On-line microwave slurry sample digestion using flow systems for the spectrophotometric determination of iron in seafood†

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A method for the spectrophotometric determination of iron in seafood slurry samples based on microwave assisted digestion has been developed. The stabilized slurry (200 µl) is introduced in a flow system and transported by an air carrier stream to a digestion coil positioned inside the microwave oven. After the digestion step (10 or 20 min at maximum power) the flushing solution is collected in a calibrated flask. A 200 µl-digested sample is introduced in a flow injection system and the iron determined at 512 nm with 1,10-phenanthroline. The proposed method features a linear range from 50 to 200 µg l^{-1} ($r > 0.999$) presenting a precision, expressed as RSD, of 3.7% ($n = 10$) for repeatability and 5.0% ($n = 30$) for reproducibility. Accuracy was assessed by using Standard and Certified Reference Materials.

Keywords: *On-line microwave digestion; flow system; spectrophotometry; seafood; iron*

Analytical techniques requiring the introduction of liquid samples, such as atomic and molecular spectrometry among others, are widely employed by analysts almost every day. In this way, a great number of sample dissolutions need to be performed in chemical laboratories, making this one of the most common and sometimes slowest operations within the overall analytical procedure.[1]

Despite the importance and widespread applicability of sample dissolution, most conventional digestion procedures are labor-intensive, and a number of them are potentially hazardous, requiring skilled analysts.

The use in the laboratory of tedious and dangerous sample preparation procedures suggests the need for a more modern alternative, particularly if it is considered that, in the last years, the objective of the analytical chemist is to obtain the greatest amount of information in the shortest time interval, at reduced effort, and making the analyses less expensive.[2]

Although sample preparation is a critical step in chemical analysis, it seems that the great advances in analytical instrumentation have not yet been accompanied by a similar break-through in sample preparation methods. In addition, recently the new status of analytical chemistry has created the need to prepare an increasing number of samples in a fraction of the time previously required. This demand has been addressed by the use of microwave ovens coupled to flow systems. These systems may aid the digestion and further accelerate the reaction through rapid heating, making it possible to generate reaction products quickly and in larger quantities.[3] Some problems, such as fume production during wet digestion and losses of volatile compounds, can be overcome or minimized while the personal security of the analyst is improved. The potentiality of the microwave oven-flow injection hyphenation has been already demonstrated.[3–5]

Although a significant number of microwave flow systems for sample digestion coupled to FAAS, ETAAS or ICP techniques appear in the literature,[6–27] the same is not true for UV/VIS.[28,31] Table 1 summarizes work published employing a microwave-flow system approach to sample pre-treatment. As can be seen in this Table, the proposed methods for microwave sample treatment coupled to UV/VIS spectrophotometry employed only water or liquid effluents.

In this way, the purpose of the present work was to design and test a flow system for on-line microwave slurry sample preparation, as simple as possible, that permits the total destruction of the organic matter present in complex matrices (oyster, mussel and fish) with posterior off-line flow injection spectrophotometric determination of iron. The method was validated employing standard and certified reference materials.

Experimental

Standards, reagents and samples

All solutions were prepared with analytical quality chemicals (Merck, Darmstadt, Germany) and distilled/deionized water. The nitric and hydrochloric acids used were distilled in a quartz sub-boiling still (Marconi, Piracicaba, Brazil). A 1000 mg l^{-1} Fe^{III} solution was prepared as stock standard solution, from which working standard solutions containing 50.0–200 µg l^{-1} Fe^{III} were prepared daily by sequential dilution with 0.014 mol l^{-1} HNO_3 or 0.15 mol l^{-1} HNO_3 + 0.35 mol l^{-1} HCl (*aqua regia*), depending on the oxidant mixture used to digest the sample.

The 1.0% (w/v) ascorbic acid and 0.25% (w/v) 1,10-phenanthroline solutions were also prepared daily. The acetate buffer was prepared by mixing ammonium acetate and acetic acid in order to obtain a pH of 3.7 in the flow system. The flow injection parameters used are shown in Table 2.

