Simple and rapid electrochemical quantification of water-stabilized HgSe nanoparticles of great concern in environmental studies

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ABSTRACT

The sensitive monitoring of mercury (II) selenide nanoparticles (HgSe NPs) is of great potential relevance in environmental studies, since such NPs are believed to be the ultimate metabolic product of the lifesaving mechanism pathway of Hg detoxification in biological systems. In this context, we take advantage of using gold-nanostructured screen-printed carbon electrodes (SPCE-Au) for the rapid, simple and sensitive electrochemical quantification of engineered water-stable HgSe NPs, as an advantageous alternative to conventional elemental analysis techniques. HgSe NPs are first treated in an optimized oxidative/acidic medium for Hg^{2+} release, followed by sensitive electrochemical detection by anodic stripping voltammetry (ASV). To the best of our knowledge, this is the first time that water-stable HgSe NPs are quantified using electrochemical techniques. The low limit of detection achieved (3.86×10^7 HgSe NPs/mL) together with the excellent repeatability (RSD: 3%), reproducibility (RSD: 5%) and trueness (relative error: 10%), the good performance in real sea water samples (recoveries of the analytical signal higher than 90%) and the simplicity/low cost of analysis make our method as ideal candidate for HgSe NPs monitoring in future environmental studies.

Graphical abstract
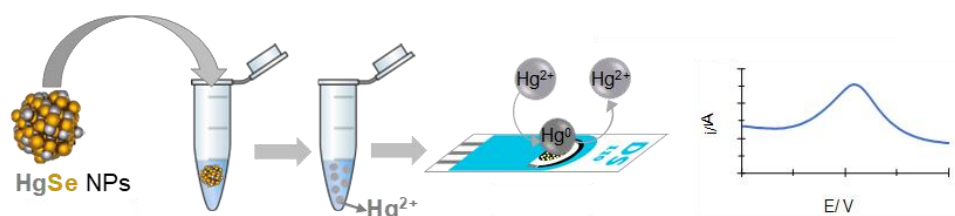
Keywords

Electrochemical detection; Anodic stripping voltammetry; Mercury selenide nanoparticles; Gold-nanoparticles; detoxification

1. Introduction

Mercury (Hg) is a widely studied metal due to its high toxicity, since it is present in the environment in diverse forms with specific biogeochemical transformation and ecotoxicity [1]. The evaluation of the toxicity of Hg species inside a biological organism is under comprehensive research, largely due to the capability of methylmercury-containing compounds to cross the blood-brain barrier causing highly neurotoxic effects [2]. Moreover, antagonistic interactions between Hg species and the selenium (Se) present in Se-containing biomolecules have been reported [3,4]. Se compounds provide, owing to Se-Hg antagonism, effective pathways for converting both inorganic and organic Hg-based compounds to HgSe granules or nanoparticles (NPs) [4].

Although the precise pathway of Hg detoxification is not yet known, HgSe NPs have been reported as the ultimate metabolic product of such lifesaving mechanism in biological systems [5]. In this sense, different studies have addressed the presence of HgSe NPs in tissues of animals [6-8] and in human brain tissue [2]. Recently, the *in vivo* formation of natural HgSe NPs in different tissues of pilot whales has also been reported [9]. Furthermore, engineered water-stabilized HgSe NPs have recently been synthesized and characterized with the aim of using these nanoparticulated standards for understanding the Hg detoxification mechanism as well as for studying their formation, behaviour and interactions in living organisms [10].

The analytical determination of mercury generally involves both spectroscopic and spectrometric methods [11], such as cold atomic fluorescence spectroscopy (AFS) [12,13], atomic absorption spectroscopy (AAS) [14,15], inductively coupled plasma-mass spectrometry (ICP-MS) [16,17] and surface enhanced raman scattering spectroscopy (SERS) [18]. However, these powerful methods have some practical

limitations, especially when routine analysis of complex samples requiring high sensitivity is needed, mostly related to the cost of analysis and the use of non-portable devices. In this context, electrochemical methods have emerged in the last years as a worthwhile alternative for analysis of Hg taking advantage of cheaper procedures suitable for *in field* analysis [19,20]. Low thermal conductivity, good electrical conductivity and ability to form amalgams are among the unique properties of Hg that made it the object of extensive electrochemical studies [21].

