MICROWAVE DIGESTION FOR MERCURY DETERMINATION IN FISH TISSUES AND BOTTOM SEDIMENTS BY AUTOMATED COLD VAPOR ATOMIC ABSORPTION SPECTROMETRY

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Lyophilized fish tissues and bottom sediments were decomposed in a microwave oven. The behavior of the inner pressure was observed for two different digestion solutions in absence of samples, by varying the number of digestion vessels. For fish analysis, effects of fat content, sample amount, and volume of HNO_3 were investigated. Decomposition of the sediment samples was studied by varying sample amount, carbon content and the volume of the digestion solution. In all situations, pressure profile curves were built up. Samples were analyzed by flow injection cold vapor atomic absorption spectrometry. Two programs were checked by using standard reference material (IAEA-350) for fish muscular tissue and laboratory intercomparison (Laboratory of Biofisica, UFRJ/RJ) for sediment samples. The fish fat content had no influence on the yield of the solubilization process, but the percentage of carbon in the sediments influenced the behavior of the sample digestion. In both cases, recoveries higher than 90% were calculated. The procedure took into account the routine analysis, according to an automated mercury determination.

Keywords: microwave digestion; mercury; fish; sediment; flow analysis.

INTRODUCTION

Both in open and closed systems, sample decomposition has been considered a difficult step in analytical chemistry[1]. Time is often the limiting factor[2] and can also affect the detection limit in elemental analysis[3].

The use of microwave preparation methods[4] including those for biological[5] and geological materials[3] has allowed progress in this area. However, these procedures are strongly dependent on the experimental conditions, and are primarily influenced by the type of equipment, the kind of material being digested, sample mass, type and number of vessels on the turntable, and the concentration and volume of the digestion solution[6].

Besides working in a closed system, care should be taken when volatile elements are to be determined in order to match the element volatilization with safety pressure operating conditions inside the Teflon reaction flask. This is of utmost importance for mercury determination.

Since fish and sediments are considered compartments of the biosphere[7], it is important to have a reliable method for solubilization of these matrices which will also permit the analysis of a large number of samples as usually requested by biogeochemical research.

In this work, experimental conditions related to fish and sediment digestion in a microwave oven were investigated in connection with automated mercury determination by cold vapor atomic absorption spectrometry. To assess for the behavior of microwave interaction on the acids used for solubilization, an assay with the digestion solutions was carried out prior to the sample decompositions. Precision and accuracy were verified by using 12 digestion vessels which is the maximum allowed for the instrument configuration.

EXPERIMENTAL

Apparatus

A CEM MDS-81D 670 ± 70W microwave system with 120-ml Teflon PFA vessels was used. The pressure controller module (CEM) was always operated according to the manufacturer recommendations, in order to monitor the pressure profile under different chemical conditions and also to ensure safety during sample solubilization. An upper pressure limit value of 120psi was selected to allow digestion up to 200mg of lyophilized fish tissue and up to 500mg of dried sediment.

To assess the microwave energy absorption inside the cavity, six opened teflon vessels, each with 50g of water, were uniformly positioned over the turntable which always operated when the microwave power was on. The water was heated for four minutes at 100% power (100% P). Thereafter, the remaining water was weighted and the percentage of weight loss calculated. Six repetitions were carried out for each position. The microwave power output was measured at full power, and calculated to have an effective oven power of 635W.

Solutions

All chemicals were of analytical-reagent quality and only double distilled-deionized water was used. Hydrochloric and nitric acids were obtained by subboiling distillation. All glassware was maintained overnight in 1+1 (v/v) nitric acid and before use, repeatedly washed with water.

Mercury standard solutions (1.0 - 10.0 μgl^{-1}) were prepared daily from a $HgCl_2$ stock solution (1000 mg $Hg.l^{-1}$) either in 1.4N nitric acid for fish or in water diluted (1:12.5) *acqua regia*,

for sediments analyses. Tin (II) chloride solution was prepared by dissolving 10g of $SnCl_2.2H_2O$ in 10ml of hot (ca 80°C) hydrochloric acid, followed by dilution with water to 100ml.