The fish samples were purchased at a local market and only the flesh portions were ground with a household blender (Arno, São Paulo, Brazil) to make the homogenate. After freezing for three days, the resulting homogenates were lyophilized by freeze-drying (Labconco, Model Freeze Dryer 8, Kansas City, Missouri, USA) at 6 Pa for 48 h to constant mass. The resulting samples were ground with a mortar and pestle and then sieved through a standard sieve (Bertel, Caieiras, Brazil) in order to obtain < 53 µm particle size. Also some Reference Materials such as Oyster Tissue (NIST SRM, 1566a), Fish Homogenate (IAEA SRM, MA-A-2 1062/TM) and Mussel (NIES CRM) were used. Table 2 summarizes the digestion conditions applied to samples employed in this work.

Instruments and apparatus

A schematic diagram of the instrumentation used in the on-line microwave digestion system is shown in Fig. 1(a). The flow

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system employed a peristaltic pump (Ismatec IPC, Glattbrugg, Switzerland) and a home-made three piece injection commutator device.[32] The valve, transmission lines and the digestion coil (2.0 m long, 2 mm id) used in the digestion system were made of poly(tetrafluorethylene) (PTFE). Acidiflex tubes were used to pump the acid. A household CCE model MW-1350 Turn Table microwave oven (São Paulo, Brazil), equipped with a magnetron of 2450 MHz with a nominal power of 700 W and operated at maximum power was used.

In the spectrophotometric determination of iron [Fig. 1(b)] the transmission lines of the flow system were of polyethylene. A FEMTO model 432 spectrophotometer (São Paulo, Brazil) set at 512 nm as well as a Micronal B 292 (São Paulo, Brazil) recorder were used to measure the absorbance of the 1,10-phenanthroline–Fe^{II} complex.

A Branson (Danbury, USA) ultrasonic bath was employed to homogenize the slurries prior to injection into the flow system for microwave digestion.

Table 1 Analytical applications of on-line microwave digestions reported in the literature

Matrix	Element(s)	Technique*	Remarks	Ref.
Solid samples	Cu, Mn, Cr, Co, Fe, Ni	FAAS	Coil length, 1 m; mixture, HNO_3/H_2O_2	6
Whole blood	Zn, Cu	FAAS	MW time, 20 s (100% power); coil length, 2 m; mixture, HNO_3/HCl/EDTA	7
Whole blood	Fe, Cu, Zn	FAAS	MW time, 25 s; coil length, 50 cm; mixture, HCl/HNO_3	8
Biological tissue (SRM)	Zn, Cd	FAAS	MW time, 8 min; acid, HNO_3	9
Soil	Hg	AFS	MW time; 1 min	10
Organic and inorganic compounds; blood and CRM	Hg	CVAAS	Coil length, 20 m; mixture, $KMnO_4$/KBr/$KBrO_3$	11
Waters, effluents and sediments	Hg	CVAAS	Coil length, 1 m; mixture, HCl/HNO_3; $KMnO_4/H_2SO_4$; HCl	12
Environmental	Hg	CVAFS	MW time, 50 s; coil length, 4 m	13
Water, urine and CRM	Bi, Sn, Pb, As	HGAAS	Coil length, 10.2 m; mixture, KBr/$KBrO_3$/HCl; $(NH_4)_2O_8$/HNO_3; $K_2S_2O_8/H_2SO_4$	12
Drinks and fruits	Pb	HGAAS	Coil lengths, 1.5–5 m; acid, HNO_3	14
Waters	As	HPLC–HGAAS	Coil length, 1.5 m; oxidizing agent, $K_2S_2O_8$	15
Cocoa powder and CRM as slurries	Cu, Fe	ICP-AES	MW time, 5 min; coil length, 10 m; Slurry preparation in Triton X-100 and HNO_3	16
Whole blood	Co	ETAAS	MW time, 50 s; coil length, 5 m; mixture, HNO_3/EDTA	17
CRM (botanical and biological tissue) as slurries	Pb	ETAAS	MW time, 25 s; coil length, 2 m; mixture, HCl/HNO_3	18
Shellfish	Se	ETAAS	MW time, 4 min (600 W); acid, HNO_3	19
Soil samples	Pb	FAAS	MW time, 5 min (250 W); mixture, HNO_3/H_2O_2	20
Water	Chemical oxygen demand and chromium	UV/VIS and FAAS	Coil length, 15 m; mixture, $K_2Cr_2O_7/H_2SO_4/HgSO_4$	21
Sewage sludge	Zn, Cu, Pb, Cd, Ni, Cr	ICP-AES	MW time, 6 min; slurry preparation in HNO_3 1.5 mol l^{-1}	22
Tap water	Se	HGAAS	MW time, 1 min; mixture, HBr/$KBrO_3$; coil length, 4 m	23
Rock, soil, sediment, sewage sludge, mussel tissue and rice flour	Ni	ETAAS	Mixture: *aqua regia*/HF/H_2O_2; MW time, 10 min	24
Silicate rocks	Mg	FAAS	Coil length, 3 m; MW time, 10 s; mixture, HF/HNO_3	25
Waters	Pb, Co, Mn^{II}, Fe^{III}	UV/VIS	MW time, 4–6 min; mixture, HNO_3/H_2O_2	26
Vegetables	Cr, Co, Ni	ETAAS	MW time, 2 min; mixture, HNO_3/H_2O_2; coil length, 4 m	27
Waters	Chemical oxygen demand	UV/VIS (λ = 445 nm)	MW time, 3 min; coil length, 10 m; mixture, $K_2Cr_2O_7/H_2SO_4$	28
Waters	Urea as NH_3	UV/VIS (λ = 635 nm)	Mixture, $H_2SO_4/K_2SO_4/HgSO_4$	29
Waters	P	UV/VIS	Coil length, 6 m; mixture, HNO_3/$HClO_4$; $NH_4Ce(SO_4)_2/HClO_4$; $(NH_4)_2S_2O_8/HClO_4$	30
Liquid effluents	P	UV/VIS	Coil length, 7.2 m; acid, HNO_3	31

