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Review

# Continuous flow systems for the determination of trace elements and metals in seafood

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**Abstract**

A review is presented to show the advantages involved in the use of continuous flow systems for the determination of trace metals in seafood samples. The flow injection methods proposed for the determination of metals in seafood are described and compared according to the used detection technique. On-line sample dissolution and on-line separation techniques, analytical figures of merit and interferences are also discussed. © 2001 Elsevier Science Ltd. All rights reserved.

*Keywords:* Flow injection; Metals; Seafood

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## 1. Introduction

Trace elements and metals are continuously released in the aquatic environment via natural and anthropogenic influx. The determination of these analytes in seafood has interest because of the importance of many of these elements to human health. This interest arises from two areas of concern, nutritional and toxicological. Nutritional because trace metals such as Ca, Fe, Mg, Zn, Cu, Co and Al are necessary for maintenance of optimum health and toxicological since certain metals such as Pb, Cd, As and Hg are detrimental to optimum health. Furthermore, it is an important aspect of environmental analysis because mussels are used as bioindicator organisms to assess bioavaility contaminant concentrations in coastal waters. Interest in the bioaccumulation of trace metals in marine bivalve molluscs, as the common mussel *Mytilus edulis*, stems mainly from their use as bioindicators of coastal contamination and from lead to an evaluation of their suitability for human consumption as seafood.

Determination of trace metals in seafood requires the use of sensitive and selective techniques such as atomic spectrometry (AS). These analytical techniques require the introduction of liquid samples, and 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. Most conventional digestion procedures are labor-intensive and a number of them are potentially hazardous. The advances in analytical instrumentation have not been accompanied by significant changes in sample preparation methods. Besides, trace analysis usually requires sample preconcentration, above all when a flame atomic absorption spectrometer is used as detector. Therefore, on-line sample dissolution, and continuous separation have evolved into two of the most prominent contributions of the flow-injection (FI) technique to atomic spectroscopy. Automation of sample pretreatment and manipulations within the manifold have increased sample throughput and decreased the potential for sample contamination, which is critical in trace analysis. There is no systems that incorporate both processes, some incorporate a continuous digestion process and other an on-line preconcentration or separation step.

In the present bibliographical survey, the FI methods proposed for the determination of trace elements and metals in seafood samples are described and compared.

The most important details of the published FI procedures for seafood analysis, following a chronological order, are contained in Table 1 (trace elements) and Table 2 (contaminant metals). In the following paragraphs, some points observed in these tables are highlighted due to their interest.

## 2. Discussion

The analytical process consist of three essential stages: preliminary operations, measurement and transducing of the analytical signal and data collection and processing. The first step, preliminary operations: sampling and sample preservation, dissolution-desegregation, separation, analytical reaction and transport to the detector has so far scarcely been automated despite the great interest in reducing human participation in such time consuming and tedious activities, which are also the source of major errors and are occasionally even hazardous. *On-line microwave digestion* and *on-line separation procedures* solve these drawbacks, and have been shown to be very effective to increase sampling frequency and througput as well as ease of preliminary operations automation.

### 2.1. On-line microwave digestion

Although direct analysis of solid samples and slurries has made significant progress, most spectroscopic techniques require liquid samples. In addition seafood samples are solids with organic matter, so the digestion of this type of samples, in order to obtain appropriate solutions for their analysis by flow-injection-spectrometry can be carried out in a simple way by classical acid or dry ashing treatments. After dissolution, flow injection is a very useful approach for on-line preconcentration of the trace elements to be determined. However, in recent years the development of slurry introduction procedures with on-line microwave-assisted sample treatments offers many advantages over the conventional methods including reduction in digestion time, digestion of difficult matrices and dissolution in what is essentially a closed environment, which reduces volatile analyte loss and atmospheric contamination. Besides, the FI methodology avoid analyte loss and potential errors from volumetric transfers.

