http://dx.doi.org/10.21577/0103-5053.20170037

J. Braz. Chem. Soc., Vol. 28, No. 10, 1953-1959, 2017.
Printed in Brazil - ©2017 Sociedade Brasileira de Química
0103 - 5053 $6.00+0.00

Article

New Design of Mini PTFE Vessels for Sample Preparation in Micro Scale: Determination of Cd, K, Mg and Na in Biological Sample

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This paper presents a study involving the development of a new mini polytetrafluoroethylene (PTFE) vessel project for biological sample preparation in micro scale in closed system with microwave radiation for subsequent determination of Cd, K, Mg and Na by atomic spectrometry techniques. All experiments were carried out in micro quantities of samples (up to 20 mg) and reagents (300 μL of HNO_3) for 5 min at 350 W. Certified reference materials of biological samples (bovine muscle and liver) were used to evaluate the accuracy of the proposed system and the results obtained were in agreement with the certified value at 95% of confidence (*t*-test). Relative standard deviations (RSD) were lower than 8.0% for all elements. The proposed method is not only easy and fast, but it is also based on the use of an inexpensive system for sample digestion.

Keywords: micro digestion, mini PTFE vessels, biological samples, atomic spectrometry techniques

Introduction

Information regarding elemental composition in biological samples, such as food, is very important for both consumers and health professionals. Recent legislation on food labeling in Brazil has also limited the presence of some compounds such as metals (toxic or not). Thus, the determination of inorganic contaminants in the food has been highlighted in chemical analysis. At the same time, since foods are complex matrices which usually require extensive sample preparation and/or extraction procedures for instrumental analysis, they are the focus of great number of studies.[1]

Among the conventional methods of sample preparation, the wet decomposition in presence of acids with oxidizing properties aided by heating using a digester block is commonly used. However, these conventional methods have some disadvantages such as time of analysis, increased risk of contamination, loss of analyte and reagent by evaporation because it is an open system, in addition to the high consumption of corrosive and dangerous reagents.[1,2]

Several alternative methods of sample preparation are being developed in order to avoid or minimize these potential drawbacks. Among the alternatives that allow the elimination of sample treatment, such as those involving decomposition steps, the analyte extraction and solubilization before the instrumental analysis are techniques of direct solid sample analysis.[2,3] These methods are interesting because there is little sample manipulation, which shows many advantages over the conventional methods, such as increased frequency rate, reduced contamination risks, and decrease of possible losses of analyte by volatilization.[4-6] Studies have also described alternative methods such as slurry sampling, which involves the partial or complete solubilization of the sample matrix using both acid and alkaline reagents.[7-9] However, few types of equipment allow direct solid sample analysis and/or suspensions, which may lead to a significant increase of the risk caused by sample matrix interference. Recently, decomposition procedures in biological samples using reflux systems have been presented as a promising alternative to conventional methods such as acid decomposition open systems. These systems use smaller quantities of reagent, and therefore, reduce considerably the risk of contamination. Also, they avoid reagent and sample losses by evaporation, as well as present lower costs related to the analysis.[10-13]

Alternatives such as micro digestion procedures are also considered a promising way to sample preparation because they use low amounts of acids and samples by

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passing the main problem of conventional decomposition methods, which use substantial amounts of both sample and reagents, interfering directly with the cost of analysis and waste generation.[14-18] A great number of these studies was performed in centrifugation micro tubes made of polypropylene, which limits time and temperature decomposition. Consequently, a micro tube deformation and rupture occurs, affecting the efficiency and repeatability of the procedure.

Therefore, this study aimed to develop and investigate the use of a mini vessel made of polytetrafluoroethylene (PTFE) for sample preparation in a closed system with microwave heating as well as to demonstrate its effectiveness for the preparation of biological samples for later determination of Cd, Mg and Na by atomic spectrometry techniques.

