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Highly Sensitive EQCM Sensor for Mercury Determination by Coupled Stripping Voltammetry

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An electrochemical quartz crystal microbalance (EQCM) can be used as electrochemical stripping sensor for mercury (II) detection. Due to the frequency changes of EQCM electrode were used as analytical signal instead of currents, the influence of interference current would not be considered. To improve the sensitivity and signal response range, a QCM electrode, modified with Nafion film and Au-nanoparticles (Au-NPs), was employed as a stripping detector for mercury (II) determination in 0.1 M perchloric acid. The cooperation of Au-NPs and Nafion on the gold electrode surface provided an environment for the enhanced electrodeposition of mercury (II). The prepared sensor exhibited a wide linear response to mercury (II) in the concentration range of 3-300 nM (correlation coefficient of 0.9968), with a detection limit of 0.15 nM. The Nafion film was placed between the Au-NPs and the QCM gold-based electrode, which prevented the direct deposition of mercury onto the QCM gold-based electrode surface, and resulted in the easier regeneration of composites modified electrodes. On the other hand, the roughness of electrode surface increased owing to the modification of Nafion film, which would provide much more area for the immobilization of Au-NPs.

Keywords: electrochemical quartz crystal microbalance, mercury, Au nanoparticles, Nafion

1. INTRODUCTION

The requirements of chemical sensors for specific target cations and anions detection are continuously increasing, especially for toxic heavy metal ions [1]. Mercury ion is a well-known chemical pollutant, which causes serious threats to human health and environment. For example, methylmercury, yielded by microbial biomethylation of mercury (II), accumulates in bodies through the food chain, leading to the brain damage and other chronic diseases [2, 3]. Therefore, it's urgent for us to determine the concentration of mercury in human body and environment. Common methods employed for mercury (II) analysis include cold vapour atomic absorption spectrometry, potentiometric stripping analysis, and differential pulse anodic stripping voltammetry [4, 5]. Recently, several novel mercury (II) detection assays in aqueous media have been developed [6-22]. A fluorescence resonance energy transfer (FRET) sensor for mercury (II) was designed by using a mercury-specific oligonucleotide (MSO) probe labeled with fluorophore quencher units [21]. In addition, conjugated polymers [10] and DNA zymes [12, 14] were also exploited to couple with this MSO probe for mercury (II) detection. However, most of these methods rely on optical techniques, such as colorimetry [9, 13, 22], fluorescence [7, 8, 11, 14], atomic absorption spectrometry [23] and fluorescence polarization [24], which cannot exempt from the time and cost demanding sample pretreatment step. However, electrochemical devices may be more impressively cost-effective and portable [25-27].

Quartz crystal microbalance (QCM) is a simple and high-resolution mass sensing technique [28]. However, it has no specific selectivity. When no sensing element is added, QCM is just a transducer. As a result, various chemicals and biomaterials have been used to modify the QCM electrode surface in an effort to obtain selectivity [29]. Combining by the electrochemistry and QCM is denoted as an electrochemical quartz crystal microbalance (EQCM) assay. In recent decades, this kind of EQCM sensors has approved widespread applications in the analysis of clinical targets [30], the monitoring of environmental contaminants such as pathogen, bacterial and the detection of biomolecular interaction [31]. This ensued thanks to its attractive performance, such as high sensitivity, low cost, compatibility with microfabrication technology and rapidness of detection. At same time, another technique combining electrochemical stripping analysis and QCM, has also been reported to obtain selective sensor in trace metal ions analysis. In general, mercury (II) quantitative analysis, using EQCM, was performed at deposition stage, by detecting the frequency changes vs. the mass of deposited mercury from solution [4]. However, it takes a relatively long measure time. Another way for mercury (II) analysis is at the stripping stage. In this stage, mercury can be stripped relatively quickly while measuring the frequency. In this way it is possible to eliminate the inevitable interfering drift in the crystal frequency during the long deposition period. Quartz crystal gold electrodes are used in stripping analysis of trace elements, but different interactions between deposited material and electrode material complicate the analysis in some cases. Gold is found to be the superior substrate for EQCM working electrode owing to its high affinity for mercury, which enhances the preconcentration effect. However, the preconcentration can cause the mercury to diffuse into the gold electrode, resulting in the incomplete stripping of mercury. Moreover, the EQCM gold electrode cannot be renewed by mechanical pretreatments. Therefore, many efforts have been focused on renewable EQCM gold electrodes for the detection of mercury (II). To the best knowledge of authors, there are few studies which take advantage of nanoparticles modified EQCM electrodes for stripping analysis of the mercury (II).