* FAAS flame atomic absorption spectrometry; CVAFS cold vapor atomic fluorescence spectrometry; CVAAS cold vapor atomic absorption spectrometry; HGAAS hydride generation atomic absorption spectrometry; HPLC–HGAAS high performance liquid chromatography–hydride generation atomic absorption spectrometry; ICP-AES inductively coupled plasma atomic emission spectrometry; ETAAS electrothermal atomic absorption spectrometry; UV/VIS spectrophotometry; MW microwave; SRM standard reference material; CRM certified reference material.

Table 2 Summary of the digestion and flow system conditions employed

Oyster tissue	Conditions
Slurry preparation	250.0 mg sample + 20 ml concentrated HNO_3 + 5 ml 30% (v/v) H_2O_2 (15 min ultrasonic bath) and diluted to 25 ml
Slurry sample loop [used in Fig. 1(a)]	200 µl
Air carrier flow rate	8 ml min^{-1}
Digestion time	10 min
MW power	100%
Final volume (digested sample)	10 ml
Digested sample loop [used in Fig. 1(b)]	200 µl
Buffer conditions	2 mol l^{-1} acetic acid + 2 mol l^{-1} ammonium acetate (1 + 1)
Carrier	0.014 mol l^{-1} HNO_3
Mussel and fish	
Slurry preparation	250.0 mg sample + 4.5 ml *aqua regia* +0.5 ml 30% (v/v) H_2O_2 (15 min ultrasonic bath) and diluted to 5 ml
Slurry sample loop [used in Fig. 1(a)]	200 µl
Air carrier flow rate	8 ml min^{-1}
Digestion time	20 min
MV power	100%
Final volume (digested sample)	Mussel: 10 ml Fish: 5 ml
Digested sample loop [used in Fig. 1(b)	Mussel: 130 µl Fish: 200 µl
Buffer conditions	2 mol l^{-1} acetic acid + 2 mol l^{-1} ammonium acetate (1 + 9)
Carrier	0.15 mol l^{-1} HNO_3 + 0.35 mol l^{-1} HCl