The digestion conditions or degree of dissolution in the microwave oven were governed by three variables: acid concentration, digestion time and microwave power. Hence, the interaction of the acid solution and microwave energy with the slurry sample influences the destruction of organic matter as demonstrated by Kuss (1992). Dilute acids absorb microwave power more strongly than do concentrated acids, the difference is attributed by Kingston and Jassie (1988) to the larger fraction of water present in more dilute acids. On the other hand, high acid concentrations expedite the attack of sample matrices in microwave ovens, but tend to produce excessive gas pressure. Because equilibrium is never reached in flow systems, completely avoiding uncontrolled high pressures within the manifold is difficult. In addition, sample digestion should be achieved with the lowest possible acid mixture concentration and shortest digestion time in order to minimize potential hazards.

Table 1
FI methods for trace element determinations in seafood samples[a]

| Analyte | Sample | Detection | Sample dissolution | Separation technique | Interferences | DL ($\mu g\ l^{-1}$) | SF ($h^{-1}$) | RSD (%) | R (%) | Reference |
|---|---|---|---|---|---|---|---|---|---|---|
| Zn, Cu, Mn, Fe, Cr(III) | Mussel | FAAS ETAAS | Off-line wet digestion | On-line ion-exchange | Al, Ti | 0.14–2.1 | 13 | 0.7–1.7 | No data | Hirata et al. (1989) |
| Ca, Fe, Mg, Zn | Mussel | FAAS | On-line MW digestion | No | No data | No data | 30–40 | $<4.5$ | 94–107 | Haswell et al. (1992) |
| Cu | Mussel | ETAAS | Off-line wet digestion | on-line sorbent extraction | No | 0.05 | No data | 2 | 87–107 | Ma et al. (1994) |
| Al | Mussel | ETAAS | on-line MW digestion | No | No | 10 | 3 | 4.3 | 90 | Arruda et al. (1995) |
| Se | Mussel | ETAAS | On-line MW digestion | No | No | 2 | No data | 6.8 | No data | Arruda et al. (1996) |
| Fe | Mussel | UV/VIS | On-line MW digestion | No | No data | 7.5 | 3 | 3.7 | No data | Pereira et al. (1998) |
| Co | Fishs Mussel | UV/VIS | On-line MW digestion | No | No | 0.0017 | No data | 2.4 | No data | Pereira-Filho and Arruda (1998) |
| Cu Co | Mussel | FAAS | Off-line wet digestion | On-line sorbent extraction | No | 0.3 6.0 | 90 | 3.0 2.2 | No data | Ali et al. (1999) |
| Zn | Fish tissue | ETAAS | Off-line wet digestion | On-line sorbent extraction | No | 3 | 40 | 2.5 | No data | Jorgensen, Cassella et al. (1999) |
| Se | Fish tissue | AFS | Off-line wet digestion | on-line hydride generation | No data | 0.0087 | No data | 2.1–8.4 | 90.5–109 | Wei et al. (1999) |

[a] DL, detection limit (defined on the basis of 3 times the standard deviation of the blank); SF, sampling frequency; RSD, relative standard deviation; R, recovery; UV/VIS, spectrophotometry; FAAS, flame atomic absorption spectrometry; ETAAS, electrothermal atomic absorption spectrometry; AFS, atomic fluorescence spectrometry; MW, microwave digestion.

Table 2
FI methods for contaminant metals determinations in seafood samples[a]