Herein, we fabricated a novel EQCM sensor for mercury (II) detection by coating Au-NPs and Nafion (Au-NPs /Nafion) film on a QCM gold electrode surface. Au-NPs provided high surface area for electrochemical deposition of mercury. Nafion was used to increase the amount of Au-NPs and eliminate the directed interaction between the gold substrate electrode and deposited mercury. The EQCM sensor was used to detect mercury by an electrochemical stripping method. Due to the mass changes of EQCM electrode were used as analytical signal instead of currents, the influence of interference current would not be considered. The results demonstrated that the sensitivity of the sensor could be significantly improved through Au-NPs -based signal amplification, which leads to an amplification factor of more 2-fold than only using gold film electrode.

2. EXPERIMENTAL

2.1. Reagents and apparatus

Mercuric chloride ($HgCl_2$), perchloric acid ($HClO_4$), chloroauric acid ($HAuCl_4 \cdot 4H_2O$) and sodium borohydride ($NaBH_4$) were purchased from Shanghai Chemical Reagents Co. (Shanghai, China). Nafion was obtained from Dupont Co. (USA). All organic solvents and the other chemicals were of analytical grade or above. Deionized water was used in all experiments.

The EQCM and electrochemical stripping experiment was performed by using a quartz crystal microbalance electrochemical workstation (CH Instruments 440A, Shanghai Huachen Ltd. Co.). A 7.995 MHz AT-cut quartz crystal (13.7 mm diameter) with gold electrodes of 5mm diameter was installed horizontally in a Teflon holder between two O-rings so that one side of the crystal contacted solution. A three electrodes system, including a bare or Au-NPs/Nafion film modified gold electrode as a working electrode, a saturated calomel electrode (SCE) as reference electrode, and platinum wire electrode as counter electrode, was used for electrochemical measurements.

The reagent solutions used in the determination of mercury by Cold Vapour Atomic Absorption Spectrometry (CV-AAS) were 0.4% $NaBH_4$ in 0.5% NaOH and 5.00 M HCl. The two components of the Fenton’s reagent (Fe(II) + H_2O_2) used in the digestion of organic mercury in water samples were a saturated solution of ammonium iron(II) sulphate (Shanghai, China) and a 30% H_2O_2 solution.

Aqua Regia solution for acid digestion of soil and sediment samples was prepared by mixing 3:1 of concentrated HCl and HNO_3.

The size and morphology of Au-NPs were observed by Scanning electron microscopy (SEM) using a model S-4800 (HITACHI, Japan) at 5.0 KV.

2.2. Synthesis of Au-nanoparticles

Au-NPs were prepared according to the literature [32] with a little modification. $HAuCl_4$ (5mL, 0.01 M) and sodium citrate (5 mL, 0.01 M) were added to 180 mL of purified water and stirred vigorously at room temperature. Then 5 mL of fresh 0.1 M $NaBH_4$ was added to above solution. And the solution color changed from light yellow to wine red. After the stirring was stopped, the solution was stored at 4 ℃ before use.

2.3. Preparation of Au-NPs /Nafion/EQCM sensor

The bare quartz crystal gold electrodes were cleaned with phiranha solution (H_2O_2/H_2SO_4=1:3 in volume) for 1min. Subsequently, the electrode was rinsed with copious amounts of water and blown dry with a gentle flow of nitrogen. The above treatment aimed to remove grease and other pollutants from the surface of the quartz crystals and gold electrodes. After that, a 5 µL 0.01% (w/v) alcoholic Nafion solution was spread over the quartz crystal gold electrode and dried to get a film by hot air blowing method. And then, a 10 µL Au-NPs solution was added onto the surface of Nafion film. Finally, the electrode was dried in the air.

