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Methylmercury determination using a hyphenated high performance liquid chromatography ultraviolet cold vapor multipath atomic absorption spectrometry system☆

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ABSTRACT

The present work investigates the use of a multipath cell atomic absorption mercury detector for mercury speciation analysis in a hyphenated high performance liquid chromatography assembly. The multipath absorption cell multiplies the optical path while energy losses are compensated by a very intense primary source. Zeeman-effect background correction compensates for non-specific absorption. For the separation step, the mobile phase consisted in a 0.010% m/v mercaptoethanol solution in 5% methanol (pH = 5), a C_{18} column was used as stationary phase, and post column treatment was performed by UV irradiation (60 °C, 13 W). The eluate was then merged with 3 mol L^{-1} HCl, reduction was performed by a $NaBH_4$ solution, and the Hg vapor formed was separated at the gas–liquid separator and carried through a desiccant membrane to the detector. The detector was easily attached to the system, since an external gas flow to the gas–liquid separator was provided. A multivariate approach was used to optimize the procedure and peak area was used for measurement. Instrumental limits of detection of 0.05 μg L^{-1} were obtained for ionic (Hg^{2+}) and $HgCH_3^+$, for an injection volume of 200 μL. The multipath atomic absorption spectrometer proved to be a competitive mercury detector in hyphenated systems in relation to the most commonly used atomic fluorescence and inductively coupled plasma mass spectrometric detectors. Preliminary application studies were performed for the determination of methyl mercury in sediments.

1. Introduction

Since its introduction, cold vapor atomic absorption spectrometry (CV AAS) has become the most popular method for total mercury determination at trace levels. Its sensitivity, simplicity and low cost have contributed to its wide acceptance for total mercury determination in biological and environmental samples. However, in relation to mercury speciation analysis, due the very low concentrations of some mercury species, the limits of detection (LOD) obtained with CV AAS were not always sufficient. This is specially the case for hyphenated techniques, considering the low sample volumes associated with chromatographic techniques, implying the use of pre-concentration strategies [1–3]. Other more sensitive detectors, such as inductively coupled plasma mass spectrometry (ICP-MS) and atomic fluorescence spectrometry (AFS) have become the choice in speciation studies [4–12]. Moreover, ICP-MS brings the possibility of isotopic discrimination [13], which can be of great importance in special cases [14–19]. Nevertheless, ICP-MS is still expensive to purchase and run, and in speciation analysis running costs are stressed by the long analysis time. Hence, the adoption of a sensitive but more affordable Hg detector is desirable. In this sense, CV AFS appeared as a natural choice [5,9–12], since it is more sensitive than CV AAS, and much less expensive than ICP-MS. Consequently, CV AFS has been used for the determination of mercury species at very low levels in several environmental studies [20–25]. However, much care must be taken in relation to the possibility of quenching: Water vapor must be strictly avoided and the maximum sensitivity is attained with the use of high-purity gases, such as argon or preferably helium. CV AAS has found its way to the scene of sub ng L^{-1} determination of Hg since the introduction of a dedicated mercury detector, using a multipath absorption cell and Zeeman-effect background correction [26], whose sensitivity is in the same range of AFS detectors. Thus, the present work investigates the hyphenation of this detector with high performance liquid chromatography for methylmercury determination.

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2. Experimental

2.1. Instrumentation

The measurements were performed with a high performance liquid chromatography-uv-cold vapor atomic absorption spectrometry (HPLC-UV-CV-AAS) assembly (Fig. 1) consisting of a GT1310A isocratic pump (Agilent, Böblingen, Germany), an injection valve (Rheodyne, California, USA) with a 200 µL stainless steel sample loop, a reversed-phase analytical column packed with Exsil ODS (RP C18, 250 mm × 4.6 mm, 5 µm), an UV speciation heated coil (S570U100, PS Analytical, Orpington, Kent, England), operating at 13 W and 60 °C, a ME gas liquid separator (PS Analytical) and a RA-915$^+$ mercury spectrometer (LUMEX, Saint Petersburg, Russia). The chromatographic separation was performed at room temperature. The reducing and acidifying solutions were merged with the column eluate using peristaltic pumps type ISM787A (Ismatec, Glattbrugg, Switzerland), and Tygon tubes (1.14 mm). The mixing chemifold and the reaction coils (length = 93 cm, inner diameter = 0.8 mm) were made of Teflon^R. The mercury vapor was dried with nitrogen using a MD-110-12FP-4 desiccant membrane, 27.5 cm long (Perma Pure, Toms River, USA). The RA-915$^+$ mercury spectrometer was used as detector. In this instrument, a multipath cell increases the optical path to a length equivalent to 3 m. A very intense Hg discharge lamp contributes to a stable baseline and a magnetic field applied to the lamp together with a polarizer permits the use of Zeeman-effect background correction. Since the mercury spectrometer has been operated in the liquid analysis mode, the flow promoted by its internal pump should be equilibrated by an extra flow of gas in order to keep the liquid inside the gas–liquid separator at the right level. For practical reasons, argon (AGA, Rio de Janeiro, Brazil) was used, but nitrogen or even air could also have been used. This operation was manually made, taking the liquid level inside the gas–liquid separator as the reference for finding the proper flow of the external gas. All gases were made mercury free by passing them through gold traps conveniently positioned in the system. A personal computer was used to collect the data which were treated using Excel (Microsoft, Redmond, USA). For the extraction of methylmercury, a Thornton model T50 ultrasonic bath (Inpec Eletrônica Ltda, Vinhedo, Brasil) was used, operating at 40 kHz.