| Analyte | Sample | Detection | Sample dissolution | Separation technique | Interferences | DL ($\mu g\ l^{-1}$) | SF ($h^{-1}$) | RSD (%) | R (%) | Reference |
|---|---|---|---|---|---|---|---|---|---|---|
| Cd | Mussel | FAAS* | Extraction with Tris–HCl buffer | No | Cu, Zn | No data | No data | No data | No data | High et al. (1992) |
| Cd Pb | Mussel | ETAAS | Off-line wet digestion | On-line sorbent extraction | No | 0.003<br>0.04 | No data | 2 | 87–107 | Ma et al. (1994) |
| As | Mussel | ICP-OES | Off-line dry ashing | On-line liquid-liquid extraction coupled to a hydride generator gas-liquid separator | No | 2 | No data | 11 | No data | Cervera et al. (1995) |
| Cd | Copepoda | UV/VIS | Off-line wet digestion | On-line ion-exchange | No | 0.11 | 25 | 2.26–2.72 | 99.1–111.2 | Gomes Neto et al. (1995) |
| Hg | Fish | CVAAS | Off-line wet digestion | On-line Hg cold vapor generation | No data | 0.0053 | 6 | 5 | No data | Canela et al. (1996) |
| Hg | Tuna Fish | CVAAS | Off-line MW digestion | On-line Hg cold vapor generation | Ag, Se | 0.2 ng $g^{-1}$ | 20–30 | 5 | No data | Murphy et al. (1996) |
| Cd | Mussel | FAAS | Off-line MW digestion | On-line ion-exchange | No | 0.56 | 40 | 1.4-6.6 | 98.8–100.6 | Enríquez-Domínguez et al. (1997) |
| Hg | Fish | CVAAS | Off-line MW digestion | On-line Hg cold vapor generation | No | 0.34 | 30 | 0.95 | No data | Bauzá de Mirabó, Thomas, Rubi, Fortiza and Cerda (1997) |
| Hg | Fish | CVAAS | Off-line wet digestion<br>off-line MW digestion | On-line Hg cold vapor generation | No data | 7.7 ng $g^{-1}$<br>19.4 ng $g^{-1}$ | No data | 1.1<br>2.6 | No data | Aduna de Paz et al. (1997) |
| Pb | Cod tissue | ETAAS | Off-line wet digestion | On-line sorbent extraction | Cu, Cd | 0.0048 | 31 | 2.1 | No data | Yan and Adams (1997) |
| Hg | Fish tissue | CVAAS | Off-line tetramethyl ammonium hydroxide digestion | On-line Hg cold vapor generation | No | 0.1 | 100 | 1.3 | No data | Tao et al. (1998) |
| Cd | Mussel | FAAS | Off-line MW digestion | On-line ion-exchange | No | 0.56 | 40 | 1.4 | 98.8–100.6 | Yebra and Enriquez (1998) |
| Methyl Hg<br>Ethyl Hg<br>Phenyl Hg<br>Inorganic Hg | Fish | CVAAS | Solid phase extraction | HPLC on-line Hg cold vapor generation | No | 0.009<br>0.006<br>0.010<br>0.005 | No data | 3.6<br>5.5<br>10.4<br>7.6 | 94<br>106<br>92<br>102 | Yin et al. (1998) |
| Methyl Hg | Dogfish muscle | FSV | No data | Electrochemical | No | 112.6 | No data | No data | No data | Lai et al. (1998) |
| Methyl Hg<br>Inorganic Hg | Mussel | CVAAS | Off-line US extraction | On-line Hg cold vapor generation | No | 0.25<br>0.17 | No data | 5–10 | 92–98 | Río-Segade et al. (1999a) |
| Cd | Mussel | FAAS | Off-line wet digestion | On-line sorbent extraction | No | 0.5 | 90 | 1.4 | No data | Ali et al. (1999) |

(*continued on next page*)

Table 2 (*continued*)

| Analyte | Sample | Detection | Sample dissolution | Separation technique | Interferences | DL (μg $l^{-1}$) | SF ($h^{-1}$) | RSD (%) | R (%) | Reference |
|---|---|---|---|---|---|---|---|---|---|---|
| Total Hg<br>Inorganic Hg | Mussel | CVAAS | Off-line MW digestion<br>off-line US extraction | On-line Hg cold vapor generation | No | 0.52<br>0.30 | No data | 0.74–1.40 | No data | Río-Segade et al. (1999b) |
| As | Fish tissue | AFS | Off-line wet digestion | On-line hydride generation | No data | 0.009 | No data | 0.4–5.8 | 92.6–113.4 | Wei et al. (1999) |

[a] DL, detection limit (defined on the basis of 3 times the standard deviation of the blank); SF, sampling frequency; RSD, relative standard deviation; R, recovery; UV/VIS, spectrophotometry; FAAS, flame atomic absorption spectrometry; CVAAS, cold vapor atomic absorption spectrometry; ETAAS, electrothermal atomic absorption spectrometry; ICP-OES, inductively coupled plasma optical emission spectrometry; FAAS*, atomic absorption spectrometry with a thermospray–microatomizer interface; HPLC, high performance liquid chromatography; AFS, atomic fluorescence spectrometry; FSV, fast-scan voltammetry; MW, microwave digestion; US, ultrasound.