2.4. Experimental procedures

The analysis procedure was typically as follows. The Au-NPs /Nafion/EQCM sensor was installed horizontally in the holder. A magnetic stir bar was placed in the three electrode cell containing 10 mL 0.1 M $HClO_4$. The solution in the cell was deoxygenated by purging with purified nitrogen for 10 min. The nitrogen flow was maintained over the solution through the whole experiment. Before starting the experiment the EQCM was electrochemically treated: ten cycles of cyclic voltammetry (CV) scanning were first run between 0.0 V and 1.3 V at a scan rate of 0.1 V/s, then more scanning of CV with a scan rate of 0.05 V/s in the same potential range. The crystal frequency was then measured with a background scan in the 0.1 M $HClO_4$ solution. If the frequency drift was less than 1 Hz, the electrode could be used in the further experiments. Otherwise, the electrode had to be scanned repeatedly.

A quantity of the stock solution was added the cell with a micro-injector under the protection of nitrogen. The plating potential was set at 0.0 V for 10 min. After a 15 s rest period without stirring, the deposited mercury was anodically stripped and then the frequency change of QCM was measured. The stripping scan rate was run at 0.05 V/s from 0.0 V to 0.9 V. Prior to deposition of mercury in next sample the plating potential was set at 1.0 V for 10 s, which was used for electrochemical eliminating of mercury on the Au-NPs /Nafion/EQCM electrode surface.

3. RESULTS AND DISCUSSION

3.1. Characterization of Au-NPs /Nafion nanocomposites

The immobilization of Au-NPs/Nafion nanocomposite on the gold electrode was examined by SEM images. The surface morphology of Nafion film solely on the gold electrode was investigated by SEM, as shown in Figure 1A. It was found that the surface roughness increased with respect to that of the plain gold electrode, which would provide much more area for the further immobilization of Au-NPs. Figure 1B showed the typical SEM image of the synthesized Au-NPs, which demonstrated that the Au-NPs could be uniformly decorated on surface of Nafion film. The particles were relatively homogeneous spherical shape with the nominal diameter of 30 ± 5 nm, while the formation of gold film or agglomerates was not observed.

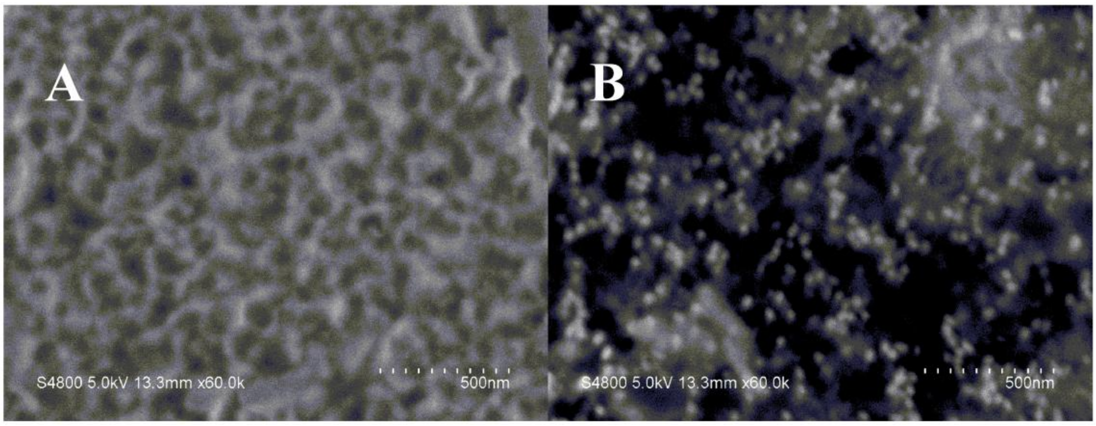

Figure 1. SEM images of the surface immobilized Nafion (A) and Au-NPs/Nafion (B) film on the gold-based EQCM electrodes

On the basis of the above observation, cyclic voltammetry (CV) was taken to further characterize the Au-NPs/Nafion nanocomposites modified gold-based EQCM electrode in a 0.1 mol/L $HClO_4$ solution. The CV curves of Nafion film modified electrode and bare gold electrode could be seen in Figure 2 a and b, respectively. Lines (c) and (d) represented the CV curves of Au-NPs/Nafion/EQCM sensor modified with different amount of the Au-NPs, obtained by placing 10 and 15 μL of Au-NPs suspension on the Nafion coated EQCM sensor surface.

