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Review

Focused-microwave-assisted strategies for sample preparation☆

Joaquim A. Nóbrega[a,*], Lilian C. Trevizan[a], Geórgia C.L. Araújo[a,b], Ana Rita A. Nogueira[b]

[a]*Grupo de Análise Instrumental Aplicada, Departamento de Química, Universidade Federal de São Carlos, Caixa Postal 676, São Carlos, SP 13560-970, Brazil*

[b]*Embrapa Pecuária Sudeste, São Carlos, SP, Brazil*

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Abstract

In this work a general discussion is presented about extraction and digestion procedures, assisted by focused-microwave radiation. Applications involving inorganic, organic, and organometallic analytes in different types of samples are presented, taking into account recent literature data. The main advantages of using focused-microwave radiation are highlighted, such as safety, versatility, control of microwave energy released to the sample, and programmed addition of solutions. All these features can be applied properly in sample preparation for speciation analysis. New routes of development are discussed considering partial digestion by acid-vapor and gradual addition of a liquid sample to hot concentrated acids. Some preliminary results using these strategies are presented to demonstrate their potentiality.

Keywords: Focused-microwave; Digestion; Extraction; Vapor-phase digestion

1. Introduction

One of the milestones in the development of sample preparation strategies has been the evolution of microwave technologies, mainly after the 1980s [1]. Nowadays this technology is being applied not only in analytical chemistry but also in organic synthesis, inorganic reactions, preparation of catalysts, and other fields [2].

Microwave ovens have successfully found a road out of the kitchen [3] and MEC was recently proposed as a new acronym, standing for microwave-enhanced chemistry [4]. Even considering that microwave technology has improved some traditional operations in chemistry, there is still a long route ahead since only some 10% of the laboratories in the world are equipped with laboratory-designed microwave ovens [5].

Most experiments are carried out using cavity- or focused-microwave systems. Usually they are referred to as closed- or open-vessel systems, respectively, but this terminology is not correct as the so-called open vessels in focused systems are not completely open, and as it is also possible to

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*Corresponding author. Fax: +55-16-260-8350.

E-mail address: djan@terra.com.br (J.A. Nóbrega).

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operate closed vessels in a focused microwave, as will be shown later. On the other hand, the use of domestic microwave ovens in laboratory experiments must be strongly discouraged taking into account safety reasons and performance.

The aim of this review is to highlight applications based on focused-microwave systems. Although less frequently used, when compared to cavity-microwave ovens, there are analytical procedures that could be better carried out using focused systems. In most situations that require the digestion of large amounts of organic material, which will result in the generation of a huge amount of gas, or when multiple additions of concentrated acids is required during digestion, the use of a focused-microwave system is of advantage.

It is not the purpose of this paper to discuss applications based on cavity-microwave system or present a comparison between this and the focused-microwave oven. The performance of this former system is well discussed by Kingston and Haswell [2], and a wealth of applications was reviewed by Smith and Arsenault [6].

The literature review on focused-microwave assisted sample preparation presented here is not comprehensive. The authors wanted to concentrate on papers published after the review of Mermet in a chapter dedicated to focused-microwave-assisted reactions in Ref. [2]. Hence, most papers discussed here were published after 1996.

The main characteristics of commercially available focused-microwave technology are:

- Safety due to operation at atmospheric pressure;
- Handling of large samples that can generate a huge amount of gas mainly when working with organic materials;
- Use of various types of materials to construct reaction vessels, such as borosilicate glass, quartz, and PTFE;
- Programmable addition of reagents (or samples as it will be discussed later on) at any time during the digestion, which allows sequential acid attack;
- Low-power focused-microwave field can be employed either to accelerate leaching of organometallic species without affecting carbon–metal bonds, or to extract organic compounds (specific examples will be discussed). The focused nature of the microwave energy confers high efficiency and avoids the application of high power;
- Multiple methods for different samples can be simultaneously applied owing to the possibility of operating each reaction vessel independently.

This last aspect can be better explained considering that commercial units are available that have 2 or 6 reaction vessels, and each one can be operated independently due to autonomous control of both reagent addition and temperature. In the past, one equipment with only one reaction vessel, and another one with six cavities and six magnetrons were available. However, in spite of some advantages, both were discontinued due to merging of companies. The equipment with one magnetron for each reaction vessel allowed a better distribution of microwave radiation, however it was more expensive. The distribution of microwave radiation is controlled using a waveguide and slots. Infrared sensors measure the temperature in each cavity and, based on a feedback system, interact with the magnetron to adjust the microwave radiation incidence in each vessel.

The main difficulties of focused-microwave are the distribution of radiation between all cavities, when they are simultaneously operated [7], and the elevated acid concentration of the digestates. The former aspect is not so critical because the control of the release of microwave radiation to each cavity is based on the temperature of the reaction vessel. On the other hand, the acid concentration is high, because usually a large volume of concentrated sulfuric acid is required at atmospheric pressure operation in order to reach high temperatures. This aspect can be overcome by changing the usual procedure that recommends the programmed addition of concentrated acids to the sample. As will be discussed later, concentrated acids can be heated by microwave energy, and sample aliquots can be gradually added to this aggressive medium.

In the following sections applications of focused-microwave will be discussed for extracting organic and inorganic compounds in speciation

Table 1
Characteristics of the focused-microwave ovens

System	Manufacturer[a]	Maximum applied power (W)	Maximum sample size (g)	Flask volume (ml)
A301	Prolabo	200	2	100
M301	Prolabo	200	1	100
M401	Prolabo	300	10	250
M350	Prolabo	300	10	250
Microdigest 3.6	Prolabo	250	–	250
Soxwave 100	Prolabo	300	–	–
STAR 2	CEM	630	5	250
STAR 6	CEM	950	5	250
7400 Spex	Spex	–	–	250

[a] Prolabo, Paris, France; CEM, Matthews, NC, USA; Spex, Metuchen, NJ, USA.

analysis and for digesting samples. Finally, two new strategies, based on acid-vapor extraction and sample addition to hot reagents will be presented.

2. Typical applications

All microwave-assisted procedures reviewed in this work used one of the equipments described in Table 1. These microwave ovens can be operated using an applied power in the range of 200–950 W. The maximum volume of the reaction vessel is 250 ml, allowing digestion of up to 10 g of sample, or even more, depending on the adopted procedure and the sample characteristics.

