A Green Photometric Method for Determination of Mercuric Ions in Saline Samples by Single Drop Micro Extraction Technique

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Present study describes a rapid, simple and sensitive spectrophotometric method for determination of Hg(II) ions in saline sample by single drop micro extraction (SDME) technique. The method is based on the extraction of dithizone (DTZ) complex of mercury(II) into the undecanol droplet which serves as the organic phase and then, the absorbance of the colored complex is measured at 490 nm by using micro volume quartz cuvette. This procedure provides a simple, rapid, cost efficient and most of all a green method for detecting mercuric ions by minimizing the organic solvent consumption. Variety of parameters affecting the signal such as pH, DTZ concentration, sample and extraction solvent volume, extraction time and temperature and salt effect were optimized. Under optimized conditions the linear range was found between 3.2 \times 10^{-8} – 5.0 \times 10^{-7} \text{ molL}^{-1} (6.4 - 100.8 \mu g L^{-1}) and the detection limit was calculated as 9.6 \times 10^{-9} \text{ molL}^{-1} (1.9 \mu g mL^{-1}) attained by high enrichment factor (EF) of 203. The performance and accuracy of the method were compared with that of atomic fluorescence spectrometry (AFS). Validation of the proposed method was performed for determination of mercury in saline samples including sea water, mineral water, thermal spring water, table and rock salts which is difficult to be analyzed by conventional methods.

Introduction

Mercury is one of the most toxic elements for all living organisms and its monitoring in biological, environmental and industrial samples is extremely important. Both organic and inorganic forms of mercury are highly toxic to humans; but, most people are exposed to organic mercury through the consumption of fish and shellfish which can accumulate in the brain damaging the central nervous system. Inorganic mercury taken by food is not as easily absorbed by the human body but, elemental mercury vapor can cause acute pneumonia in case of inhalation in large concentrations. Divalent mercury can cause kidney damage and leukemia.

Inorganic mercury occurs naturally in the environment and also released as a consequence of human activities. Surface waters in vicinity of the industrial areas are the important indicators for mercury pollution. The amount of total mercury usually present in a range of 0.5 - 5 ngmL^{-1} in natural and fresh waters and increases to several mgmL^{-1} levels in contaminated waters. For drinking water, EPA recommends a limit of 2 ngmL^{-1}.

Therefore, analytical monitoring of trace amounts of mercury in surface waters is of great significance for the public health safety and environmental pollution control.

Numerous analytical techniques have been used for the determination of trace level of mercury in water samples, including atomic absorption spectrometry (AAS), cold vapor atomic absorption spectrometry (CV-AAS), atomic fluorescence spectrometry (AFS), inductively coupled plasma with optical emission spectrometry (ICP-AES), and mass spectrometry (ICP-MS) along with X-ray fluorescence spectrometry (XRF).

Although these methods provide a sensitive tool for mercury determination in environmental samples, their application to saline samples is cumbersome due to the impairment of the direct analysis by high ionic strength of such samples. For this purpose, in situ solid-phase preconcentration methods were utilized for avoiding interferences and improving the sensitivity of the method.

Electrochemical methods provide a more economical and yet sensitive tool for mercury determination in the saline samples. A study carried out in this lab has revealed that mercury content...
of table salt samples can be determined at a gold film electrode by anodic stripping voltammetry (ASV) [2]. The interference arose from high chloride content was eliminated by applying a medium-exchange after the deposition step.

Spectrophotometric methods, on the other hand, are one of the most common methods due to their simplicity and inexpensive instrumentation with reasonable sensitivity for determination of mercuric ions [24-26]. However, mercuric ions usually exist in the environment at trace levels in a rather complicated matrix, thus extraction and pre-concentration procedures are essential in photometric detection [2]. After complexation of mercury(II) with different type of reagents, the complex have been extracted either in chloroform, toluene, xylene and carbon tetrachloride prior to the measurement [21]. However, the use of these solvents is avoided due to their toxicity, and they display some drawbacks such as large consumption of reagent, high volatility and low enrichment factor. As a result greener and faster methods for monitoring the trace levels of mercury are being searched.