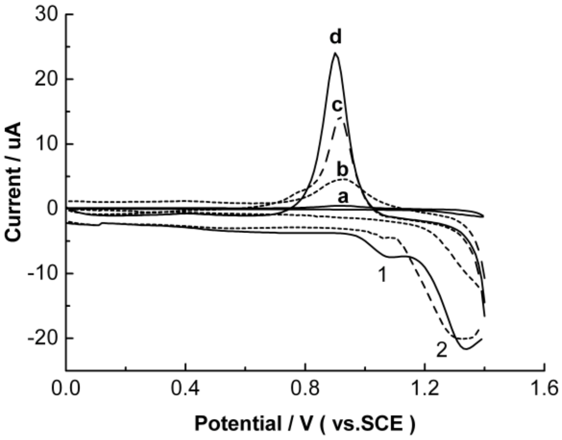

Figure 2. Cyclic voltammograms of (a) 0 μL, (c)10 μL (d) 15 μL of 0.05 mg/mL Au-nanoparticles modified Nafion/EQCM sensors and (b) bare EQCM gold-based electrode in 0.1 M $HClO_4$ with scan rate of 0.1 V/s.

The CVs anodic peaks between 1.1 - 1.4 V and cathodic peaks in 0.91 V, caused by oxidation and reduction of gold surface. Two anodic peaks, labeled 1, 2 in Figure 2, appear in the voltammograms of the Au-NPs/Nafion/ EQCM electrode, while only a single anodic peak is observed in the CV of a bare planar gold electrode. The clean cathodic peak was observed at 0.91 V corresponding to the reduction of Au^{3+} on the Au-NPs/Nafion/ EQCM electrode surface. The shape and potentials of the anodic peak 1 (1.08 V) and broad peak 2 (1.33 V) on the cyclic voltammograms (curves c and d) are consistent with the oxidation for polycrystalline Au (111) and Au (100), respectively [32, 33]. Based on the CV curves outlined in Figure.2, the real surface area of Au-NPs supported on the EQCM sensor was estimated. This was conducted based on the amount of charge consumed during the reduction of the gold surface oxide monolayer with a reported value of 400 $\mu C/cm^2$ [32]. The real surface area ware calculated to be 0.18 and 0.32 cm^2 for the Au-NPs/Nafion/ EQCM sensor. This was more than 4.5 times of the geometric surface area of the gold electrode (0.07 cm^2), which indicated the higher electroactive sites of the Au-NPs/Nafion /EQCM sensor.

3.2. Comparison of gold-based EQCM and Au-NPs/Nafion coated EQCM

Stripping analysis and the QCM were combined to investigate the performance of the Au-NPs/Nafion/EQCM sensor in trace mercury (II) detection. Figure 3 displays the frequency response of (A) Au-NPs/Nafion EQCM sensor and (B) gold-based EQCM electrode for detecting mercury (II) at a concentration of 4.5×10^{-7} M, respectively. The stripping of preconcentrated mercury was a relatively rapid and quantitative process at the Au-NPs/Nafion EQCM sensor (Figure 3A). In addition, the stripping frequency response was remarkably enhanced (about 55%) at the Au-NPs/Nafion EQCM sensor compared to gold film EQCM electrode (Figure 3B), which may be attributed to the larger effective surface area and better stripping ability resulting from small size of Au-NPs modified on the Nafion film coated gold-based EQCM electrode surface.

