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Reflux open-vessel digestion system can overcome volatilization loss in mercury speciation analysis

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ABSTRACT

Volatilization loss of analytes in open-vessel digestion is a concern for mercury speciation analysis. Loss of Hg^0 vapour can be overcome by using oxidative digestion media, while loss of aerosols can be circumvented by complete condensation under sub-boiling-point heating. Using a simple system that consisted of a block heater and culture tubes, two digestion protocols were developed for determination of total Hg (tHg) and inorganic Hg (iHg). The resulting Hg^{++} was selectively reduced by $SnCl_2$ to Hg^0, which was detected by atomic fluorescence spectrometry (AFS). $MeHg^+$ was oxidized to Hg^{++} to yield tHg and calculated from the difference. These methods were validated using certified reference material (CRM) DORM-4, and applied to seafood analysis with 3.6 and 3.2 ng g^{-1} (dry weight) limit of detection for iHg and tHg, respectively. Using only off-the-shelf components, these methods gained cost, simplicity, and parallel operation advantages.

1. Introduction

$MeHg^+$ in seafood was identified as the cause of Minamata Disease [1] five decades ago. Notorious toxicity and the ability to bioaccumulate [2] render $MeHg^+$ the focus of regulatory monitoring [3]. The Food and Agriculture Organization/World Health Organization (FAO/WHO) Codex Alimentarius Commission has adopted recommendations on guideline levels of 1 mg kg^{-1} $MeHg^+$ for predatory fish, and 0.5 mg kg^{-1} for non-predatory fish [3]. Consequently, quantification of total mercury (tHg) [4,5] no longer meets regulatory requirements. However, many technical challenges still endure in Hg speciation analysis. Though rapid and sensitive detection is possible by atomic spectrometry or inductively coupled plasma-mass spectrometry (ICP-MS), sample preparation remains the bottleneck in food and biological analysis.

Extraction of Hg from biological matrices is hindered by its strong affinity to sulfhydryl groups [6,7]. To decompose proteins and cleave the Hg-S bond, strong acids like HCl [8], $HClO_4$ [9], HNO_3 [10], H_2SO_4 [11] or their mixtures; or bases like KOH [12] or tetramethylammonium hydroxide (TMAH) [13,14] are typically used. In these aggressive media, interspecies conversion may compromise the integrity of speciation analysis [15,16]. To facilitate digestion, agitation [11], heating [9], ultrasound [15], or microwave radiation [10,17] are often applied, among which microwave-aided extraction (MAE) is considered the most effective [17,18]. Closed-vessel MAE was developed to minimize volatilization loss, speed up digestion, and minimize potential contamination [17]. Use of closed vessels enables heating beyond the boiling points of the digestion media and hence enhancing reaction kinetics. To facilitate condensation and avoid overpressurization, however, a relatively large vessel is required even for a small sample size; sample counts and throughput thus suffer. Venting mechanisms needed for safety further raises the concern of analyte loss *via* aerosol escape. Analyte loss also occurred in open vessel MAE in both alkaline [19] and acidic media [14]. Finally, fluorinated polymers are good insulators, slow cooling offsets MAE's speed advantage.

Popularity of closed-vessel MAE is in part due to the preconception that loss of volatile analytes, such as Hg and As, can be minimized [20]. However, in Hg analysis in 8 of 10 commercial seaweeds, reflux digestion using open polypropylene tubes rendered better recovery and precision than either closed-vessel MAE, or high-pressure, high-temperature heating using closed polytetrafluoroethylene (PTFE) vessels or stainless steel-PTFE bombs [21]. In arsenic analysis, open-vessel heat-assisted extraction resulted in quantitative (94–102%) recoveries in a control plant sample and mostly > 80% recoveries in all plant matrices *vs.* 8–56% recoveries by closed-vessel MAE [22]. Mass balances (80–108%) further indicated no analyte loss during open-vessel extraction [22]. Such observations contradicted the presumption that closed-vessel digestion minimizes analyte loss.

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In one case of Hg analysis, the causes of analyte loss were attributed to incomplete digestion or adsorption on vessel material [21]. But PTFE is commonly considered as the best material to store trace-level Hg standards [23]. On the other hand, when HNO_3 is used in digestion, interference from NO_2^- and NO_2 [24] should not be overlooked; and pathways of analyte loss in open-vessel systems warrant further investigation. In theory, analyte loss occurs primarily *via* escape of Hg^0 vapour or aerosols. To minimize formation of Hg^0, the digestion medium must be oxidative enough to prohibit Hg^{++} reduction. To avoid aerosol escape, mass transport must be inhibited by suppressing effervescence and fulfilling complete aerosol condensation.

A typical open-vessel digestion system consists of heater, reactor, and condenser. The heater can be a hot plate, block heater, water bath, or microwave oven. The reactor can be a flask [25], or test tube [26,27]. The condenser designs vary widely. To enhance throughput, parallel operation was achieved using a water bath that accommodated twenty 125 mL Erlenmeyer flasks [28], each covered with a marble; or using a block heater that accommodated multiple test tubes, each coupled loosely to an air-cooled tube with a bulge [27] or a water-cooled cold finger to improve condensation [29]. These reflux systems were applied to tHg determination in sediments [30], biological CRMs [29], or crude oil [31]. Parallel operation is possible with compact reactor-condenser designs, but cumbersome with bulky, complicated designs. In this investigation, a culture tube coupled to a straight condenser was initially used. Later, it was found that under certain conditions, an open-vessel digestion system could be simplified to just a block heater and multiple culture tubes; the latter functioned as both reactors and condensers. This simple, low-cost design facilitated parallel operation, minimized analyte loss, and reduced interference from volatile nitrogen oxides (VNOs).

