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Microwave Oven Digestion Procedures and Flow Injection for the Analysis of Biological Samples

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SUMMARY

The fundamentals of the use of microwave ovens for sample decomposition, the modifications proposed to adapt the commercially available microwave ovens to the laboratory work requirements and the possibilities and applications of those methods in flow injection analysis are discussed.

INTRODUCTION

Biological samples are not completely soluble either in water, nor in organic solvents. So, the analysis of this kind of samples involves organic matter decomposition as a previous treatment.

Both, dry ashing (at temperatures from 350 to 500 °C) (1) or wet ashing techniques (using different acids or mixtures of acids (2)) are generally used for the destruction of the matrix in the determination of metallic elements in biological samples (3).

Dry ashing treatment allows a minor dilution of samples and moreover it avoid the problems of the use of strong acid solutions in measurement methods (4). However, bad recoveries are obtained for elements as iron, aluminium and chromium and also an appreciable loss of volatile elements, such as mercury, arsenic, selenium and lead (5) takes place. On the other hand, often, samples of tissues are mineralized with difficulties by dry ashing (6).

The use of wet ashing techniques decreased the risk of volatilization losses (7). However, a higher attention kindness from the operator and a higher dilution of the samples are required.

In summary, a lot of problems are derived from the use of each one of these techniques. The use of a combination of both, carrying out a dry ashing at low temperature using the addition of mineral acids (8), allows to avoid some of these problems, however it does not avoid the slowness and time-consuming of this kind of tecniques.

The development of digestion procedures under pression (9-10) or the acid extraction methods under energic pression and temperature conditions (11) has provided a drastic reduction in the sample preparation time required. However, the minimum time for the acid decomposition or acid extraction is about 1 or 2 hours. This relative long time required and the pressing need to use of hermetic containers have difficulted the

automatization of these procedures.

Other alternative methods, such as the use of alcoholic solutions of tetramethylammonium hydroxide (TMAH) allows the solubilization of small quantities of several tissues at low temperature. However, this method requires at least two hours for the decomposition of 1 g of tissue (12). The use of a microwave oven permits a very fast digestion of biological samples, which decreases the time required until to be one or several minutes and allows rapid decomposition methods in batch susceptible to automatize.

FUNDAMENTAL OF THE MICROWAVE OVEN DIGESTION

Microwave radiation consists of alternating electric and magnetic fields. The frecuency of the microwaves are of the order of gigahertzs, having the most commonly used ovens at 2.45 GHz generator.

In a microwave oven the radiation generated in the magnetron is guided to the oven cavity where it is absorbed by the molecules of samples and solvents.

Only the polar molecules absorb the microwave energy (13,14). In the electric field generated by the radiation, the dipole moment of polar molecules attemps to align with the field and, due to the rapid changes in the electric field directions (2.45×10^9 times per second) produced when the microwave passes through a polar molecule, a series of aligning and realigning takes place. So the frictionel effects produces the heating of the sample. In consequence the polarity has an important role in the temperature attempted for a substance in a microwave oven and, for example, it has been noticed (15) that the temperature of 100 mL of water increases 70°C in 80 seconds while the temperature of 100 mL of CCl_4 only increases 2°C in 140 seconds. For the same volume of methyl palmitate an increase of 30°C is obtained in 140 seconds.

The heating of a sample in a microwave oven increases against the power supplied and the irradiation time and decreases with the mass of and, in general, with the load of the oven (16,17).

Owing to the influence of the dipole character of the molecules, in the absorption of the microwaves it is necessary to carry out the digestion samples in the presence of an acid or mixture of acids. The dielectric liquids heated in contact with the dielectric particles will generate the heating of the surface molecules of a sample. This can create a large thermal convenction currents which agitate and destroy the surface layers and this fresh surface is exposed to the acid solutions providing a very efficient sample dissolution. So the heating in a microwave oven is not produced by an external source of heat but by interaction between the microwave radiation and the sample molecules. The rapid uptake of radiation throughout the sample solution eliminates the heat conduction stage and the sample-acid mixture acts as a lossy dielectric in which an internal heating is produced as a consequence of the mechanical stress induced by the alignment of the polarized molecules.