Food mineralization can be accomplished with various acids including nitric, hydrochloric, sulfuric and perchloric, but sulfuric acid attacks PTFE tubes at high temperatures and perchloric acid is potentially explosive in contact with organic materials. Therefore, the most frequently used acids for continuous digestion of foods in microwave ovens are hydrochloric and nitric acids.

### *2.2. On-line separation procedures*

On the other hand, the combination of classical preconcentration procedures provides an increase in sensitivity of the detection technique. Because of the extremely low concentrations of metals in matrices such as food samples, a preliminary preconcentration step and/or selective separation from interfering matter is usually necessary before their determination. FI on-line preconcentration systems play an important role in today's analytical chemistry. They offer the possibility of determining lower analyte concentrations and avoiding matrix effects by effective separation of the analyte from interfering matrix components. In this respect, flow systems combined with ion-exchange or sorbents extraction techniques carried out into minicolumns have been used successfully in atomic absorption spectrometry to preconcentrate trace metal ions from seafood samples.

For metal ions determination in seafood samples, still there is no systems proposed that combine both on-line processes, some incorporate a continuous digestion process and other an online preconcentration step.

The reported FI procedures for the determination of trace elements and metals have been grouped to discussion according to the employed detection technique. The FI systems proposed are mainly based on flame (FAAS) and electrothermal atomic absorption (ETAAS) detectors, while for the determination of mercury cold vapor atomic absorption spectrometry (CVAAS) and for arsenic determination inductively coupled plasma-optical emission spectrometry (ICP-OES) has been used.

### *2.3. Description of the methods according to the used detection technique*

#### *2.3.1. Flame atomic absorption methods*

One of the drawbacks of FAAS is its limited sensitivity. Therefore, only majority metallic ions can be determined directly without a preconcentration step. In this way Hirata, Honda and Kumamaru (1989) concentrated diverse metal ions on a microcolumn packed with Muromac A-1, which is an iminodiacetate chelate resin. The digestion procedure for the biological samples analyzed (mussels) is carry out by decomposition in a Teflon beaker on a hot-plate by nitric acid/ hydrofluoric acid/perchloric acid digestion. Haswell and Barclay (1992) proposed a flow injection system for on-line microwave digestion of slurried samples in 5% v/v nitric acid with direct determination of Ca, Fe, Mg and Zn. Bubble formation during digestion was controlled by post-digestion cooling and pressure regulation. High, Azani, Fazekas, Chee and Blais (1992) described and optimized a micro-atomization interface (made from quartz tubes) which is fuelled by hydrogen for the detection of cadmium on a FI system. Enríquez-Domínguez, Yebra-Biurrun and Bermejo-Barrera (1998), and Yebra and Enríquez (1998) proposed the determination of cadmium in mussels by FAAS. Mussel samples were dissolved off-line by acid digestion with microwave heating in high-pressure teflon bombs, and then the metal was continuously preconcentrated on a minicolumn packed with a poly(aminophosphonic acid) chelating resin (PAPhA) and eluted with dilute hydrochloric acid into the nebuliser–burner system of an atomic absorption spectrometer. Ali, Yin, Shen, Ye and Gu (1999) proposed an on-line determination of copper, cadmium and cobalt by use of 1,10-phenanthroline as complexing agent and preconcentration on RP-$C_{18}$ material contained in a microcolumn with a FI-FAAS system. The mussel samples were dissolved off-line by wet treatment with nitric acid and hydrogen peroxide. Jorgensen et al. (1999) developed an on-line procedure where Zn is preconcentrated from acidic medium as its thiocyanate complex onto a plyurethane foam minicolumn. The elution step is performed with a stream of 30% acetone in 2% nitric acid and the zinc displaced is introduced directly into the nebulizer of a flame atomic absorption spectrometer. The biological certified reference materials (fish tissues) were dissolved off-line by wet treatment with nitric acid and perchloric acid.