The ability of quickly pre-concentrate different metals on the electrode surface also makes electrochemical techniques highly suitable to detect low concentrations of Hg. Hg pre-concentration can be highly enhanced by modifying the electrode surface with materials with high affinity for it [22], being gold (Au) an ideal candidate for this purpose. Au can form reduced Hg monolayers at a potential higher than its redox potential (underpotential deposition process, UPD) [23]. For this reason, Au-based structures, mainly those nanostructured, have been widely used as electrode modifiers for Hg determination [24-26]. The advantages of Au nanostructures for the detection of Hg have also been combined with the easy to use and low-cost screen-printed carbon electrodes (SPCEs) for its sensitive determination [27].

In this work, we take advantage of using Au-nanostructured SPCEs (SPCE-Au) for the rapid, simple and sensitive electrochemical quantification of engineered water-stable HgSe NPs. The proposed methodology opens the way for further environmental applications for HgSe NPs monitoring in environmental samples which would be of great relevance for detoxification studies. In addition, and to the best of our knowledge, this is the first time that water-stable HgSe nanoparticles have been quantified using electrochemical techniques.

2. Experimental section

2.1. Chemicals and equipment

The precursors used for the synthesis of HgSe NPs were selenium powder (99.99%) from Sigma-Aldrich (Spain), mercury (II) acetate (≥98%) from Panreac (Spain) and ethylenediaminetetraacetic acid disodium salt dihydrate (EDTA, ≥99%) from Fluka (Switzerland). Sodium hydroxide and absolute ethanol were obtained from Merck (Germany) and Sigma-Aldrich (Spain), respectively. Water-stabilization of HgSe NPs was achieved by coating them with dihydrolipoic acid (DHLA). For this purpose, DL-Thioctic acid (98%), sodium bicarbonate and sodium borohydride (≥96%) were purchased from Acros Organics (Belgium), Merck (Germany) and Fluka (Switzerland), respectively. Potassium tert-butoxide (≥98%), anhydrous methanol and anhydrous toluene were acquired from Sigma-Aldrich (Spain).

Gold standard (HAuCl$_4$ · 3H$_2$O in 12.7% HCl), fuming hydrochloric acid (37%) and hydrogen peroxide (30%) were obtained from Merck (Germany). Nitric acid (65%) was purchased from VWR International (Spain).

All chemical reagents used in experiments were of analytical grade and used as received without further purification. All the solutions were prepared in ultrapure water (18.2 MΩ) obtained with a Millipore Direct-Q® 3 UV purification system from Millipore Ibérica S.A (Spain).

Fluorescence emission spectra were recorded with a LS-50B Luminescence Spectrometer from Perkin Elmer (United States of America), using a fixed excitation wavelength of 415 nm with both excitation and emission slit width of 10 nm. Conventional Quartz SUPRASIL cuvettes obtained from Hellma Analytics (Germany) were used for such measurements. High resolution-transmission electron microscopy (HR-TEM) images were obtained using a JEM-2100F transmission electron microscope

purchased from JEOL (Japan) on a copper grid, using an accelerating voltage of 200 kV. Elemental measurements were performed using a 8800 ICP-MS/MS from Agilent Technologies (USA) equipped with a MicroMist nebulizer. Hg was measured in on-mass mode ($^{202}Hg^+ \rightarrow ^{202}Hg^+$). Water-stabilized HgSe NPs were completely digested in *aqua regia* before ICP-MS measurements. External calibration using Merck (Germany) certified 1000 mg L^{-1} standard of Hg was used in the ICP-MS analysis (see **Figure SI-I in the supporting information**).

Nanoparticle acidic treatment for electrochemical measurements was done in an Elmasonic P 30 H ultrasonic bath from Elma Schmidbauer GmbH (Germany). Electrochemical measurements were performed with an Autolab PGSTAT-10 from Eco Chemie (The Netherlands), controlled by Autolab GPES software from Metrohm (Switzerland). Both screen-printed carbon electrodes (SPCEs, ref. DRP-110) and their connector to the potentiostat (ref. DRP-DSC) were purchased from DropSens S.L (Spain). The conventional three-electrode configuration of SPCEs includes both carbon working and counter electrodes and a silver pseudoreference electrode. All measurements were carried out at room temperature.