The microwave do not penetrate the metallic containers and increases slighly the temperature of pyrex or plastic containers. The water molecules are the most absorbing species of the microwaves energy.

MODIFICATION OF MICROWAVE OVENS FOR LABORATORY USE

Microwave ovens have found widespread use in commercial kitchness and in homes. These can defrost, heat and cook foods very fast and, if they are used properly, do not constitute hazards to operators.

However, when the use of the commercial available ovens for laboratory is wanted, it is necessary to consider some points to avoid the magnetron damage and the potential risks of operation.

It is important to note that when samples are treated in an oven the load of this decreases as samples approaches complete dryness, and when the oven losses its loads the energy that still being supplied is not absorbed, the magnetron tends to arc and destroy itself (18). Because of this, it is suitable to place a small beaker containing water to prevent that magnetron operate dry.

A potential risk exists for an explosion when organic compounds are heated and vapours are produced around electrical equipment (15). On the other hand, highly acidic materials should not be dried in a microwave oven, since the acid fumes mat attack the cavity walls (17). Thus, to carry out wet digestions in a microwave oven it is important to assure a convenient system to avoid the stay of acid or solvent vapours in the cavity.

To carry out the evacuation of the acid fumes formed during the wet digestion, the most general strategie proposed in the bibliography consist of introducing the samples inside a closed recipient, connected with the exterior of the oven.

Abu Samra and colleagues (19) have used a plexiglas box, Barrett et al (20) a pyrex rectangular chromatographic jar and Tsukada et al (21) a heat-resistant glass vessel with a 1 cm diameter hole. These recipients allow a protection of the interior cavity of the oven and also to accommodate an exhaust pipe which is connected with a water aspirator or with a fumes trap.

Pougnet et al (22) have proposed the use of a digestion vessel assembly integrated by a dessicator (in which erlenmeyer flasks containing the samples were placed) connected with an erlenmeyer containing KOH (in which acid fumes are neutralized) and a beaker containing water. This assembly is introduced into the oven. The presence of an amount of water avoids the magnetron damage and prevents the acid attack of the oven. In this latter case it is not necessary to modify the commercially available oven.

In general it seems preferable to extract the acid fumes but Lamothe and col. (23) recommended to purge the oven with compressed air during the heating cycle of samples in pressurized vessels introduced in a tupperware bottle rack. The purge is assured by different plastic tubes inserted through the ventilation holes of the side of the oven.

When the samples digestion is carried out under pression the use of a closed container seems a sufficient caution to avoid the oven damage (24). In this case, pressurized vessels allow higher sample-solution temperature, however acid vapours are transparents to microwaves and only the liquid phase continues to absorb the energy.

The choice of both appropiate vials and reactors is also other problem to take into account.

Metallic materials are impervious to microwave and because of this,

they can not be used. However, teflon, pyrex glass and some plastics such as polycarbonate and polyethylene are suitable as vessels in the samples attack. Moreover, teflon and pyrex glass can be used in the treatment under pressure. However, pyrex vessels gain in heat quickly and so teflon vessels are used more frequentely.

Plastic materials could be recommended for use as digestion vessels in microwave oven. However, polystyrene becomes unstable above 70°C and cannot be used.

Matthes et al (26) and Lamothe et al (23) have been recommended the use of polycabonate pressurized vessels, because polycarbonate is transparent to microwave and it is a high tenside strength and acid resistant plastic with a melting point of 135°C. However, when a mixture of strong mineral acids is used to dissolve samples in a microwave oven yellowing of the plastic and gradual frosting of the bottle walls are normal after four runs.

USE OF MICROWAVE OVEN DIGESTION IN BATCH

The microwave ovens have been used to dry biological tissues (27) and inorganic samples (17) and a greater speed and cost economy than thermal ovens are gotten.

