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Utility of ultrasound assisted-cloud point extraction and spectophotometry as a preconcentration and determination tool for the sensitive quantification of mercury species in fish samples

Nail Altunay

Cumhuriyet University, Faculty of Sciences, Department of Chemistry, TR-58140 Sivas, Turkey

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ABSTRACT

The current study reports, for the first time, the development of a new analytical method employing ultrasound assisted-cloud point extraction (UA-CPE) for the extraction of CH_3Hg^+ and Hg^{2+} species from fish samples. Detection and quantification of mercury species were performed at 550 nm by spectrophotometry. The analytical variables affecting complex formation and extraction efficiency were extensively evaluated and optimized by univariate method. Due to behave 14-fold more sensitive and selective of thiophene 2,5-dicarboxylic acid (H_2TDC) to Hg^{2+} ions than CH_3Hg^+ in presence of mixed surfactant, Tween 20 and SDS at pH 5.0, the amounts of free Hg^{2+} and total Hg were spectrophotometrically established at 550 nm by monitoring Hg^{2+} in the pretreated- and extracted-fish samples in ultrasonic bath to speed up extraction using diluted acid mixture (1:1:1, v/v, 4 mol L^{-1} HNO_3, 4 mol L^{-1} HCl, and 0.5 mol L^{-1} H_2O_2), before and after pre-oxidation with permanganate in acidic media. The amount of CH_3Hg^+ was calculated from difference between total Hg and Hg^{2+} amounts. The UA-CPE method showed to be suitable for the extraction and determination of mercury species in certified reference materials. The results were in a good agreement (with Student's *t*-test at 95% confidence limit) with the certified values, and the relative standard deviation was lower than 3.2%. The limits of detection have been 0.27 and 1.20 μg L^{-1}, for Hg^{2+} from aqueous calibration solutions and matrix-matched calibration solutions spiked before digestion, respectively, while it is 2.43 μg L^{-1} for CH_3Hg^+ from matrix-matched calibration solutions. A significant matrix effect was not observed from comparison of slopes of both calibration curves, so as to represent the sample matrix. The method was applied to fish samples for speciation analysis of Hg^{2+} and CH_3Hg^+. In terms of speciation, while total Hg is detected in range of 2.42–32.08 μg kg^{-1}, the distribution of mercury in fish were in range of 0.7–11.06 μg kg^{-1} for CH_3Hg^+ and in range of 1.72–24.56 μg kg^{-1} for Hg^{2+}.

1. Introduction

The main threats to human health from heavy metals are associated with exposure to mercury, lead, cadmium, and arsenic. From these heavy metals, the emitted mercury both naturally and anthropogenically is in an inorganic form predominantly metallic vapor, which is carried off to great distances by winds and eventually falls in water bodies. In aquatic environments, inorganic mercury, Hg^{2+} is microbiologically transformed into lipophilic organic compounds, generally methylmercury, CH_3Hg^+ [1]. Due to their toxicity and bio-accumulation, a maximum permissible intake limit of 1.6 μg kg^{-1} per week for methylmercury and of 5.0 μg kg^{-1} per week for total Hg, respectively, was established by JECFA in 2003 [2]. Thus, biomonitoring of toxic elements such as mercury in marine foods is crucial. The seafood can represent a major source of this element to human dietary, and the amount of mercury entered the body leads to health problem depending on the consumption of these samples. The maximum allowed mercury concentrations in fish are in the range of 500 to 1000 μg kg^{-1} [3]. In order to control the effectiveness of these legal provisions and to ensure the safety of aquatic organisms for consumption, it is highly desirable to develop a sensitive, selective, accurate and reliable analytical method for the detection and quantification of trace and/or ultra-trace amounts of each species of mercury in aqueous environment.

Determination of toxic metals in real samples is a task frequently asked by analytical chemists and food engineers, for the food safety, human health, the evaluation and phenomenon interpretation of ecosystems. Also, direct determination of toxic mercury in fish samples at sub-microgram per liter levels is limited because of its low level of concentration and matrix interference [4]. It is evident that the application of separation and preconcentration procedures is still necessary for elimination of interfering matrix component before the determination step, despite instrumental advances [5,6]. In recent years, various procedure has been reported for the separation and preconcentration of

E-mail address: naltunay@cumhuriyet.edu.tr.