2.2. Materials, reagents, solutions and samples

All reagents were of analytical reagent grade. Ultra pure water (resistivity > 18.0 MΩ cm), obtained from a Master System apparatus (Gehaka, S. Paulo, Brazil) was used throughout. Analytical grade HNO_3 and HCl (both Vetec, Rio de Janeiro, Brazil) were purified by sub-boiling distillation using a PTFE sub-boiling apparatus (Hans Kuerner, Rosenheim, Germany). The 1000 mg L^{-1} mercury (Hg II) standard solution was prepared by diluting Titrisol (Merck, Darmstadt, Germany) ampoules with 0.2% v/v HNO_3. A 1000 mg L^{-1} methylmercury (as Hg) stock solution was prepared from its chloride salt (Carlo Erba, Milan, Italy): The salt was accurately weighed (±0.0001 g) and dissolved with 50% v/v ethanol; further dilutions to the indicated concentrations were made with water; 1.000 µg L^{-1} intermediary solutions of both mercury species were prepared daily and used to prepare the calibration solutions. The calibration solutions were prepared just before their introduction into the injector, in order to avoid losses. For the mobile phase methanol (Vetec, Duque de Caxias, Brazil), and mercaptoethanol (Vetec) were used. Acetic acid (Vetec) and ammonium acetate (Vetec) were used for buffering. Sodium borohydride (Vetec), dissolved in 0.5% NaOH (Vetec) was used as reducing agent. This solution was prepared daily. Dichloromethane and KOH (both Vetec) were used for methylmercury extraction. For the preliminary application studies a sediment certified reference material (IAEA 405, IAEA, Vienna, Austria), as well as surface sediment samples were used. They were collected with an Ekman grab sampler, conditioned in plastic bags, labeled and taken to the laboratory. They were kept frozen until the analysis. All plastic and glassware were washed with tap water, immersed in neutral Extran (48 h), rinsed with tap and deionized water, and immersed in 20% v/v HNO_3 for, at least, 24 h. Before use, these materials were thoroughly rinsed with ultrapure water and oven dried at 40 °C, avoiding any contact with metallic surfaces and dust contamination. Contamination was always checked by a strict blank control.

2.3. Procedures

2.3.1. Instrumental determination of methylmercury

The mobile phase consisted in a 0.01% v/v mercaptoethanol solution in 5% v/v methanol, with the pH adjusted to 5 by the addition of 0.06% v/v acetic acid + 0.15% ammonium acetate. The mobile phase flow rate was 1.4 mL min^{-1}. Calibration and sample solutions were manually injected (200 µL) using a hypodermic syringe; 1.5% $NaBH_4$ in 0.5% NaOH was used as reducing agent and 3 mol L^{-1} HCl as acidifying solution with a flow rate of 2.2 mL min^{-1}. The dry nitrogen flow through the desiccating membrane was 80 mL min^{-1}. An argon flow of 240 mL min^{-1} was necessary to keep the liquid in the gas–liquid separator in the right level. Aqueous standards, prepared just before their injection by adequate dilutions of the 1.000 µg L^{-1} solutions were used for calibration. Since each run took about 20 min (for methylmercury determination only), this procedure had no deleterious effect in the total analysis time. Peak area was used for measurement. Data collected in the personal computer were transferred to Excel data sheets, and chromatogram profiles were obtained. Integration consisted in localizing the peaks by the retention time and summing up the absorbance of the individual data in the integration interval. At least 200 points were counted, relative to the smallest (blank) peaks.

2.3.2. Sediment characterization and methyl mercury extraction and determination

The organic matter content in the sediments was determined after calcination at 400 °C for 6 h. The procedure for methylmercury extraction from the sediments was based on the method of Ramalhosa et al. [27]. Sample masses up to 300 mg were weighed in conical ended screw capped 50-mL tubes (Sarstedt, Nümbrecht, Germany). Two mL of a 25% m/v KOH solution in methanol were added and the

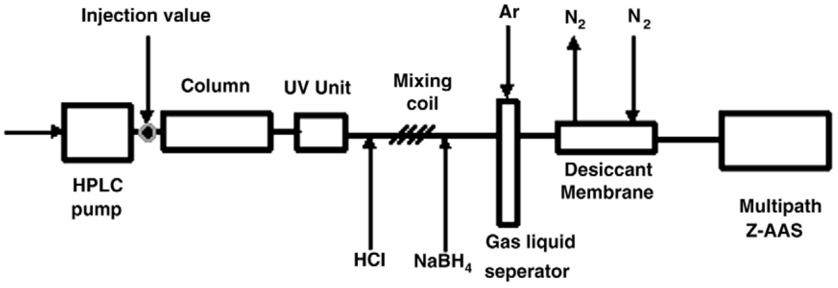

Fig. 1. Block diagram of the hyphenated system.