When biological samples are dried into a microwave oven, the highest temperature which samples get is 95-100°C if oven is operating at minimum power for 15 minutes. However, in foods digestion using HNO_3 and H_2SO_4 a temperature of 120°C is gotten in 5 minutes (21). That is why microwave oven is appropiate for both dry up and digestion of samples.

Since the publication in 1975 of the work of Abu Samra et al (19), several authors have applied this technique to acid digestion of samples. Table I summarize the papers published until now. Matrix, elements determined, technique used and digestion conditions are indicated.

It can be observed that in most cases the works look at the determination of elements by atomic absorption spectroscopy and plasma emission and all kind of elements have been determined (transition metals, alkalis and alkaline earths, volatile elements and other of small solubility, phosphorus and sulphur, etcetera).

The geater part of the samples are biological tissues (19, 22,24,25), foods (21,30,31) and plants (29) coal and sediment samples (24) also have been digested.

Commercial ovens with power of the order of 650 W are used in all the works published.

The weigh of the sample used ranges from 20 mg to 10 g, being between 200 and 500 mg the more frequently amount used.

Digestion time is generally in order of a few minutes, but some authors recommend treatments of 30 minutes or yet more. However, it seems that smaller times are enough to assure total recovery of elements to be determined.

Losses of the elements during the digestion process generally do not happen (31). Using standard solutions it may be proved that the greater part of elements do not volatilize during their digestion (24, 25, 28). Only a losse of 26% of chromium and 20% of lead is found when standard solutions are treated by 10 mL of acqua regia and 5 mL of HF for 5 minutes at 625 W (24).

TABLE I Use of microwave oven wet digestion in batch

MATRIX	ELEMENTS DETERMINED	TECHNIQUE	MICROWAVE OVEN	DIGESTION VESSEL	DIGESTION CONDITIONS	REFERENCE
BIOLOGICAL SAMPLES	Pb,Cu,Zn,As,Se,Co, Cr, Ni	Neutron activation,AAS	MWS	125 mL flasks	0,5g with 10 mL HNO_3.$HClO_4$ (3 minutes) 1-5mg with 100 mL HNO_3 and 100 µL H_2O_2 (15 minutes)	(19)
BIOLOGICAL SAMPLES COAL, SEDIMENTS	Al,Ba,Be,Ca,Co,Cr, Cu,Fe,K,Li,Mg,Mn, Na,Ni,P,Si,Sr,Ti, V,Zn,As,S	ICP	Sears Kenmore 625W	teflon or polycarbonate beakers	200mg with 5 mL HNO_3.HCl and 2 mL HF (3 minutes at 625W), add H_3BO_3	(24)
BIOLOGICAL SAMPLES ZOOPLANCKTON	Fe,Cu,Cd	Electrothermal AAS	Penney RE 705 TC 700W	polyethylene or polypropylene cups	with 100 µL HNO_3, 5 minutes at 700 W	(25)
BIOLOGICAL SAMPLES AND PLANTS	Ca,Mg,P,Al,Fe,Mn, Zn,Cu,Na,K,Sr	ICP	SYARP R 6950 E 650 W	erlenmeyer flasks	250 mg with 1mL $HClO_4$ and 2 mL HNO_3, 3 minutes at 650W	(22)
URINARY CALCULI	Ca,Mg,P,Al,Cu,Fe, K,Li,Mn,Mo,Na,Pb, S,Sr,Zn	ICP	SHARP R 6950 E 650 W	50 mL erlenmeyer flasks	250 mg with 2mL HNO_3 and 1 mL $HClO_4$, 3 minutes at 650W	(28)
PLANTS	Ba,Ca,Mg,Mn,P,K, Na,S,Zn	ICP	MDS 81 600 W	100 mL Kohlrausch flasks	0,5g with 10mL HNO_3, 30 minutes at 540 W , with 10 mL HNO_3 and 1 mL H_2O_2, 30 minutes at 540 W	(29)
FOODS	As	Electrothermal AAS	TOSHIBA ER 500 1kW	erlenmeyer flask	0.1-2 g with 4mL Ni sol. 15-100mL HNO_3 and 0.5mL H_2SO_4, 10-30 minutes.	(21)
FOODS	Zn,Cu	Electrothermal and flame AAS			0.5 g (dry) with 10-40 mL HNO_3 and 5-15 mL $HClO_4$, 107-250 minutes.	(30)
FOODS	Zn,Cu,Mn,Pb,Cd	AAS	TOSHIBA ER 500 1 kW	erlenmeyer flasks	20 mg-1 g with 15-75 mL HNO_3 and 0.1-0.5 mL H_2SO_4 (10-30 min) at 1000 W	(31)
FISH	Pb,Hg	Could vapour AAS	600 W.	125 mL erlenmeyer	5-15 mg with 300 µL HNO_3.$HClO_4$.H_2SO_4 (10 minutes) 5-10 g with 20 mL HNO_3.H_2SO_4 (3 minutes)	(20)