2.1. Extraction procedures

Speciation analysis has become an important aspect in environmental and analytical chemistry [8]. Speciation was defined by IUPAC as the analytical activities of identifying and/or measuring the quantities of one or more individual chemical species in a sample [9].

A successful leaching (or extraction) procedure prior to speciation analysis requires the preservation of all original compounds, such as organometallic compounds. These compounds can be extracted using focused-microwave systems that allow a careful control of the energy delivered to the reaction medium. It is imperative to control both the power applied and the exposure time to avoid the degradation of any compound as well as the formation of artifacts. The integration of dissolution, extraction, and derivatization in a focused-microwave system may bring new dimensions in sample preparation for speciation analysis [10].

Organic, organometallic, and inorganic analytes can be determined in environmental, industrial residues, and clinical samples after a preliminary step of sample preparation based on the use of focused-microwave system. For example, this system can be used instead of conventional Soxhlet procedures, allowing a fast, simple, and reliable sample preparation, using lower volumes of organic solvents, and consequently, generation of less hazardous residues that are expensive to discard.

2.1.1. Inorganic and organometallic analytes

Focused-microwaves are extensively used for sample preparation before the determination of inorganic and organometallic analytes. Extraction procedures can be carried out either on-line or off-line as can be seen in Table 2.

On-line procedures were implemented by using flow-injection to hyphenate focused-microwave ovens with chromatographic and spectroanalytical techniques [12–18,24,27]. Se speciation analysis in urine can for example be performed in an on-line microwave-HPLC system, operated at low microwave radiation power. The extracted forms of selenium were determined using hydride generation atomic absorption spectrometry [13].

Table 2 also shows that most papers dealt with sample preparation as a preliminary step in speci-

Table 2
Focused-microwave-assisted extraction of inorganic and organometallic compounds (1996–2002)

Sample	Element(s)	Sample size	Microwave system	MW conditions	Technique for determination	Reference
Biological samples	Fe and Co	30 mg	STAR 6	Vapor-phase partial digestion: 150 μl 30% v v^{-1} H_2O_2 or water Ramp time: 4 min, 115 °C, 6–10 min (Co) Ramp time: 4 min, 115 °C, 15–60 min (Fe)	ETAAS	[11]
Human urine	Se speciation	50 μl (on-line)	M301	Mobile phase A: 0.01 mol l^{-1} ammonium acetate buffer solution (pH 4) in 0.5% v v^{-1} methanol + 10^{-5} mol l^{-1} didodecyldimethy-lammonium bromide (DDAB) Mobile phase B: 0.1 mol l^{-1} ammonium acetate solution (Ph 6.5) with 0.5% v v^{-1} methanol + 10^{-5} mol l^{-1} DDAB 47% v v^{-1} HBr + 1.5×10^{-2} mol l^{-1} $KBrO_3$ 15% power, 1 min	HPLC-HG-AAS or HPLC-ICP-MS	[12,13]
Human urine	Se speciation	100 μl (on-line)	M301	0.1 mol l^{-1} ammonium acetate buffer solution (pH 4.5) 48% v v^{-1} HBr + 1.5×10^{-2} mol l^{-1} $KBrO_3$ 15% power, 1 min	HPLC-HG-AAS	[14]
Citric fruit juice and geothermal waters	Se(IV) and Se(VI)	0.5 ml (on-line)	M301	3.0 ml min^{-1} 0.5% w v^{-1} $NaBH_4$ 40% power—Se(IV) 95% power—Se(VI)	HG-AAS	[15]
Spiked tap water	Se speciation	500 μl (on-line)	M301	47% v v^{-1} HBr + 1.5×10^{-2} mol l^{-1} $KBrO_3$ + H_2O 15% power, 1 min	HG-AAS	[16]
Urine	Se speciation	100 μl (on-line)	M301	3% w v^{-1} $K_2S_2O_8$ + 3% w v^{-1} NaOH 10 mol l^{-1} HCl 90 W	HG-AAS	[17]
Spiked water	Se speciation	On-line	M301	HBr conc. 1.5×10^{-2} mol l^{-1} $KBrO_3$ 15% power, 1 min	HPLC-HG-AAS	[18]

Table 2 *(Continued)*

Sample	Element(s)	Sample size	Microwave system	MW conditions	Technique for determination	Reference
Biological tissues	Hg speciation	0.1–0.5 g	A301	5 ml extractant solution (tetramethyl ammonium or methanolic KOH solution) 20–80% power, 1–4 min	HPLC-ICP-MS	[19]
Fish	Hg speciation	0.2 g	M301	5 ml tetramethyl ammonium hydroxide 20 W, 20 min	MIP-AES	[20]
Mussel	As speciation	0.5 g	A301	20 ml solution (methanol + water 1:1 v v^{-1}) (a) 40 W, 5 min (b) 50 W, 5 min	HPLC-HG-ICP-MS	(a) [21] (b) [22]
Soil and sediments	As speciation	0.3 g	M301	50 ml 2 mol l^{-1} H_3PO_4 20–30% power, 10–20 min	HPLC-ICP-MS	[23]
Solutions of organoarsenic species	As speciation	On-line	M401	2% v v^{-1} L-cysteine + 0.2 mol l^{-1} HNO_3	HG-ICP-MS	[24]
Sediments (IAEA 356 and CRM 580)	Hg speciation	1 g	A301	10 ml 2.0 mol l^{-1} HNO_3 60 W, 3 min	QFAAS	[25]
Sediments (BCR S19 and BCR 580)	Hg speciation	1–2 g	A301	10 ml HNO_3 or HCl 60 W, 3 min	QFAAS	[26]
Diatomaceous earth	Hg	0.1 g (on-line)	M 301	5×10^{-3} mol l^{-1} HCl, 5% w v^{-1} $SnCl_2$ in 15% v v^{-1} HCl 30 W, 1 min	AFS	[27]
Sediment	Sn speciation	0.25 g	STAR 2	10 ml extraction solution (2.5×10^{-3} mol l^{-1} sodium 1-pentanesulfonate, 5% v v^{-1} acetic acid and 90% v v^{-1} methanol in water) Ramp time: 1 min, 60 °C, 3 min	HPLC-ICP-MS	[28]
Biological materials	Sn speciation	0.1–0.2 g	A301	100 μl Pr_3SnCl + 5 ml acetic acid + 1 ml nonane + 3 ml 2% w v^{-1} $NaBEt_4$ 40 W, 3 min	FPD/AAS	[29]

