

Development of a modified medium pressure microwave vapor-phase digestion method for difficult to digest organic samples

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A previously developed microwave heated vapor-phase digestion method for biological samples was modified to enable digestion of difficult to digest organic samples. Organic samples containing *ca.* 100 mg of organic carbon were digested using volume calibrated quartz inserts inside second generation type medium pressure microwave vessels. As digestion reagents, 98% sulfuric acid, 70% nitric acid and 30% hydrogen peroxide were used. The accuracy of the method was tested with six certified reference materials. Elements Ca, Fe, K, Na, Mg, P and Zn were determined from NIST-SRM 8433 corn bran. Elements Al, Fe, Cd, Cu, and Zn were determined from NRCC DOLT-2 dogfish liver. The element Cd was determined from IRMM-VDA Cd in polyethylene No. 001–004 reference materials. These elements were determined from digested samples by ICP-OES. The results were close or within certified limits. The modified method could digest nearly all the materials tested, including the above mentioned reference materials, 2-nitrobenzoic acid (2-NBA), 4-NBA and copper(II) phthalosyanine-3, 4',4'',4'''-tetrasulfonic acid tetrasodium salt (CPS). The method could not digest 3-NBA.

Introduction

Nitric acid, hydrogen peroxide, sulfuric acid and perchloric acid are strong oxidizing reagents used generally for digestion of organic samples. The use of these reagents is thoroughly discussed in the literature.^{1–3} Nitric acid is preferred mainly in sample digestion due to its compatibility with instrumental methods, safety and ease of purification. Complete (>99%) oxidation of biological organic materials such as bovine liver with nitric acid requires high temperatures (~300 °C). This results in high pressure (~1500 psi) in closed digestion vessels due to the vapor-pressure of nitric acid and gaseous digestion products (NO_x, CO₂).^{4–7} Development of the multimode microwave vessels can be categorized in three generations according to Walter *et al.*¹ Although the third generation type, Teflon made, multimode microwave vessels can withstand pressures up to 1500 psi, at temperatures close to 300 °C they withstand only much lower pressure due to heat softening of the Teflon. Therefore, the complete oxidation of biological material is not achieved in practice with nitric acid alone, due to the high temperatures needed. The oxidation strength of the digestion solution can be increased by adding hydrogen peroxide, perchloric acid or sulfuric acid to the digestion mixture. Still, incomplete oxidation of biological organic materials may occur as reported in several publications.^{8–10} The organic residue resulting from nitric acid digestion of biological material constitutes mainly of isomers of nitrobenzoic acids (NBAs).^{11–13} If present in small amounts, most instrumental methods, *e.g.* ICP-OES, FAAS and GFAAS can be used to measure analyte concentrations without interferences due to the presence of these resistant organic compounds. However, problems may exist, depending on the degree of oxidation, the analyte, the analytical method and the sample matrix.^{12,14–18} Therefore, oxidation of the sample material should be as complete as possible to avoid interference. More challenging than, for example, bovine liver, are materials such as different polymers and high molecular mass organics, because even

dissolving of the sample material can be difficult. These types of materials are usually digested by heating with sulfuric acid to char the sample, followed by oxidation with nitric acid and/or hydrogen peroxide.^{19,20} In the charring step, the temperature of Teflon-made vessels must be monitored carefully because the boiling point of sulfuric acid exceeds the melting point of all Teflon types. In open focused microwave systems and reflux type conductively heated digestion techniques where glass or quartz vessels are used, sulfuric acid can be used without any trouble, allowing the use of higher charring temperatures. When the sample contains low-levels of analytes, contamination becomes a limiting factor, especially when open systems are used. In open systems and normal microwave digestion methods, sample and reagents are directly mixed with each other, therefore very pure, and thus expensive reagents are needed. Vapor-phase digestion methods with both conductive^{21–26} and microwave^{26–31} heating result in very low contamination and even relatively impure reagents can be used because isopiestic distillation of the reagents during digestion minimizes contamination from the reagents. In our previous work, we developed a vapor-phase digestion method that was applied to digestion of biological samples.³² In this work the method is modified to enable digestion of difficult to digest organic samples, such as different types of polymer samples.