Sample preparation

For fish analysis, the dissolution program was initially set as follows: 20% P (power) for 8 min, 40% P for 8 min and 60% P for 4 min. Based on the recommendation of Vermeir[5], 50 to 200mg of lyophilized sample were solubilized in 2.5-10.0ml of concentrated nitric acid. When the samples were digested, the final solution was 1.4N in nitric acid and the final volume was 100ml. Because of the high pressure possibilities in the vessels during digestion, the Teflon vessels were sealed using the proper torque setting for the CEM vessel capping system, at high power. Recovery was performed by spiking 0.25 and 0.5μg Hg to 200mg samples. The fat content in the muscular fish tissue was determined[8] as 2.6 and 25.8% (w/w) for "porquinho" (Balistes capriscus), and "sardinha" (Sardinella brasiliensis) respectively. In addition, a reference material IAEA-350 (tuna fish) was also analyzed.

For sediment analysis, samples from the Ibitinga Reservoir, Tietê River, São Paulo, Brazil were collected through a 30cm long sediment core. Their carbon content, as determined with a Carmographo 12A and varied from 0.32 to 3.24% (w/w). Also, bottom dried marine sediments, available in the laboratory was analyzed. The method described for biological material was also employed here: 250 and 500mg of 200-mesh sieved sediment was added to in the digestion vessel and the dissolution program set as recommended by Van Delft[3] (30% P for 1 min, 80% P for 4 min and 100% P for 10 min). The volume of the digestion solution (acqua regia plus water) varied from 9 to 18 ml, and the volumetric proportion of acid to water was fixed at 0.8[3]. Sediment samples were sent to the Laboratório de Biofísica, Rio de Janeiro University (UFRJ), for analytical comparison. After digestion, samples were cooled to room temperature, and the volume completed to 50ml with water, by using a volumetric pipet.

For most samples, the cooling time was around 20 min. The pressure control flask should be opened before reaching room temperature, otherwise the internal volume would increase as cooling leads to a decrease in the inner pressure of the flask, as a consequence of this the water in the pressure control tube would spill into the connected flask. It was observed that, after cooling, the internal volume of the Teflon flask initially containing 2.5ml HNO_3, increased until 7.5ml.

The flow system

Directly from the digestion vessel, samples were aspirated by an mp13GJ4 Ismatec peristaltic pump to fill a 500μl sampling loop (L = 100cm) and injected into the C carrier stream (1.4 N HNO_3 for fish, or water for sediment) flowing at 10.6ml.min⁻¹ towards the detector (Fig.1). At the x_1 confluence, 30cm downstream, the sample zone met with a 10% (w/v) stannous chloride stream (1.0ml.min⁻¹). At x_2 confluence, 30cm further downstream, met the argon flow (200ml.min⁻¹) and on through a 10cm analytical path with 0.8mm i.d. polyethylene tube, until the separation chamber (SC, Perkin-Elmer B019-3772). During phase separation, the depleted solution was wasted at 12.3ml.min⁻¹. The mercury vapor was then directed through 50-cm polyethylene tubing, towards a 10 x 150mm, quartz window flow cell (Perkin-Elmer B009-7693)(D). The transient signal was detected by a Perkin-Elmer model 503 AAS spectrometer at 253.7nm and recorded as a peak with height being proportional to the Hg content of the sample[9]. When the injector commutator (I) was displaced to the other permissible position, the next sample was selected and another cycle was initiated. With this approach, up to 40 samples could be analysed per hour.
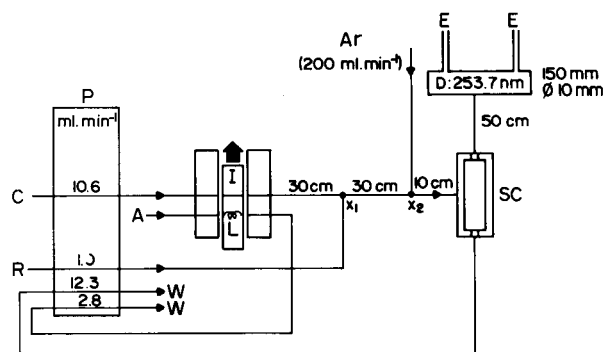

Figure 1. FIA manifold employed for mercury determination in digestion solutions of fish tissues and sediments. Where: Ar - argon gas flow; C - sample carrier stream; D - window quartz flow cell; E - Exhaust; I - Injector commutator; L - sample loop; P - peristaltic pump; R - reagent stream; SC - separation chamber; x_1 and x_2 - three-way confluence points and W - waste. For details see the text.