2.2. Methods

2.2.1. HgSe NPs synthesis and water stabilization

HgSe NPs were synthesized following a procedure previously reported by our group [10]. Briefly, Se powder was dissolved in a 5 M NaOH solution and the resulting mixture was irradiated with ultrasounds via a direct-immersion of a high-intensity probe for 30 min. Then, an equimolar amount of Hg^{2+} in a 0.1 M EDTA solution was slowly added. After that, the solution was left irradiating during 3 h at room temperature. The final suspension was centrifuged, and the black precipitate was washed in sequence with volumes of 0.1 M EDTA, water and absolute ethanol. The washed powder was left to

air-dry. Finally, HgSe NPs were surface-modified with dihydrolipoic acid (DHLA) following the procedure described elsewhere **[10,28]** to render them compatible with aqueous media.

2.2.2. Hg^{2+} release from HgSe NPs for electrochemical measurements

Hg^{2+} was released from DHLA-capped HgSe NPs suspension through an acidic treatment. NPs suspension was treated in the optimized oxidative/acidic medium and then sonicated for a determined time in an ultrasonic bath (frequency: 30-100 Hz, temperature: 25 °C).

2.2.3. SPCEs modification with gold nanostructures

Gold nanostructures were *in situ* generated on the working area of the SPCEs by the electrochemical reduction of $AuCl_4^-$, following a method previously optimized in our group **[29]**. Briefly, an aliquot of 40 µL of 1 mM $HAuCl_4$ solution (in 0.1 M HCl) was dropped on the electrode surface and a constant current intensity of -100 µA was applied during 180 s, leading to the formation of nanostructures (electrode covered with monodisperse AuNPs with a mean diameter around 75 nm).

2.2.4. Electrochemical quantification of mercury

Electrochemical quantification of Hg was based on the Hg underpotential deposition (UPD) process onto SPCE-Au, according to a procedure previously reported by our group **[30]**. Briefly, Hg was preconcentrated by placing 40 µL of the appropriate solution on the electrode surface and applying a constant potential of + 0.30 V for 60 s. Then, the potential was switched between + 0.30 V and + 0.50 V using square-wave voltammetry (SWV) at a frequency of 80 Hz, an amplitude of 30 mV and a step potential of 4 mV.

All measurements were performed by triplicate without removing oxygen from the solution. A new electrode was used for each measurement. Repeatability of the method was evaluated by performing different measurements of the same sample with the same instrument, while reproducibility was estimated by evaluating different samples with the same concentration using different instruments.

2.2.5. *Spike and recovery protocol*

Spike and recovery experiment is an important method for validating and assessing the accuracy of an analytical technique for particular sample types. It was done to determine whether HgSe NPs quantification is affected by a real sample matrix when compared with the one used in the standard calibration curve (Milli-Q water). Sea water samples were taken from two coastal cities of the Cantabrian Sea: Gijón and Llanes (Asturias, Spain) for such purpose. This experiment was performed by spiking both sea water samples with 5.14×10^9 and 5.14×10^8 HgSe NPs/mL (n = 3 for each sample). Then, the spiked samples were electrochemically evaluated. After that, the % recovery of the analytical signal in the real matrix sample was calculated.

3. RESULTS AND DISCUSSION

3.1. Characterization of the water-stable HgSe NPs

The quality of the synthesized water-stable HgSe NPs was first evaluated by high resolution-transmission electron microscopy (HR-TEM). Quasi-spherical HgSe NPs were observed, as shown in **Fig. 1a** and **b**, demonstrating the successful NPs formation. Nanoparticle average diameter of 23 ± 2 nm (**Fig. 1c**) was obtained after counting HR-TEM images (n = 50) using *ImageJ* image processing software. HgSe NPs were also optically characterized by evaluating their fluorescent properties. A fluorescence emission centered at 575 nm when excited at 415 nm was noticed, as shown in **Fig. 1d**.