Among the currently available extraction and preconcentration methods, miniaturized preconcentration methods have been attempted for liquid liquid extraction (LLE) systems based on single-drop microextraction (SDME) [27-29], solidified floating organic drop microextraction (SFODME) [30], dispersive liquid liquid microextraction (DLLME) [31] for the determination of mercuric ions in different samples. These techniques have recently aroused a great interest, due to their favorable characteristics of simplicity, rapidity, cost effectiveness and minimized toxic and flammable organic solvents consumption [31]. High enrichment factor can be easily obtained by SDME since microliter volume of liquid drops are used [31]. Previously, organic solvents like carbon tetrachloride, cyclohexane, toluene, m-xylene, n-octane have been used in SDME as the extracting phases [21,32]. However, the use of such solvents in SDME is limited due to their high rate of dissolution and evaporation in addition to the emulsion formation during extraction [31]. Recently, low volatile compounds such as ionic liquids (IL) [33,34] were used instead of toxic, flammable and water miscible organic solvents. As an alternative to ionic liquids, a greener solvent; undecanol was used as the extracting phase in a recent study [32]. Mercuric ions were complexed with dithiodyldithiocarbamate and then, extracted into fine droplets of undecanol phase.

Optimization Studies

Preliminary experiments comprise the optimization studies of the method in which a micro volume quartz cuvette is utilized for absorbance measurement of the dithizonate complex of mercuric ions extracted into fine droplets of undecanol phase. Fig 1 shows the absorption spectra of the free DTZ at 420 nm has shifted to 490 nm in the presence of mercuric ions indicating Hg(II)-DTZ complex formation extracted into undecanol phase. It can also observed from the color change of colorless undecanol phase to light green in the presence of DTZ and then, to orange upon addition of mercuric ions to the solution (Fig 1 inset). Since the extraction efficiency depends on different experimental parameters, such as medium pH, volume of undecanol and sample, DTZ concentration, extraction temperature and time, stirring rate and salt amount, were optimized. The experiments were performed in triplicate analysis.

The effect of pH on the extraction efficiency was investigated by using 0.01 M Britton Robinson (BR) buffer solutions in a wide range of pH. As shown in Fig. 2, the absorbance of the extracted solution has given a maximum in the pH range of 6.5–8.0 and therefore, pH 7.2 was chosen. Buffer type and concentration were also studied. Phosphate buffers at the same pH were tested and no significant difference was observed in a concentration range of 0.03 -0.1 mol L⁻¹. Therefore, 0.1 mol L⁻¹ phosphate buffer at pH 7.2 was finally selected.
For accurate and precise analysis, extraction time should be optimized since mass transfer of the complex between water and undecanol phases is time dependent. The effect of the extraction time on the signal was investigated between 5–45 min and plotted against the measured absorbance (Figure 4). Extraction efficiency has substantially increased with increasing extraction time up to 15 min and then has given a plateau after 20 min. Hence, the experiments were carried out by 20 min optimal extraction time.

The dependence of the extraction efficiency on the solvent volume was investigated in a range of 5–100 µL of undecanol nanodrop picked from a solution containing 7.5 x 10^{-6} M Hg(II) and 1.5 x 10^{-5} M DTZ. A stable and relatively high signal was observed in the range of 5–20 µL (Fig 5). Considering that mass transfer of the complex into the organic phase occurs only by diffusion, a concentration gradient is produced in the drop for non-equilibrium conditions, thereby explaining the lower extraction efficiency observed for larger volumes.

In the next step, the sample volume was changed in the range of 1.0–20 mL by keeping the undecanol volume constant as 15 µL and the extraction efficiency was increased with sample to organic phase volume ratio as shown in Fig 6. Here, 10 mL of sample volume was chosen since the reassembling of the microdrop distributed into higher sample volumes is getting more challenging.
It is well known in the liquid phase micro extraction that the addition of salt to the sample solution can increase the mass transfer of hydrophobic compounds into the extract phase (salting-out effect). On the other hand, the salt dissolved in the sample solution can change the physical properties of the Nernst diffusion layer altering the extraction kinetics. Therefore, salt concentration in the sample solution should be optimized. In this study, salting out effect was investigated by addition of NaCl to the sample in the range 0.02–0.50 mol L\(^{-1}\). As shown in Fig 7, the extraction efficiency of Hg(II)-DTZ complex was increased until 0.1 mol L\(^{-1}\) NaCl concentration and at higher NaCl concentration no significant change was observed by the addition of NaCl. On the basis of these results, further experiments were performed at 0.1 mol L\(^{-1}\) NaCl concentration.