RESULTS AND DISCUSSION

The average water evaporation was 13.78% ranging from 10.86 to 14.05% as can be observed in table 1. Depending on the sample matrix, oxidant reagent and time schedule for solubilization, these variations can lead to a deterioration in analytical precision. Considering these results, it should be noted that when a good recovery result was obtained for a certified sample, the time digestion program should be increased for some minutes to ensure a complete digestion of all the samples in the microwave oven.

Table 1. Average water losses (%) for six open teflon vessels uniformly positioned over the turntable (4 min and 100% P).

Position on the turntable	Average water loss (%)	s.d. (n = 6)
1	13.23	1.29
2	14.05	1.27
3	12.50	1.11
4	13.25	1.07
5	12.79	1.25
6	10.86	1.45

The rate of the pressure increase depends on the power and time programmed for the oven, the number of vessels on the turntable, the volume of acid solution and also the type of acid used for digestion (Figs. 2a-c).

As can be seen in the figures 2a and 2b, when the number of vessels was increased, the pressure increased at a slower rate. Two exceptions were observed, namely for nitric acid (one vessel curve) and acqua regia (12 vessels curve), where a plateau was established. For nitric acid, the rate of pressure increase was quite different for that observed for acqua regia. The energy absorption in the acqua regia medium demonstrates another curve shape due to the change in the dielectric behavior of the hydrochloric/nitric acid mixture. The absorption pattern is similar to that of hydrochloric acid, because there is three times more hydrochloric acid than nitric acid in the final solution. Considering one flask on the turntable, the pressure related to nitric medium is almost twice than in acqua regia. For 6 and 12 vessels, the power supplied was probably not enough to warm up the solutions.

At the first step (20% P), when only one teflon vessel is on the turntable, the pressure controller presents a positive value on the dial. Changing P to 40%, an almost stable situation is established for acqua regia medium, with the three curves obtained following the same tendency. After half time of this
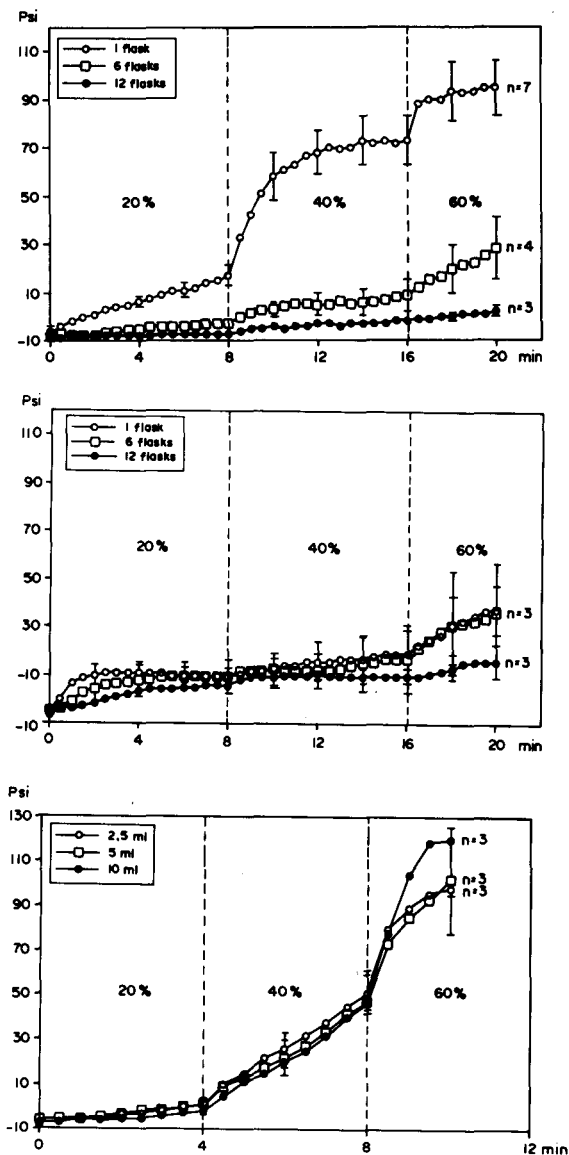
Figure 2. Pressure profile curves for 2.5ml acid medium, obtained in a 120ml Teflon PFA vessel exposed to 20% P/8min, 40% P/8 min and 60% P/4 min, using 1, 6 and 12 vessels (Fig. 2a HNO₃, Fig. 2b acqua regia). In Fig. 2c, 2.5, 5.0 and 10.0ml HNO₃ are used in only one vessel and the program is reduced by half in time. n indicates number of replicates.