However, in the actual samples metallic elements are not present in pure ionic form but as different compounds and results published on the analysis of certified samples have been demonstrated that relative volatile elements such as arsenic, selenium, chromium and mercury are not lost from the biological materials during the microwave digestion (19,20).

Low recovery values have been obtained in the determination of iron in plants and however very good results are obtained for bovine liver (22). This fact has been interpreted in basis of the formation of volatile reaction products between this element with other components of the samples digested or on the retention on the small residue obtained when microwave digestion is carried out.

Recoveries of the zinc, copper, manganese, lead and cadmiun in the analysis of foods when samples are digested in a microwave oven with HNO_3 and H_2SO_4 during 10 to 30 minutes are between 84.3 and 113 per cent with a coefficient of variation of 0.1 to 6 per cent (31).

The determination of highly volatile elements such as lead and mercury in fish samples (20) and arsenic in foods (21) could be carried out by previous digestion with mineral acids at high power level during 10 to 30 minutes. A significant decrease in the blank level for lead has been observed, due to the decrease in the exposure time of the sample to ambient having not found any loss of lead and mercury during the microwave digestion. In the determination of arsenic the results obtained for the treatment of different arsenic (III) and arsenic (V) compounds in a microwave oven during 30 minutes reveals that arsenic is not lost from any type of compounds. However, for the digestion of actual samples the addition of 400 mg of nickel is recommended to prevent the formation of volatile compounds.

The treatment of mineral samples is not so easy as the biological samples or foods.

For the determination of metallic elements in coal samples low results are obtained for barium, chromium and titanium, due to the presence of chromite or titanium minerals in the sample, and for the determination of a series of elements in geological samples low values are obtained for chromium using a mixture of acqua regia with HF to extract the elements (24), in the determination of magnesium and titanium in the analysis of certified samples, low results are obtained likewise.

Other application of microwave digestion is the sulfur removal from coal and fly ash by extraction of the pyrite sulfur. However, organic compounds such as benzyldisulfide are not affected by the microwave heating (23).

To prevent losses of the elements to be determined and to obtain more strong conditions, the wet digestion of samples in a microwave oven could be carried out under pressure. In Table II the applications of this technique are summarized.

In the digestion of biological samples the use of pressurized vessels allows the complete recuperation of metallic elements in 1 minute at 700 W with a mixture of H_2SO_4 and HNO_3 (32). Other matrices more acid resistants could be attacked and elements be extracted.

Sample weighed is of the order of 100-1000 mg, the volume of the reactors is about 60 - 250 mL, and material of these is usually teflon. Digestion time required is between 1 to 10 minutes, except for some geological samples where Fischer (33) recommends use of low power and long time (even 5 hours).

When pressurized vessels are used the recovery of chromium, and those of the boron, lead or silicon are very good. However, aluminium recovery from aluminosilicate minerals, and silicium recovery, from samples containing quartz, are very bad. The recovery of the latter element increasing when the particle size of the sample decreases (33). In general minerals as quartz, corundum chromite, zircon and rutile are not dissolved by a mixture of HNO_3, HCl and HF working in a microwave oven with sealed polycarbonate bottles during 15 minutes at 650 W.