Experimental

Instrumentation

For Cd determination, integrated absorbance signals were measured in a PinAAcle 900z atomic absorption spectrometer (AAS, PerkinElmer, Walthan, USA), equipped with autosampler (AS-900 model) and longitudinal Zeeman-effect background correction. Operational conditions were used according to the manufacturer, such as wavelength (228.8 nm) and width of spectral band (0.7 nm). Hollow cathode lamp source (HCl's) (PerkinElmer, Walthan, USA) was used operating at 8 mA. Argon, 99.996% (Linde, São Paulo, Brazil) was used as purge and cooling gas. All determinations were carried out using pyrolytic graphite coated tubes with platforms. Measures were performed with a volume of 20 μL of samples and standard solutions that were introduced into the graphite tube by the autosampler and submitted to heating program. Table 1 shows the time and temperature program optimized in the presence of chemical modifiers, used for Cd determination.

A flame atomic absorption spectrophotometer (FAAS) AA-6300 (Shimadzu, Japan) equipped with autosampler (ASC-6100 Model) was used for Mg determination. For this analysis, a flame composed of air-acetylene and background correction with deuterium arc lamp and a hollow cathode lamp Mg (Shimadzu, Japan) operated at 8 mA were used. Operational conditions recommended by the equipment manufacturer, such as wavelength (285.2 nm) and width of spectral band (0.7 nm) were employed. All analyzes were performed by using a micro sample injection system adapted to the pneumatic nebulizer which is available in this equipment by the manufacturer. This system allowed microinjection of small volumes of sample solution into the flame (5 to 200 μL), which results in a transient signal. The recording was performed on an integrated area.

Table 1. Graphite furnace temperature program for Cd determination in biological samples using micro digestion

Program stage	Temperature / °C	Ramp / (°C s^{-1})	Hold time / s	Gas flow / (L min^{-1})
Drying	110	10	30	250
Drying	130	10	30	250
Pyrolysis	500	10	20	250
Atomization	1200	0	5	0
Cleaning	2450	1	3	250

Na and K determinations were carried out in a flame photometer Model B462 (Micronal, São Paulo, Brazil) that was operated under the following conditions for both elements: sample aspiration rate (5 mL min^{-1}), compressed air (9 L min^{-1}) at a pressure of 1 kgf cm^{-2} and butane gas flame.

Samples were weighed in an analytical balance with a resolution of 0.1 mg and maximum weight of 210 g (Ohaus Adventurer, Model AR 2140, USA). For micro acid digestion of the samples, a microwave oven (28.0 L) manufactured by Panasonic (Manaus, Brazil, NN-ST571WRU Model) with operational frequency of 2450 MHz and variable power up to 750 W (indicated) was used.

Considering that the decomposition procedure was done in a conventional microwave oven with a security level lower than the scientific equipment, it is essential to work on a miniaturized scale and use reaction vessels designed for this purpose, avoiding unnecessary risks to the analyst. The actual power of the equipment must be known in order to employ the amount of energy required for the mineralization of biological samples avoiding excessive heating and high pressures within the mini vessels. Thus, to study the actual power, optimization was performed for the microwave oven, as described by Rosini *et al*.[19] Furthermore, in order to preserve the useful life of the apparatus by preventing the microwave return and damaging the magnetron, a beaker with water was placed in the oven cavity to absorb the excess of radiation. The results of this optimization are presented on Table 2.

Figure 1 shows the mini vial developed for micro digestion procedures, which were produced with inert material, PTFE, since it is resistant to the action of strong oxidizing reagents, tolerates high temperatures (melting point of 327 °C), and is transparent to microwaves. The internal cavity of the mini vial has a total volume of 2.0 mL. For an efficient fitting, the sealing cap is threaded through the system that further comprises the addition of an O'ring.