### 2.3.2. *Electrothermal atomic absorption methods*

Combination of FI sorbent extraction with ETAAS can reduce the sample loading time of the extracted metal amount by two to three orders of magnitude thanks to the higher sensitivity of ETAAS detection. However, ETAAS is a discrete non-flow-through technique with limited sample injection volume and low tolerance to matrix interference, which makes the combination more difficult. Two types of on-line sample introduction techniques are generally involved: thermospray sample deposition and direct sample injection through the autosampler capillary in a volume-based or time-based mode at very low flow rates. In the former technique, larger sample volumes can be introduced, while the cycle time of ETAAS measurement without a drying step is significantly reduced. In the latter technique, the autosampler is controlled manually or modified to be controlled automatically.

Ma, Van Mol and Adams (1994) developed a FI sorbent extraction system for determination of trace and ultratrace cadmium, copper and lead by ETAAS. Using ammonium diethyldithiophosphate (DDPA) as complexing agent with citrate as masking agent, the analytes were selectively preconcentrated with effective removal of the matrixes. The mussel samples were dissolved off-line in standards digestion bombs by wet treatment with nitric acid and perchloric acid. Arruda, Gallego and Valcárcel (1995) proposed a flow-injection system incorporating a microwave oven for the digestion of food samples for the determination of aluminium by ETAAS. Samples as slurries were digested on-line with dilute nitric acid and were collected in an autosampler cup. The chemical modifier was 0.01 mol $l^{-1}$ $Mg(NO_3)_2$ and was placed in another autosampler cup. A similar flow system was reported by the same authors (Arruda et al., 1996) for the determination of selenium in shellfish tissue. These systems are characterized by their simplicity because no special device is needed to process evolved gases during sample digestion. Yan and Adams (1997) developed a more selective FI on-line sorption separation and preconcentration system with a knotted reactor for ETAAS determination of lead in the presence of alkali, alkaline earth and heavy metals using diethyldithiophosphate as complexing agent and citric acid as masking agent. The collected analyte complex was eluted quantitatively with ethanol and all the eluate was directly introduced into the graphite tube without a L'vov platform by an air flow. Cod muscle samples were dissolved off-line in standards digestion bombs by wet treatment with nitric acid and perchloric acid.

### 2.3.3. *Cold vapor atomic absorption methods*

Cold vapor atomic absorption spectrometry (CV-AAS) is the most wide spread technique for the determination of mercury because of its high sensitivity, absence of spectral interferences, relatively low operation costs, simplicity, and speed. Therefore, several articles for mercury determination and speciation in fish and mussel tissues involving CVAAS and FI methodology were reported. Canela, Jardim and Rohwedder developed an automatic FI system for the determination of mercury. In this method, Hg(II) and a reductant solution ($SnCl_2$/5% $HNO_3$) were mixed in a sample introduction device. The resultant Hg vapor was swept in $N_2$ via a gas/liquid separator into a quartz tube packed with Au wire protected with a precolumn packed with soda lime. The quartz tube was heated electrically, and once cooled, the displaced Hg was determined by cold vapor AAS. Murphy, Jones and Hill (1996) developed a simple and accurate method for the determination of total Hg. The method consists of an off-line microwave digestion stage followed by analysis using a FI system with detection by cold vapor atomic absorption spectrometry. Aduna de Paz, Alegría, Barberá, Farré and Lagarda (1997) studied a FI system CV-AAS preceded by a wet digestion in a microwave oven for the determination of Hg in fish. Bauzá de Mirabó, Thomas, Rubí, Forteza and Cerdá (1997) proposed a sequential injection analysis system for the determination of Hg by CV-AAS. The sample was subjected to an acid digestion with sulfuric and nitric acid in a microwave oven with high-pressure pumps. Tao, Willie and Sturgeon (1998) developed a simple, rapid and reliable method for the determination of total Hg in biological samples such as certified marine biological reference materials. These samples were solubilized using tetramethylammonium hydroxide. Thus, the organically bound Hg was cleaved and converted to inorganic Hg by on-line addition of $KMnO_4$. The decomposed Hg together with inorganic Hg originally present in samples was determined by FI CVAAS after reduction to elemental Hg vapour using $NaBH_4$. Yin, Frech, Hoffmann, Lüdke and Skole (1998) described a fully automated system for the direct determination of methylmercury, ethylmercury, phenylmercury and inorganic mercury. It is based on solid phase extraction preconcentration incorporated in a FI system, high performance liquid chromatography (HPLC) separation, reduction combined with thermolysis and determination by CVAAS. Río-Segade and Bendicho (1999a) proposed an ultrasound-assisted extraction method with hydrochloric acid for mercury speciation in fish tissues, and centrifuged extracts were directly injected into a FI-CVAAS system. First, methylmercury was separately determined using sodium tetrahydroborate as reducing agent after selective extraction with dilute hydrochloric acid. Inorganic mercury was determined by selective reduction with stannous chloride in dilute hydrochloric acid extracts containing both mercury species. Total mercury could not be determined in the sonicated acid extracts using sodium tetrahydroborate as reducing agent because the methylmercury and inorganic mercury sensitivities were different. Total mercury, calculated as the sum of methylmercury was compared with the total mercury content determined after microwave digestion. The same authors (Río-Segade et al. (1999b) developed a method based on FI-CVAAS using selective reduction for the separate determination of inorganic and total mercury in mussel samples. Mineralization procedures (nitric acid-hydrogen peroxide) in Parr-type high-pressure reactors heated by microwave irradiation were used for the decomposition of mussel samples to determine total mercury with sodium borohydride as reducing agent. Inorganic mercury was determined in acid extracts obtained by sonication, and stannous chloride in acid medium was used as reducing agent because it was the most selective, sensible and precise, and it had the best detection limit for inorganic mercury determination.