Other authors prove that microwave oven digestion is applicable for

TABLE II Microwave oven digestion under pression for the analysis in batch

MATRIX	ELEMENTS DETERMINED	TECHNIQUE	MICROWAVE OVEN	DIGESTION VESSEL	DIGESTION CONDITIONS	REFERENCE
BIOLOGICAL SAMPLES PLANTS	Cu,Fe,Zn,Cd,Cr,Pb	AAS	700 W	60 mL pressurized teflon vessels	250 mg with 1.5 mL H_2SO_4 and 1.5 mL HNO_3, 60 seconds at 700 W	(32)
GEOLOGICAL SAMPLES	Si,Al,Fe,Mg,Ca,Na,K, Ti,P,Mn,Ag,As,Au, B,Ba,Be,Bi,Cd,Ce,Co, Cr,Cu,Ga,Ge,In,La,Li, Mo,Nb,Ni,Pb,Sb,Sc,Sn, Sr,Tl,V,W,Y,Yb,Zn,Zr	ICP	650 W	250 mL polycarbonate pressurized bottels	0.1 g with 2mL HNO_3 and 5mL 7:3HCl:HF,2.5 minutes 650 W, add H_3BO_3, 10 minutes at 650 W	(23)
GEOLOGICAL SAMPLES	U,Pb	isotopic solution	MDS 81 600 W	60 mL teflon closed vessels in a 300 mL teflon vessel	500mg with 1mL HNO_3,3 mL HF, 0,5 mL $HClO_4$ and 1.2 mL isotopic sol., 5 minutes at 15%P, 15 minutes at 23% P; add 4 mL HF, 1-5 hours at 23% P	(33)
SULFIDE MINERAL	Ni,Cu	flame AAS	TOSHIBA ER 800 BTC 720 W	150 mL closed teflon vessel	0.5-1 g with 1.5 g $KClO_3$ 10 mL HNO_3 and 5 mL HF 3 minutes at 477 W	(34)
STEEL	Al,Mn,P,Cu,Ni,Cr,V, Mo,Sn,Si,Ti,As,W	DCP	SEARS 625 W	60 mL closed teflon	1 g with 3 mL HNO_3, 3 mL HCl and 2 mL HF, 80 seconds at 625 W	(35)

the determination of nickel and copper in minerals (34) and for lead and uranium in some geologic matrixes including less stable zircons (33).

In summary, digestion by microwave oven seems a general procedure for preparation of biological samples for theirs analysis and it can be use for other matrixes. However, there are very few applications described. In particular, we do not have found any application to the quantitative determination of anions.

THE USE OF MICROWAVE OVEN DIGESTION IN FLOW INJECTION ANALYSIS

The digestion of biological samples for their analysis by flow injection and atomic spectroscopy could be carried out both in batch and in the flow system.

They are a limited number of precedings on the use of this digestion system in flow injection analysis.

For the digestion of samples previously to their injection in the carrier flow, methods are proposed for the analysis of iron and copper in powdered milks (36) and lead in hair (37-39).

3 mL of milk are digested by 1 mL of HNO_3 and 1 mL of water during 5 minutes, using a power of 700 W and pyrex glass vials of 50 mL. After digestion , Triton X 100 is added to solubilize the remained milk fat and 100 µL are injected in the carrier stream.

In the analysis of hair samples, 100 mg of sample can be digested by 2 mL of a 1:1 HNO_3: $HClO_4$ mixture during 15 minutes, using a 700 W power. A system consisting of a serie of test tubes closed in a glass container coupled to a water pump which can be incorporated to a manifold flow injection analysis by a system of selective aspiration (Fig. 1) has