Table 2. Values of indicated power and actual power of microwave conventional oven

Level	Indicated power / W	Actual power / W
1	150	52.3
2	200	139.6
3	300	195.4
4	400	279.1
5	500	348.9
6	550	401.3
7	650	488.5
8	750	558.3

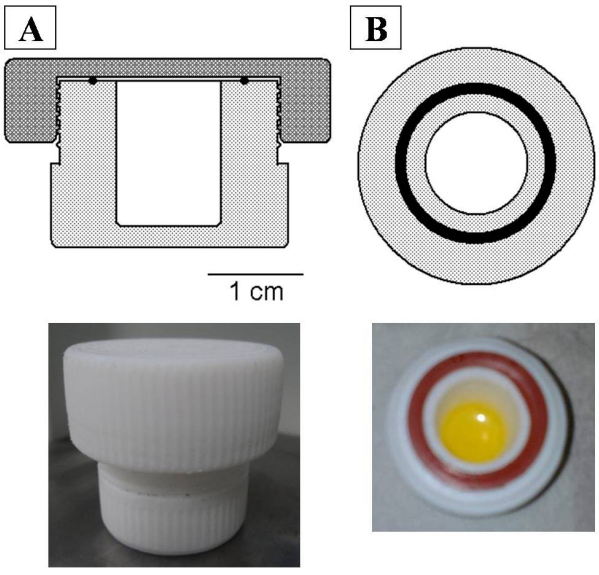

Figure 1. (A) Mini bottle and cap made of PTFE; (B) cavity of mini vial with fitting O'ring.

Consequently, it avoids losses of reagents and analytes and allows the determination of volatile elements, such as Cd.

The dimensions of the minis vessels are: (*i*) the outer part of the mini vial measures 23 mm high and 28 mm in diameter, and the internal cavity measures 18 mm deep by 13 mm diameter; (*ii*) the outer part of the cap measures 17 mm high and 40 mm in diameter, and the inside measures 12 mm deep by 28 mm diameter; (*iii*) O'ring measures 20 mm in diameter and 2 mm in thickness.

Solutions, reagents and reference materials

All reagents used were of analytical grade. All solutions were prepared by using deionized water obtained by a water distiller MA078 (Marconi, Piracicaba, SP, Brazil) and subsequently deionized by passing through a column CS1800 (Permution, Curitiba, PR, Brazil). Nitric acid 65% (v/v) (Synth, Diadema, SP, Brazil) was purified by doubly sub-boiling distillation in a quartz system MA-075 (Marconi, Piracicaba, SP, Brazil). Glass containers and plastic material were washed and immersed in 10% v/v HNO_3 for at least 48 hours and thoroughly rinsed with ultrapure water prior to use. The mini vessels used in micro digestions were decontaminated in a bath of 50% (v/v) HNO_3 for at least 24 hours, then rinsed with deionized water and dried at room temperature.

Standard solutions of Cd, Mg and Na were daily prepared through dilutions from a stock solution containing 1000 mg L^{-1} (Fluka, Steinheim, Germany) of each element in deionized water. KCl solution was used to minimize interference (ionization) related to Mg atomization process by F AAS. A solution of Pd (Sigma-Aldrich, Darmstadt, Germany) was used as chemical modifier, with the addition of 5 µg in graphite furnace (GF) for Cd measurements.

The following certified reference materials (CRM) were used in this work for method development and to assess the accuracy: DOLT-4 (dogfish liver) from the National Research Council Canada (NRCC, Ottawa, ON, Canada) and SRM 8414 (bovine muscle powder); SRM 2976 (muscle tissue) and SRM 1577c (bovine liver), both produced by the National Institute of Standards and Technology (NIST, Gaithersburg, MD, USA).

Sample treatment

Biological samples were weighed directly into mini vessels which contained a mass of up to 20 mg of sample. For this amount, 300 µL of HNO_3 bidistilled was added. After the vial closure with the cap, the system was introduced into the microwave oven cavity near a beaker containing 200 mL of deionized water, and then it was subjected to heating with application of microwaves at a power of 350 W for 5 min.