Such emission is characteristic of the water-stable HgSe NPs [10], being an evidence of high quality HgSe NPs formation.
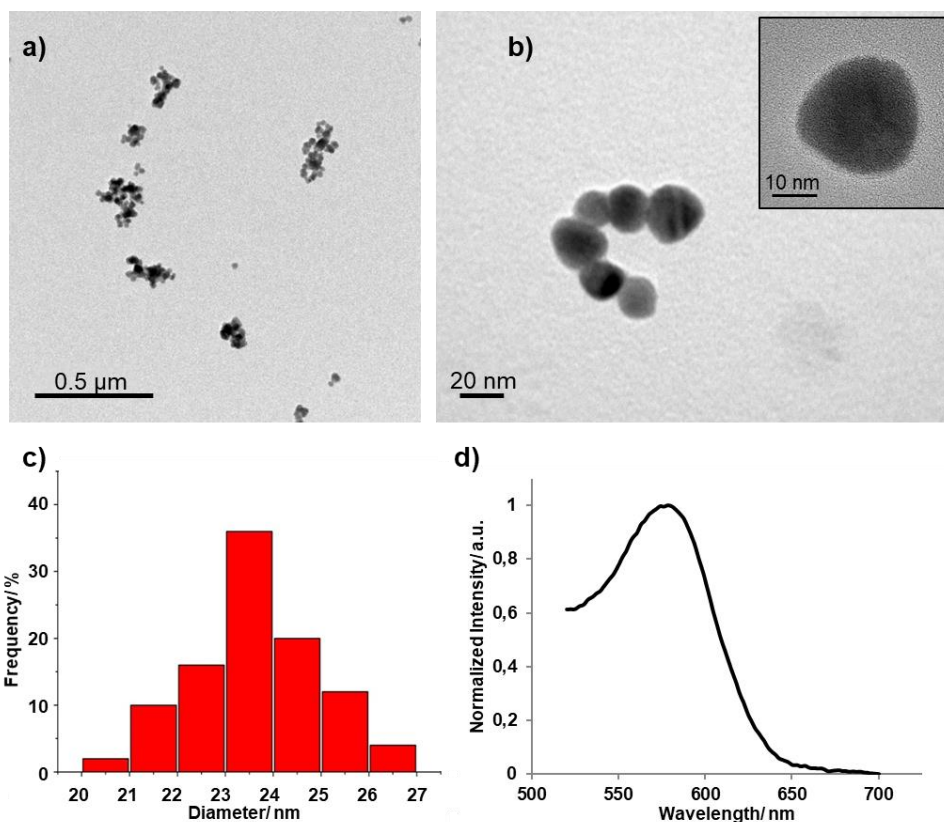

Fig. 1. (a, b) HR-TEM images at different magnifications and (c) nanoparticle size distribution histogram of the obtained HgSe NPs. (d) Fluorescence emission spectrum of the HgSe NPs in water media ($\lambda_{ex} = 415$ nm).

Nanoparticle concentration is a critical and very important parameter to be measured since is evident that toxicological effects would depend on the number of NPs and their size polydispersity rather than on the simple NP mass. Therefore, HgSe NP concentration in the stock suspension containing the water-stabilized HgSe was determined by ICP-MS. Taking into account the Hg/Se molar ratio in the NP, determined by ICP-MS, that is 1.04 [10], from a simple Hg quantification (total Hg was measured after acidic treatment using external calibration, see **Fig. S1 at the supporting information**, and turned out to be 350 ± 10 μM (n = 3) it is possible to obtain the mass of HgSe in the sample. Then, considering the HgSe density (i.e. 8.23 g cm^{-3}) the total volume of the HgSe NPs in the solution is calculated. Nanoparticle

concentration in the measured sample could then be estimated dividing such total NP volume by the individual NP volume (water solubilized HgSe NPs in the measured sample were spherical with an average diameter of 23 nm as shown in **Fig. 1**). A nanoparticle concentration of 1.80×10^{12} HgSe NPs/mL was finally obtained.