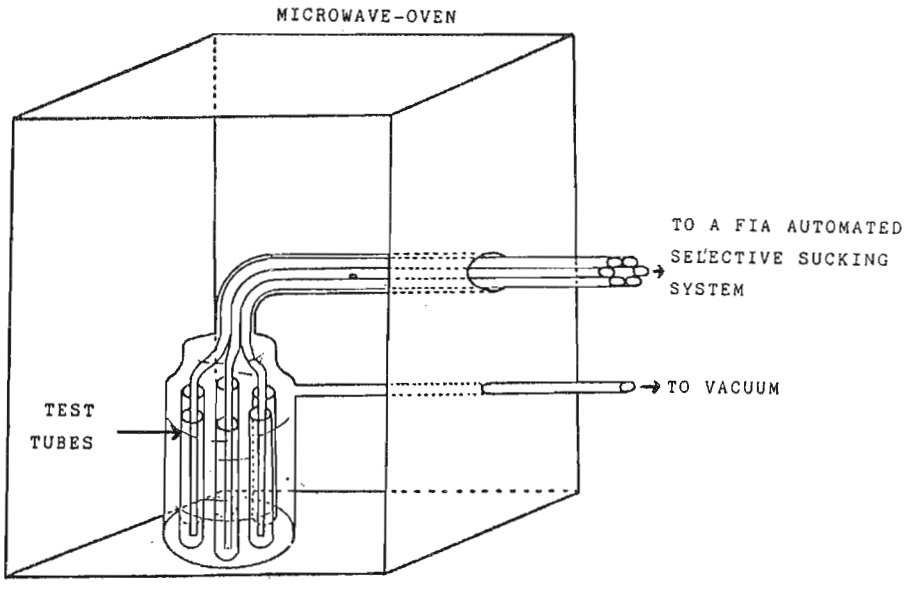

Fig. 1 Microwave-oven assembly for coupled to a FIA/spectrometric system designed for the analysis of biological tissues

been proposed.

The brief digestion time consuming in the sample attack by a microwave oven allows the automation of the process. So, 1 g of tissue can be digested in 5 minutes at 200 W, using 10 mL of HNO_3 10M, and the total time consuming in the analysis of the sample is about 8 minutes (40). Figure 2 shows the configuration used in the determination of cadmium and zinc in kidney and liver tissue by microwave acid digestion, flow injection analysis and atomic absorption spectroscopy.

To carry out the digestion, test tubes with samples and acid are placed in the pyrex jar. Then, it is covered and placed inside the microwave oven. The oven is operated at low power (200 W) for 2 minutes (during the digestion time valve 9 is opened to assure the evacuation of the acid fumes and all valves 4 are closed (Figure 2).

To aspirate one sample from the digestion assembly the corresponding valve 4 and valves 2 and 3 are opened. After the aspiration of the sample in the collector tube, valves 2 and 3 are closed, while valves 5, 7 and 8 are opened. The digested sample is pumped into the flowing sample collector and therefore within the closed flow system (valves 7 and 8 having previously opened).

A plug of digested sample is introduced into the carrier stream by the injection valve, and at least 5 injections of each sample are carrying out.

To wash the system after the analysis of each sample, the tygon tube is disconnected after valve 7 from the flowing sample collector, valves 4

