High-throughput, multi-batch system for the efficient microwave digestion of biological samples

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Abstract

In this paper, we proposed a high-throughput microwave digestion system based on multi-batch reactors (three quartz test tubes inside commercial PTFE vessels). This original configuration was validated by ICP-MS analysis of several elements in biological certified reference materials (fish tissues and plankton). The proposed system was proved to be free from contamination showing very low LODs. The improved hardware configuration is therefore highly beneficial for the detection of trace elements in microsamples from the marine food web.

Keywords

High-throughput microwave digestion, biological samples, trace elements, marine food web, microsamples.
Introduction

Microwave-assisted sample digestion has become an important routine technique to dissolve solid samples for the analysis of inorganic and organic matrices\textsuperscript{1–4} ensuring fast dissolution, limited to no loss of volatile species and very low contamination levels.\textsuperscript{5}

As a general procedure, a mixture of pure acids is added to the sample in a digestion vessel which is sealed and irradiated by microwaves (MW) to reach the desired temperature.

Existing and conventional MW digestion systems may be modified and improved with the aim of i) increasing sample throughput, ii) reducing contamination processes and iii) decreasing the consumption of chemical reagents. Vessels, rotors and the entire procedure have to be suitably designed to tackle these issues.

Concerning the sample holder, fluoropolymers (e.g. Polytetrafluoroethylene, PTFE) are the material of choice thanks to their stability, chemical inertia and transparency to MW radiation.\textsuperscript{6} However, sample contamination may occur because of infiltration and trapping of gases or other phases into the polymer during the digestion treatment.\textsuperscript{7,8} The employment of highly pure quartz may be a simple strategy to overcome this problem.\textsuperscript{9} The only restriction to the utilization of this material concerns the impossibility to use hydrofluoric acid.

Besides the nature of the material, also the vessel appearance and design (size, shape, etc.) can be adapted to meet particular analytical necessities. For instance, the insertion of a small quartz cup inside a commercial PTFE closed vessel allowed to design vessel-inside-vessel systems which ensure a low dilution factor and the digestion of limited amount of sample (tens of milligrams).\textsuperscript{9–14} The employment of such small sample holders is advantageous when the mass of the samples to be analyzed is very limited or when very low analyte concentrations are expected. The analysis in these cases can be, in fact, challenging considering the high volume of acid mixtures (8–10mL) commonly used for the digestion in
commercial vessels.\textsuperscript{15,16} Moreover, lowering the volumes used for MW mineralization shows additional benefits such as reduced contamination and consumption of chemical reagents, leading to a more sustainable, clean and cost-effective sample treatment procedure.

As further evolution of vessel-inside-vessel systems, Millos et al.\textsuperscript{17} designed multi-batch reactors with a “vertical” configuration (i.e. three Perfluoroalkoxy alkanes (PFA) vials accommodated one over the one in a conventional vessel) enabling an increase of the sample throughput keeping the mineralization efficiency and simplicity of operation unaltered. These features are of key importance especially for research fields involving the analysis of a large number of specimens (e.g. environmental and biological monitoring campaigns), considering that the sample preparation is often the bottleneck in the whole analytical procedure.

In consideration of these benefits, a commercial version of multi-batch system was recently proposed too.\textsuperscript{18} However, no comprehensive validation for trace elements of this product is available.

For this reason, in this work we propose an alternative low-cost multi-batch system for high-throughput MW digestion of biological samples based on the insertion of 3 small quartz test tubes inside a commercial PTFE vessel. This system allows to simultaneously digest 30 samples using a maximum of 2 mL of digesting solution to dissolve small amounts of samples. A careful evaluation of contamination process and a systematic validation over around 20 trace elements were performed using three certified reference materials (CRMs) from the marine food web (fish tissues and plankton) to demonstrate the effectiveness of the proposed system.

**Experimental**

*Reagents*
Pure nitric acid produced by sub-boiling distillation\(^9\) from commercial HNO\(_3\) (Carlo Erba, 65% pure) was used for sample digestion and for the acidification of diluted solutions and standards.

Ultrapure water used for dilution was produced by a Sartorius Arium mini UV Lab Water System.

Three CRMs were used for the validation of the procedure: ERM®-BB422 (fish muscle) and BCR 414 (plankton) from the Institute for Reference Materials and Measurements in Belgium and DOLT-5 (dogfish liver) from the National Research Council in Canada.

Monoelemental stock solutions of 1000 mg/L of V, Cr, Mn, Co, Ni, Cu, Zn, As, Se, Sr, Mo, Ag, Cd, Sn, Pb, Fe, Sc, In and Hg (TraceCert®, Sigma-Aldrich) were properly diluted by weight to obtain multi-standard solutions (containing all the elements to be analyzed other than Hg) and single standard solutions containing only Hg.