**Fig 5** Effect of undecanol volume on the absorbance of Hg(II)-dithizonate complex. Conditions: Sample volume: 10 mL, DTZ concentration: 1.5×10\(^{-5}\) mol L\(^{-1}\), Hg(II) concentration: 7.5×10\(^{-6}\) mol L\(^{-1}\), agitation rate: 250 rpm, extraction temperature: 45°C, extraction time:20 min, light path:0.2 mm.

**Fig 6** Effect of sample volume on the absorbance of Hg(II)-dithizonate complex. Conditions: undecanol volume: 15 µL, DTZ concentration: 1.5×10\(^{-5}\) mol L\(^{-1}\), Hg(II) concentration: 7.5×10\(^{-6}\) mol L\(^{-1}\), agitation rate: 250 rpm, extraction temperature: 45°C, extraction time:20 min, light path:0.2 mm.

**Fig 7** Effect of salt amount on the absorbance of Hg(II)-dithizonate. Conditions: sample volume: 10 mL, undecanol volume: 15 µL, DTZ concentration: 1.5×10\(^{-5}\) mol L\(^{-1}\), Hg(II) concentration: 5.0×10\(^{-6}\) mol L\(^{-1}\), agitation rate: 250 rpm, extraction temperature: 45°C, extraction time:20 min, light path:0.2 mm.

**Fig 8** Effect of agitation rate on the absorbance of Hg(II)-dithizonate complex. Conditions: sample volume: 10 mL, undecanol volume: 10 µL, DTZ concentration: 1.5×10\(^{-6}\) mol L\(^{-1}\), Hg(II) concentration: 1.0×10\(^{-7}\) mol L\(^{-1}\), extraction temperature: 45°C, extraction time:20 min, light path:1.0 mm.
In terms of sensitivity, instrumental parameters were also taken into consideration and the light path was changed from 0.2 mm to 1 mm to improve the absorbance signal for low Hg(II) concentration. On the other hand, agitation of the sample solution allows enhancing the extraction kinetics as a result of the reduction of the Nernst diffusion film. Agitation of sample reduces the time required to reach the equilibrium between the sample solution and undecanol phase. In this work, the effect of the agitation rate was studied in the range 50–250 rpm. The extraction efficiency has increased with increasing stirring rate up to 250 rpm (Fig 8). Larger agitation rates were avoided since the fine undecanol droplets dispersed into the solution cannot be easily collected. Thus, 250 rpm was selected as a compromise between sensitivity and the practicality of the method.

Last parameter to be optimized is the DTZ concentration which is expected to have a direct influence on the extraction efficiency of Hg(II) complex. Generally, low ligand concentrations result in inefficient complex formation but, high concentrations would also lower down the absorbance signal as the free ligand tends to dissolve in undecanol phase. Therefore, the effect of DTZ concentration was examined in the range of 1.0×10⁻⁶–2.0×10⁻⁴ molL⁻¹ and absorbance at 490 nm plotted against DTZ concentration after baseline correction (Fig 9). The signal has given a maximum at 1.5×10⁻⁶ molL⁻¹ and this concentration was selected for further studies.

Analytical figures of merit
Under the optimal extraction conditions, linearity, limits of detection (LOD) and limit of quantification (LOQ), intra-day repeatability, inter-day reproducibility and enrichment factor of the proposed method were summarized in Table 1. Three replicate measurements were performed at each level. A good correlation coefficient (R² = 0.9989) was obtained in the working range. The detection (LOD) and quantification (LOQ) limits were calculated according the IUPAC approach as 3s/m and 10s/m (s being the standard deviation of 10 blank measurements and m the slope of the calibration line), respectively.

The repeatability of the method, expressed as relative standard deviation (RSD), was evaluated by extracting seven consecutive aqueous samples spiked at 1.0×10⁻⁷ molL⁻¹ with Hg(II). Furthermore, the interday reproducibility was estimated by performing the calibration procedure over five consecutive days.