it is desirable to consider the lower part of the curve as the effective pressure inside the flasks, and to adapt the digestion program by increasing slowly the power or time interval for each step. When considering volatile elements, for the upper part of the curve, care should be taken to set the pressure control where loss of the acid vapor is to be avoided[6].

When the volume of nitric acid was varied up to four times, no differences for the first two steps of the program were noted (Fig. 2c). The pressure was different for 10ml HNO_3, in the last step, at 60% P. While for 2.5 and 5.0ml the pressure rose until 100psi, for 10.0ml, it was as high as 120psi. It is possible to point out that when a time interval of 8 min was used, for 40% P (Fig. 2a), the pressure reaches a maximum in 4 min and stays in a plateau until the change of power. Consequently, in the experiment related in figure 2c, the time was reduced to a half, in order to maximize the efficiency of the microwave oven.

Microwave behavior for fish digestion

In the earliest stage of decomposition, fresh tissue has a better interaction with microwaves due to the water molecules (Fig. 3), demonstrating that water content of the sample is an important parameter to be considered. During solubilization, the water was diluted with acid, and the final pressure was close to that observed for 200mg of dry tissue decomposition. As humidity in the fresh fish tissue is around 80%[10], these samples have the same amount of muscular tissue. Although the pressure digestion curves show little advantages for fresh fish, in terms of microwave energy absorption, lyophilized fish tissue was preferable over fresh fish because it is easier to homogenize and to keep under laboratory conditions.
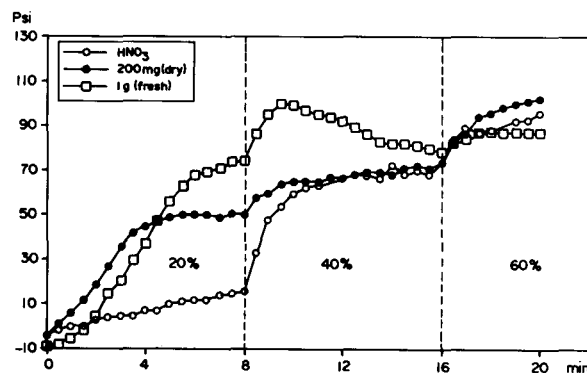

Figure 3. Pressure profile curves obtained in the solubilization of 1g of fresh and 200mg of lyophilized fish tissue in 2,5ml HNO₃. Data are related to three replicates.

stage, the 12-vessels curve can be distinguished from the others. While the pressure is increasing for 1 and 6 vessels, up to 40psi (60% P), it continues at 12psi for 12 vessels (Fig. 2b). The similarity between the 1 and 6-vessel curves could be due to the high standard deviations observed, emphasizing the non uniform distribution and interaction of microwave energy, although the turntable alternatively rotates 360° clockwise and anticlockwise.

Figure 2a indicates that the digestion program for 12 vessels should be altered when the inner pressure of the reaction flasks approaches 0, which would impair the sample solubilization and miss the main advantage provided by a closed system. An analogous effect was observed during the first 8 minutes when 6 vessels were used.

In all situations, large standard deviations in the pressure profiles were observed, the standard deviations being higher for acqua regia relative to nitric acid medium. Variations became worse during the second stage of the program which can be related to the distribution of the microwave within the oven. For a better digestion efficiency, and to improve reproducibility,

The influence of the amount of fish on the development of pressure is clearly observed in figure 4a, even considering that it was only used half the time of the initial program. Although the pressure was kept close to zero during the first stage, it reached values higher than 10psi in the vessel with 100mg, and 90psi for 500mg of sample. Using 500mg of fish tissue, maximum pressure was attained in the earlier stage of the second step of the program while using 100mg maximum pressure was achieved only at the interchange between second and third steps. Following this a stable pressure was maintained in both cases, indicating the good interaction between the reagent with the matrix. Moreover, the temporal increase in pressure time shows that microwave absorption is much higher when the sample amount increases for the same volume of the digestion solution, which leads to a higher CO_2 production. When a 500mg sample is used, the digestion time is increased, as the time to diminish the inner pressure inside the reaction vessel is longer and the sample solubilization is not satisfactory.