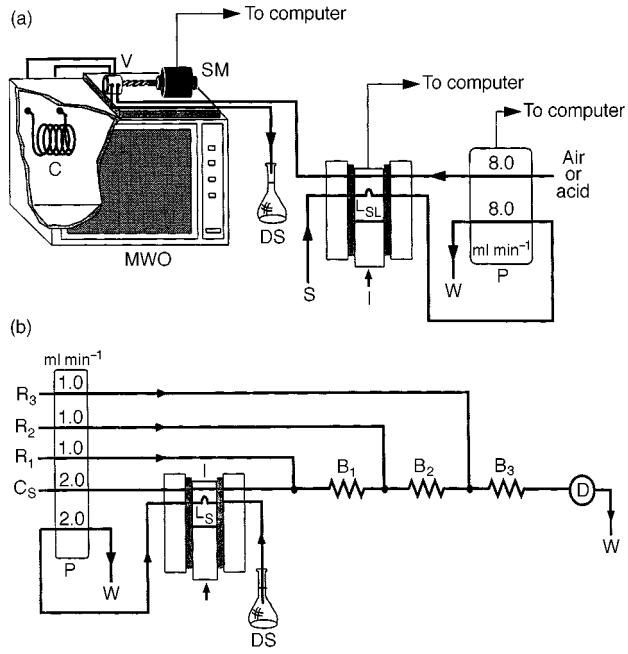

Fig. 1 (a) Flow diagram of the on-line sample digestion system. P, peristaltic pump; W, waste; L_{SL}, slurry sample loop (200 µl); S, slurry sample; I, injector commutator; DS, digested sample; SM, stepped motor; V, digestion valve; C, digestion coil (2 m long) and MWO, microwave oven; DS, (b) Flow diagram for FI spectrophotometic determination of iron. L_S, sample (200 µl); C_S, carrier; R_1, ascorbic acid; R_2, 1,10-phenanthroline; R_3, acetate buffer; B_1, B_2 and B_3, mixing coils of 10, 25, and 30 cm, respectively; D, detector (512 nm). Other symbols as Fig. 1(a).

Procedure

In order to perform the microwave sample digestion [Fig. 1(a)], a stabilized oyster tissue slurry (53 µm particle size in concentrated HNO_3 + 5 ml 30% v/v H_2O_2) was introduced in the flow system and transported by air carrier stream to the digestion coil located inside the microwave oven. The air carrier stream was used here in order to permit the expansion of the gases formed during the sample digestion. About 35 s after slurry sample injection, the peristaltic pump was automatically stopped and the valve (built-in Teflon) switched in order to trap the sample inside the digestion coil and produced the necessary pressure to sample digestion. After the digestion time (10 min at maximum power), the valve was switched again, the pump re-started and the sample was collected in a calibrated flask (10 ml). A solution of 0.014 mol l^{-1} HNO_3 was introduced *via* the same tube as the air to wash the manifold and to complete the volume in the calibrated flask. In order to initialize the new digestion cycle once more the air was pumped to eliminate the acid solution present in the transmission lines. At this time, a new sample could be injected. After the microwave sample preparation and collection of the digested sample, it was introduced to an off-line flow injection system [Fig. 1(b)] for spectrophotometric determination of iron with 1,10 phenanthroline.[33]

The commutator, peristaltic pump and digestion valve employed in the on-line microwave digestion system were controlled by a microcomputer employing a PCL 711-S Advantech interface and by using a program written in Visual Basic 3.0. The valve used in the microwave flow system [Fig. 1(a)] was built-in Teflon and the connection of the transmission lines to this valve was made using four screws (also in Teflon) each one containing a hole for passing through the connection tubes. The end of this tube was flanged and connected by screwing it in the valve.

Results and discussion

Safety considerations

No modifications were made to the domestic microwave oven to use it as a sample digestor. The same safety requirements as those for cooking, stated by the supplier, were followed. In addition, to avoid problems related to radiation leakage and exposure of the operator to the microwave radiation, the vent holes of the oven were used for the entrance and exit points of the digestion coil. Also, no microwave leakage (monitored with a Microwave Leakage Detector, Micronta, Hong Kong, China) $\geqslant$ 5 mV cm^{-2} was found at a distance of 5 cm from the oven.

Microwave pattern investigation

According some papers related to microwave oven-flow injection hyphenation,[34,35] it is of the utmost importance to know the best position of the digestion coil relative to the magnetron because of the pattern of the microwaves inside the oven when the target microwaves are stopped.[1,36] For this purpose, 10 glass beakers were placed in different positions in the oven [Fig. 2(a)], each one containing 40 g of water. In this experiment the microwave power applied was 700 W for 2 min and the test was made in triplicate, with the results of the weight loss being calculated by the differences between the weights of the water present in the beakers before and after microwave action, which ranged from 0.8 to 2.2 g. The best position found (beaker A positioned at 7.2 and 28.9 cm, for the *x* and *y* axes, respectively) was obtained based on microwave power spatial distribution and shown in Fig. 2(b). In addition, a 120 ml water beaker located at the minimum microwave point (27.2 and 28.9 cm, *x* and *y* axes, respectively) was always used in order to avoid damage to the magnetron during the microwave actuation

because of low volumes (μl) employed during the digestion. The beaker was always filled to 120 ml with water after each digestion cycle to maintain the same digestion conditions.