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mercury, such as dispersive liquid-liquid microextraction (LLME) [7], solid phase microextraction (SPME) [8], ultrasound assisted extraction (UAE) [9], single drop microextraction (SDME) [10], cloud point extraction (CPE) [11] and hollow fiber supported liquid phase membrane microextraction (HF-LPME) [12]. Among these procedures, ultrasonic radiation has been used in frequently, although it could be a powerful tool for accelerating various steps in the analytical process [13]. Ultrasound radiation is of great help in the pretreatment of samples. The effects of extremely high temperature and pressure were at the interface of the sonicated solution and the solid matrix, along with the oxidative power of strong acids, results in high extractive power [14]. The use of an ultrasonic device is also a good alternative to minimize the disadvantages of conventional extraction procedures in terms of number of analytical steps, time, extraction efficiency and reagent consumption by facilitating and accelerating pretreatment process of various biological and environmental samples. The efficiency of UAE for the extraction or preconcentration of target substances has been evaluated for different sample matrices [15,16].

After extraction, in the last five years, various detection techniques for determination of mercury in seafood are currently available, such as cold vapor-atomic emission spectrometry (CV-AES) [17], cold vapor atomic absorption spectrometry (CV-AAS) [18], graphite furnace atomic absorption spectrometry (GFAAS) [19], direct thermal decomposition AAS [20], atomic absorption spectroscopy (AAS) [21], inductively coupled plasma mass spectrometry (ICP-MS) [22], inductively coupled plasma optical emission spectrometry (ICP-OES) [23], cold vapor atomic fluorescence spectroscopy (CV-AFS) [24], spectrofluorimetry [25], cold vapor generation inductively coupled plasma mass spectrometry (CVG-ICP-MS) [26]. However, these techniques are expensive and complicate instruments. Unlike them, spectrophotometric method is very simple, fast, and relatively lower cost alternative for more routine applications.

Thiophene-2,5-dicarboxylic acid (H_2TDC), as a member of the multicarboxylate ligands containing S-donors, obeys the $4n + 2\pi$ electron rule, so it is generally considered to be aromatic, but is relatively rarely used. As is well known, the rigid thiophene ring possesses unique physical and chemical activities [27]. Because of the bigger radius of the S atom than the C, N and O atoms, its lone pair of electrons can be more easily delocalized within the heterocycle, and the ligand exhibits good charge-transfer ability. H_2TDC, is capable of diverse binding modes and can function variously as a monodentate, bidentate, tridentate or bridging ligand in its metal complexes, it can also bridge or chelate too [28]. Because of these rich bridging modes, H_2TDC complexes are useful building blocks in the construction of coordination polymers [29]. As a result, even if there is more sensitive and selective chelating agents for determination of mercury in real samples, as a molecular sensor, sulfur of thiophene could offer high sensitivity and selectivity in mixed micellar media for the detection of soft heavy metals such as Hg^{2+} or Pb^{2+}.

In the current work, UA-CPE was combined with spectrophotometry simultaneously to determine Hg^{2+} and CH_3Hg^+ in contaminated fish samples. Because spectrophotometry as a detection tool is a simple, easy to use, low-cost and accessible instrument in analytical research labs, the proposed method is very practical in routine analysis. Fish bought from local supermarkets (Sivas, Turkey) were analyzed to demonstrate the applicability of the proposed preconcentration method. In extraction step, SDS in presence of Tween 20 as extractant as a result of a change of in cloud point, CMC, micellar size and micelle aggregation number in case of binary surfactant mixture was used as synergistic auxiliary agent, so as to protect and stabilize chelate complex against possible matrix components. In this case, when UA-CPE is used to extract and preconcentrate Hg^{2+} and CH_3Hg^+ from fish samples, the trace amounts of Hg^{2+} in fish samples pretreated by ultrasound assistance in acidic media, due to act more selective of chelating agent, H_2TDC, to Hg^{2+} than CH_3Hg^+ at pH 5.0 was monitored and determined by spectrophotometry. The CH_3Hg^+ contents of samples were determined from differences between Hg^{2+} and total Hg after pre-oxidation of CH_3Hg^+ to Hg^{2+} with permanganate in acidic medium.