For a constant mass-to-volume ratio of 1:20 (Fig. 4b), no differences in slopes of the curves were observed, indicating that the influence of total mass in the microwave energy absorption is minimized. However, for 50mg of sample, after 1 min at 40% P, the curve was parallel to abscisse, increasing again when the power was set at 60% P. This pressure digestion curve should be similar to the others, but the interaction between sample and acid solution was modified: 2.5ml of solution did not totaly cover the sample.

With fat contents of 2.5 and 25.8% (w/w), no variation in pressure profile curves suggests the same thermodynamic behavior for decomposition (Fig. 5). As the fat content variation was so wide, the method can be employed without restrictions to fish belonging to distinct trophic levels, and this is important for mercury research within an aquatic food chain[11].
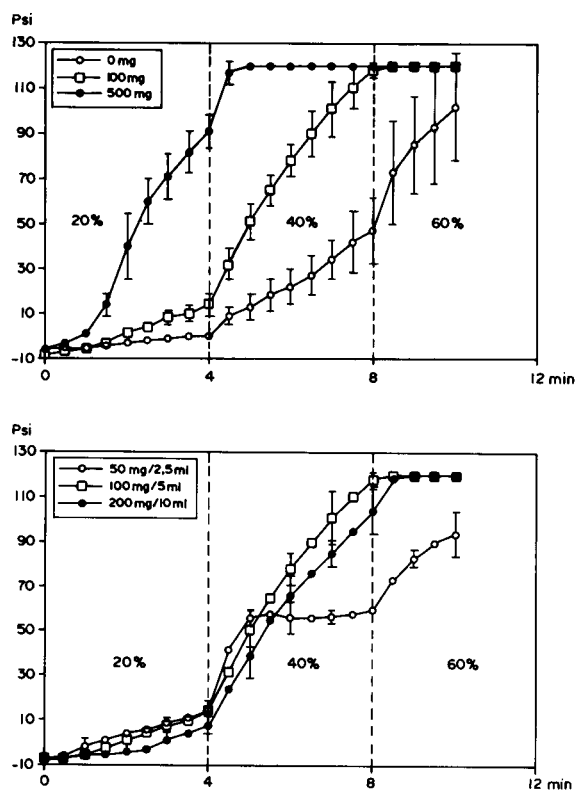

Figure 4. Pressure profile curves obtained in the solubilization of different mass of lyophylized fish tissue in 5ml HNO₃ (Fig. 4a) and in different mass and volume of HNO₃, keeping the same mass to volume ratio as 20 (Fig. 4b). Data are related to three replicates.
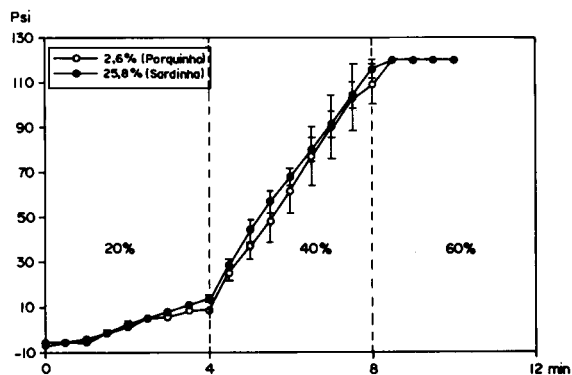

Figure 5. Pressure profile curves as a function of the fat content in fish muscular tissue obtained in the solubilization of 100mg fish tissue in 10ml HNO₃. Data are related to three replicates.

Microwave behavior for sediment digestion

Addition of water to the digestion mixture produced a difference between digestion volumes which was not observed with only *acqua regia*, although curve shapes are similar. Water content also leads to more reproducible results (Fig.6a). It was also mentioned that the water addition to *acqua regia* increased the percentage of mercury extracted from sediment samples[12]. Using *acqua regia*, the pressure was higher than in the digestion mixtures, probably because the production of nitrosyl chloride was faster in the undiluted media[6].