3.2. Electrochemical detection of HgSe NPs

3.2.1. Detection principle: Hg^{2+} release and voltammetric monitoring

A key step for the electrochemical detection of mercury relies on the Hg^{2+} release from HgSe nanoparticles through acidic treatment. After that, Hg^{2+} is reduced to Hg^0 and preconcentrated on the surface of the SPCE-Au by applying a constant potential of + 0.30 V for 60 s, which is facilitated by the underpotential deposition process of mercury on gold. Finally, Hg^0 is re-oxidized to Hg^{2+} by scanning from + 0.30 V to + 0.50 V using square wave voltammetry (SWV), being the full process known as anodic stripping voltammetry (ASV). The voltammetric peak current at approximately + 0.40 V corresponding to Hg^{2+} re-oxidation is considered as the analytical signal. The process of Hg^{2+} release from HgSe NPs and electrochemical detection is illustrated at **Fig. 2**.
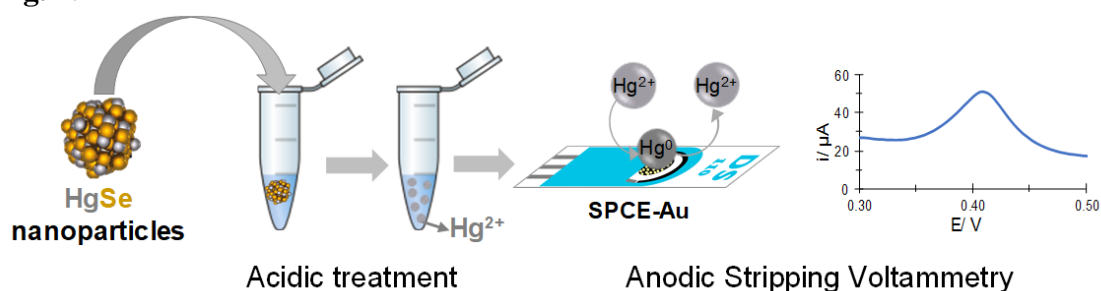

Fig. 2. Scheme of the procedure of Hg^{2+} release from HgSe NPs and electrochemical detection by anodic stripping voltammetry.

3.2.2. Optimization of the HgSe NPs acidic treatment

With the aim of extracting the maximum amount of Hg^{2+} from the HgSe NPs in a reproducible way, the use of HCl in combination with oxidative HNO_3 was considered.

Mixtures of different concentrations of both acids in the range 0.10-0.50 M were evaluated. Higher acid/hazardous concentrations were avoided. As shown in **Fig. 3a** for a fixed concentration of HCl the analytical signal (peak current) increases with the concentration of HNO_3 as expected, due to the oxidative power of HNO_3. The higher signal is observed for the use of 0.50 M HCl in combination with 0.50 M HNO_3, being such ratio of concentrations considered as optimum. Notably, such highest signal (20.10 μA) is very close to that obtained using 0.25 M HCl and 0.50 M HCl (19.80 μA), which seems to indicate that we already approached the optimum plateau.
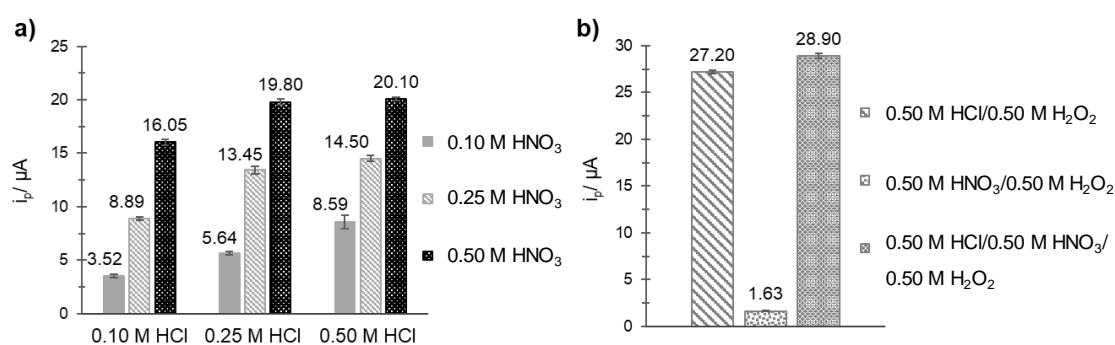

Fig. 3. Comparative study of the analytical signals obtained for a solution of 5.14×10^9 HgSe NPs/mL after treatment during 60 min with (a) mixtures of different concentrations (0.10-0.50 M) of HCl and HNO_3 and (b) 0.50 M H_2O_2 in combination with 0.50 M HCl and 0.50 M HNO_3 both individually and together.