The enrichment factor, defined as the ratio of the final analyte concentration in the extracting phase to the initial aqueous sample concentration. Hg(II) content of the 5x10⁻⁸ M Hg(II) solution was determined by AFS method prior to and after the procedure was applied. The enrichment factor was calculated as 203 which can be ascribed to the high sensitivity of the photometric method.

### Table 1 Analytical merits of the method

<table>
<thead>
<tr>
<th>Linear working range</th>
<th>3.2-50×10⁻⁸ (molL⁻¹)</th>
<th>6.4-100.8 (µgL⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linear equation</td>
<td>A = 1.61x10⁻¹[C] - 0.0116</td>
<td></td>
</tr>
<tr>
<td>R²</td>
<td>0.9989</td>
<td></td>
</tr>
<tr>
<td>LOD</td>
<td>9.6×10⁻⁹ (molL⁻¹)</td>
<td>1.9 (µgL⁻¹)</td>
</tr>
<tr>
<td>LOQ</td>
<td>3.2×10⁻⁸ (molL⁻¹)</td>
<td>6.4 (µgL⁻¹)</td>
</tr>
<tr>
<td>RSD % (Intraday) (n=7)</td>
<td>8.5</td>
<td></td>
</tr>
<tr>
<td>RSD % (Interday) (n=7)</td>
<td>13.4</td>
<td></td>
</tr>
<tr>
<td>Enrichment factor</td>
<td>203</td>
<td></td>
</tr>
</tbody>
</table>

**Interference Studies**
In order to investigate the selectivity of the proposed method, the effect of interfering ions that are usually present in saline samples was studied. On the other hand DTZ is a versatile chelating agent; interferences may occur due to the competition of some heavy metal ions for DTZ and their subsequent co-extraction with Hg (II). The tolerance limit (M/M) was studied until 5000 the ion/Hg(II) ratio and defined as the concentration of the interfering ions added causing a relative error within ±10% in the true absorbance of Hg (II)–dithizone complex and the results were listed in Table 2. According to Table 2, the major ions in the saline sample matrices have no significant interferences on the analysis. Meanwhile, a few metallic ions including Cu(II) and Pb(II), which can compete with Hg(II) for
the chelating agents, have displayed much less tolerable limits at pH 7.2. One possible solution for that is to use higher concentration of DTZ but, background signal increases accordingly.

Table 2 Tolerance limits of interfering ions for the determination of Hg(II) (1.0 × 10^{-7} mol L^{-1}).

<table>
<thead>
<tr>
<th>Foreign ion added</th>
<th>Interference/metal ratio (M/M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zn^{2+}</td>
<td>1</td>
</tr>
<tr>
<td>Cu^{2+}</td>
<td>5</td>
</tr>
<tr>
<td>Pb^{2+}</td>
<td>50</td>
</tr>
<tr>
<td>Ni^{2+}, Co^{2+}, Ca, Cd, Mg</td>
<td>500</td>
</tr>
<tr>
<td>Cd^{2+}, Cr^{3+}</td>
<td>5000</td>
</tr>
<tr>
<td>Al^{3+}, Bi^{3+}, Fe^{2+}, Fe^{3+}, K^{+}, Mn^{2+}, Br^{-}, NO_{3}^{-}, SO_{4}^{2-}</td>
<td>No interference</td>
</tr>
</tbody>
</table>

Table 3 Mercury content and recovery values of the saline samples analyzed by SDME and AFS methods.