Slurry particle size

As the literature[37,38] states that slurry analytical results depend on the slurry particle size, a study was made utilizing a 250 to 53 μm particle size range. It was observed that if the particle size used was greater than 100 μm the transmission lines were clogged and high pressure was noted with the 0.8 mm id transmission lines. It was also observed that using < 53 μm particle size, a higher pressure in the system was also achieved depending on the matrix employed (*e.g.*, fish). So, the transmission lines were changed to 2 mm id in order to avoid this problem and with the smaller particle sizes chosen (< 53 μm) a better precision (6–10% RSD) of the digestion procedure was achieved.

Using < 53 μm particle sizes, studies were made to cover a 5–25 min[19,34,38] homogenization time of the slurry samples by the ultrasonic bath. Using time intervals of 5 or 10 min, the sample was not stabilized (precision *ca.* 15% RSD) and the introduction to the microwave flow system was more difficult. On the other hand, for ⩾15 min time intervals this problem was solved. So, a sonication time of 15 min was chosen as a compromise between slurry homogeneity and analytical frequency.

Slurry sampling loop

After the coil position inside the oven and the best particle size were established to make the slurry samples, an experiment was performed in order to find out the optimal digestion conditions of the samples. In this way, a Oyster Tissue 1566a standard reference material was prepared according to the Experimental section and injected (200 μl) into the flow digestion system [Fig. 1(a)]. This volume was selected because it was sufficient for sensitivity of the spectrophotometric method, allowing a good expansion of the gases formed during the digestion. In addition, the use of larger volumes (300 or 400 μl) resulted in recoveries that were not acceptable, indicating that the destruction of the organic matter was not efficient. Preliminary experiments showed that, even with 200 μl injected volume, it was necessary to use the maximum power in the oven in order to achieve sample dissolution.

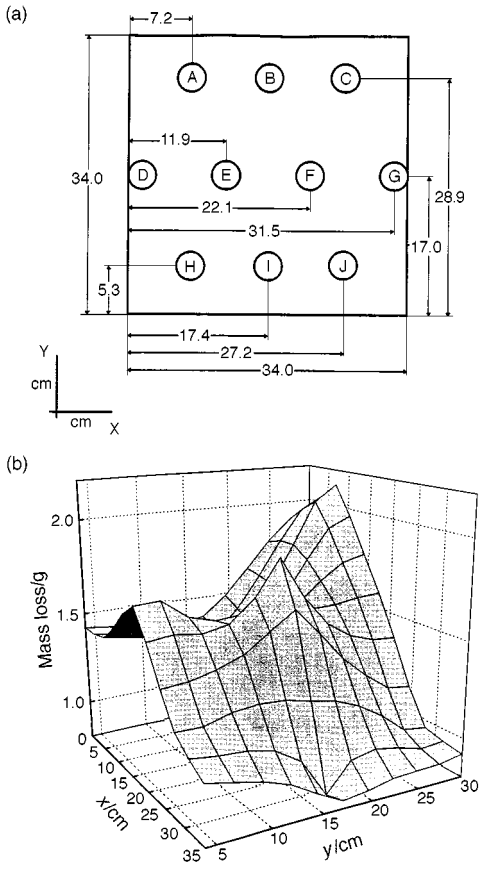

Fig. 2 Influence of the sample position relative to the microwave magnetron on mass loss. (a) Different positions (A–J) of the sample inside the microwave oven. (b) Response surface for the influence of axes *x* and *y* on mass loss.

Sample digestion conditions

To begin the experiments related to the optimization of the acid concentration (HNO_3) its initial concentration (6 mol l^{-1}) was the same as in previous work.[34, 35] The maximum power was fixed for sample digestion, and the acid concentrations were varied at 6.0, 8.0 and 15.6 mol l^{-1} while the digestion times were varied between 5 and 25 min. Longer times were not tested because the analytical frequency would be decreased. The situation without microwave actuation was also tested using the oyster tissue and unacceptable recoveries were obtained (*ca.* 472.3%), due to the contribution of the matrix color that was not eliminated. When nitric acid was used in the concentrations mentioned above (6, 8 and 15.6 mol l^{-1}), the iron recoveries were 256.3, 229.0 and 130.2%. The results of these experiments showed that the organic matter was not completely eliminated when only nitric acid was used and, therefore, volumes of 3 or 5 ml of 30% (v/v) H_2O_2[1] were also added to the slurry sample in order to increase the oxidizing power and to eliminate the organic matter, improving the digestion conditions and recoveries. At maximum power, the time required to digest the oyster standard reference material was only 10 min, and an acceptable recovery value of *ca.* 108% was obtained when 20 ml of concentrated HNO_3 + 5 ml of 30% (v/v) H_2O_2 as digestion solution was used (Table 2).

