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Mercury determination in urine samples by gold nanostructured screen-printed carbon electrodes after vortex-assisted ionic liquid dispersive liquid–liquid microextraction

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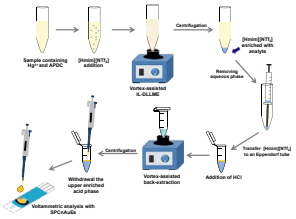
HIGHLIGHTS

- Mercury determination in non-digested urine samples.
- Vortex-assisted DLLME and back-extraction as sample preparation methodologies.
- SPEs are employed for the first time for mercury determination in urine samples.
- Limit of detection lower than threshold level for normal content of mercury in urine.

GRAPHICAL ABSTRACT



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ABSTRACT

A novel approach is presented to determine mercury in urine samples, employing vortex-assisted ionic liquid dispersive liquid–liquid microextraction and microvolume back-extraction to prepare samples, and screen-printed electrodes modified with gold nanoparticles for voltammetric analysis. Mercury was extracted directly from non-digested urine samples in a water-immiscible ionic liquid, being back-extracted into an acidic aqueous solution. Subsequently, it was determined using gold nanoparticle-modified screen-printed electrodes. Under optimized microextraction conditions, standard addition calibration was applied to urine samples containing 5, 10 and 15 μg L^{-1} of mercury. Standard addition calibration curves using standards between 0 and 20 μg L^{-1} gave a high level of linearity with correlation coefficients ranging from 0.990 to 0.999 (N = 5). The limit of detection was empirical and statistically evaluated, obtaining values that ranged from 0.5 to 1.5 μg L^{-1}, and from 1.1 to 1.3 μg L^{-1}, respectively, which are significantly lower than the threshold level established by the World Health Organization for normal mercury content in urine (i.e., 10–20 μg L^{-1}). A certified reference material (REC-8848/Level II) was analyzed to assess method accuracy finding 87% and 3 μg L^{-1} as the recovery (trueness) and standard deviation values, respectively. Finally, the method was used to analyze spiked urine samples, obtaining good agreement between spiked and found concentrations (recovery ranged from 97 to 100%).

1. Introduction

Mercury is a highly toxic element whose adverse health effects depend on several factors such as chemical form, route of exposure,

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dose and personal features [1]. Inhalation exposure mainly corresponds to elemental mercury (i.e., Hg^0) due to its high vapor pressure. Occupational exposure to Hg^0 vapors occurs in mining and fossil-fuel processing activities, manufacture of amalgams, manipulation of mercury-containing fungicides, waste incineration or chloralkali plants. Hg^0 is oxidized to Hg^{2+} in most body tissues and can be retained and accumulated, especially in the brain and kidneys. Oral intake is the main source of inorganic mercury (i.e., Hg^{2+}), although its absorption from gastrointestinal tract occurs only to a limited extent [2]. Cutaneous absorption has been proposed as another less significant route of exposure, since dermal penetration of Hg^{2+} can occur through use of skin-lightening cosmetic products containing mercuric salts. Once in the body, Hg^{2+} accumulates mainly in the kidneys. Methylmercury (i.e., $MeHg^+$) is the most toxic and frequent form of organic mercury. $MeHg^+$ exposure mainly occurs through a diet high in fish and marine mammals. In contrast to Hg^{2+}, $MeHg^+$ is rapidly and extensively absorbed through the gastrointestinal tract and accumulates predominantly in the brain [2].

Urine and blood have been broadly employed for risk assessment of mercury exposure and health risk prevention. Mercury content in urine generally reflects recent exposure to inorganic and/or elemental mercury. However, Hg^{2+} accumulates in the kidneys and is slowly excreted through urine, therefore, urinary mercury can also reflect long-term exposure in the past [2]. $MeHg^+$ is mostly eliminated by demethylation and excretion in the feces and it is not typically found in urine [1]. Urinary mercury levels are normally expected to be lower than 10–20 $\mu g\ L^{-1}$ in an unexposed population.

Different publications report mercury determination in urine using cold-vapor atomic absorption [3] or fluorescence [4] spectrometry, electrothermal absorption spectrometry [5], UV–Vis spectrophotometry [6], inductively coupled plasma atomic emission [7] or mass [8] spectrometry. Besides spectrometric techniques, electrochemical techniques have also been proposed [9–13]. Electrochemistry offers sensitivity, simplicity, rapid response and inexpensive instrumentation with miniaturization and portable options. In this respect, screen-printed electrodes (SPEs) [14] have gained widespread interest. SPEs are size-reduced devices designed to analyze low-volume samples, which also allow de-centralized testing. In addition, SPEs are mass-produced at a low cost and are thus disposable. In this work, screen-printed carbon electrodes modified with gold nanoparticles (SPCnAuEs) have been employed as electrochemical transducers for mercury determination. Gold nanoparticles exploit the properties of gold as a high affinity material for mercury, with the advantages of including nanosized particles, such as high active surface area, enhanced mass transport and signal to noise ratio [15]. In addition, mercury undergoes a process named underpotential deposition (UPD) on gold electrodes. The presence of gold promotes the adsorption of mercury atoms on the surface once the ionic metal is reduced, forming an amalgam (Au–Hg). This adsorption is usually limited to a monolayer. Due to the strong interaction between gold substrate and reduced mercury, the deposition of mercury is favored energetically and takes place at a less negative potential than the reversible Nernst potential for bulk deposition.

