materials, comparing the target and experimental values, and obtaining mean relative error less than 5%. Additionally, the recovery studies were carried out, obtaining recoveries of 102 ± 2%. Detection limit and characteristics mass were 0.30 ng Hg/L and 4.4 ng, respectively. Liberated mercury concentrations found in artificial saliva did not show significant statistically differences respect to the types of trituration employed. CVAAS-based method for the direct determination of Hg in artificial saliva was accuracy, precise and free from interferences.

Key words: Artificial saliva; cold vapor atomic absorption spectrometry; dental amalgam; liberated mercury; mercury determination.

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Determinación de mercurio liberado de amalgamas sumergidas en saliva artificial por espectrometría de absorción atómica con vapor frió

Resumen

El mercurio puede ser liberado a partir de las amalgamas dentales en cantidades tóxicas para los seres humanos, lo cual evidentemente representa un riesgo para la salud. En este trabajo se presenta la determinación directa *in vitro* del mercurio liberado de la amalgama sumergida en saliva artificial utilizando la espectrometría de absorción atómica con vapor frío (CVAAS). La saliva artificial se preparó con KCl, KSCN, $NaHCO_3$, $NaH_2PO_4.H_2O$ y ácido láctico. Las amalgamas se prepararon empleando diferentes tipos de trituración: poca (P), normal (N) y excesiva (E), se sumergió en 10 mL de saliva artificial en los tubos de 2,5 x 9,0 cm con tapa de rosca y se colocaron en el baño termostatado a 37°C. Las porciones totales de prueba de saliva se tomaron cada 24 h los primeros tres días y luego cada tres días hasta completar 30 días; retirando el diente tallado de la saliva y guardando la solución a 4°C hasta el análisis espetrométrico. La precisión se evaluó determinando Hg en 4 muestras de saliva artificial preparadas por triplicado y, los resultados mostraron DER promedios <5%. La exactitud se verificó analizando dos materiales certificados, obteniéndose un error relativo menor al 5% entre el valor teórico y experimental. Adicionalmente, se llevó a cabo un estudio de recuperación, obteniéndose una recuperación de 102 2%. El límite de detección y la masa característica fueron 0,30 ng Hg/L y 4,4 pg/0,0044 s^{-1}, respectivamente. Las concentraciones encontradas del mercurio liberado en la saliva artificial no mostraron diferencias estadísticamente significativas con respeto a los diferentes tipos de trituración empleados. El método basado en la CVAAS para la determinación directa de Hg en saliva artificial fue exacto, preciso y libre de interferencias.

Palabras clave: Amalgama dental; determinación de mercurio; espectrometría de absorción atómica con vapor frío; mercurio liberado; saliva artificial.

Introduction

The possibility that mercury (Hg) might be liberated from dental amalgam in toxic amounts for humans has been indicated to be a health risk (1-3). Amalgams are in fact mixtures of various substances used to fill cavities and include 45-52 percent mercury, 30 percent silver and small amounts of zinc, tin and copper (4, 5). Several authors have estimated the amount of mercury-released daily from dental amalgam restorations in an individual (2, 6-8). The rate of release of mercury from amalgam into saliva and the total and occlusal surface areas of amalgam restorations in an individual are positively related (9-12). Studies in the last decades have confirmed that amalgam fillings release mercury vapor into the air in the oral cavity. The rate of release is greatly exaggerated by chewing. Dental amalgams have been estimated to be the major source of background (non-occupational) exposure to mercury vapor

It was found that mercury release from fillings increases dramatically by 15-fold whenever the fillings are stimulated by chewing, brushing, hot fluids, bruxism, etc. (4). Other investigators have worked using solutions of artificial saliva for determination of mercury and its relationship with dental amalgams (13-15). For further insight into the relationships between amalgam fillings, mercury concentration in the saliva a large-scale field study using saliva analyses was carried out (13-15).