Different digestion conditions were required when mussel or fish samples were processed. The digestion of these samples was tried using the same acid and oxidizing agent, as well as microwave time mentioned above, but the conditions were not sufficient to destroy the organic matter and recoveries of about 470% were obtained. For this reason *aqua regia* and reversed *aqua regia* (Leffort mixture) solutions[1] plus 10% (v/v) H_2O_2 were tried in order to increase the oxidizing power. Good results were obtained with the recovery values being 103.6 and 100.5% for mussel and fish certified samples, respectively, when *aqua regia* and H_2O_2 was used as the digestion mixture. For these samples a microwave time of 20 min at maximum power was necessary.

An experiment was also performed in order to discover the influence of digestion time over the blank. In this way, the HNO_3 concentration range varied between 6, 8 and 15.6 mol l^{-1} + 30% H_2O_2 and the microwave was fixed to the maximum power for 20 min. In all cases, the blank values (about 8 μg l^{-1} Fe) were not significant, indicating that no contamination problems occurred using the proposed system.

Table 2 summarizes the digestion conditions applied to the different samples used in this work.

Optimization of the flow injection system for iron determination

During the studies related to digestion conditions, the effect of some chemical and physical parameters of the flow injection system, used for iron determination, were evaluated. The first studies aimed to find out the effect of the buffer solution. It was

found that, for each sample digest, it was necessary to change the ratio between the components of the buffer. Using concentrated acid for sample digestion, the final acid concentration in the digested sample provokes double peaks in the flow injection signals if the same buffering conditions as previously described are used.[33] This fact indicates that two distinct pH regions are formed in the flow system. To overcome this effect, the acid buffer component was diluted in order to guarantee a pH of 3.7 for reaction development. So, when the oyster samples were employed a ratio of 1 : 1 between acetic acid and ammonium acetate was used, while this ratio was 1 : 9 for mussel or fish samples. The carrier stream flow rate was decreased to 2 ml min^{-1} in order to avoid baseline drift problems and to maintain the best pH conditions for the reaction. Table 2 shows the best conditions for the spectrophotometric flow injection determination of iron. Other parameters employed were the same as used in the previously described system.[33]

Figs. 3(a) and 3(b) shows the peak profiles obtained with the flow system of Fig. 1(b) for digested samples.

Analytical characteristics

The proposed method covered the 50.0–200 μg l^{-1} iron concentration range ($r > 0.999$; $n = 5$) and presented detection and quantification limits of 7.5 and 24.4 μg l^{-1}, respectively, according to the IUPAC recommendations.[39] Precision, expressed as RSD, was 3.7% ($n = 10$) for repeatability and 5.0% ($n = 30$) for reproducibility using the Oyster Tissue standard reference material.

Analysis of real samples

Various fresh fish samples were analyzed after lyophilization. The results, expressed in μg g^{-1} Fe, and their precision ($n = 5$) are showed in Table 3. The accuracy was assessed by using NIST 1566a Oyster Tissue, IAEA MA-A-2 1062/TM Fish Homogenate Standard Reference Material and NIES no. 6 Mussel Certified Reference Material. By applying the unpaired *t*-test the results were found to be similar at the 95% confidence interval, indicating that the proposed method is fairly accurate for these types of samples.