Hydrogen peroxide (H_2O_2) was also added to the HCl/HNO_3 mixture for enhancing the Hg^{2+} release, thanks to its oxidative character. HgSe NPs treatment with 0.50 M H_2O_2 in combination with 0.50 M HCl and 0.50 M HNO_3 both individually and together was tested, as shown in **Fig. 3b**. The signal measured after the treatment with HNO_3 and H_2O_2 was very low, whereas in presence of HCl the signals were significantly higher. This suggests that the presence of HCl is crucial for achieving an effective Hg^{2+} release, probably thanks to the stabilization effect of the chloride ions over the Hg^{2+} ions released. A slightly higher signal was measured after the treatment with the three-component mixture in comparison with the use of the HCl/H_2O_2 solution,

which means that the combined oxidizing power of HNO_3 and H_2O_2 is highly advantageous. In addition, using the 0.50 M HCl/0.50 M HNO_3/0.50 M H_2O_2 mixture, a 92 % of efficiency releasing the Hg^{2+} from HgSe nanoparticles has been achieved. Therefore, that mixture was selected as the optimum solution and it was used for further quantification studies.

Notably, the addition of 0.50 M H_2O_2 to the HCl/HNO_3 mixture still enhanced Hg^{2+} release from the NPs as indicated by the significantly higher signal obtained (28.90 µA) in comparison to the use of the acid mixture alone. Considering the low purity of available H_2O_2 solutions, higher H_2O_2 concentrations were not evaluated to avoid contaminations that could interfere in the measurements.

3.2.3. Quantification of HgSe NPs

The effect of the HgSe NPs concentration on the analytical signal (voltammetric peak current at + 0.43 V) under the optimized NPs treatment conditions (0.50 M HCl/0.50 M HNO_3/0.50 M H_2O_2; ultrasounds for 60 min) was evaluated. Well-defined voltammetric peaks were obtained, being the peak current higher for increasing concentrations of HgSe NPs in the range between 5.14×10^7 HgSe NPs/mL and 5.14×10^9 HgSe NPs/mL (**Fig. 4**).
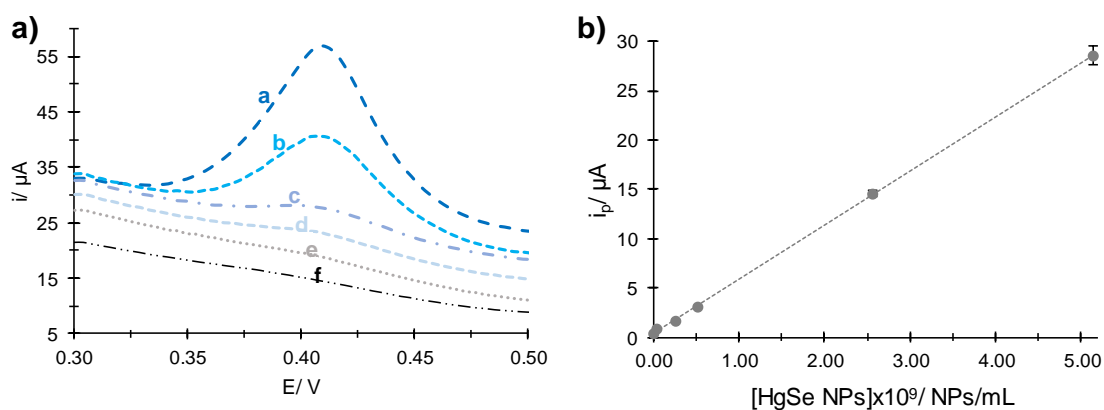

Fig. 4. a) Square wave voltammograms from + 0.30 V to + 0.50 V corresponding to different concentrations of HgSe NPs (a: 5.14×10^9, b: 2.57×10^9, c: 5.14×10^8,

d: 2.57×10^8, e: 5.14×10^7, f: 0 HgSe NPs/mL) after deposition at + 0.30 V for 60 s. Frequency: 80 Hz. Amplitude: 30 mV. Step potential: 4 mV; b) Effect of HgSe NPs concentration on the analytical signal following the optimized experimental procedure: measurements after treatment during 60 min with 0.50 M HCl/0.50 M HNO_3/0.50 M H_2O_2 in an ultrasonic bath. Data are given as average ± SD (n = 3).