2. Experimental section

2.1. Reagents and solutions

ACS reagent grade concentrated (68% w/w) HNO_3, 30% (w/w) H_2O_2, and $SnCl_2{\cdot}2H_2O$ were purchased from Sigma-Aldrich (Milwaukee, WI, USA). ACS reagent grade 37% (w/w) HCl was purchased from Fisher (Hampton, NH, USA). $Hg(NO_3)_2$ standard, 1000 μg mL^{-1} Hg in 12% HNO_3, and MeHgCl standard, 1000 μg mL^{-1} Hg in water, were from Fluka (Milwaukee, WI, USA) and Alfa Aesar (Ward Hill, MA, USA), respectively. A reductant solution, 2.0% (w/v) $SnCl_2{\cdot}2H_2O$ in 10% (v/v) HCl, was prepare by first dissolving 20.00 g $SnCl_2{\cdot}2H_2O$ in 100 mL of 37% HCl before mixing with 0.9 L of deionized water (DIW), and subsequently purged with argon. Addition of 30 μL of Antifoam A (Sigma-Aldrich) was done shortly before detection to prevent foaming. HNO_3 at 4 mol L^{-1} was prepared by diluting 36.0 g 68% HNO_3 with DIW to a total volume of 100 mL. Reagent blank solution for the detector is created by mixing 92.4 g 68% HNO_3 with DIW to a total volume of 1 L. All working standard solutions were prepared daily. CRM dogfish DORM-4 was purchased from National Research Council Canada (Ottawa, Ontario, Canada). Glassware was cleaned and soaked with a Micro-90 (Cole-Parmer, Vernon Hills, IL, USA) solution, rinsed, placed in a 15% (v/v) HNO_3 bath overnight, and rinsed thoroughly with DIW before use. A Barnstead E-pure system (Dubuque, IA, USA) was used to make DIW for standards and reagent solutions.

2.2. Reactor-condenser design

Screw-capped Pyrex culture tubes, 16 × 125 mm (*od* × *l*) (CLS-4208-10, Chemglass, Vineland, NJ, USA), were used as reactors. Initially, the culture tubes were coupled *via* PTFE joints to (4–10) × (100–500) mm (*id* × *l*) sections of Pyrex tubes, with 1 mm wall thickness, as condensers (Fig. 1). The inert PTFE joints also shielded phenolic caps from acid exposure. Experimentally, it turned out later that the condensers, screw caps, and joints were unnecessary under certain conditions and hence were removed from the final protocols.

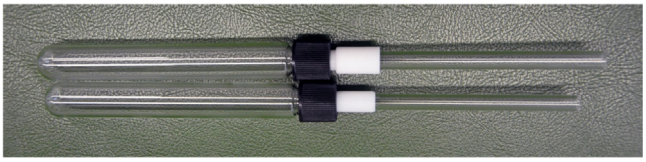

Fig. 1. Initial design: culture tubes with condensers.

2.3. Sample preparation

2.3.1. Pretreatment of fish samples

Seafood samples, purchased from local food stores, were homogenized using a Blixer 2 processor (Robot Coupe, Ridgeland, MS, USA), and oven dried at 40 ± 1 °C until constant weight was achieved. Each dried sample was ground to fine powder using a small coffee grinder (F203, Krups, Mexico), transferred to amber jars, and stored at −20 °C.

2.3.2. Digestion apparatus

Heating was performed in a fume hood using a dry bath heater (6785-DB, Corning Life Sciences, Lowell, MA, USA) equipped with two 12-position aluminium heat blocks (480125, Corning Life Sciences).

2.3.3. Digestion protocols for tHg and Hg^{++} determination

The final extraction protocols for tHg and Hg^{++} determination are shown in Fig. 2. The resulting supernatants were used for AFS measurement.

2.4. Cold vapour generation (CVG)

Hg^0 cold vapour was generated using a Millennium Merlin atomic fluorescence spectrometer (P S Analytical, Kent, UK) under the control of Millennium software (P S Analytical). Sample was mixed with

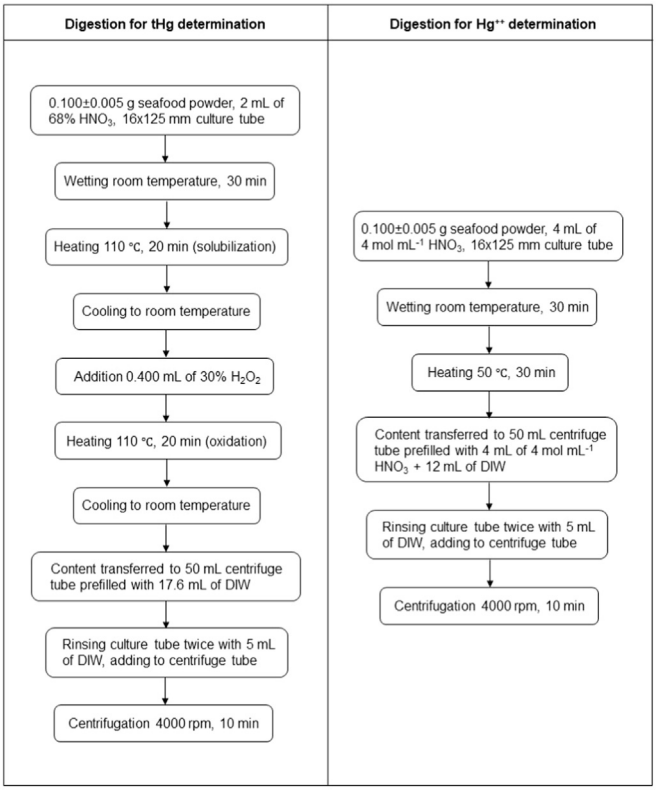

Fig. 2. Final digestion protocols for tHg and Hg^{++} determination.