After cooling at room temperature, the vessels were opened and the solution was transferred into Eppendorf tubes that were previously calibrated and adjusted to 2.0 mL with deionized water. This procedure was performed in triplicate for all samples, as well as for the analysis of analytical blank.

Methodology

For Cd quantification in biological material samples, both optimization of the temperatures of pyrolysis and atomization were made to avoid interferences during the atomization process. This study allows the choice of a working condition, which enables a higher efficiency in removing the matrix during the pyrolysis step, and also

allows a higher sensitivity during atomization. For the pyrolysis and atomization curves, the analysis was carried out in matrix and aqueous medium with the addition of 0.5 μg L^{-1} of Cd, employing 5 μg of Pd as a chemical modifier, which was added to samples and standards during the injections into the graphite tube.

All atomic spectrometry instruments were calibrated by the construction of analytical curves, using standard solutions and employing the same reagents used in the sample preparation step. The accuracy of the proposed method was evaluated for each analyte by analysis of two CRM of biological materials from different natures, which were already described in Solutions, reagents and reference materials section.

Results and Discussion

Development of mini PTFE vessels

During the initial studies for the use of sample preparation in micro scale, mini commercial vessels (Eppendorf) made of polypropylene with 2.0 mL of capacity were used, as previously reported.[14-18] Some problems were not reported by these studies, such as a deformation caused when the vessels reached high temperatures during the reactional process. Also, the increase of pressure led to the cap projection of the polypropylene vessel, opening the system and then leading to losses of analytes and reagents. Also, larger tubes were tested, such as centrifuge tubes type, with volumes of 15 or 50 mL, which solved the problem of high pressure inside the tubes. However, the vessel deformation persisted in some cases.

These difficulties led to the search of a vessel that would circumvent these drawbacks faced in the preliminary tests with biological samples. Thus, the development of new mini reaction vessel for decomposition using the conventional microwave oven was based in the model of the larger vessels which are used in microwave ovens destined for sample preparation. The vessels were constructed of PTFE because of its chemical resistance, purity, high temperature tolerance, and transparency to microwave radiation. In our first prototype, the pressure inside the vessel let the vapors escape from the cap, which was subsequently solved by the addition of an O'ring, resulting in the final design shown previously in Figure 1.

A previous study to evaluate the ideal conditions for a complete digestion of the samples was carried out, searching a shorter sample irradiation time, as well as the more efficient microwave power. For this, the CRM DOLT-4 was used and different irradiation times (2, 5 and 10 minutes) and actual power (52.3, 348.9 and 558.3 W) were tested. After the digestion step, the final volume of 2.0 mL was adjusted with deionized water and the samples were taken for the quantification of Na and K in a flame photometer and the concentrations obtained were compared with the certified values. The exact same conditions optimized for Na and K were applied for the determination of the other elements. Tables 3 and 4 showed the results obtained from the optimization of irradiation time and power level, respectively, used in the microdigestion process of the biological samples.

Using the conditions of irradiation time (2 min) and minimum power (150 W), an incomplete digestion of the samples was observed. When the maximum power was used and the samples were irradiated for a longer time (10 min), the concentrations obtained for Na and K in the CRM DOLT-4 presented values below the informed values. This is probably directly related to the high turbulence of the sample solution inside the digestion vessels under these conditions. Thus, the biological samples analyzed were digested at a medium power of 348.9 W during 5 min of irradiation time.

Pyrolysis and atomization temperature optimization

As mentioned above, the graphite furnace program optimization was performed for the solution of SRM 1577c and standard solution, which was constructed on the basis of the absorbance signal. Results are shown in Figure 2. It was

Table 3. Variation of irradiation time for complete digestion of DOLT-4 using mini PTFE vessel. Actual power: 348.9 W. Concentrations values in mg kg^{-1} (n = 3)

time / min	Na			K		
	Informed value / (mg kg^{-1})	Found value / (mg kg^{-1})	RSD / %	Informed value / (mg kg^{-1})	Found value / (mg kg^{-1})	RSD / %
2		[a]	–		[a]	–
5	6800	6979 ± 188	2.7	9800	9591 ± 379	3.9
10		6608 ± 170	2.6		9259 ± 381	4.1

[a]Incomplete digestion of the sample.