Table 2 *(Continued)*

Sample	Element(s)	Sample size	Microwave system	MW conditions	Technique for determination	Reference
Sediments and biomaterials	Sn speciation	0.1–0.2 g	A301	Sediment: 10 ml 50% v v^{-1} acetic acid 60 W, 3 min Biomaterials: 5 ml 25% v v^{-1} tetramethyl ammonium hydroxide 60 W, 3 min	ETAAS	[30]
Sediments and biomaterials	Sn speciation	0.1–0.2 g	A301	Sediments: 100 μl Pr_3SnCl + 100 ml 50% v v^{-1} acetic acid 60 W, 3 min Biomaterials: 5 ml 25% v v^{-1} tetramethyl ammonium hydroxide 60 W, 3 min	MIP-AES	[31]
Coal	Hg, As, and Se	2 g	Soxwave 100	50 ml HNO_3 65% power, 3 min	AFS	[32]
Tea	Al, Ca, Mg and Mn	0.1–0.5 g	STAR 6	20 ml 1% v v^{-1} HNO_3 Ramp time: 2 min, 95 °C, 3 min	FAAS and ICP-OES	[33]
Sediments and biotissues	Hg speciation	Sediments: 1 g Biotissues: 0.1–0.5 g	A301	Sediments: 10 ml HNO_3 (2 or 6 mol l^{-1}) 60 W, 3 min Biotissues: 5 ml 25% v v^{-1} tetramethyl ammonium hydroxide 60 W, 2 min	GC-QFAAS	[34]
Edible mushroom	As speciation	0.1 g	MX350	5 ml of methanol + water (1 + 9 v v^{-1}) 75 W, 8 min	HPLC-ICP-MS	[35]

AFS, atomic fluorescence spectroscopy; ETAAS, electrothermal atomic absorption spectroscopy; FDP, flame photometric detection; GC, gas chromatography; HG-AAS, hydride generation atomic absorption spectroscopy; HPLC, high performance liquid chromatography; CP-MS, inductively coupled plasma mass spectrometry; ICP-OES, inductively coupled plasma optical emission spectroscopy; MIP-AES, microwave induced plasma atomic emission spectroscopy; QFAAS, quartz furnace atomic absorption spectroscopy.

ation analysis. However, there are also a few papers that proposed procedures for the quantitative extraction of analytes without a complete destruction of the organic matrix [11,27,32,33]. The main advantage of these procedures is the better control of the energy transferred to the reaction medium using microwave-assisted heating.

Most papers listed in Table 2 involve biological materials, such as urine, soils, and sediments. In all cases the sample solution is microwave-heated, using a low power of 20–90 W, to reach low temperatures, 60–115 °C. More than 90% of the cited papers dealt with the speciation analysis of As, Hg, Se and Sn. The significantly different toxicity of various compounds of these elements can explain the focus on them. Extraction of As compounds from mussel samples, for example, can be easily performed in a focused-microwave oven using a mixture of methanol and water ($1+1$ v v^{-1}) and applying a 50-W power for 5 min [22].

Considering all the previously highlighted advantages of focused-microwave ovens, the outstanding characteristic that should be emphasized when considering most papers included in Table 2, is the possibility of sample preparation for speciation analysis without degradation of labile compounds. It should also be mentioned that considering all the recent evolution of hyphenated techniques, it seems that some strategies present enough sensitivity for the accurate determination of trace elements and their compounds, but the sample preparation step still needs further progress to improve the reliability of the results. Focused-microwave-assisted sample preparation could be a suitable alternative to overcome these difficulties in speciation analysis.

2.1.2. Organic analytes

Representative papers dealing with extraction of organic compounds published during the review period are compiled in Table 3. The most frequently investigated organic compounds are polynuclear aromatic hydrocarbons (PAHs), organochlorine pesticides (OCPs), polychlorine pesticides (PCBs), and alkanes [36–38].

Several papers presented in the literature showed that microwave-assisted heating in closed or open vessels reduced drastically, solvent consumption and leaching times. In addition, recoveries of analytes increased when using focused-microwave heating compared to other extraction techniques, such as Soxhlet, using conventional heating, sonication, or supercritical fluid extraction [8]. However, the formation of artifacts by thermal degradation should be investigated in spite of the suitable control of energy delivered by the magnetron. As previously mentioned, focused-microwave heating can be applied to carry out simultaneous extractions using multi-vessel systems. Vessels can be built using borosilicate glass, quartz, or PTFE. Microwave heating can also be applied as a clean-up procedure before chromatographic analysis [39,40].

The most critical parameters for method optimization are chemical characteristics, volume of solvent, applied power, particle size distribution, extraction time, and sample moisture [41,43,45,46,48]. An experimental design was applied for establishing the most critical parameters [45,49]. Moisture was pointed out as a determinant factor in analyte recovery [41,43,45,46,48]. According to García-Ayuso and Luque de Castro [45], the strong absorption of microwave energy by water dipole molecules increases sample temperature, causing both water evaporation and rupture of the analyte–matrix bonds.

Letellier and Budzinski [46] showed that samples with smaller particle sizes resulted in lower relative standard deviations. Thus, particle size distribution also affects analyte recoveries and a better contact between sample and solvent results in an efficient diffusion of the analyte out of the matrix [45,46,48].

Focused-microwave techniques have also been applied to promote derivatization reactions, such as ethylation of organotin compounds [29] and hydrolysis [54,56].