<table>
<thead>
<tr>
<th>Sample Type</th>
<th>Hg(II) found by</th>
<th>Spiked Hg(II) (molL^{-1})</th>
<th>Recoveries (%)</th>
<th>LOD ng mL^{-1}</th>
<th>RSD (%)</th>
<th>Enrichment factor</th>
<th>Literature</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sea Water</td>
<td>&lt; LOD</td>
<td>0.19 µgL^{-1}</td>
<td>2.0×10^{-8}</td>
<td>88.9 ± 7.2</td>
<td>0.01</td>
<td>6.1</td>
<td>770</td>
</tr>
<tr>
<td>Thermal Spring Water</td>
<td>&lt; LOD*</td>
<td>&lt; LOD*</td>
<td>1.0×10^{-7}</td>
<td>100.4 ± 5.1</td>
<td>1.0</td>
<td>430</td>
<td>38</td>
</tr>
<tr>
<td>Mineral Water</td>
<td>&lt; LOD</td>
<td>0.23 µgL^{-1}</td>
<td>2.0×10^{-8}</td>
<td>86.6 ± 3.7</td>
<td>5.0</td>
<td>37</td>
<td>27</td>
</tr>
<tr>
<td>Table Salt</td>
<td>0.78 µgkg^{-1}</td>
<td>0.64 µgkg^{-1}</td>
<td>2.0×10^{-8}</td>
<td>112.4 ± 5.5</td>
<td>5.0</td>
<td>182</td>
<td>36</td>
</tr>
<tr>
<td>Iodized Table Salt</td>
<td>7.73 µgkg^{-1}</td>
<td>8.14 µgkg^{-1}</td>
<td>2.0×10^{-8}</td>
<td>98.6 ± 3.8</td>
<td>5.0</td>
<td>22.8</td>
<td>34</td>
</tr>
<tr>
<td>Rock Salt</td>
<td>0.15 µgkg^{-1}</td>
<td>0.12 µgkg^{-1}</td>
<td>2.0×10^{-8}</td>
<td>102.3 ± 6.8</td>
<td>5.0</td>
<td>64</td>
<td>28</td>
</tr>
</tbody>
</table>

Application of the method to saline samples

The developed method was applied for the determination of Hg(II) content of edible salt and natural saline water samples. Standard addition method was used in the sample analysis. SDME sample preparation technique was employed for the blank samples and for the standard spiked samples. For this purpose the Hg (II) standard solutions were added into sample to be 2.0×10^{-6}, 1.0×10^{-7}, and 5.0 × 10^{-7} mol L^{-1} and their recovery values were calculated. The recovery assays were replicated three times and acceptable recovery values were obtained (Table 3). The same samples were also analyzed with a reference AFS method for the verification of the accuracy of the method. The results were presented in Table 3. As can be seen from the results, mercury content of the samples found by proposed method was in good agreement with AFS results.

Method comparison with other studies

Table 4 lists the methods developed for trace determination of Hg(II) in aqueous samples by microextraction preconcentration techniques.

<table>
<thead>
<tr>
<th>Method</th>
<th>Sample type</th>
<th>Sample prep. technique</th>
<th>Extraction solvent type</th>
<th>LOD ng mL^{-1}</th>
<th>RSD (%)</th>
<th>Enrichment factor</th>
<th>Literature</th>
</tr>
</thead>
<tbody>
<tr>
<td>ETV-AAS</td>
<td>River water</td>
<td>MDME</td>
<td>n-xylene</td>
<td>0.01</td>
<td>6.1</td>
<td>770</td>
<td>1</td>
</tr>
<tr>
<td>ETV-AAS</td>
<td>Water fish</td>
<td>HS-SDME</td>
<td>Thiourea APDC</td>
<td>5.0</td>
<td>3.3</td>
<td>430</td>
<td>2</td>
</tr>
<tr>
<td>ETV-AAS</td>
<td>Mineral Tap water</td>
<td>SFODME</td>
<td>Undecanoic acid</td>
<td>0.07</td>
<td>2.1</td>
<td>38</td>
<td>38</td>
</tr>
<tr>
<td>ETV-ICP-MS</td>
<td>Water</td>
<td>SDME</td>
<td>Ionic liquid</td>
<td>0.0098</td>
<td>5.2</td>
<td>50</td>
<td>34</td>
</tr>
<tr>
<td>CV-AAS</td>
<td>Sea water</td>
<td>HS-SDME</td>
<td>Ionic liquid</td>
<td>0.01</td>
<td>4.6</td>
<td>75</td>
<td>35</td>
</tr>
<tr>
<td>CV-AFS</td>
<td>Human saliva</td>
<td>SFODME</td>
<td>Undecanol</td>
<td>0.025</td>
<td>4.1</td>
<td>182</td>
<td>27</td>
</tr>
<tr>
<td>HPLC</td>
<td>Tap River Waste water</td>
<td>SDME</td>
<td>Ionic liquid</td>
<td>22.8</td>
<td>11.6</td>
<td>3</td>
<td>36</td>
</tr>
<tr>
<td>Jv-VIS</td>
<td>Tap River</td>
<td>SDME</td>
<td>Carbon tetrachloride</td>
<td>0.2</td>
<td>4.9</td>
<td>34</td>
<td>32</td>
</tr>
<tr>
<td>Jv-VIS</td>
<td>Water</td>
<td>DLLME</td>
<td>Ionic liquid</td>
<td>5.9</td>
<td>6.7</td>
<td>18.8</td>
<td>39</td>
</tr>
<tr>
<td>Jv-VIS</td>
<td>River Waste Sea water</td>
<td>SFME</td>
<td>Ionic liquid</td>
<td>0.7</td>
<td>0.94</td>
<td>4.3</td>
<td>16</td>
</tr>
<tr>
<td>Jv-VIS</td>
<td>Drinking River Sea water</td>
<td>DLLME</td>
<td>Carbon tetrachloride</td>
<td>3.3</td>
<td>1.9-5.8</td>
<td>34</td>
<td>28</td>
</tr>
<tr>
<td>Jv-VIS</td>
<td>Saline samples</td>
<td>SDME</td>
<td>Undecanol</td>
<td>1.9</td>
<td>8.5</td>
<td>303</td>
<td>This work</td>
</tr>
</tbody>
</table>