A 10 µg/kg solution of In was used as internal standard for all the measurements.

**Instrumental setup and digestion protocol**

A ETHOS One (Milestone MLS) MW digestion system equipped with 10 PTFE commercial vessels (internal volume \(~100\) mL) was used for acid digestion.

Home-made PTFE inserts were realized to host 3 quartz test tubes inside each vessel (see details in technical drawings reported in Figs. S1a-c). Each test tube was closed with a PTFE cap (see Fig. S1d).

Sample digestion was performed as follows. The adequate sample mass was weighted and transferred into the test tubes: 250 mg of DOLT-5, 100 mg of BCR-414 and 200 mg of ERM®-BB422 (minimum masses to be used accordingly to the certificates of the CRMs). 2 mL of distilled HNO\(_3\) were added to each test tube, whereas 1 mL of ultrapure water was
transferred into the PTFE vessel. The temperature programs reported in Fig.S2 were used for the digestion.

After mineralization samples were transferred in low density polyethylene (LDPE) bottles, diluted to 30 g with ultrapure water and then diluted 1:10. LDPE bottles were decontaminated through a 3-stage process: i) washing with ultrapure water and immersion in a 0.4% w/w detergent solution (Nalgene L900) for one week; ii) rinsing with ultrapure water and soaking in a HNO₃ solution (2%, w/w) for one week; iii) rinsing with ultrapure water and soaking in a second HNO₃ solution (2%, w/w) for one week. The bottles were finally rinsed with ultrapure water before use. After each digestion batch, quartz test tubes were cleaned in a 0.4% Nalgene L900 solution under heating at ~70°C for 4 hours, soaked in a 2% HNO₃ bath for at least 24 h and rinsed with ultrapure water before use.

Diluted solutions were analyzed using a Thermo Scientific ICAP Q inductively coupled plasma mass-spectrometer (ICP-MS) using a He-collision cell in kinetic energy discrimination (KED) mode, under the operating conditions summarized in Table S1. Trace element quantification was performed by external calibration, except for mercury for which the standard addition method was used: indium was used as the internal standard for both procedures.

Results and discussion

Description of the hardware configuration

As shown in Figs. 1a-b, commercial PTFE vessels were modified inserting home-made PTFE holders for three quartz test tubes. The PTFE support was designed with the aim of keeping as stable as possible the test tubes in vertical position (the hole hosting the vial is deep almost as the vial length, see Fig. 1b), which is one of the main practical criticism of the
commercial multi-batch system. These inserts can be easily introduced and removed thanks to the handle present in the upper part (see the triangular-shaped handle on top of the insert in Fig. 1b). Each insert can host up to three quartz test tubes allowing to triple the throughput of the digestion batch (up to 30 samples may be digested using 10 commercial vessels). One of the test tubes holders was specifically designed to host the thermocouple used to control the temperature inside the vessel during the digestion process (see technical drawing in Fig. S1c). The monitoring of the temperature is accordingly performed outside the test tubes thus avoiding a possible contamination source. The possibility to control the temperature is of the utmost importance to correctly address the digestion process. This represents one of the main advancements achieved with respect to the previously proposed multi-batch digestion configuration in the literature.

Narrow quartz test tubes with an approximate capacity of 8 mL were fabricated to work with up to 2 mL of digesting solution avoiding liquid spills due to gas development during the mineralization. Test tubes were capped with PTFE covers for the same purpose and, primarily, to limit as much as possible contamination processes. The samples should be in fact protected from the drip of liquids condensed on the cap of the PTFE vessel, but gas evolution from the sample must be allowed to avoid the breakage of the quartz tubes caused by the increasing pressure.

As reported in Fig. 1a, a small volume of ultrapure water (1 mL) was introduced in the bottom of the vessel to further minimize the probability of cross-contamination related to volatile species. The presence of water should in fact generate a little counter-pressure during the digestion process and it should reduce sample evaporation.

*Evaluation of contamination processes and detection capabilities*
As described in the previous paragraph, the multi-batch system was designed paying particular attention to the limitation of potential contamination and cross-contamination processes (i.e. contamination between samples inside the same PTFE vessel). Therefore a careful evaluation of the occurrence of such phenomena is needed.

MW digestion cycles were performed loading in the same PTFE vessel test tubes containing one blank solution (i.e. only 2 mL of distilled HNO₃) and two sample aliquots to be digested (an example of the results in Table S2). These experiments were performed employing the DOLT-5 (dogfish liver) and BCR-414 (plankton) to assess the possible effect of the two different temperature programs. The analysis of the blank solutions allowed to assess the occurrence of both contamination and cross-contamination processes with only one experiment, as any of the two contamination processes would result in appreciable element concentrations in the blank.