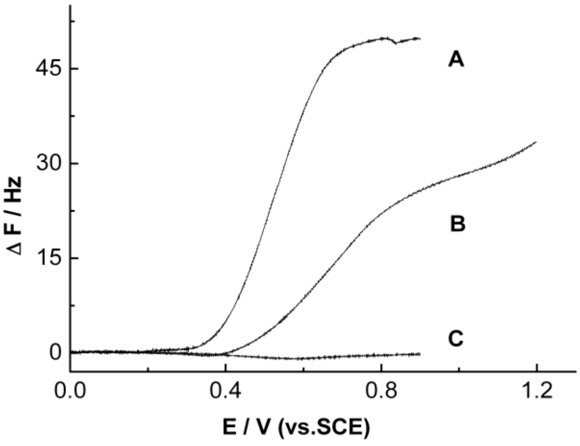

Figure 3. Frequency responses of (A) Au-NPs/Nafion/EQCM sensor and (B) EQCM gold-based electrode in the same mercury (II) concentration (450 nM), (C) Au-NPs/Nafion/EQCM electrode in absence of mercury (II) solution.

At gold film EQCM electrode for mercury (II) detection, mercury was absorbed on the gold electrode surface during deposition and some of the mercury was retained even after stripping and cleaning, that was, the nature of the gold electrode was fundamentally altered by irreversible deposition of mercury. The electrochemical preconditioning step may not produce a fresh gold surface, resulting in the loss of reproducibility. Due to the formation of Hg-Au amalgam, preconcentrated mercury could not be stripped completely from the sensor surface, leading to the decrease of sensitivity. Mercury of Hg-Au amalgam was stripped in the range 0.40-0.90 V [34]. A mercury monolayer cannot be mixed with gold to form these compounds, but multilayer deposits of mercury can be diffused into the bulk of the gold electrode forming Hg-Au amalgam. This was confirmed that the deviation from linearity originates from kinetic or experimental parameters [1]. Because of the high surface area of Au-NPs, mercury film deposited on the Au-NPs should be much thinner than that on bare gold electrode under the same preconcentrated conditions. Therefore, the amount of produced Hg-Au amalgam should decrease.

3.3. Optimum experimental parameters

The EQCM frequency responses were compared with the electrodes that loadings different amount of Au-NPs in 1.5×10^{-8} M mercury (II) solution. Theoretically, the Au-NPs films of EQCM will have much more active-centers and larger surface area with the loadings increment. In this work, we found that the electrode modified with 15 μL Au-NPs solution (2.5×10^{-5} mol/cm^2 Au-NPs on the sensor surface) resulted in the biggest frequency responses. Deficient loadings deposited on the electrode may result in less active-centers and smaller surface area. Furthermore, excess loadings of Au-NPs may affect the reproducibility and sensitivity of the mercury (II) detection. Thus, we choose 2.5×10^{-5} mol/cm^2 Au-NPs as the optimum amount in our experiments.

At a sensor for mercury (II) detection, a great advantage of the Au-NPs/Nafion/EQCM electrode is the possibility of mass production of the EQCM sensors with a regenerated surface. Usually, solid gold electrodes can be regenerated by mechanically polishing with alumina powder. Due to the QCM electrode is a gold film electrode (gold film thickness, 6 μm), this treatment should irreversibly damage the EQCM electrode. Our EQCM sensor was modified with a Nafion film between the Au-NPs layer and the EQCM gold-based electrode. Nafion film prevented the direct deposition of mercury on the EQCM gold-based electrode surface, resulting in the easier regeneration of composites modified electrodes. On the other hand, the roughness of electrode surface increased owing to the modification of Nafion film, which would provide much more area for the immobilization of Au-NPs. Herein, a 5 μL Nafion solution (0.01 % (w/v)) was selected as the modified quantity.

It is known that longer deposition time led to higher sensitivity. The frequency responses increased with the increase of the deposition time in the range of 0-40 min. A deposition time of 10 min was sufficient to achieve satisfactory stripping efficiency, though the maximum was not reached. Considering the total detection time, the deposition time was set at 10 min.