2.2. Digestion procedures

Focused-microwave ovens can be employed to assist the total digestion of organic and inorganic samples. Some advantages of this system have been highlighted before. The efficiency of the

Table 3
Focused-microwave-assisted extraction of organic compounds (1996–2002)

Sample	Substances	Sample size	Microwave system	Extraction conditions	Technique for detection	Reference
Soil	AL, AE, FL, PHE&AN, FLT, Pyr, BaA, C&T, BbF, BkF, BaP, IP, DahA and BghiP	1 g	Soxwave 100	20 ml (cyclohexane, hexane, acetone, dichloro-methane or benzene) 90 W, 10 min	GC-MS	[41]
Sediment	Phenol, 2-nitrophenol, 4-nitrophenol, 2,4-dinitrophenol, 4-methylphenol, 2,4-dimethylphenol, 2-chlorophenol, 2,4-dichlorophenol, 2,4,6-trichlorophenol and pentachlorophenol	10 g	Soxwave 100	50 ml methanol + water 4:1 v v^{-1} + 2% v v^{-1} triethylamine 75–90 W, 30–40 min	LC-APCI-MS	[42]
Sediments and soil	P, A, Fluo, Pyr, BaA, Chry, Trip, Bbp, BjF, BkF, BeP,BaP Per, IP, BP, DacA and DahA	0.1–1 g	Soxwave 100	30 ml (CH_2Cl_2 + toluene or CH_2Cl_2 or acetone + hexane 1:1 v v^{-1} or 4:6 v v^{-1} or acetone) 30–210 W, 5–30 min	GC-MS	[43]
Sewage sludge	PCBs	1 g	Soxwave 100	30 ml hexane + acetone 1:1 v v^{-1} 30 W, 10 min	GC-MS	[44]
Olives	Volatile matter content	4 g	A Soxhlet modified to assistance of A301	100 ml hexane (heated with an electrical isomantle in refluxed) Recovering time (7 cycles): 90 W, 25 s each cycle		[45]
Sediment	P, Flu, Pyr, BaA, Chry, BbF, BeP, BaP, Per, IP, B(ghi)P and DaA	0.3–10 g	Soxwave 100	30 ml dichloromethane + 30% w w^{-1} moisture (water per sediment) 30 W, 10 min	GC-MS	[46]
Soil	*n*-dodecane, *n*-tridecane, *n*-tetradecane, BaA, BkF, BeA, BkF, Pyr, BaP, BeP, dichlorobenil, trifluraline, dinitramine, alachlor, metribuzine, terbutrine, simazine and nitrofen	7 g	A Soxhlet modified to assistance of A301	1 or 1.5 ml water + 30 ml DCM and benzene 100 W, 15 s each cycle Alkanes: 8 cycles PAHs: 10 cycles Herbicides: 10 cycles	Alkanes: GC-FID PAHs: GC-ITMS Herbicides: GC-ECD	[47]

Table 3 *(Continued)*

Sample	Substances	Sample size	Microwave system	Extraction conditions	Technique for detection	Reference
Plants	Withaferin A, iochromolide and with acnistin	100 mg	Soxwave 100	5–30 ml (methanol + water or water or methanol or ethanol or dichloro-methane or dichloromethane + water or hexane) 25–250 W, 40 s to 10 min	HPLC-UV	[48]
Sediment	P, AN, Flu, Pyr, BaA, Chry, Trip, BbF, BjF, BkF, BeP, BaP, Per, IP, B(ghi)P, DacA and DahA	1 g	A301	10–30 ml (dichloromethane or heptane + ethanol 80:20 v v^{-1}) 20–140 W, 2–10 min	GC-MS	[49]
Soil	N, AL, AE, FL, P, NA, Fluo, Pyr, BaA, Chry, BbF, BkF, BaP, IP and B(ghi)P	5 g		40 ml (CH_2Cl_2 or CH_2Cl_2 + 20 % v v^{-1} water or CH_2Cl_2 + acetone or CH_2Cl_2 and acetone + 20% of water) 30 W, 10 min	GC-MS	[50]
Soil	BaP, BeP, BaA, BeAc, BkF and B(ghi)P	5 g of soil or 0.1 g CRM	A301	100 ml of acetonitrile (4 aliquots of 25 ml) 60 W, 4–5 min each cycle (the number of cycles depends on the kinetics of the target sample)	HPLC-FDP	[51]
Seeds	TGD, DG, FA and OxTGM	2 g	A301	100 ml *n*-hexane 25–90 W, 30–90 min	GC-FID	[52]
Soil	4,4′-DDT, 4,4′-DDD and 4,4′-DDE	1 g	Microdigest 3.6	5 ml 0–2 mol l^{-1} sodium chlorate + 2 ml iso-decane 98 °C, 15 min	GC-AED	[53]
Milk	FAME, polymeric compounds and non-polar triacylglycerols	10 ml	A301	Hydrolyses: 30 ml 6 mol l^{-1} HCl 200 W, 10 min Dryness: 60 W, 1 min Extraction: 100 ml *n*-hexane 3 cycles of 200 W, 50 min (total time)	GC-FID HPSEC LC-FID	[54]
Strawberries	Pesticides: carbendazim, diethofencarb, azoxystrobine, napropamide and bupirimate	25 g	Soxwave Map microwave oven adapted (Prolabo)	15 ml water (2 aliquots) 30 W, 7 min	SPME-HPLC-DAD	[55]

Table 3 *(Continued)*

Sample	Substances	Sample size	Microwave system	Extraction conditions	Technique for detection	Reference
Cheeses	TAG and polymers	2.5 g	A301	Hydrolyses: 40 ml HCl solution 200 W, 10 min Dryness: 60 W, 1 min Extraction: 100 ml *n*-hexane 9 cycles of 180 W, 85 s	GC-HPSEC TLC-FID	[56]

AE, acenaphthene; AL, acenaphthylene; NA, anthracene; BeAc, benzo[*e*]acenaphtene; BaA, benzo[*a*]anthracene; BaP, benzo[*a*]pyrene; BbF, benzo[*b*]fluoranthene; BeA, benzo[*e*]acephenanthrylene; BeP, benzo[*e*]pyrene; B(ghi)P, benzo[*ghi*]perylene; BjF, benzo[*j*]fluoranthene; BkF, benzo[*k*]fluoranthene; DBT, Bu_2SnCl_2; TBT, Bu_3SnCl; MBT, $BuSnCl_3$; Chry, chrysene; DaA, dibenz[*a*,*h*]anthracene; DacA, dibenzo[*a*,*c*]anthracene; DG, diglycerides; Ddot, Do_2SnCl_2; MdoT, $DoSnCl_3$; FAME, fatty acid methyl ester; FA, fatty acids and polar unsaponificable matter; Fluo, fluoranthene; FL, fluorene; IP, ideno[1,2,3-*cd*]pyrene; N, naphthalene; PHE, *N*-phenanthrene; DocT, Oc_2SnCl_2; MocT, $OcSnCl_3$; OCPs, organochlorine pesticides; OxTGM, oxidized triglyceride monomers; Per, perylene; DPhT, Ph_2SnCl_2; TphT, Ph_3SnCl; P, phenanthrene; MPhT, $PhSnCl_3$; PCBs, polychlorinated biphenyls; PAHs, polynuclear aromatic hydrocarbons; TPrT, Pr_3SnCl; Pyr, pyrene; TMAH, tetramethyl ammonium hydroxide; TAG, triacylglycerols; TGD, triglyceride dimers; Trip, triphenylene; AED atomic emission detector; ECD electrons capture detector; FID flame ionisation detector; FPD flame photometric detector; ITMS ion trap mass spectrometry; GC-MS gas chromatography mass spectrometry; UV, Ultraviolet; HPSEC, high-performance size exclusion chromatography; LC-FID, liquid chromatography flame ionization detector; APCI-MS atmospheric pressure chemical ionization mass spectrometry; SPME, solid-phase micro extraction; DAD, diode array detector; TLC-FID, thin-layer chromatography flame ionization detection.