In comparison to these techniques, the method proposed in this study offers an inexpensive and rapid way for determining trace amounts of Hg(II) content in various samples. Attention was paid to all saline samples including sea water, thermal spring and mineral water, rock and table salt which can be complicated with other methods even with those employing expensive and sophisticated instruments.

The method also bears a potential to exploit for field analysis by using miniaturized photometer coupled with a compact extraction system with a greener solvent. The LOD level is well below the limits and allows us to use the method for screening of pollution in a number of environmental samples without need of a sophisticated system. In comparison to other micro extraction techniques, this method has the advantage of extracting in a micro drop which does not require a time consuming freezing and melting steps as is the case for SFODME. By using the multi vessel extraction system, a set of 6 samples can be simultaneously analyzed in less than 30 min.

Experimental

Reagents

All chemicals were of analytical reagent grade and working solutions were prepared in ultra pure water (Milli-Q 18.2 MΩ cm, Millipore System Inc.). NaH₂PO₄·2H₂O, EDTA di-Sodium (Triplex III) Dehidrate and tin chloride were supplied from Merck (Darmstadt, Germany). A standard 0.1 mol L⁻¹ solution of Hg(II) ion was prepared in 0.1 mol L⁻¹ HCl solution by dissolving a weighed portion of HgCl₂ (Merck Darmstadt, Germany) immediately before use. Working standard solution of Hg(II) was prepared by appropriate dilution of the stock standard solution with 0.1 mol L⁻¹ pH 7.2 phosphate buffer solutions. Standard DTZ solution (7.5×10⁻⁴ mol L⁻¹) was prepared daily by dissolving appropriate amount of the reagent in ethanol. Universal Britton Robinson buffer solutions (BR) were prepared by mixing equal molar (0.04 mol L⁻¹) of phosphoric, boric and acetic acid solutions and by drop wise addition of 0.2 mol L⁻¹ NaOH to provide a wide range (2-10) of pH. All standards and extracted samples were stored at 4°C in the dark. Tin chloride reducing agent (3% w/v) was used in AFS studies. All standards and extracted samples were stored at 4°C in the dark. All glassware were soaked in 10% nitric acid for at least 24 h before use and then rinsed with ultra-pure water.

Apparatus

A transonic 460/H ultrasonic bath was used for preparation of DTZ solution. Jenway ion analyzer (model 3040 ion) with a combined glass electrode was used for pH measurements. Samples were placed into a 20 mL clear glass screw vials with a PTFE coated cap (Agilent G1888A). Laboratory made double walled glass cell connected to a circulator of water bath (Nüve BS402) for temperature controlling. Undecanol was pipetted into the aqueous samples with a commercially available 25-µL glass syringe (Hamilton, model 1702). Heidolph Rotormax 120 model orbital shaker was used for extraction purpose. The droplet of undecanol on the surface of water was collected by glass pasteur pipette. Undecanol droplet was santrifugated in 250 µL pulled-point glass inserts. Mini centrifuge purchased from Combi-Spin FVL 2400 N (Boeco, Germany) was used for removing water at 2400 rpm.