The form of mercury released is significant with respect to its potential toxicity (2). The mercury is an extremely toxic metal; it is overly the most dangerous environmental pollutant, not only for the graveness of the illnesses that causes, but for the irreversible effects that it provokes in the human beings (16, 17). The mercury is broadly distributed in the atmosphere and its toxic effects are known from the beginnings of the civilization (17). The toxicity of the Hg is observed for the most part of the human vital systems such as nervous central, renal, digestive, reproductive and breathing (18, 19).

Numerous analytical methods are currently available for the determination of Hg in biological materials, which are based on several techniques such as atomic absorption spectrometry (7, 16), inductively coupled plasma mass spectrometry (20-23), high resolution liquid chromatography (24-26), and cold vapor atomic absorption spectrometry (CVAAS) (23, 27, 28-37). However, the latter is preferentially employed because of its extremely high sensitivity, the absence of background attenuation-type spectral interferences and the relatively low operating costs (33). Measurement of Hg by CVAAS requires oxidation of the concomitants (i.e., organic and inorganic) present in the sample to liberate the analyte element from its chemical bonding; hence, a single labile mercury species (i.e., Hg^{II}) is produced; being afterwards reduced quantitatively to Hg^0 for spectrometry evaluation (assuming that volatilization losses of mercury are eliminated) (33).

In this work we present the analytical method for the direct determination of *in vitro* liberated mercury from amalgam submerged in artificial saliva by cold vapor atomic absorption spectrometry. Artificial saliva sample was directly reacted with sodium tetrahydroborate without any pre-treatment to obtain the mercury species required in the redox reaction, which is the chemical principle of the CVAAS technique.

Experimental

Instrumentation

A mechanical amalgamator Vivadent Model Silamat S4 (Schaan, Liechtenstein, Austria) was used for the trituration of the amalgams. A Perkin-Elmer Model 460 atomic absorption spectrophotometer (Norwalk, CT, USA), equipped with a mercury hollow cathode lamp operated at 6 mA (spectral bandwidth of 0.7 nm) and at a resonance wavelength of 253.6 nm, was used throughout this work. A Perkin-Elmer Model MHS-10 mercury/hydride system was attached to the spectrophotometer to generate the mercury vapors. Nitrogen (inlet pressure of 255 kPa) was the purge gas. Absorbance was measured in the instrumental mode of peak height, with a measurement time of approximately 45 s by test portion analyzed. The flow rate of reducing agent was 17 mL/ min.

Reagents and standard solutions

All chemical were of analytical grade. Artificial saliva were prepared with KCl (Merck, Darmstadt, Germany), KSCN (Merck), $NaHCO_3$ (Merck), $NaH_2PO_4.H_2O$ (Merck) and lactic acid (Merck) in grade I (as established by the American Society for Testing and Materials, ASTM, electrical resistivity 16.6 MΩ/cm at 25°C (24)) triply-distilled and deionized water. A SDI (Southern Dental Industries, Australia) gs-80 non gamma 2 admix of spherical and lathe cut alloy particles containing silver (40%), tin (31.3%) and copper (28.7%) and mercury in predosed capsules were used. The sodium tetrahydroborate solution (3% m/v) was prepared by dissolving sodium tetrahydroborate powder (Riedel de Haën, Hannover Germany) in appropriate amounts of grade I ASTM triply-distilled and deionized water, then stabilized with 1% m/v sodium hydroxide (J.T. Baker, Phillipsburg, NJ, USA). This solution was prepared daily before use. Concentrated nitric acid (Riedel de Haën) was used during the digestion procedures. The

stock solution (1,000 mg/L of Hg) was prepared from Titrisol (Merck) concentrates. Standard solutions were freshly prepared by serial dilution of the stock with 0.01 M nitric acid.

The evaluation of the accuracy of the method was carried out through the analysis using a standard reference material from the National Institute of Standards and Technology (NIST, Gaithersburg, MD, USA) and for the recovery studies. The materials were: Albacore Tuna (RM 50, NIST, USA) and Venezuelan Commercial Tuna, which was previously analyzed and reported its target concentration of Hg (24).