Figure 6a also indicates a decrease in slope by doubling the digestion mixture volume, from 9 to 18ml. The presence of sample modifies the energy absorption in both situations, but no differences were observed when 250 or 500mg of samples were decomposed in the same volume (Fig. 6b). With a 9ml solution, samples stayed longer at the highest permissible pressure. If the efficiency of the digestion was not hindered, a reduction in the time of the proposed program[3] could be achieved.

When 500mg of sediment samples, with carbon contents between 0.32 and 3.24% were decomposed in a same volume of solution (Fig.6c), a tendency for the inner pressure to increase with the organic matter was observed. Due to the presence of carbon, pressure reaches the plateau of 120psi after *ca* 3 min of the second stage. With 250mg sediment, the standard deviation of the measurements was lower than that obtained for 500mg, indicating the influence of sample amount on the energy absorption.

CONCLUSIONS

Fish analysis

The program was set for 200mg of fish in 10ml of HNO₃, keeping a mass-to-volume ratio of 1:50. A lower ratio should not be compatible for the required sensitivity level and, at higher ratios a deterioration of the solubilization process would occur, leading to an increase in the time consumed for the whole procedure. Under these conditions, and considering 12 flasks on the turntable, the program was defined in only two steps: 50% P/8 min and 100% P/16 min, and was suitable for fish routine analysis. A noteworthy feature is the reproducibility of the results which is improved, since the established digestion conditions stay longer in the plateau where the standard deviations are much lower than those obtained in the "lagphase" of the pressure/time curve.

The first phase of the program permits the control of the initial fast increase of pressure observed when organic matter decomposition is carried out[6] and the probable loss of mercury vapor through the safety valve is strongly reduced. This problem is minimized when samples stay overnight in nitric acid.

The similarity of recovery rates for "porquinho" and "sardinha" fish (Tab.2), shows that the fish fat content had no influence on the yield of the solubilization process. Our result is in good agreement with the standard reference value IAEA-350.

Sediment analysis

In general, almost the same program already described by Vermeir[5] can be used. Two exceptions should be considered: the first is that instead of using 18ml of digestion solution, 9ml was considered sufficient, giving improvements in the digestion efficiency and reagent economy. The same 0.8 ratio of *acqua regia* to water is maintained. Although the inner pressure variation presents a higher variation for 500 than 250mg sample, 500mg of sieved sediment was used, as sample solubilization was satisfatory for 9ml of digestion solution and also by considering the sensitivity for mercury detection. The second is related to the mercury detection by the flow injection analysis procedure. With 18ml of digestion solution, the final volume should be completed to 100ml instead of 50ml, otherwise,
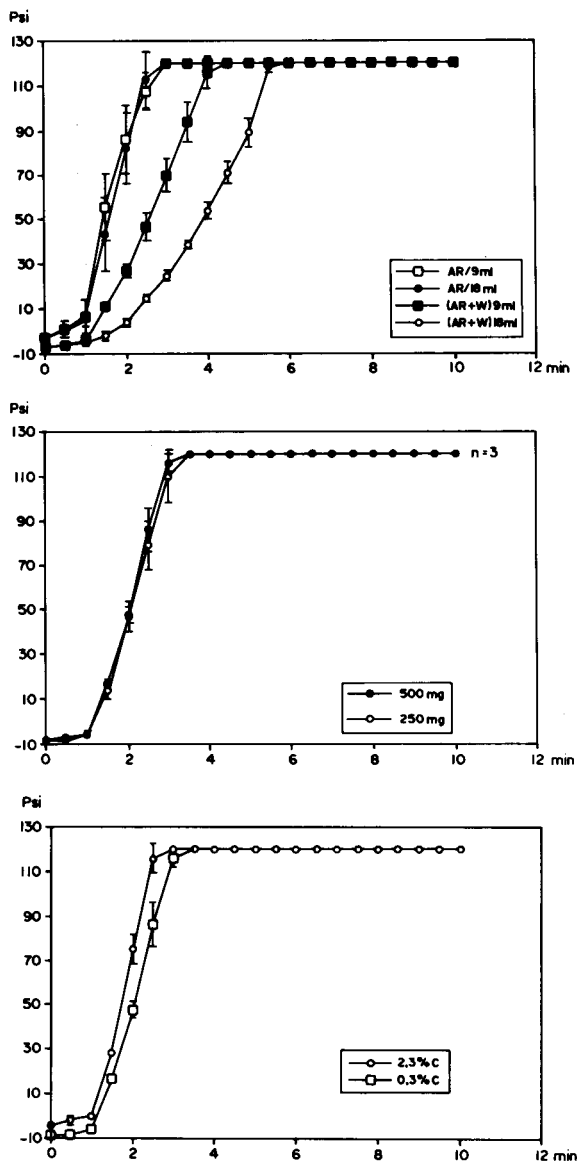
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Figure 6. Pressure profile curves obtained for acqua regia (AR) and acqua regia plus water (AR + W), where the total volumes were 9 and 18ml, keeping the ratio acqua regia: water as 0.8 (Fig. 6a). Using 9ml of acqua regia plus water, 250 and 500mg sediment were digested (Fig. 6b). In Fig. 6c, 500mg of sediments containing 0.3 and 3.2% C were digested in 9ml of digestion solution (acqua regia plus water). Data are related to three triplicates.