digestion can be improved by the sequential addition of different reagents during the procedure.

Organic samples, such as oil, generate large amounts of gas during acid digestion [7]. The use of focused microwave ovens makes possible to digest a large sample mass without any safety problems. The digestion efficiency can be improved by the addition of H_2SO_4 to reach temperatures as high as 250 °C.

The feasibility of working with a large sample mass was also exploited for the determination of radio nuclides in soils, which are in this case required due to the trace level of analytes. Different digestion methods can be optimized using focused-microwave ovens. A typical procedure for digestion of 2 g of mineral soil was proposed by Torres et al. [62,63].

Sample digestion can also be performed on-line. A fully automated on-line system comprised by a focused-microwave system, pre-concentration and matrix separation, and ICP-MS measurements, has been described [68] for the digestion of blood and serum, employing an oxidant mixture containing $HNO_3 + H_2SO_4$ ($1+1$ v v^{-1}). The main advantages of on-line systems are the reduction of both, analysis time and consumption of reagents, and the low blank values since all reactions occurred in a system isolated of the laboratory environment.

Digestion procedures carried out using focused-microwave ovens are summarized in Table 4. The most critical aspect of some of the listed procedures is the amount of acids employed for sample digestion. The use of high volumes of concentrated acids (see e.g. Refs. [58,67,69,70,75,77]) generates digestates with elevated concentrations of acids that generally require extensive dilution before measurement when spectroanalytical techniques with conventional sample introduction systems are used. The high amount of acids can be necessary to proportionate a complete digestion and to allow temperature increase at atmospheric pressure. The temperature increase is generally reached by adding concentrated H_2SO_4 because of its high boiling point. The excess of acid could be removed in part by evaporation. However, the boiling point of H_2SO_4 makes this operation difficult for digestates containing large amounts of this acid, making sample preparation tedious. An alternative procedure could be adopted to decrease the volumes of concentrated reagent as will be discussed following chapter.

3. New routes

3.1. Acid-vapor-phase digestion

Acid-vapor-phase digestion was successfully implemented using closed vessels [79,80]. Recently, this same procedure was adapted to an open-vessel system [11]. The main advantages of using this strategy are the reduced concentration of acid in the digestate, the possibility of using a technical grade acid without any deterioration of analytical blank, and the reduction of blank values due to the purification of reagent during microwave-assisted evaporation. The acid-vapor formed is condensed in the upper part of the reaction vessel and partially collected in the PTFE cups containing up to 50 mg of sample. Depending on the size of the sample vessel, 3 or 4 samples can be treated simultaneously in each microwave reaction vessel.

It should be mentioned that each PTFE cup is at a different reaction condition considering its position in the reaction vessel (Fig. 1). The focused microwave radiation reaches only the lowest sample cup and this implies that this recipient is at a higher temperature and the digestion conditions are more aggressive. The microwave radiation does not reach the upper cup, as was shown by inserting a thermo-sensitive paper inside the reaction vessel and applying microwave radiation for 1 min. Fig. 2 might be interpreted as a rough photography of the microwave radiation distribution. This effect caused non-quantitative recoveries in all sample cups, and best recoveries were always attained in the lowest cup. The digestion conditions in each cup can be improved by adding a small volume of hydrogen peroxide to the sample [11]. However, despite its successful application for Co and Fe when using a 6–10 and 15–60 min heating program for biological materials, respectively, the heating time for quantitative recoveries of Fe does not appear to be attractive. We have tried to apply a 25-min heating program for partial digestion of bovine liver for determining Ca, Cu, Fe, Mg, Mn and Zn. A difference in the recoveries between

Table 4
Focused-microwave-assisted digestions of organic and inorganic samples (1996–2002)

Sample	Element(s)	Sample size	Microwave system	MW conditions	Technique for determination	Reference
Cosmetics	Hg	0.25 g	M301	1 ml HNO_3 + 1 ml H_2O_2 + 1 ml H_2SO_4 170 W, 45 min	HG-AFS	[57]
Solid environmental samples	Hg	0.25 g	M301	Biological samples: 5 ml HNO_3 + 6 ml H_2SO_4 20 W, 5 min 20 W, 15 min cool: 10 min 2 ml H_2O_2 20 W, 10 min Sediment samples: 5 ml HNO_3 + 3 ml H_2SO_4 20 W, 5 min cool:10 min 2 ml H_2O_2 20 W, 5 min	CV-AFS	[58]
Plants: pine needles (NIST 1575), rye grass (BCR 281), beech leaves (BCR 100) and an aquatic plant (BCR 596)	Cr	0.50 g	M301	5 ml HNO_3 30 W, 10 min, 40 W, 5 min 5 ml HF + $HClO_4$ (2 + 1 v v^{-1}) 30 W, 10 min, 50 W, 5 min, 60 W, 15 min 10 ml 10% v v^{-1} HNO_3 20 W, 5 min	ETAAS	[59]
Feed additive	Cr	0.5 g	A301	3 ml HNO_3 10% power, 5 min 2 ml H_2SO_4 15% power, 5 min, 25% power, 5 min, 35% power, 5 min cool: 3 min 1 ml H_2O_2 40% power, 5 min	ETAAS	[60]
Oil	RCC	1 ml	STAR 6	10 ml HNO_3 + 10 ml H_2SO_4 4 ml HNO_3 ramp time: 1 min, 90 °C, 0.5 min 4 ml HNO_3 ramp time: 3 min, 150 °C, 0.5 min 5 ml HNO_3	ICP-OES	[7]