Procedures

Artificial saliva were prepared mixing calculated masses of KCl, KSCN, $NaHCO_3$, $NaH_2PO_4.H_2O$ and lactic acid in grade I ASTM triply-distilled and deionized water at pH 6.7-6.8 in calibrated glass flasks (ca. 500 mL). A specimens of a commercial, containing dental amalgam were prepared and tested. The pre-capsulated alloy and mercury were triturated according to the manufacturer's instructions and condensed following the ADA Specification No. 1. The amalgams capsule were prepared with different types of trituration (mean wet approximately 0.5212 g): few (F), normal (N) and excessive (E), were submerged in 10 mL of artificial saliva in tubes of 2.5 x 9.0 cm with cap and put into thermostated bath at 37°C. The trituration time into mechanical amalgamator employed was 8 second in according to manufacturer. In this study, it time was normal, few and excessive were obtained added and deducted 4 seconds. The total portions of test of saliva were taken every 24 h the first three days and then every three days until completing 30 days; moving away the carved tooth of the saliva and storing this to 4°C until their spectrometric analysis using CVAAS.

Previous to the spectrometric analysis, the certified material was mineralized with the purpose of obtaining the Hg in its maximum state of oxidation. Thus, a test portion was placed (ca. 0.040 mg of certified material) and 2.5 mL of concentrated HNO_3 in a Teflon capsule. The closed capsule of Teflon was introduced in the transparent body of the reactor and the closed reactor was put inside to the microwave oven. The system was irradiated with microwaves by 70 s at 100% of power (ca. 600 MHz). Each sample was made up to at 10 mL with a mixture of 1.0 M $HNO_3/HClO_4$. Similar procedure was applied to the Venezuelan Commercial Tuna. The quantification of Hg in each sample of mineralized materials was carried out using calibration curves prepared with aqueous standard solutions in 0.01 M nitric acid.

Mercury determination by CVAAS

An aliquot of the artificial saliva and digestion solution materials certified samples (volume ranged between 2 and 5 mL depending on mercury concentration) was placed into the generator vessel of the mercury/hydride system. Nitric acid solution (1.5% v/v) was added to obtain a final volume of 20 mL. Sodium tetrahydroborate solution was added and the mercury vapors generated were directed to the optical cell. The absorbance reading was taken at the maximum reached. Working curves obtained by adding 10, 20, 50 and 100 µL of a 1.0 mg/L Hg solution to the reaction flask of the mercury/hydride system, which represented 10, 20, 50 and 100 ng of Hg, respectively.

Statistical analysis

Statistical analyses were carried out by conventional methods using commercial statistical programs (i.e., Origin 6.0, Excel® 2000, etc). Differences were considered statistically significant when $P < 0.05$.

Results and Discussion

Evaluation of the analytical parameters

Aqueous mercury standards were prepared daily in order to avoid losses due to metal adsorption on the container walls and volatilization. The linear range was found to be 10-100 ng of Hg. Most of the samples analyzed were within this linear range. Peak height absorbance readings (A_p) increases linearly in relation to the equation $A_p = 0.0010$ C (correlation coefficient 0.9999).

The within- and between-run precisions of the developed methods were evaluated by means of the Hg content in 3 real samples of artificial saliva (Table 1). Three aliquots of each real sample were analyzed (five runs each) using the instrumental and operational conditions previously indicated. The obtained results show average RSD of 3.97% for both the within-and between-run precision. These results can be considered adequate for this kind of analyses.

The accuracy was verified by mean of the evaluation of two (2) standard reference materials: Albacore Tuna (SRM 50, NIST, USA) and Venezuelan Commercial Tuna (VCT). These materials were decomposed by using the reported mineralization procedure (23). Under these conditions, the total mercury concentrations found by CVAAS for the reference materials were statistically indistinguishable ($P > 0.05$) from the certified or target values, furthermore, this study are doing with the standar adition method obtaining recoveries of 102±2%. Results are shown in Table 2 and 3 and verifies the excellence of the analytical method.