Both parameters are adjusted to a linear relationship within that range (correlation coefficient of 0.9999), according to the following equation:

$$i_p (\mu A) = 5.50 \times 10^{-9} [\text{HgSe NPs}] (\text{NPs/mL}) + 0.41$$

A limit of detection (LOD, calculated as three times the standard deviation of the intercept divided by the slope) was found to be 3.86×10^7 HgSe NPs/mL. Such limit of detection is similar to the previously achieved with ICP-MS (4×10^7 NPs/mL) [10]. It is worthy to highlight that the procedure here described represents a faster and lower-cost way to quantify mercury from HgSe nanoparticles, very suitable for further *in field* environmental applications.

The method shows a repeatability (RSD) of 3% (n = 3) and a reproducibility (RSD) of 5% (n = 3) for a HgSe NPs concentration of 2.57×10^8 HgSe NPs/mL. The trueness of the method was estimated in terms of % of relative error also for 2.57×10^8 HgSe NPs/mL, considering that:

% of relative error = [(real value-experimental value)/real value] x 100

According to the equation of the calibration plot, an experimental NPs concentration of 2.31×10^8 NPs/mL is estimated when a concentration of 2.57×10^8 HgSe NPs/mL is analysed. From these data, a 10% of relative error is estimated.

3.2.4. HgSe NPs analysis in real samples: spike and recovery

While analysis in MIlli-Q water is important, analysis in sea water is the main goal for demonstrating the good performance of the HgSe NPs analysis method in the

presence of the potential interferences that may be found in a real scenario. Spike and recovery experiment was performed as detailed at the experimental section. The data shown in **Table 1** summarizes the results of such experiment. The high recovery rates of the analytical signal at levels above 90% demonstrate that our methodology is not affected by the real matrix in a significant extent, opening the way to an accurate HgSe NPs determination in sea water samples for environmental studies.

Table 1. Spike and recovery assay data. The study was done by spiking 5.14×10^9 and 5.14×10^8 NPs/mL of HgSe NPs in sea water samples (n = 3 for each sample) and calculating the % recovery of the analytical signal when compared with the results in Milli-Q water.

Samples	Spiked HgSe NPs/ NPs/mL	Current in Milli-Q water/ µA	Current in real samples/ µA	Recovery/ %
Sea water 1	5.14×10^9 5.14×10^8	28.90 3.09	27.19 2.80	94.08 90.75
Sea water 2	5.14×10^9 5.14×10^8	28.90 3.09	27.25 2.87	94.30 92.88

4. Conclusion

Water-stabilized mercury (II) selenide nanoparticles (HgSe NPs) have been synthesized, characterized and quantified for the first time using anodic stripping voltammetry (ASV). Voltammetric detection takes advantage of the Hg underpotential deposition process (UPD) on the gold contained in gold-nanostructured screen-printed carbon electrodes (SPCE-Au). Hg^{2+} release conditions from HgSe NPs have been carefully optimized, finding as optimum a treatment with 0.50 M HCl/0.50 M HNO₃/0.50 M H₂O₂ with ultrasounds for 60 min. Under such conditions, HgSe nanoparticles can be quantified over two orders of magnitude ($5.14 \times 10^7 – 5.14 \times 10^9$ HgSe NPs/mL) with a good repeatability (RSD: 3%), reproducibility (RSD: 5%) and trueness (relative error: 10%), obtaining a limit of detection of 3.86×10^7 HgSe NPs/mL. Additionally, analysis of sea water samples gave recoveries of the analytical signal at levels around 90%, demonstrating the suitability of our method for real

environmental analysis. These analytical characteristics make this technique as very useful for further *in field* environmental applications being simpler, faster and cheaper than conventional methods for the quantification of Hg.

Acknowledgments

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.talanta.2019.03.038.

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