3.4. EQCM performance of Au-NPs/Nafion/EQCM sensor for mercury (II) detection

Under the above optimal parameters, Au-NPs/Nafion/EQCM sensor was used to detect mercury (II) by the combination of stripping analysis and EQCM. Figure 4 displayed the stripping frequency responses recorded in 0.1 M $HClO_4$ with a plating time of 10 min containing different concentration of mercury (II) by Au-NPs/Nafion/EQCM sensor. When using anodic stripping voltammetry (ASV), anodic stripping peaks observed in the range 0.4 - 0.9 V were concluded to result from Hg-Au intermetallic compounds. For a quantity of deposited Hg much less than the equivalent of one monolayer, a single stripping peak was observed at approximately 0.8 V [35]. As shown in Figure 4 b, c, and d, the starting value of the deposited mercury stripping potential was observed at +0.71 V corresponding to the oxidation of mercury, indicating the formation of monolayer mercury on electrode surface. It was obvious that the stripping potential in curves e, f, and g all shifted towards cathodic direction. And the stripping peak in curve h shifted to +0.40 V demonstrated the production of multilayer mercury [1, 32]. Stripping potential changes was unimportant since the frequency change covers total mercury stripping regardless of whether it was deposited as a monolayer or a multilayer [1]. The insert is the calibration curve of the detection system. The frequency responses are linear with mercury (II) concentration ranging from 3 nM to 300 nM (the correlation coefficient (r^2) was 0.9968) with the detection limit of 0.15 nM (3σ), which indicates a good sensitivity of Au-NPs/Nafion/EQCM sensor for mercury (II) detecting.

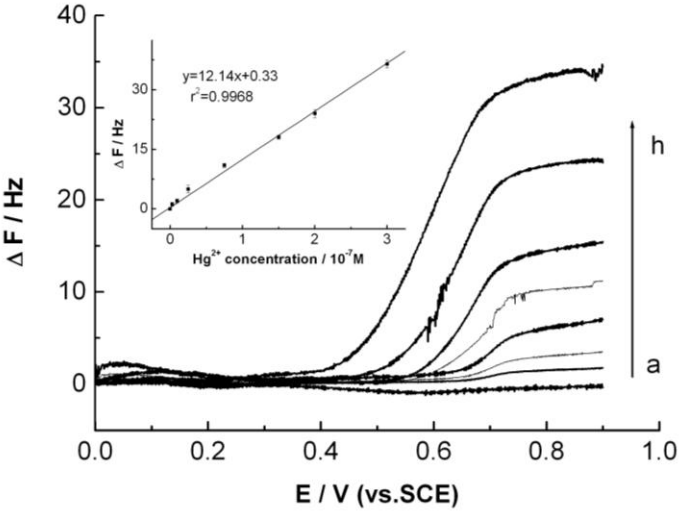

Figure 4. Frequency responses for increasing concentration of mercury (II) at Au-NPs/Nafion/EQCM sensor: (a) blank, (b) 3 nM, (c) 10 nM, (d) 25 nM, (e) 75 nM, (f) 150 nM, (g) 200 nM and (h) 300 nM

3.5. Interferences experiments

In order to test the selectivity of the Au-NPs/Nafion/EQCM sensor for practical application, the control experiments were performed with 1.5×10^{-7} M mercury (II) in 0.1 M $HClO_4$ in the absence (f) and in presence of 5.0×10^{-5} M Cu (II), Pb (II), Cr (III), As (III), and Cd (II) inorganic ions (a-e). The changes of frequency were 3, 2, 1, 0.5, and 1.5 Hz, respectively. When the above mentioned ions (2.5×10^{-5} M, each) were added in1.5×10^{-7} M mercury (II) solution, only produced a slight frequency change (<3 Hz) (Figure.5), and the other inorganic cations in sample did not interfere with detection, which indicated the high selectivity of the Au-NPs/Nafion/EQCM sensor for mercury (II) detecting.