### *2.3.4. Inductively coupled plasma optical emission spectrometry methods*

ICP-OES has been used for the determination of As in a great variety of matrices since the technique is free of chemical interference and offers a wide dynamic range. Nevertheless, this technique presents a relatively high instrumental detection limit and for the analysis of foods, a spectral interference study must be carried out. An alternative method for improving the low sensitivity of As determination by ICP-OES is in combination with hydride generation. A method to eliminate interferences is extract As in an organic phase before hydride generation. This causes that the matrix interference will be eliminated and, As is determined without the need to resort to long and tedious back-extraction processes. Thus, Cervera, Montoro, Sánchez, Menéndez and Sanz (1995) proposed the determination of As in foods (beer, tomatoes, mussels and liver paste). The organic matter was destroyed off-line by dry ashing, and the white ash was disolved in hydrochloric acid. The FI system consists of two-step separation procedure: first, the liquid-liquid extraction of $AsI_3$ into xylene, and then, the continuous generation of As, on-line with the ICP, from the organic phase. Arsine gas and the organic phase were separated in a gas–liquid separator. The hydride was fed continuously into the ICP where As was determined at the 228.8 nm line.

### *2.3.5. Spectrophotometric methods*

Gomes Neto, Bergamin Filho, Sartini and Zagatto (1995) proposed a FI system with a minicolumn packed with a strongly basic anion exchanger for the spectrophotometric determination of cadmium based on the formation of a cadmium iodide–Malachite Green associate. Since the system proposed was incompatible with samples solubilized with perchloric acid, a sample mineralization with nitric acid and hydrogen peroxide was used.

Although a significant number of microwave flow systems for solid sample digestion coupled to FAAS, ETAAS or ICP techniques apperar in the literature, the same is not true for UV/VIS spectrophotometry. However, Pereira, Rohwedder and Arruda (1998) developed a method for the spectrophotometric determination of iron in seafood slurry samples based onthe on-line microwave assisted digestion. Into de FI system, the digested sample reacts with ascorbic acid, and $Fe^{3+}$ was reduced to $Fe^{2+}$. Then, this reacts with 1,10-phenanthroline and the absorbance of the complex formed was continuously determined at 512 nm. Pereira, Filho and Arruda (1999) proposed a mechanised system for on-line slurry food sample microwave digestion and an off-line cobalt determination. Cobalt was determined by spectrophotometry, and this determination are based on the catalytic effect of cobalt on the oxidation of Tiron.

### *2.3.6. Other detection techniques*

Lai, Huang, Zhou and Wipf (1998) proposed the only example of an electroanalytical FI determination of trace elements and contaminant metals in seafood samples. They developed a selective determination of methylmercury by flow-injection fast-scan voltammetry.

A thin Hg film was formed at a Pt microelectrode prior to the measurement. Detection of methylmercury is carried out by measuring the oxidation of methylmercury radicals that were generated at the Hg microelectrode.