Non-spectral interferences study was carried out by comparing the slopes of the working curves with those obtained by the method of standard additions, for the Hg determinations in artificial saliva by CVAAS. The equations obtained for standard addition and calibration curves were: $A_p = 0.00105\,c$, $r = 0.9999$) and $A_p = 0.00100\,c$, $r = 0.9999$, respectively. The mean relative error between slopes was 5%. These results implied the absence of non-spectral interferences in the CVAAS analyses by proposed method for Hg and permitted the use of either the calibration graphs or the standard additions methods for metal quantification.

Table 1
Study of precision in the determination of mercury in artificial saliva for CVAAS.

		Within-run		Between-runs	
Samplesa	c	SD (ng/L)	RSD (%)	SD (ng/L)	RSD (%)
Saliva 1	148	6	4.05	5	3.38
2	163	8	4.90	7	4.29
3	148	6	4.05	5	3.38
X ± SD 3.97 ± 0.51					

[a]Samples prepared for triplicate and read by pentaplicate.

Table 2
Accuracy study for the determination of the Hg in standard reference materials by CV-AAS.

Certified material	Concentration of Hg (mean ± DS)		Relative Error (%)
	Certified μg/g	Found μg/g	
Venezuelan Commercial Tuna	1.45 ± 0.01	1.47 ± 0.01 μg/g	1.4
Albacore Tuna SRM N° 50, NIST*	0.95 ± 0.10	1.00 ± 0.04	5.0

* National Institute of Standards and Technology, USA.

Table 3
Recovery study for the determination of the Hg in artificial saliva by CV-AAS

Sample	Concentration (ng/L)	Added	Expected	Found	Recovery (%)
Artificial saliva	24.1	5	29.1	30.1	103
		10	34.1	35.6	104
		15	39.1	39.1	100
				X ± SD	102 ± 2

Table 4
Mercury concentration (mean ± SD, ng Hg/L) in artificial saliva by CVAAS

	Type of trituration		
Day	F	N	E
1	123 ± 17	136 ± 14	124 ± 22
2	158 ± 33	168 ± 14	213 ± 11
3	305 ± 38	283 ± 28	374 ± 44
6	394 ± 118	559 ± 72	527 ± 96
9	267 ± 36	137 ± 18	252 ± 9
12	268 ± 7	414 ± 18	304 ± 11
15	303 ± 11	273 ± 14	408 ± 40
18	436 ± 29	496 ± 11	459 ± 20
21	366 ± 17	285 ± 14	416 ± 19
24	412 ± 11	109 ± 20	236 ± 15
27	373 ± 92	244 ± 21	385 ± 59
30	313 ± 34	442 ± 51	354 ± 22

F: few trituration, N: normal trituration, E: excessive trituration.

The characteristic mass was 4.4 ng/0.0044 s^{-1} of Hg for 3 mL of solution undergoing analysis. The detection limit, defined as two times the standard deviation of the blank, for all samples analyzed was 0.30 ng/L.

Mercury levels in artificial saliva

Table 4 shows the mean concentrations of mercury determined by CVAAS in artificial saliva, relating with the different types of trituration applied to the amalgams: few (F), normal (N) and excessive (E). The analysis of the results permited to observe the continue liberation of mercury in the artificial saliva due to the progressive increment of the concentration of the analyte in the samples by to the applied freatment, which considered the exposition time (in days) and type of trituration (F, N and E). It is important to highlight that from the day 18 a reduction of the Hg levels is observed, this can explain due to the internal thermal conditions of the bakelite tube, which was to 37°C during this time. However, inside the tube high pressures can be generated which increase the inner temperature of the tube; in this way port of the gaseous mercury is relea-

sed to the environment. The tap of the bakelite tubes were adjusted again but the release of mercury continued increasing in the artificial saliva (i.e., days 21, 24, 27, 30). Liberated mercury concentrations found in artificial saliva did not show significant statistically differences respect to the types of trituration employed. This experiment allows demonstrated that the release of Hg is constantly in an amalgams have been observed by others authors (1, 2).

Conclusions

CVAAS-based method for the determination of Hg in artificial saliva was accuracy, precise and free from interferences. This experimental development could be applied in future work related to the odontological areas to evaluate the possible Hg intoxication of the dental patients.

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