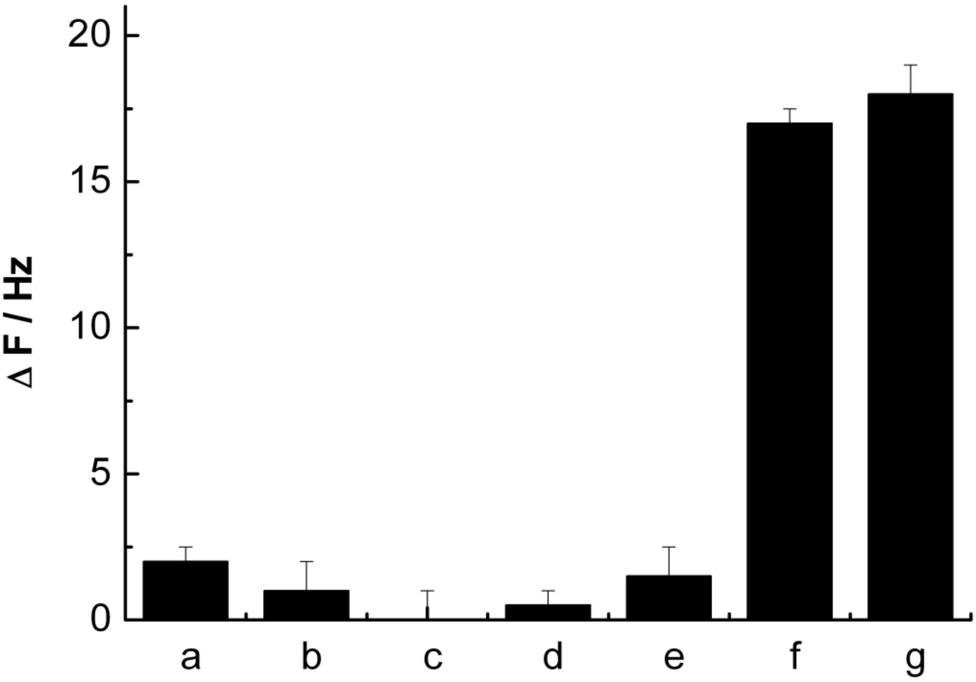

Figure 5. Selectivity of the Au-NPs/Nafion/EQCM sensor has been prepared with 5.0×10^{-5} M (a) Cu^{2+}, (b) Pb^{2+}, (c) Cr^{3+}, (d) As^{3+}, (e) Cd^{2+}, (f) 1.5×10^{-7} M $Hg^{2+}$and g. the mixture of Cu^{2+}, Pb^{2+}, Cr^{3+}, As^{3+}, Cd^{2+}(2.5×10^{-5} M, each), and Hg^{2+} (1.5×10^{-7} M), respectively.

3.6. Applications

The original water samples were gray in color and contained organic and inorganic particulate matter. The EQCM analysis of the filtered original water samples and digested water, sediment and soil samples revealed the presence of Hg (II) (Table 1). The interference of these common cations was eliminated in CV-AAS determination of Hg(II) by the addition of hydrazine sulfate and potassium iodide in concentrations 4 and 30 $mg\cdot L^{-1}$, respectively. Both the original and digested samples analyzed by EQCM and CV-AAS were filtered prior to analysis, while those analyzed by the proposed EQCM method were injected directly into the Teflon cell. The results obtained indicate that in the freshwater samples mercury in the form of organic mercury compounds (Table 1, freshwater-digested) was twice as much as inorganic dissolved mercury (Table 1, freshwater-original) . Very good agreement for all samples was observed between the EQCM and CV-AAS results (Table 1).

The Au-NPs/Nafion/EQCM sensor was used several times over a period of 2 months, and the RSD was found to vary by less than 7 % for mercury (II) detection.

Table 1. EQCM and CV-AAS results for Hg(II) samples

Sample	Concentration of Hg(II) ± S.D.[a] ($\mu g \cdot l^{-1}$)	
	EQCM	CV-AAS
Freshwater (original)	1.67 ± 0.5	1.60 ± 0.7
Freshwater (digested)	4.82 ± 1.1	5.11 ± 0.8
Sediment (digested)	175 ± 1.0	174 ± 2.1
Soil (digested)	146 ± 1.2	148 ± 2.0

[a] Standard deviation calculated on the basis of three replicate measurements.

4. CONCLUSIONS

A novel kind of Au-NPs/Nafion/EQCM sensor with the combination of stripping analysis and the QCM was successful for the detection of trace mercury. The material of nanocomposite, Au-NPs/Nafion, exhibited large surface area, which facilitated the deposition and stripping of mercury. The sensitivity of the Au-NPs/Nafion/EQCM sensor has been investigated by the EQCM method. The results showed that the Au-NPs/Nafion/EQCM sensor exhibited high sensitivity for the detection of trace mercury in solution and real samples. The results obtained from this work demonstrated that the proposed sensor holds great promise for the detection of trace mercury in various agricultural products and environmental samples.

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