Very low concentrations were obtained for all the investigated analytes using both temperature programs evidencing that no contamination process occurs during the mineralization, independently on their nature (i.e. contamination inside the test tubes or cross-contamination). In details, the average concentrations of the investigated trace elements were not distinguishable from the ones observed in 2% distilled HNO₃, i.e. the blank levels due to nitric acid only (refer to reference¹⁹ for the latter values, see Table S2 for representative actual values). Zinc should be mentioned as the only one element showing appreciable higher concentrations (around 0.3 µg/kg as the average values in blanks) with respect to the ones reported in¹⁹: some limitation is to be expected when ultratrace concentrations of zinc are to be determined, although its contribution is negligible in the present case.

The detection capabilities were assessed by estimating the limits of detection (LODs) for all the investigated elements. LODs (reported in Table 1) were calculated by tripling the
standard deviation of the concentrations measured for 12 blank solutions obtained during three different digestion batches. The obtained values were then corrected for the dilution factor (1:10) and divided by the sample weight (100 mg, i.e. the lowest sample mass employed in this study). Accordingly, these LODs are the lowest detectable concentrations in the biological samples.

For the sake of comparison, the levels of trace elements in blank solutions obtained using the proposed system were compared with the ones obtained using PTFE vessels without insert (i.e. standard hardware configuration). No significant differences in terms of elemental composition were observed and all the experimental concentrations were lower than the estimated LODs.

**Validation of the method using certified reference materials**

The validation was performed by digesting three biological CRMs (DOLT-5, BCR 414 and ERM-BB422) following the procedures reported in the experimental section.

The numerical results obtained for the analysis of the CRMs are reported in Table S3 (BCR-414), S4 (DOLT-5) and S5 (ERM-B422) and are depicted in Fig. 2a-c. Experimental data are represented as percent recoveries to allow a more clear interpretation of the results (see the very different concentrations in the CRMs, e.g. in DOLT-5 cobalt and iron concentrations are 0.27 and 1070 mg/kg, respectively).

The absence of bias in the results was assessed by the procedure proposed by the Institute for Reference Materials and Measurements (IRMM). This statistical test takes into account the values of the concentrations and their uncertainties comparing the difference between the certified and measured mean values (Δm) with the expanded uncertainties (UΔ). No statistical differences were found between certified and experimental concentrations for all the
elements, except for Sc, Zn and Pb in BCR-414, as suggested also by Fig. 2a. The statistically significant difference found for Sc may be considered reasonable since the Sc content in BCR-414 (experimental concentration = 0.46 mg/kg, see Table S3) is approximately equal to the determined limit of quantification (LOQ) value (LOQ = 0.39 mg/kg; i.e. three times the LOD value reported in Table 1). On the other hand, the bias in the result found for zinc and lead in BCR-414 cannot be hitherto explained and are still the subject of investigation. Nonetheless, recoveries higher than 80% were obtained also for these elements.

Regarding the precision of the measurements, satisfactory results were achieved, taking into account that trace element determinations are involved and that the precision was estimated using data from three different digestion batches. Briefly, relative standard deviations (RSD%) higher than 10% were found only for six analytes (Hg in BCR-414, Cr, Ni, Sn, Pb and Hg for DOLT-5). These higher values can be easily explained by observing that the determined concentrations are close to or even lower than the estimated LOQ (compare Table 1 and S3, S4 for numerical values).

Conclusions

The addition of home-made inserts hosting three quartz test tubes in commercial PTFE vessels was proved to be a simple and cost-effective way to convert a low-throughput system (i.e. rotor equipped with 10 PTFE vessels) into a high-throughput one keeping unaltered the efficiency of MW digestion and the extent of contamination. The low-cost and ease to manufacture of the proposed hardware configuration make it a valid practical alternative to the expensive commercial offer for routine analysis.

Microsamples (i.e. < 100 mg) can be easily digested and analyzed using the developed system thanks to the employment of reduced volumes of chemical reagents.
The proposed hardware configuration was validated by digesting and analyzing three different biological samples from the marine environment CRMs: BCR-414 (plankton), DOLT-5 (Dogfish liver) and ERM-BB422 (fish muscle). As a result, the absence of bias was demonstrated for all the elements in the three matrices, besides Sc, Pb and Zn in the plankton CRM.

The proved applicability to biological CRMs paves the way for the digestion of microsamples from biological monitoring campaigns: at present, the procedure is applied to the investigation of trace element concentrations in biopsies from sharks and plankton samples (~10 mg samples).

The applicability of the proposed system to microsamples from other research fields (e.g. valuable cultural heritage, tissues of clinical relevance) as well as the possibility to employ hydrofluoric acid (introducing fluoropolymeric test tubes, like PFA) will be subject of future works.
References

1. M. F. Mesko, C. A. Hartwig, C. A. Bizzi, J. S. F. Pereira, P. A. Mello, and E. M. M. Flores, *Int. J. Mass Spectrom.*, 2011, 307, 123.