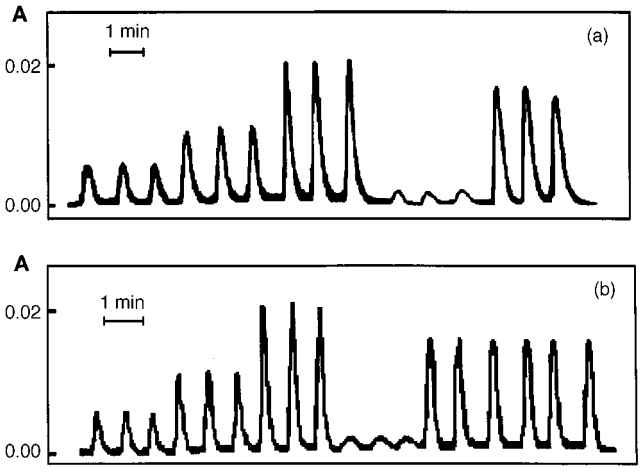

Fig. 3 Peak profiles obtained after microwave digestion for iron spectrophotometric determination. (a) From left to right, 50, 100 and 200 μg l^{-1} Fe standards solutions in triplicate followed by the blanks and oyster tissue; (b) as Fig. 3(a) followed by the blanks, mussel and fish reference materials, also in triplicate.

Table 3 Iron content in the samples as determined by on-line microwave digestion

Fish	Scientific name	Concentration/ μg g^{-1}*
Cação	*Squalus fernandinus* Mol.	89 ± 5
Pescadinha	*Cynoscion leiarchus* (Cuv. & Val.)	162 ± 19
Merluza	*Merlucius gayi*	79 ± 3
Oyster tissue†	—	567 ± 32
Fish homogenate‡	—	54 ± 2
Mussel§	—	167 ± 6

* Mean ± standard deviation ($n = 5$). † NIST SRM Oyster Tissue 1566a (certified value: 539 ± 15 μg g^{-1}). ‡ IAEA SRM MA-A-2 1062/TM (certified value: 54 ± 1 μg g^{-1}). § NIES CRM Mussel (certified value: 161 ± 12 μg g^{-1}).

Conclusion

With the proposed on-line microwave assisted digestion *ca.* 10 min are necessary to digest the oyster samples and 20 min to digest mussel and fish samples, giving an analytical frequency (sample preparation + analyses) of *ca.* 5 and 3 sample h^{-1}, respectively. When compared with other work, involving mechanization, the analytical frequency obtained here seems to be lower. However, it is necessary to consider that the total digestion of the sample must be achieved in the present case due to the use of the UV/VIS spectrophotometric method. In addition, this frequency was established taking into account the complete analytical process and not only the sample measurements.

Although the sample is trapped during the digestion time and concentrated acids and oxidizing agents were used, no problem with high pressure was detected because the inner volume of the digestion coil was increased by using 2 mm id tubes. In addition, because only few microliters of sample and acid are necessary for sample digestion, problems of damage to the analyst and/or environment are minimized. In this way, the proposed method is appropriate for samples in which the available volume is restricted, such as spinal liquid, body fluids and others.

Only small modifications in the on-line digestion and flow injection systems are necessary to adjust the systems to the different samples employed here, permitting its utilization in the routine analysis.

Finally, this work pointed out the possibility of a more complete mechanization of the analytical process, including sample preparation with subsequent spectrophotometric analyses.

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References

1 Kingston, H. M., and Jassie, L. B., *Introduction to Microwave Sample Preparation—Theory and Practice*, American Chemical Society, Washington, DC, 1988, p. 263.
2 Valcárcel, M., Luque de Castro, M. D., and Tena, M. Y., *Extracción con Fluidos Supercríticos en el Processo Analítico*, Editorial Reverté S. A., Barcelona, 1993, p. 469.
3 Burguera, M., and Burguera, J. L., *Quím. Anal.*, 1996, **15**, 112.