Table 4 *(Continued)*

Sample	Element(s)	Sample size	Microwave system	MW conditions	Technique for determination	Reference
				ramp time: 3 min, 220 °C, 5 min ramp time: 2 min, 250 °C, 0 min 20 ml 30% v v^{-1} H_2O_2 200 °C, 10 min		
Bovine liver	RCC	0.25 g	HPA-FM	3 ml HNO_3 350 W, 5 min	Elemental analyser	[61]
Soil	^{90}Sr	(a) 1–2 g (b) 4 g	(a) A301 (b) M401 and A301	(a) HNO_3, HF, $HClO_4$, H_2O_2 10–75% power, 5–20 min each step (b) HNO_3, HF, $HClO_4$, H_2O_2 10–70% power, 5–20 min each step	Cerenkov technique	(a) [62] (b) [63]
Algae matrix	Cu, Mn and Ni	0.5 g	M401	2 ml HNO_3 10, 30 and 40% power, 5 min 2 ml HNO_3 40% power, 10 min	ETAAS	[64]
Pharmaceutical samples (nutritional supplements and shampoos)	Se	1.5–2.5 ml (nutritional samples) and 0.5 g shampoo	M301	5 ml HNO_3 + 2 ml H_2O_2 25% power, 5 min 10 ml 6 mol l^{-1} HCl 75% power, 5 min	HG-AFS	[65]
Marine sediments (IAEA 135) and Mediterranean sediment	Pu, Am, U, Th and Sr	2–5 g	A301 and M401	HNO_3, HF, $HClO_4$ 20–70 W, 86 min total digestion time	XRF	[66]
SRM (citrus leaves, bovine liver and oyster tissue	P and N	0.5–1 g	MX350	20 ml H_2SO_4 60 W, 2 min, 120 W, 2 min, 210 W, 6 min 0 W, 3 min 6–12 ml 30% v v^{-1} H_2O_2 270 W, 10 min	UV–vis. Kjeldahl	[67]
Blood and serum	Fe, Cu, Ni, Pb and Zn	0.5 ml (on-line)	MX350	Blood: 0.8 ml HNO_3 + H_2SO_4 (1:1 v v^{-1}) Serum: 0.4 ml HNO_3 + H_2SO_4 (1:1 v v^{-1}) 300 W, 2 min	ICP-MS	[68]
Seafood (oyster and mussel)	As	500 mg	A301	10 ml HNO_3 40 W, 5 min, 70 W, 10 min 5 ml H_2SO_4 120 W, 10 min	HG-AFS	[69]

Table 4 *(Continued)*

Sample	Element(s)	Sample size	Microwave system	MW conditions	Technique for determination	Reference
Mussel tissue	As	0.5 g	A301	10 ml HNO_3 40 W, 5 min, 70 W, 10 min 5 ml H_2O_2 60 W, 10 min 3 ml HNO_3 40 W, 15 min	ICP-MS	[70]
Water and wastewater	COD	20 ml	Microdigest 3.6	0.5 g mercuric sulfate + 10 ml dichromate solution + 5 ml H_2SO_4 150 °C, 8 min	Titrimetric method	[71]
Human brain and bovine liver	Trace elements	0.15–0.25 g	A301	7 ml HNO_3, 40 W, 10 min 50 W, 3 min 3 ml 30% v v^{-1} H_2O_2 40 W, 5 min, 60 W, 2 min 4 ml HNO_3 50 W, 10 min 1 ml 30% v v^{-1} H_2O_2 50 W, 3 min, 60 W, 5 min, 80 W, 7 min	ICP-MS	[72]
Soluble coffee	Mineral nutrients and toxic elements	(a) 1–5 g (b) 1–2 g	7400 Spex	(a) 6 ml HNO_3 105 W, 10 min cool: 5 min 0.5 ml 30% v v^{-1} H_2O_2 105 W, 10 min cool: 5 min (b) 15 ml HNO_3 or H_2SO_4 105 W, 10 min 10 ml 30% v v^{-1} H_2O_2 105 W, 5 min	ICP-OES	(a) [73] (b) [74]
Soluble coffee	As and Se	5 g	7400 Spex	30 ml HNO_3 30 min ambient temperature 105 W, 5 min cool: 5 min 105 W, 5 min cool: 5 min 2.5 ml 30% v v^{-1} H_2O_2 105 W, 5 min cool: 5 min	HG-ICP-OES	[75]

Table 4 *(Continued)*

Sample	Element(s)	Sample size	Microwave system	MW conditions	Technique for determination	Reference
Sewage sludges and incineration ashes	Cd, Co, Cr, Cu, Fe, Mg, Mn, Ni, Pb, Zn	0.5 g	A301	10 ml $HCl + HNO_3$ (3:1 v v^{-1}) or HF 10 min	ICP-OES, FAES and ETAAS	[76]
Solid waste (fly ash and filter cakes)	Major and minor elements	1–2 g	A301	25 ml HCl or HNO_3 40 W, 15 min	ICP-OES and QFAAS	[77]
Antarctic Krill	Cd, Cu, Fe, Mn, Pb, Zn	0.5 g	M401	5 ml HNO_3 45 W, 10 min, 150 W, 10 min 40 ml 30% v v^{-1} H_2O_2 45 W, 7 min, 150 W, 30 min dryness: 45 W, 7 min 150 W, 12 min	ICP-OES and GFAAS	[78]

CV, cold vapor; XRF, X-ray fluorescence, FAES, flame atomic emission spectroscopy, COD, chemical oxygen demand; RCC, residual carbon content; HPA-FM, high-pressure asher focused microwave.

cups 1 and 2 was observed even when adding 200 μl H_2O_2 (30% v v^{-1}) to the sample (Fig. 3). The addition of 100 μl of sodium hypochlorite solution (2.1% v v^{-1} active chlorine) to the sample was tested as well. The hypothesis was that hypochlorite could generate Cl_2, a strong and reactive oxidant, when condensed acid is collected in the sample cup. The generation of chlorine from hypochlorite in acid medium is a well-known chemical process:

$$ClO^- + H^+ \rightarrow HClO \quad (1)$$

$$3\ ClO^- \rightarrow ClO_3 + 2Cl^- \ (\text{fast}) \quad (2)$$

$$HClO + H^+ + Cl^- \rightarrow Cl_2\ (g) + H_2O \quad (3)$$

Results obtained based on hypochlorite addition are shown in Fig. 4. As expected, recoveries for all elements were close to 100% in both sample cups, indicating that the above processes are operative, and strong oxidant conditions are resulting in quantitative recoveries.