Experimental

Instrumentation

The elements Al, Ca, Cd, Fe, K, Na, Mg, P and Zn were determined by the Philips PU-7000 inductively coupled plasma optical emission spectrometer (ICP-OES), equipped with a Gilson® 221 autosampler. The following operating parameters were used: forward power 1.0 kW, cooling gas flow 12 L min⁻¹, nebulizer pressure 45 psi, sample uptake rate 1.1 mL min⁻¹ and integration time 3 × 3 s. The emission lines used were: Al 308.215 nm, Ca 393.366 nm, Cd 214.438 nm, Fe 259.940 nm, K 766.490 nm, Mg 280.270 nm, Na 588.995 nm,

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P 213.618 nm and Zn 213.856 nm. Carbon content of the samples was determined by a PerkinElmer series II CHNS/O analyzer 2400. Amounts of dissolved carbon in liquid samples were determined by an Astro 2001 total organic carbon analyzer. The digestion equipment consisted of a CEM MDS-81D multi-mode microwave oven equipped with CEM ACV digestion vessels (max. pressure 200 psi and temperature 200 °C) and Milestone QS-50 quartz inserts. The microwave oven was calibrated before experiments were begun, as described in the US EPA 3052 standard method.³³ Thereafter, calibration was verified weekly. No need for re-calibration was observed during the time when experiments were performed.

Reagents and reference materials

The following reagents were used for sample digestion: sulfuric acid 98% (p.a., Fisher Scientific, in sample), nitric acid 70% (intra analyzed, J.T. Baker, in sample), nitric acid 70% (p.a., Riedel de Haen, outside) and hydrogen peroxide 30% (p.a., J.T. Baker, outside). 4-nitrobenzoic acid (purum, Fluga AG) and copper(II) phthalocyanine-3, 4',4'',4'''-tetrasulfonic acid tetrasodium salt (Aldrich) were used as test materials. Calibration stock solutions (1000 mg L⁻¹) for ICP-OES determinations were prepared from Merck Titrisol® ampoules for elements Al, Ca, Cu, Na, Fe and Zn. For elements Cd, K and Mg, stock standards from Riedel de Haen were used (AAS standard 1.00 g L⁻¹). For the element P, a Reagecon stock standard was used (phosphorus AAS standard solution 1000 mg L⁻¹). Carbon stock standard (1000 mg L⁻¹) was prepared from potassium hydrogen phthalate (p.a., Merck) by dissolving an appropriate amount in distilled water. All working standards were matrix matched (acid concentration in samples) and prepared in class A volumetric flasks by serial dilution from stock standards. Distilled water was produced by an Aquatron AS-4 water distillation apparatus and ultra pure water (resistivity 18 MΩ) was made from distilled water by a Barnsted UHQ-4 water purification unit. All other reagents not specifically mentioned were at least p.a. grade. For method performance evaluation the following reference materials were used: NIST SRM 8433 corn bran, NRCC DOLT-2 dog fish liver and IRMM VDA Cd in polyethylene No 001-004.

The digestion program

Before digestion, the quartz inserts were cleaned with freshly prepared *aqua regia* (4 mL) by heating inside microwave vessels for 15 min at 106 W power (power setting for four vessels). After this, the inserts were rinsed several times with ultra pure water.

The sample was weighed (C org. ca. 100 mg) into a quartz insert, and that insert was lowered into the bottom of the digestion vessel, where a glass insert holder kept the insert approximately 2.5 cm above the bottom of the microwave vessel. Sulfuric acid (1 mL) and nitric acid (0.5 mL) were added directly into a sample. Nitric acid (3 mL) and hydrogen peroxide (0.5 mL) were added outside of the quartz insert. The digestion vessel was closed and positioned inside the microwave oven. This was repeated for up to twelve vessels. Then, the first part of the heating program was run (Table 1). After completion, the vessels were cooled to room temperature in an ice water bath, and vented carefully. The vessels were closed again and repositioned inside the microwave oven. The second part of the heating program, with higher power settings, was run. After the second heating period, the vessels were again cooled to room temperature in an ice water bath and vented carefully. The insert was removed from the microwave vessel. A clear sulfuric acid phase (~1 mL) was observed after successful digestion. Dilution was done directly in the insert to 10 mL or 20 mL with

ultra pure water. ICP-OES determinations were done directly from these solutions. For TOC determinations, the solution was transferred quantitatively to a 100 mL volumetric flask and diluted to 100 mL with distilled water. For illustration of the insert positioning inside the microwave vessel, see ref. 32.

Caution: If insert is not properly positioned inside microwave vessel, damage to the microwave vessel may occur due to overheating.

Results and discussion

Preliminary tests

Two difficult to digest organic materials were selected as the testing material used in preliminary tests. 4-NBA has been identified as a residue when the aromatic amino acid phenylalanine or biological materials containing this amino acid has been digested with nitric acid.¹¹ Digestion of this compound with nitric acid requires high temperatures (~300 °C). Therefore, it is a good indicator compound to test efficiency of the digestion. CPS is used as a refractory TOC-standard for testing of TOC-instruments.³⁴

In the preliminary tests it was found out that the previous method³² could not digest or dissolve 4-NBA or CPS. Only the modified method could digest both testing materials. The oxidation efficiency was over 99.7% ($n = 4$) for 4-NBA and 96 ± 3% ($n = 4$) for CPS.