Table 2. Percent recovery in samples of Porquinho and Sardinha fish spiked with 1250 and 2500ng Hg.g⁻¹ and sediment from Ibitinga Reservoir spiked with 200 and 500ng Hg. g⁻¹. Data are averaged values ± standard deviation based on three replications.

Sample	Added mercury ($ng\ Hg.g^{-1}$)	Obtained concentration (proposed method) ($ng\ Hg.g^{-1}$)	Recovery (%)
Porquinho (2.6 % fat)	1250	1340 ± 80	107.0
	2500	2250 ± 50	90.0
Sardinha (25,8% fat)	1250	1340 ± 80	107.0
	2500	2230 ± 50	93.2
Ibitinga sediment (3.24% C)	200.0	210.0 ± 8.7	105.0
	500.0	0579.0 ± 12.8	115.8

the measured signal is sharply reduced and the kinetic reaction conditions established for the designed FIA manifold were not followed. It should be pointed out that, in a batch method for mercury determination in soils and sediments, the quantitative mercury reduction in *acqua regia* required a longer time[13]. Even by using 9ml of digestion solution, water was used as carrier stream to provide the conditions for mercury reduction in the FIA system, without signal decrease.

In spite of the strong affinity of mercury for organic matter, good results were obtained for 3.24% carbon content for both tests, standard addition and comparison between laboratories (Tab.2, 3). Considering these results, it could be proposed that

Table 3. Mercury content ($ng\ Hg.g^{-1}$) in lyophilized fish tissues (200mg) and in dried sediment samples (500mg). (1) Standard reference value. (2) UFRJ laboratory value. Data are averaged values ± standard deviation based on three replications.

Sample	Sample concentration ($ng\ Hg.g^{-1}$)	Obtained concentration (proposed method) ($ng\ Hg.g^{-1}$)	Recovery (%)
IAEA-350	4 100 (1) (min: 3310 - max: 4420)	3 895 ± 70	95.0
Marine sediment	302.0 ± 6.8 (2)	295.0 ± 7.0	—
Ibitinga sediment (3.24 % C)	304.0 ± 7.5 (2)	321.0 ± 23.0	—

this digestion method could be employed for lower carbon content sediment samples with good efficiency. For the comparison between laboratories, sediment samples were analysed in the Radioisotops Laboratory of Carlos Chagas Filho Institute (UFRJ/RJ), using an open digestion method[14] and the determination of mercury content was carried out by a Varian cold vapor geration accessory (VGA-76) coupled with a Varian AA/1475 atomic absorption spectrometer. The good agreement with the results obtained in our loboratory proves the suitability of the proposed analitical system (Tab.3).

The proposed system is suitable for large scale sample digest for fish and sediment analysis. Results compare well for both substrates, as indicated by the reference material and the comparison between laboratories, with recoveries always higher than 90%.

Nevertheless, this paper underlines some problems encountered when utilizing a microwave oven for samples digestion. Principally, it should be pointed out that it is difficult to achieve a good digestion program for unknown sample matrices because of the complexity of the experimental conditions such as distribution homogeneity of microwaves, type and volume of the oxidant solution, vessels number, power and time schedule for solubilization.

ACKNOWLEDGEMENTS

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