It should be mentioned that all steps of the procedure are carried out in a single vessel, minimizing contamination in trace analysis. All measurements were carried out using an inductively coupled plasma optical emission spectrometer with

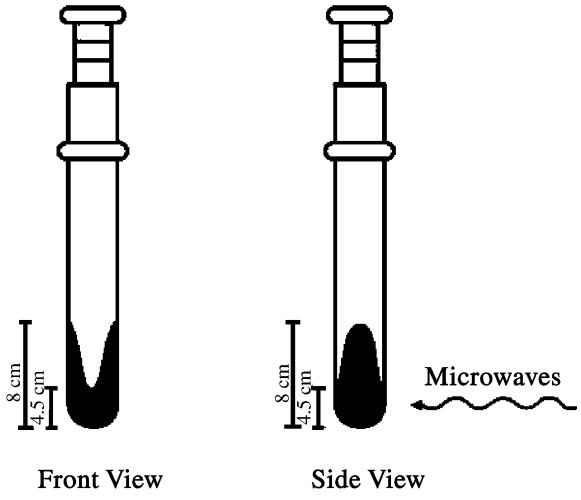

Fig. 2. Schematic of microwave radiation distribution inside the reaction vessel.

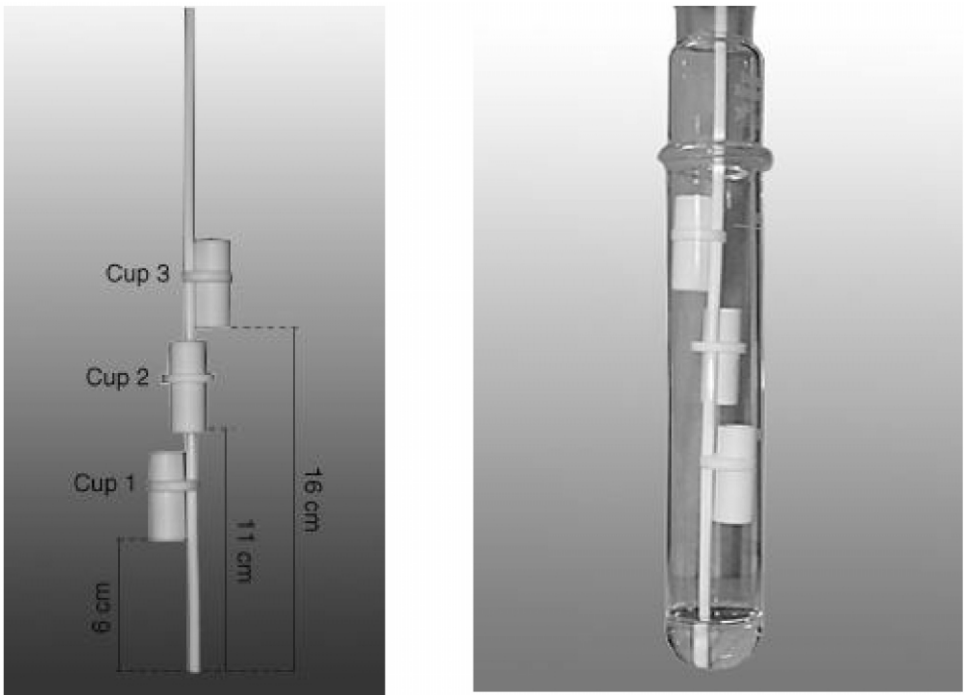

Fig. 1. PTFE cups arranged on a PTFE stick: outside and inside the glass reaction vessel.

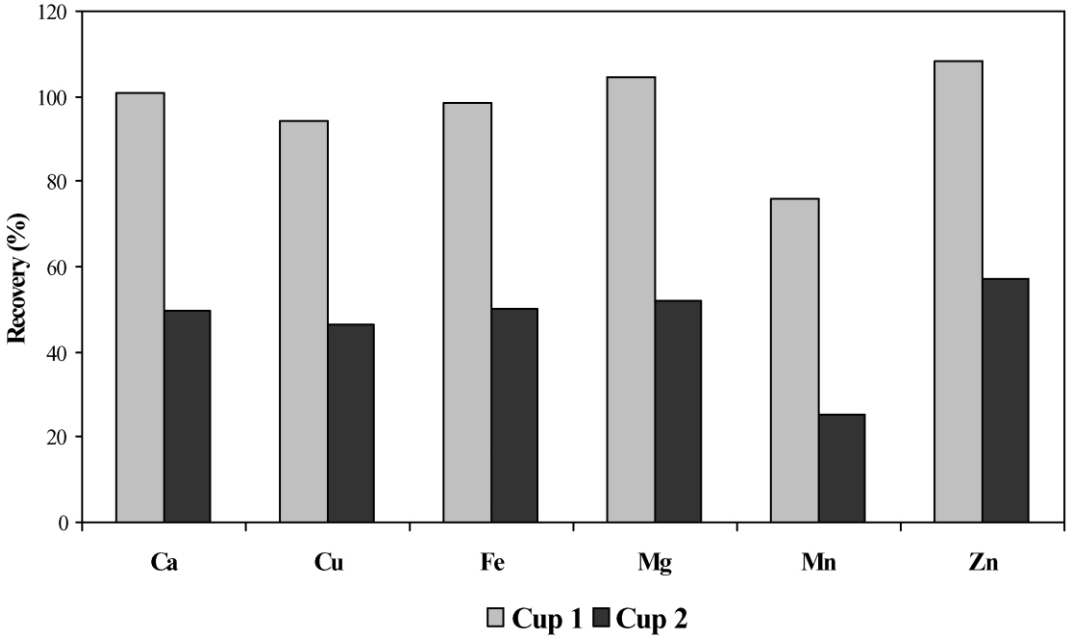

Fig. 3. Recovery of Ca, Cu, Fe, Mg, Mn and Zn in bovine liver using acid-vapor-phase digestion with the addition of 200 μl H_2O_2.