Table 4. Variation of power of conventional microwave oven for complete digestion of DOLT-4 using mini PTFE vessel and irradiation time of 5 min. Concentrations values in mg kg^{-1} (n = 3)

Power / W	Na			K		
	Informed value / (mg kg^{-1})	Found value / (mg kg^{-1})	RSD / %	Informed value / (mg kg^{-1})	Found value / (mg kg^{-1})	RSD / %
52.3 (minimum)		[a]	–		[a]	–
342.9 (medium)	6800	6979 ± 188	2.7	9800	9591 ± 379	3.9
558.3 (maximum)		6714 ± 349	5.1		9159 ± 329	3.6

[a]Incomplete digestion of the sample.

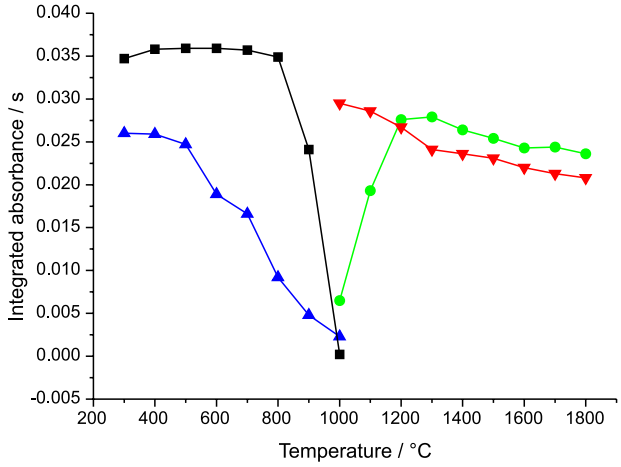

Figure 2. Pyrolysis and atomization curves for the sample solution (SRM 1577c) and standard solution (0.5 µg L^{-1}) with the addition of 5 µg Pd. Pyrolysis: sample (□) and standard solution (▲); atomization: sample (●) and standard solution (▼).

used 5 µg of Pd as chemical modifier for Cd determination and the standard concentration used was 0.5 µg L^{-1}.

Through the analysis of the curves, it is verified that the chemical modifier promoted a thermal stability of Cd at temperatures up to 500 °C for both media. Thus, this temperature was chosen to ensure greater elimination of the sample matrix during the pyrolysis step. Regarding the atomization temperature, it was set at 1200 °C, since there is a greater agreement of the analytical signal in both aqueous and matrix medium as well as better sensitivity, under this condition. The complete program of time and temperature for Cd determination by GF AAS were presented earlier in Table 1.

Analytical results

Figures of merit for the analytical curves corresponding to each analyte investigated in their respective technique based on the methodology proposed by micro sample preparation digestion are shown in Table 5. According to these parameters, the values of the correlation coefficients (R) were above 0.99, confirming the good linearity of the method. The instrumental limit of detection (LOD) was calculated as being three times the standard deviation of ten measurements of the blank divided by the slope of the analytical curve for each analyte. For the method LOD, the mass sample used and the final volume of the solution were considered. The limits of detection (LOD) reached are appropriate for the present proposal study for determination of all investigated analytes. Campos *et al.*[17] used a micro digestion procedure with autosampler cups for determination of Cd in biological samples obtaining a LOD of 0.02 µg g^{-1}. The LOD for Cd in our work was similar.

Based on the development of a new method of chemical analysis aimed at minimizing inconveniences related to the sample preparation step, studies were conducted and they prove the applicability of the proposed system of micro digestion to the objective of the work. To evaluate the efficiency of sample decomposition using the micro digestion system, the analysis of certified reference materials was performed, and results are shown in Table 6.