4 de la Guardia, M., Salvador, A., Burguera, J. L., and Burguera, M. J., *Flow Injection Anal.*, 1988, **5**, 121.
5 Arruda, M. A. Z., and Santelli, R. E., *Quím. Nova*, 1997, **20**, 638
6 de la Guardia, M., Carbonell, V., Morales-Rubio, A., and Salvador, A., *Talanta*, 1993, **40**, 1609.
7 Burguera, J. L., Burguera, M., and Brunetto, M. R., *At. Spectrosc.*, 1993, **14**, 90.
8 Burguera, M., Burguera, J. L., and Alarcón, O. M., *Anal. Chim. Acta*, 1996, **179**, 351.
9 Burguera, M., Burguera, J. L. and Alarcón, O. M., *Anal. Chim. Acta*, 1988, **214**, 421.
10 Bryce, D. W., Izquierdo, A., and Luque de Castro, M. D., *Anal. Chim. Acta*, 1996, **324**, 69.
11 Guo T., and Baasner, J., *Talanta*, 1993, **40**, 1927.
12 Hanna, C. P., and McIntosh, S. A., *At. Spectrosc.*, 1995, **16**, 106.
13 Morales-Rubio, A., Mena, M. L., and McLeod, C. W., *Anal. Chim. Acta*, 1995, **308**, 364.
14 Cabrera, C., Madrid, Y., and Cámara, C., *J. Anal. At. Spectrom.*, 1994, **9**, 1423.
15 López-Gonzálvez, M. A., Gómez, M. M., Cámara, C., and Palacios, M. A., *J. Anal. At. Spectrom.*, 1994, **9**, 291.
16 Gluodenis, Jr., T. J., and Tyson, J. F., *J. Anal. At. Spectrom.*, 1993, **8**, 697.
17 Burguera, M., Burguera, J. L., Rondón, C., Rivas, C., Carrero, P., Gallignani, M., and Brunetto, M. R., *J. Anal. At. Spectrom.*, 1995, **10**, 343.
18 Burguera, J. L., and Burguera, M., *J. Anal. At. Spectrom.*, 1993, **8**, 235.
19 Arruda, M. A. Z., Gallego, M., and Valcárcel, M., *J. Anal. At. Spectrom.*, 1996, **11**, 169.
20 Sooksamiti, P., Geckeis, H., and Grudpan, K., *Analyst*, 1996, **121**, 1413.
21 Cuesta, A., Todoli, J. L., and Canals, A., *Spectrochim. Acta, Part B*, 1996, **51**, 1791.
22 Bordera, L., Hernandis, V., and Canal, A., *Fresenius' J. Anal. Chem.*, 1996, **355**, 112.
23 LaFuente, J. M. G., Sánchez, M. L. F., Marchante-Gayón, J. M., Uria, J. E. S., and Sanz-Medel, A., *Spectrochim.. Acta Part B*, 1996, **51**, 1849.
24 Chakraborty, R., Das, A. K., Cervera, M. L., and de la Guardia, M., *Anal. Lett.*, 1997, **30(2)**, 283.
25 de Almeida, M. D., Leandro, K. C., da Costa, C. V., Santelli, R. E., and de la Guardia, M., *J. Anal. At. Spectrom.* 1997, **12**, 1235.
26 Maksimova, I. M., Morosanova, E. I., Kuz'min, N. M., and Zolotov, Y. A., *Fresenius' J. Anal. Chem.*, 1997, **357**, 946.
27 Carlosena, A., Gallego, M., and Valcárcel, M., *J. Anal. At. Spectrom.*, 1997, **12**, 479.
28 Balconi, M. L., Borgarello, M., Ferracioli, R., and Realini, F., *Anal. Chim. Acta*, 1992, **261**, 295.
29 Schmitt, A., Buttle, L., Uglow, R., Williams, K., and Haswell, S., *Anal. Chim. Acta*, 1993, **284**, 249.
30 Benson, R. L., McKelvie, I. D., Hart, B. T., and Hamilton, I. C., *Anal. Chim. Acta*, 1994, **291**, 249.
31 Williams, K. E., Haswell, S. J., Barclay, D. A., and Preston, G., *Analyst*, 1993, **118**, 245.
32 Bergamin, H. Fº, Reis, B. F., Jacintho, A. O., and Zagatto, E. A. G., *Anal. Chim. Acta*, 1980, **117**, 81.
33 Mortatti, J., Krug, F. J., Pessenda, L. C. R., Zagatto, E. A. G., and Jørgensen, S. S., *Analyst*, 1982, **107**, 659.
34 Arruda, M. A. Z., Fostier, A. H., and Krug, F. J., *J. Braz. Chem. Soc.*, 1997, **8**, 39.
35 Fostier, A. H., and Ferreira, J. R., *Quím. Nova*, 1995, **18**, 425.
36 Miller-Ihli, N. J., *J. Anal. At. Spectrom.*, 1988, **3**, 73.
37 Bendicho, C., and de Loos-Vollebregt, M. T. C., *J. Anal. At. Spectrom.*, 1991, **6**, 353.
38 Januzzi, G. S. B., Krug, F. J., and Arruda, M. A. Z., *J. Anal. At. Spectrom.*, 1997, **12**, 375.
39 Analytical Methods Committee, *Analyst*, 1987, **112**, 199.

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