Hydride vapour generation atomic absorption spectrometry is a preferred method for the determination of hydride forming elements such as arsenic and selenium, particularly at ultratrace concentration levels. Therefore, Wei, Gupta, Hernández and Farhat (1999) applied a FI hydride vapour generator-coupled atomic fluorescence spectroscopic system to replace the atomic absorption spectroscopic detector, which is used in the ordinary hydride generation technique. Membrane dryers were used in order to improve sensitivity. These membranes consist of a hygroscopic ion-exchange membrane in a continuous drying process between hydride generator (separator) and atomic fluorescence detector to selectively remove water vapour from mixed hydride gas streams.

### 2.4. Analytical figures of merit

To compare the methods studied in this review has been taken into account the detection limit, precision, accuracy and sampling frequency.

Detection limits obtained provides the determination trace elements and metals in seafood samples at $\mu g\ l^{-1}$ or ng $l^{-1}$ level, even improved by the instrumental technique or the separation process incorporated into the FI system. Concerning the precision, all methods provided a good precision and even $<10\%$, expressed as relative standard deviation. Accuracy of methods was evaluated by determining the metal content in certified reference materials (CRM) such as Mussel Tissue (supplied by the National Institute for Environmental Studies (NIES) Japan), Mussel Tissue CRM 278 and CRM 422 (cod tissue) supplied by the Community Bureau of Reference (BCR) Belgium, 1566a Oyster Tissue (supplied by the National Institute of Standards an Technology (NIST) USA), Mussel Tissue GBW 08571 (supplied by the Bureau of Oceanography, China), TORT-1 and TORT-2 (lobster hepatopancreas from the National Research Council of Canada), DORM-1 (fish liver from the National Research Council of Canada), DOLT-1 (fish muscle from the National Research Council of Canada), DORM-2 (dogfish muscle from the National Research Council of Canada), DOLT-2 (dogfish liver from the National Research Council of Canada), CRM 464 (tuna fish muscle from the Community Bureau of Reference (BCR) Belgium), SRM MA-A-2 1062 (fish homogenate), MA-A1/TM (copepoda), MA-B-3/TM (fish flesh) and and IAEA-350 (tuna homogenate) from the International Atomic Energy Agency (IAEA) Vienna. In all cases, the contents found were in good agreement with the certified values.

In all the methods the sample throughput is not calculated. However, the sampling frequency obtained in the methods cannot be compared, since in some the digestion step is included and in others it is not. It is considered only when an on-line sample digestion is employed. The methods that present a greater sampling frequency do not take into account the digestion step, and it is incorporated in those that present a minor sampling frequency. In any case, it is greater than if a sample dissolution is accomplished in the bath mode, shortening the digestion time.

## 3. Interferences and samples

The majority of the proposed methods have been applied to real samples. They were mainly applied to determine trace metals in mussels and other seafoods samples (fish and shellfish). Therefore, the study of interferences was based on the potential interferents present in these samples. Thus, $Na^+$, $Ca^{2+}$, $K^+$, $Cl^-$, $SO_4^{2-}$, $H_2PO_4^-$, $CO_3^{2-}$, $Fe^{3+}$, $Mg^{2+}$ were tested. None of these ions interferes significantly in the determination of the trace metal studied at concentrations typically present in seafoods.

The interference effects of the transition metals and hydride forming elements in an organic medium were studied for As determination. The results obtained showed that most elements did not interfere. Since the concentrations assayed are higher than commonly found in foods, the extraction of As(III) in xylene avoids interferences from most transition metals.

## 4. Conclusions

FIA is shown to be a powerful technique for the determination of trace metals in seafood samples. This methodology allows accurate, selective, and selective determinations. Furthermore, *on-line microwave digestion* and *on-line separation procedures* solve the drawbacks of batch/manual preliminary operations, and have been shown to be very effective to increase sampling frequency and througput as well as ease of automation.

Flow systems incorporating sample dissolution and sample preconcentration have still not been proposed. It is hoped that in the future are developed new FI methodologies including these two proceses to allow the total automation of the determination of trace metals in solid samples, such as seafood, when an increase of the sensitivity or the removal of matrix interferences will be necessary.

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