2. Q. Jin, F. Liang, H. Zhang, L. Zhao, Y. Huan, and Daqian Song, *TrAC Trends Anal. Chem.*, 1999, 18, 479.

3. J. R. Lill, E. S. Ingle, P. S. Liu, V. Pham, and W. N. Sandoval, *Mass Spectrom. Rev.*, 2007, 26, 657.

4. M. das G. Andrade Korn, E. S. da Boa Morote, D. C. M. Batista dos Santos, J. T. Castro, J. T. P. Barbosa, A. P. Teixeira, A. P. Fernandes, B. Welz, W. P. C. dos Santos, E. B. G. Nunes dos Santos, and M. Korn, *Appl. Spectrosc. Rev.*, 2008, 43, 67.

5. F. E. Smith and E. A. Arsenault, *Talanta*, 1996, 43, 1207.

6. G. M. Kimber and S. Kokot, *TrAC Trends Anal. Chem.*, 1990, 9, 203.

7. C. J. Mason, M. Edwards, and P. Riby, *Analyst*, 2000, 125, 327.

8. S. Recchia, D. Spanu, D. Bianchi, C. Dossi, A. Pozzi, and D. Monticelli, *Talanta*, 2016, 159, 29.

9. K. Eilola and P. Perämäki, *Anal. Chim. Acta*, 2009, 634, 205.

10. K. Eilola and P. Perämäki, *Analyst*, 2003, 128, 194.

11. K. Eilola and P. Perämäki, *Anal. Methods*, 2012, 4, 3251.

12. L. Schloske, H. Waldner, and F. Marx, *Anal. Bioanal. Chem.*, 2002, 372, 700.

13. M. Picone, F. Corami, C. Gaetan, M. Basso, A. Battiston, L. Panzarin, and A. Volpi Ghirardini, *Ecotoxicol. Environ. Saf.*, 2019, 179, 62.

14. E. Conca, O. Abollino, A. Giacomo, S. Buoso, R. Traversi, S. Becagli, M. Grotti, and M. Malandrino, *Atmos. Environ.*, 2019, 203, 153.

15. M. Bettinelli, U. Baroni, and N. Pastorelli, *Anal. Chim. Acta*, 1989, 225, 159.
16. R. C. Richter, D. Link, and H. M. “Skip” Kingston, *Anal. Chem.*, **2001**, 73, 30 A.

17. J. Millos, M. Costas-Rodríguez, I. Lavilla, and C. Bendicho, *Anal. Chim. Acta*, **2008**, 622, 77.

18. R. C. Richter, J. A. Nóbrega, and C. Pirola, “*Think Blank: Clean Chemistry Tools for Atomic Spectroscopy*”, **2016**, ed. Milestone Srl and Ikonos Srl.

19. D. Monticelli, A. Castelletti, D. Civati, S. Recchia, and C. Dossi, *Int. J. Anal. Chem.*, **2019**, 2019, 1.

20. T. Linsinger, ERM Application Note 1 - Comparison of a measurement result with the certified value, **2010**.
Captions to Figures

Fig.1

(a) Schematic representation of the proposed system (drawing not in scale) and (b) picture of the insert, quartz test tubes and the PTFE vessel. Labels: A = commercial PTFE vessel; B = PTFE insert with the slot to host the thermocouple (see technical drawings in Fig. S1c); C = quartz test tubes with PTFE caps; D = 1 mL of ultrapure water; E = caps of the commercial PTFE vessel.

Fig.2

Percent recoveries determined for different analytes after MW digestion of (a) BCR-414, (b) DOLT-5 and (c) ERM-B422. Mean recoveries (black circles, error bars: one standard deviation) are reported against the certified relative confidence intervals (grey bars).
Figures

Fig. 1
Fig. 2

(a) BCR-414

(b) DOLT-5

(c) ERM-B422
Tables

Table 1.

LOD values estimated from 12 blank solutions.

| Element | LOD/mg kg\(^{-1}\) |
|---------|-------------------|
| Sc      | 0.13              |
| V       | 0.29              |
| Cr      | 0.42              |
| Mn      | 0.30              |
| Fe      | 2.4               |
| Co      | 0.023             |
| Ni      | 0.30              |
| Cu      | 0.29              |
| Zn      | 2.8               |
| As      | 0.23              |
| Se      | 0.15              |
| Sr      | 2.8               |
| Mo      | 0.042             |
| Cd      | 0.036             |
| Pb      | 0.15              |
| Hg      | 0.33              |
Graphical Index

TRIPLED THROUGHPUT
(10 x 3 samples)

NO CROSS-
CONTAMINATION

BIOLOGICAL
MICROSAMPLES

MULTI-BATCH SYSTEM
FOR MW DIGESTION