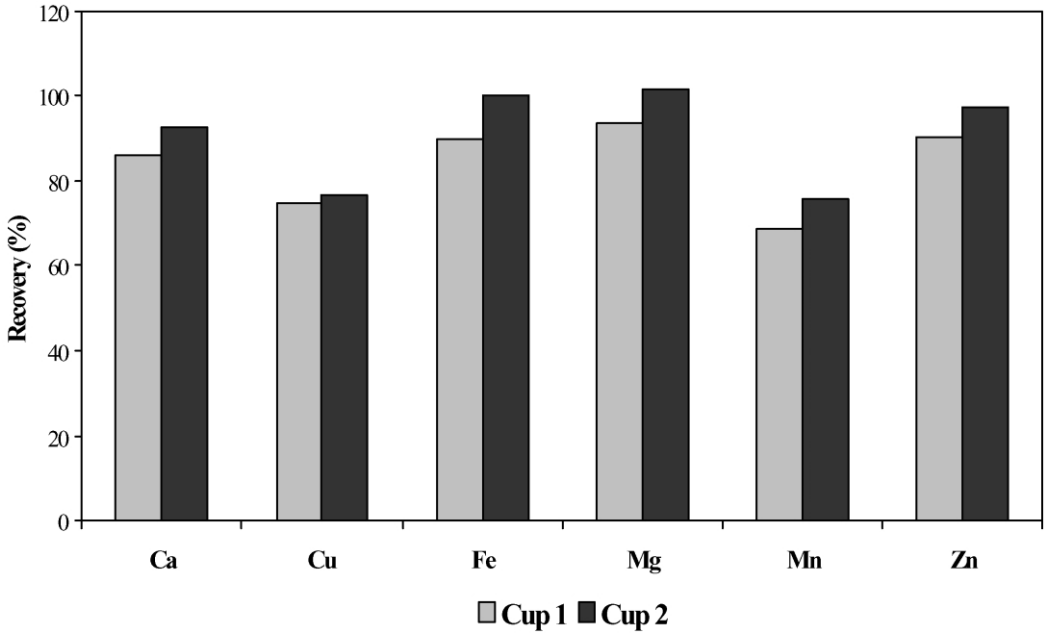

Fig. 4. Recovery of Ca, Cu, Fe, Mg, Mn and Zn in bovine liver using acid-vapor-phase digestion with the addition of 100 μl NaClO.

Table 5
Focused-microwave program for the digestion of milk using sample addition into the pre-heated reagent

Step	T (°C)	t_{ramp} (min)	$T_{plateau}$ (min)	Reagent or sample (ml)	Aliquot (ml)
1	130	5	2	2 (milk)	0.5
2	170	2	2	3 (milk)	0.5
3	170	0	10	10 (H_2O_2)	1.0

an axially viewed configuration (ICP OES, Vista AX, Varian, Australia). Measurements were made at standard operating conditions. Further experiments are in progress to apply this procedure for other elements in different types of samples.

3.2. Sample addition to hot reagent

Another alternative to decrease the final acid concentration of the digestate is to modify the conventional procedure and to add gradually, the liquid sample to a small volume of concentrated acid (<5 ml), heated by microwave action. This strategy can be easily implemented using standard equipment, and has some interesting implications:

- Digestion of a greater volume of sample, using a smaller volume of acids;
- Each sample aliquot is partially digested before adding the next one;
- Digestion is carried out in a more concentrated acid medium because the reagent is less diluted by the solvent, i.e. the acid is in excess compared to the sample during all digestion steps;
- Hot concentrated acids can generate reactive radicals that can speed up the digestion process;
- Lower blank values and better sensitivity due to lower dilution of digestates.

The feasibility of this procedure was evaluated for milk samples. The conventional procedure involves 10 ml HNO_3 plus 3 ml H_2SO_4 for digesting 2.5 ml of whole milk. Using the proposed procedure, it was possible to digest 5 ml of whole milk when sample aliquots were gradually added to a mixture of 3 ml HNO_3 plus 1 ml H_2SO_4 (see heating program in Table 5).

Results obtained for a whole milk powder (NIST SRM 8435) are shown in Table 6. Determined and certified values were in agreement at a 95% confidence level. All measurements were carried out using an ICP-OES with axially viewed configuration operated at usual conditions.

This procedure was also successfully applied to diesel oil samples. In this case, a total volume 2 ml diesel fuel was gradually added to microwave-heated HNO_3, and after that H_2SO_4 was added to reach temperatures approximately 210 °C. This procedure reduced the consumption of concentrated acids from 20 ml (conventional procedure) to 4 ml. It implied that the obtained digestates did not require an extensive dilution to proper introduction by using a conventional pneumatic nebulizer.

4. Conclusion

Focused-microwave ovens are not as disseminated as cavity-microwave ovens, however, there are some applications that could be performed better on the former system. Sample preparation for speciation analysis can be improved using a focused-microwave oven owing to a better control of the energy delivered to the sample. Extraction procedures using diluted acid or organic solvents at low temperature can be easily carried out in focused-microwave ovens. Additionally, total digestion of either large amounts of samples or samples rich in organic compounds can also be performed in an open vessel operated at atmospheric pressure. The conventional procedure can be modified by gradually adding sample aliquots to hot concentrated acid. This strategy decreases the amount of acid, and simultaneously increases

Table 6
Determined and certified values in whole milk powder (SRM 8435)

Element	Determined value	Certified value
Ba (mg l^{-1})	0.71 ± 0.09	0.58 ± 0.23
Ca (%)	0.759 ± 0.080	0.922 ± 0.049
Fe (mg l^{-1})	1.39 ± 0.48	1.8 ± 1.1
K (%)	1.78 ± 0.15	1.363 ± 0.047
Mg (mg l^{-1})	874 ± 101	814 ± 76
Na (%)	0.360 ± 0.034	0.356 ± 0.040
P (%)	0.854 ± 0.066	0.780 ± 0.049
Zn (mg l^{-1})	24.8 ± 2.4	28.0 ± 3.1

Mean $\pm$ one standard deviation ($n=3$).

the sample volume that can be digested. Finally, focused-microwave oven can also be applied for acid-vapor digestion in a single-vessel procedure. It can be concluded that the use of focused-microwave ovens probably will be extended and it could become a standard tool for sample preparation in speciation analysis.

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