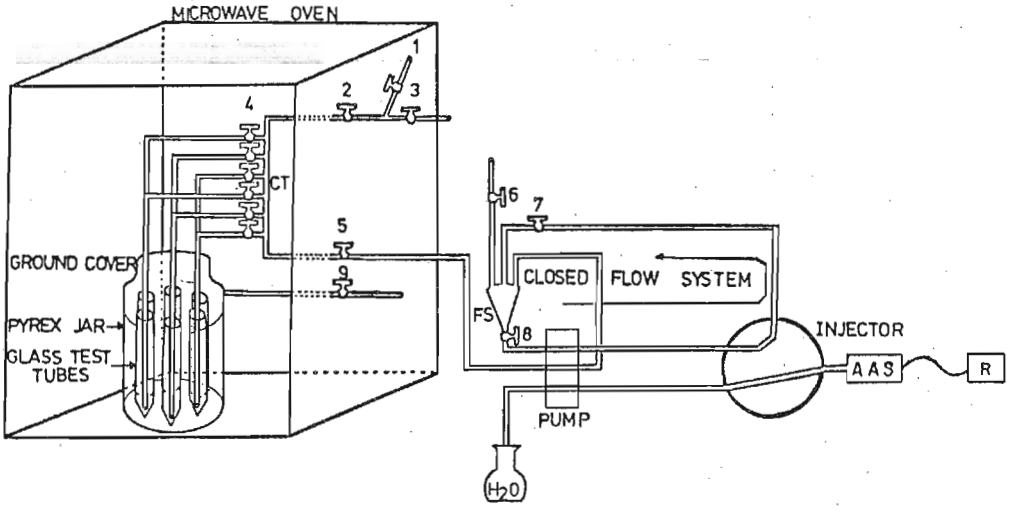

Fig. 2 Microwave-oven assembly used in the determination of cadmium and zinc in kidney and liver tissue by acid digestion, flow injection analysis and atomic absorption spectroscopy. CT = collector tube; FS = flowing sample collector; valves 1 - 8 are operated as described in the text. (Reproduced with permission of Elsevier Sci. Pu. (40)).

and 3 are closed and valves 1 and 2 are opened to introduce 10 mL of a washing solution through the collector tube into the flowing sample collector and within the closed flow system. The washing solution is pumped out of the flow system and this operation is repeated 3 times before the analysis of one other sample.

In the analysis of liquid samples without much digestion problems, such as blood samples, a simple design like Figure 3 shows can be used. To Assure an adequated stay time of the sample into the microwave oven for to get the total digestion, modifications in the length of the tube introduced into the microwave oven and the carrier flow rate can be made. So, 100 µL of blood can be digested by 100 µL of a mixture of HCl 0.3 M and HNO_3 0.4 M in 25 seconds, using a tube of 50 cm of length with 0.5 cm of interior diameter and flow of 1.8 mL/min. Then, a stay time about 15 - 26 seconds is assured (41).

This last system can be applied to digestion of solid samples by formation of a slurry from the sample dried and powdered, using mineral acids and operating in continuous.

Just as it is showed in the reviewed works, the use of a microwave oven allows a rapid digestion of the biological samples which can be automated. Therefore, this is the only digestion procedure which could be employed together with a conventional manifold in flow injection analysis.

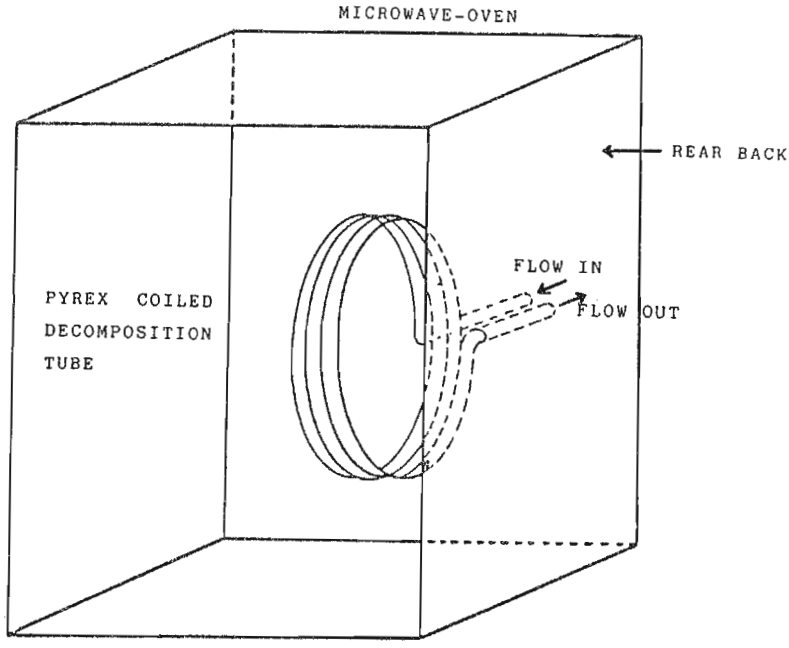

Fig. 3 Schematic representation of a microwave-oven/FIA assembly designed for the analysis of biological fluids

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