Optimization

Method optimization was carried out using CPS as the test material, as it was not fully digested in the preliminary tests. Optimized parameters were: (1) the amount of hydrogen peroxide added before the first part of the digestion, 0–2 mL, (2) the amount of hydrogen peroxide added before the second part of the digestion, 0–2 mL and (3) the amount of sulfuric acid, 1–1.5 mL. After optimization experiments, the following reagent composition was chosen as optimum: (1) 0.5 mL (2) none (3) 1 mL. With the optimized method, CPS was digested nearly completely (98.1 ± 2.0%, $n = 8$). Optimal nitric acid and hydrogen peroxide amounts are the same as the previously used reagent composition.³²

Proposed mechanism of the digestion: localized heating effect

Improvement in the digestion efficiency occurs probably due to following factors: (1.) sulfuric acid is a stronger absorber of microwave energy than the outer reagent mixture and its boiling point is much higher.¹ (2.) When the insert is lifted from contact with the outer reagents, sulfuric acid absorbs microwave energy

Table 1 Digestion program

Stage	Reagents		Power settings ^a /min W ⁻¹
	In sample	Outer	
1.	1 mL H ₂ SO ₄ 0.5 mL HNO ₃	3 mL HNO ₃ 0.5 mL H ₂ O ₂	(1.) 5 min 106 W (2.) 5 min 174 W (3.) 20 min 207 W (4.) 10 min 241 W
2.	None	None	(1.) 5 min 140 W (2.) 5 min 207 W (3.) 20 min 241 W (4.) 10 min 274 W

^a Power settings for four vessels. Between the first and the second stage vessels were cooled to room temperature and vented.

that is otherwise absorbed mostly by the surrounding outer reagents. (3.) Heat transfer between the outer reagents and the sulfuric acid is less efficient, thus sulfuric acid and sample temperature rise to a much higher level than when the insert is in contact with the outer reagents and therefore, (4.) the rise in temperature is mainly local (sample and sulfuric acid), since microwave vessels would not tolerate high pressure due to overall high temperature of the outer reagents.

Because temperature monitoring equipment for temperature measurement inside pressurized vessels was not available, the proposed localized heating mechanism was tested in an atmospheric pressure (semi closed) microwave vessel. Temperature was measured with a standard laboratory thermometer from the nitric acid–hydrogen peroxide mixture (outside the insert) and from the sulfuric acid (inside the insert). Temperature was measured from both locations when the insert holder was used and was not used. Results for temperature measurements are presented in Fig. 1. When the insert holder was used, at the end of the 5 min heating period, the sulfuric acid temperature was 192 °C, while the outer reagent temperature was 113 °C. When the holder was not used, at the end of the 5 min heating period, the sulfuric acid temperature was 110 °C and the outer reagent temperature was 110 °C. These observations support the proposed mechanism. However, exact temperatures attained in the vessels during digestion are not known. When the insert holder is used it is approximated that the sulfuric acid temperature reaches close to or above 300 °C at the end stages of the digestion, while the outer reagent mixture temperature is slightly above 200 °C. When the insert holder is not used, there is no localized heating effect and therefore the digestion temperature is approximated to be slightly above 200 °C.

Accuracy

Several certified reference materials were digested to test accuracy of the developed method.

The results for NIST-SRM 8433 corn bran, NRCC dolt-2 dog fish liver and IRMM-VDA Cd in polyethylene No 001–004 reference materials are presented in Tables 2, 3 and 4. The results obtained are mostly close or within certified values.

Oxidation efficiency

The oxidation efficiency of the developed method for tested materials and digested certified reference materials are given in Table 5. Materials 2- and 3-NBA were tested much later than other materials and it was discovered that 3-NBA could not be

digested with developed method. The oxidation efficiency of the developed method is comparable to high pressure and temperature nitric acid digestion methods.^{5–7}

Conclusions

The developed method improves the usefulness of the closed vessel, medium pressure, microwave digestion technique considerably. Difficult to digest materials can be digested with high oxidation efficiency in multimode medium pressure Teflon made microwave vessels. Vapor-pressure of the oxidizing

Table 2 Results for NIST-SRM 8433 corn bran^a

Element	Our result/mg kg ⁻¹ (n = 7--8)	Certified/mg kg ⁻¹
Ca	480 ± 30	420 ± 38
Fe	14.1 ± 0.8	14.8 ± 1.8
K	591 ± 27	566 ± 75
Mg	885 ± 50	818 ± 59
Na	464 ± 24	430 ± 31
P	169 ± 12	171 ± 11
Zn	19.5 ± 1.2	18.6 ± 2.2

^a Mean ± standard deviation reported.