According to the results, a good agreement for the analytes studied was observed between the values found and the certificates, thereby proving the accuracy of the

Table 5. Figures of merit for K, Na, Mg and Cd

	Range / (mg L^{-1})	a / (L^{-1} mg)	LOD / (µg g^{-1})	LOD / (µg L^{-1})	R
Cd	0.25-1.0 µg L^{-1}	0.0750	0.01	0.09	0.9955
K	1.0-5.0	0.1680	26.2	131.0	0.9988
Mg	0.1-0.4	1.2747	7.48	37.4	0.9963
Na	1.0-5.0	0.1671	10.6	53.0	0.9982

a: slope; LOD: limit of detection; R: correlation coefficient.

Table 6. Cd, K, Mg and Na concentrations in different certified reference materials (CRM)

Analyte	CRM	Certified value	Found value[a]	RSD / %
Cd	1577c / (μg kg^{-1})	97.0 ± 1.4	95.6 ± 1.8	1.9
	8414 / (mg kg^{-1})	0.013 ± 0.011	0.014 ± 0.001	7.1
	2976 / (mg kg^{-1})	0.82 ± 0.16	0.80 ± 0.10	12.5
K	1577c / %	1.023 ± 0.064	1.021 ± 0.027	2.6
	8414 / %	1.517 ± 0.037	1.541 ± 0.109	7.0
	DOLT-4 / (mg kg^{-1})	9800[b]	9786 ± 79	0.8
Mg	1577c / (mg kg^{-1})	620 ± 42	637 ± 32	5.0
	8414 / (mg kg^{-1})	960 ± 95	939 ± 20	2.1
	DOLT-4 / (mg kg^{-1})	1500[b]	1443 ± 69	4.8
Na	1577c / %	0.2033 ± 0.0064	0.2049 ± 0.0113	5.5
	8414 / %	0.210 ± 0.008	0.204 ± 0.011	5.4
	DOLT-4 / (mg kg^{-1})	6800[b]	6811 ± 93	1.4

[a](Average ± standard deviation) for n = 3; [b]informed value.

proposed method. In addition, the application of the Student's *t*-test showed (at a level of 95% confidence) that the results have no significant differences. Through the analysis of the results, the proposed method shows good precision, which can be observed in Table 5 by the standard deviation values (RSD) of less than 7.0%, showing the reliability of the analysis. Regarding the values of RSD obtained, the same are similar with those obtained by other works that used different micro digestion systems. This is a fundamental parameter that ensure the reliability of the analysis given for these systems.[14-17]

Conclusions

The mini vessel proposed proved to be suitable for applications in micro digestion procedures for preparation of biological samples in acidic medium under heating by microwave radiation. The procedure showed to be fast, safe and with lower consumption of reagents and samples, minimizing waste generation and providing accurate and precise results for macro and micro constituents, as well as the slightest possibility of loss by volatilization, as demonstrated for Cd.

It is important to mention that the mini PTFE vessel developed brings significant contributions to the stage of biological sample preparation, presenting itself as a promising alternative to conventional methods of analysis that already use this micro digestion procedure. Due to the high cost of microwave ovens designed for laboratory applications, we propose a procedure for sample micro digestion using a conventional microwave oven.

This procedure ensures full security to the analyst, since the sample preparation was based on the use of mini PTFE vessels, which are resistant to the temperatures and pressures reached during micro digestions, as well as inertia of chemical properties against concentrated acids. In addition, micro amounts of samples and reagents are needed, which comes as an advantage in terms of safety, cost analysis, and less waste generation.

Acknowledgments

The authors are grateful to Conselho Nacional de Desenvolvimento Científico e Tecnológico (Edital Universal 14/2014, Processes 447552/2014-7 and 447373/2014-5) for financial support through research projects and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES, AUXPE 2973/2011) for research scholarship.

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Submitted: October 25, 2016
Published online: February 22, 2017

FAPERGS/CAPES has sponsored the publication of this article.