A Varian, Cary 100 Bio UV-Vis spectrophotometry with a matched Hellma ultra-micro traycell was used for recording the UV-Vis absorption spectra. A PSA 10.004 Merlin Plus atomic fluorescence spectrometer (AFS) (Kent, UK) was used for the determination of mercury. Cold vapor atomic fluorescence (CV AFS) measurements were made with PSA 10.004 (PS Analytical, Sevenoaks, Kent, UK), which consisted of a PSA 20.099 random access model auto-sampler, continuous-flow vapor generation system and a fluorescence detector. Automated continuous- flow generation system (PSA 10.003) was used to generate gaseous mercury. The generated mercury was, then, detected by utilizing a 254 nm interference filter to achieve wavelength isolation and reduction of background scatter (Merlin, PSA 10.023). Wet gas from the gas-liquid separator was continuously dried by using a semi-permeable Nafion membrane dryer tube (Perma Pure Products, USA). The salinity of the water samples were calculated by measuring the conductivity using a Metler Toledo FG3 system.

SDME Procedure

A 10 mL aliquot of Hg(II) solution containing 0.1 molL⁻¹ phosphate buffer (pH 7.2), 0.1 molL⁻¹ NaCl and 1.5×10⁻⁵ molL⁻¹ DTZ was placed into a 20 mL screw vials. 15 µL of undecanol is added as the extracting solvent and Teflon coated cap of the vial was closed tightly. Sample vial is placed into a laboratory made thermostatic glass cell connected to a circulator of Nüve water bath at 45°C (Figure 10). Six parallel samples can be extracted simultaneously in this assembly. The assembly is placed in orbital shaker set at 250 rpm. The Hg(II)-dithizonate complex were extracted into the undecanol phase from the sample solution for 20 min and then, the droplet is carefully vacuumed in a glass posture pipette for transferring into a glass insert. Any residual water can be removed by centrifuging at 2400 rpm. Extracted sample droplet was placed on to the drop-supporting surface (pedestal) of the tray cell by Eppendorf micro pipette (0.5-5 µL). The gap was controlled by using 0.2 mm and 1 mm paths for the absorbance measurements. Background correction was performed by subtracting the absorbance of the pure undecanol at 490 nm.
**Sample Preparation**

Sea water, mineral water and thermal spring water samples were stored at 4°C and filtered through 0.45 µm pore-sized cellulose acetate filters prior to analysis. The salinity of the samples was determined by conductivity measurements and then, it was adjusted to 0.1 mol L⁻¹ NaCl by adding necessary amount of solid NaCl. Then, the medium pH was made 7.2 by adding 0.1 mol L⁻¹ phosphate solution containing 0.01 mol L⁻¹ EDTA. Upon addition of DTZ to be 1.5×10⁻⁶ mol L⁻¹ the sample was made up to 100 mL. 10 mL aliquots of this mixture were then subjected to SDME.

Iodized table salt, non-iodized table salt and rock salt samples were obtained commercially from local market and 0.6000 g of the salt samples were weighed precisely. The same procedure was applied to the salt sample except addition of NaCl. The resultant samples were then subjected to SDME and subsequently analyzed by UV–Vis spectrophotometry.

**Conclusions**

The present study provides a simple, rapid and yet sensitive spectrophotometric method for trace determination of Hg(II) in saline samples. Without any need of expensive instrumentation, spectrophotometry was coupled with single drop microextraction for enrichment of Hg(II) thus, minimizing organic solvent consumption. Analytical characteristics of the method was found comparable with sophisticated methods and trace amounts of Hg(II) in saline water and salt samples were shown to be detected with good repeatability and high recoveries. The accuracy of the method was compared with atomic fluorescence spectrometry (AFS). Consequently, the method is appropriate to automation and can be adapted to portable systems for field analysis.

**Acknowledgements**

The authors thank to the Ege University for financial support and also thank to Prof. Dr. Emür Henden and Dr. Onur Yayayırük for guidance during AFS measurements.

**Notes and references**

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