Table 3 Results for NRCC DOLT-2 dog fish liver^a

Element	Our result/mg kg ⁻¹ (n = 7)	Certified/mg kg ⁻¹
Al	23.8 ± 2.1	25.2 ± 2.4
Cd	22.0 ± 0.6	20.8 ± 0.5
Cu	27.7 ± 0.8	25.8 ± 1.1
Fe	1046 ± 18	1103 ± 47
Zn	93.0 ± 1.9	85.8 ± 2.5

^a Mean ± standard deviation reported.

Table 4 Results for Cd in IRMM-VDA Cd in polyethylene No 001–004^a

Reference material	Our result/mg kg ⁻¹ (n = 7)	Certified/mg kg ⁻¹
No 001	39.5 ± 1.0	40.9 ± 1.2
No 002	71.3 ± 1.3	75.9 ± 2.1
No 003	196.6 ± 7.5	197.9 ± 4.8
No 004	406 ± 41	407 ± 12

^a Mean ± standard deviation reported.

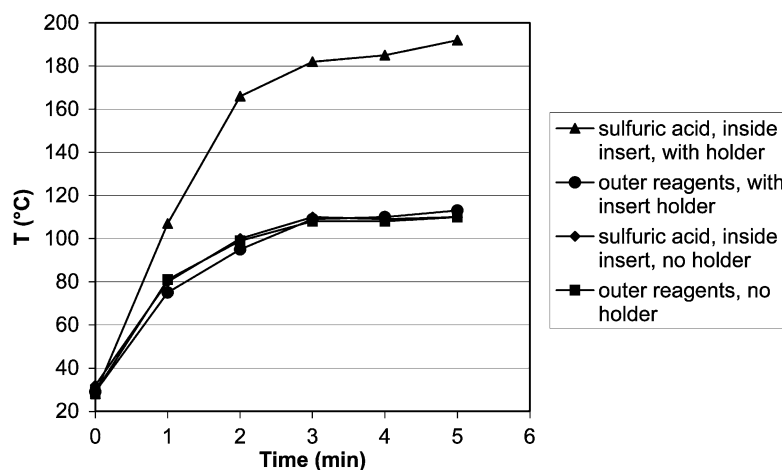


Fig. 1 Temperature inside an atmospheric pressure microwave vessel heated with 106 W power.

Table 5 Results for oxidation efficiency^a

Material	Carbon in solid sample (%) (n = 3)	Efficiency (%) (n = 4–8)
Corn bran	46.8 ± 0.1	> 99.7
Dolt-2	52.4 ± 0.2	> 99.7
Cd in PE No 001	85.42 ± 0.07	95.4 ± 2.6
Cd in PE No 002	85.56 ± 0.01	95.9 ± 2.0
Cd in PE No 003	85.4 ± 0.1	95.9 ± 2.9
Cd in PE No 004	85.4 ± 0.1	95.8 ± 1.7
CPS	^b 39.1	98.1 ± 2.0
2-NBA	^b 50.3	99.3 ± 1.5
3-NBA	^b 50.3	^c 73.7 ± 8.8
4-NBA	^b 50.3	> 99.7

^a Mean ± standard deviation reported. ^b Determined from formula. ^c Milky solution, not fully dissolved.

reagents can be minimized while maintaining high sample temperature and thus enabling high oxidation efficiency. Because oxidation takes place mainly through vapor-phase, contamination is minimized from the oxidizing reagents, allowing the use of cheaper and less pure reagents. Also, since sample handling is done completely in one vessel (insert), contamination from liquid/sample transfer steps is eliminated. To our knowledge, this is the first time that this type of digestion method is described.

The method could be improved in several ways. Improved monitoring using pressure and temperature control could be of great value during method optimization. Second generation type microwave vessels could be replaced with third generation type vessels for improved pressure capability. In this way, digestion could be probably performed in one step and also sample masses could be increased. Other methods for localized heating should be explored, such as microwave absorbing insert material as proposed by Matusiewicz.²⁷ This would eliminate the need for the addition of sulfuric acid to the sample, and thus the disadvantages of sulfuric acid usage could be avoided. These include sample contamination from sulfuric acid, analyte losses due to sparingly soluble sulfate formation, and rapid destruction of graphite tubes in GFAAS measurements. Finally, additional digestion experiments should be made with 3-NBA since it seems to be the most resistant compound of the testing materials used.

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