Due to the complexity of biological samples, including urine, sample preparation is necessary prior to electrochemical analysis. To date, the electrochemical methods proposed to determine mercury in urine samples employ initial digestion steps to decompose organic matter, which generally involve wet acid digestion [9–13]. However, these procedures constitute a risk for mercury loss and thus careful manipulation is essential to avoid analyte evaporation. In this work, dispersive liquid–liquid microextraction (DLLME) is presented as a valuable alternative for sample preparation. DLLME is a miniaturized liquid-phase extraction technique whose major advantages include: speed and ease of use, low cost, low sample volume, extremely low solvent consumption, reduced generation of wastes, high enrichment factors and affordability. Classical DLLME is based on the dispersion in tiny droplets of a water-immiscible solvent into the aqueous sample with the aid of a disperser agent [16]. Other formats of DLLME are based on vortex agitation [17], ultrasound energy [18], temperature changes [19], metathesis reactions [20] or air-assisted methodology [21]. The cloudy solution formed presents a great contact surface area between the donor and acceptor phases, thus enhancing extraction efficiency. In addition to conventional organic solvents, ionic liquids (ILs) have been employed as extractant phase in DLLME (i.e., IL-DLLME) due to their remarkable properties, such as low vapor pressure, good extractability of organic and inorganic compounds, non-flammability and adjustable hydrophobicity [22].

The purpose of this work is to present a novel method for mercury determination in urine samples, combining vortex-assisted IL-DLLME with electrochemical detection by SPCnAuEs. Mercury complexes with ammonium pyrrolidinedithiocarbamate (APDC) are directly extracted from non-digested urine samples into a water-immiscible IL using vortex agitation. Then, mercury is back-extracted into 10 μL of an acidic aqueous solution, which is finally analyzed by anodic stripping voltammetry. The proposed method is based on a previous work [23], in which mercury was determined in water samples, where some changes related with the microextraction techniques are proposed. In the previous work, mercury was extracted from water samples using an in-situ ionic liquid formation dispersive liquid–liquid microextraction [23]. This microextraction technique was not suitable for urine samples since the formation of a precipitate in the extractant phase formed in-situ hindered its retrieval. Hence, vortex-assisted IL-DLLME was adopted in order to overcome the problem. On the other hand, ultrasound-assisted back-extraction [23] has been replaced by vortex agitation in this work to assist back-extraction of mercury to the final aqueous phase, leading to shorter extraction time.

The present method synergistically combines the advantages of an environmentally friendly miniaturized sample-preparation protocol with speed, low cost, high sensitivity and selectivity of the electrochemical detection with SPCnAuEs. To our knowledge, this is the first report of an analytical method in which SPEs are employed to determine mercury in urine samples. The aforementioned method was evaluated in order to demonstrate its applicability to the analysis of real urine samples.

2. Experimental part

2.1. Reagents and samples

A stock standard solution of Hg^{2+} (1000 mg L^{-1} in 2% HNO_3) was obtained from High-purity Standards (Charleston, SC, USA). Working solutions were prepared by proper dilution of this stock standard. IL 1-hexyl-3-methylimidazolium bis[(trifluoromethyl) sulfonyl]imide ([Hmim][NTf_2]) (99%) was purchased from Iolitec (Heilbronn, Germany). The chelating agent APDC (~99%) was supplied by Sigma–Aldrich (St. Louis, MO, USA). A solution of 2 mg mL^{-1} of the chelating agent was prepared by dissolving APDC in ultrapure water. Fuming HCl (37%) was supplied by Merck (Madrid, Spain) and used to prepare HCl solution (4 M) in ultrapure water. Reactive grade NaOH (≥97%, pellets) was from ACS Scharlau (Barcelona, Spain) and used to prepare NaOH solution (0.5 M). Reactive grade NaCl was also from ACS Scharlau. The ultrapure water (resistivity of 18.2 MΩcm at 25 °C) employed for preparing all solutions was obtained with a Millipore Direct System Q5™ purification system from Ibérica S.A. (Madrid, Spain).