2. Experimental

2.1. Instrumentation

In the study, the amounts of mercury species, Hg^{2+} and CH_3Hg^+ at 550 nm were detected and determined using a Shimadzu Model UV–Visible 1800 PC spectrophotometer (Kyoto, Japan) equipped with a 1 cm quartz cell. An ultrasonic cleaner, UCS-10 model, Seoul, Korea, was used in the pretreatment and extraction of the fish samples. Extracts were centrifuged in a Universal Hettich model centrifuge (London, England). A vortex mixer, VM-96B model, (Jeio Tech, Co., Ltd., Seoul, Korea), was used for thorough the efficient mixing of solutions. A digital pH meter, a Selecta 2001, Sartorius docu-model (North America), equipped with a combined glass calomel electrode was used for pH measurements.

2.2. Reagents

Ultra-pure water (18.2 MΩ cm^{-1}) produced using a Labconco (Kansas City, USA) water purification system was used for the preparation of all solutions throughout the study. The stock CH_3Hg^+ and Hg^{2+} standard solutions (1000 mg L^{-1}) stabilizing with 0.2 mol L^{-1} HCl were prepared from their chloride salts (Sigma, St. Louis, MO, USA) by dissolving in methanol and water, respectively and completed to 1 L with ultra-pure water. The stock solutions were stored at 4 °C prior to use. All working standard solutions of Hg were prepared daily to prevent any possible species change, due to dilution of the appropriate stock solution aliquots with water. When necessary, the stock Hg solutions were titrimetrically standardized by using 0.01 mol L^{-1} EDTA solution in presence of metal ion sensitive indicator, Xylenol orange. A stock thiophene-2,5-dicarboxylic acid (H_2TDC, 1.0×10^{-4} mol L^{-1}) solution as chelating agent solution prepared by dissolving its appropriate amount (Sigma, USA) in water and diluting to the mark in a 1000 mL volumetric flask. A 1.0×10^{-3} mol L^{-1} of sodium dodecyl sulphate (SDS) and cetylpyridinium chloride (CPC) (Sigma) was prepared by dissolving its suitable amount with the water. A 5.0% (w/v) polyoxyethylenesorbitan monolaurate (Tween 20) (Sigma) was prepared by dissolving 5.0 g of Tween 20 in methanol, using an ultrasonic bath, and diluted to 100 mL with water. The buffer solution of pH 5.0 (potassium hydrogen phthalate/NaOH, 0.1 mol L^{-1}) was used to control the pH of the solutions. All vials for the storage of samples and standard solutions, all glassware was washed with 10% (v/v) HNO_3 for 24 h, rinsed with ultrapure water and dried by shaking prior to starting the experiment.

2.3. Sample Collection and Preparation

To assess the applicability of the proposed method, the fish samples (salmon, anchovy, mackerel, trout, sardine, tuna) were purchased from commercial supermarkets in Sivas, Turkey. Sample preparation is one of the most important steps for the accurate and reliable determination of mercury in different fish samples. For determination of total mercury, this step is usually needed for the decomposition of organic compounds in sample matrix prior to analysis. To accomplish this purpose, the fish samples were washed with ultra-pure water and dried in tissue paper after defrosting in the laboratory. A portion of the edible muscle tissue was removed from the dorsal part of each fish samples, was homogenized and then saved in closed glass vials and kept in a freezer until the sample treatment. The fish muscle tissue samples were mineralized by using an open digestion flask procedure for the determination of total Hg with slight modification [30,31], as described below: a part of the muscles was taken out quickly and was dried in an oven at 70 °C for one day. After grinding the dry tissue to a size of <60 μm, mercury extraction from 0.25 g dried sample was transferred to a 50-mL centrifuge flask, and 6 mL of a mixture, 4 mol L^{-1} HNO_3, 4 mol L^{-1} HCl and 0.5 mol L^{-1} H_2O_2 (1:1:1, v/v) (or 5 mL of mixture of 0.1 mol L^{-1} $HClO_4$ and 1.0 mol L^{-1} HCl (1:4, v/v) for 30 min at 40 °C for structuring