

Application of Polyaniline Nanoparticles Modified Screen Printed Carbon Electrode as a Sensor for Determination of Hg(II) in Environmental Samples

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Abstract

We have described the development of electrochemical nano sensor for the detection of mercury ions from aqueous solutions based on the formation of polyaniline nanoparticle films. Screen printed carbon electrodes were modified with polyaniline nanoparticles. Electropolymerization of polyaniline nanoparticles was performed by the pulsed potentiostatic method. The sample of polyaniline nanoparticles was prepared by repeating the potential step process three times. Structural and morphological characterization of polyaniline nanoparticles modified screen printed carbon electrode was performed using Fourier Transmission infrared (FTIR), X-Ray Diffraction (XRD) and Scanning Electron Microscopy (SEM). PANI nanoparticles were spherical shaped having an apparent diameter varying from 20 to 45 nm. Square wave anodic stripping voltammetry was used for the detection of Hg(II) on PANI NPs modified screen printed carbon electrode under optimized conditions. Hg(II) was deposited for 60 seconds by the reduction of Hg(II) in buffer solution: (citric acid and sodium chloride pH 2.30), followed by Hg stripping between 0.3 and 0.8 V at the following parameters: Scan rate: 100 mV s⁻¹, frequency: 60 Hz, amplitude: 0.025 V and increment: 4.0 mV. It was found that the PANI NPs modified screen printed carbon electrode had a highest anodic stripping peak current in solution of pH 2.30. The limit of detection value for Hg(II) was found to be 2.50 ± 0.03 ppb. The limits of detections determined are below the corresponding guideline value from the World Health Organization (WHO). In addition, the modified nano electrode exhibited excellent reproducibility and high stability. The developed method was successfully applied to determine Hg(II) in real water samples with satisfactory results.

Keywords: Electrochemical nanosensor; Polyaniline nanoparticles; Mercury sensor; Modified electrode; Voltammetry

Introduction

Nowadays the environment getting more pollution due to release of so many heavy metals which has considerable attention. Due to this more and more efforts have been put to control the metal exposure from the different fields of environment. According to this Mercury and Arsenic possess high toxic potential which are accumulated in living organisms and environmental samples.

Solvated mercuric ion, Hg(II), is one of the most stable inorganic forms of mercury [1], a widespread heavy metal pollutant distributed in air, water and soil with a high cellular toxicity and severe adverse effect on human health such as the brain, kidney, stomach, and intestines [2]. Methyl mercury is the most common organic source of mercuric ion which is generated by microbial biomethylation of Hg(II) ions [3]. Methyl mercury is a serious neurotoxin which can accumulate in the human body through the food chain [4]. The US Environmental Protection Agency (EPA) limit of Hg(II) for drinkable water is 10 nM which is much lower than the detection limit of most available assays [5]. Therefore, development of a simple, sensitive, selective, economical, practical and compatible label-free method is highly demanded for environmental monitoring, food industry and clinical diagnostics [6]. Although inductively coupled plasma mass spectrometry (ICPMS) is a powerful technique for the detection of heavy metal ions, it is complex, expensive and is thus not practical for routine monitoring of Hg(II) and As(III) [6]. Much effort has been devoted to design optical sensing systems for the detection of Hg(II) and As(III) based on organic chromophores or fluorophores, biomolecules such as proteins, antibodies, oligonucleotides, DNA, enzymes and also semiconductor quantum dots, conjugated polymers and inorganic materials. However, poor selectivity, inefficient sensitivity and stability are some limitations with most of these methods. In the past few years, functionalized gold nanoparticles were used as a colorimetric sensor for the detection of

Hg²⁺, Pb²⁺, Cu²⁺ [7]. Moreover, several colorimetric methods have been developed for the detection of Hg(II) and As(III) using gold nanoparticles (AuNPs) functionalized with various oligonucleotides such as DNA. Most of these methods involve a color change from red to blue or an extremely sharp change in melting transitions through aggregation in the presence of Hg(II) [8,9].

As a portable instrument, electrochemistry instrumentation, which can perform anodic stripping voltammetry (ASV) and cathodic stripping voltammetry (CSV) has some advantages over the various "naked-eye" arsenic test kits. These electrochemical techniques can determine mercury at trace levels within few minutes. In addition, these techniques can also distinguish between different oxidation states, are easy to operate and, compared with other instrumental techniques, are comparatively cheap. However, these methods also suffer from interferences and matrix effects, which means that voltammetric methods are rarely used for mercury analysis and determination in complex matrixes, such as food samples [10].

Electrochemical measurements can also be used to detect heavy metal ions, which have shown numerous advantages including rapid analysis speed, good selectivity and sensitivity. For metal ion analysis three conditions should be met during electrochemical measurements:

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[11] selective adsorption of metal ions; [12] enhancing electron exchange between the working electrode and the metal ions; [13] the strong electrochemical response. The nanoparticle-modified electrode can meet the three conditions. The nanoparticle modified electrodes can be divided to metal oxide nanoparticles, carbon material nanoparticles, polymer material nanoparticles and self-assembled nanoparticles [14-16]. The nanoparticle-modified electrodes were used for determination of heavy metal ions, such as Hg(II), Cr(VI), As(III), Cd(II) and Pb(II) [17]. In this study, an electrochemical nanosensor based on polyaniline nanoparticles (40-100 nm) modified screen printed carbon electrode has been applied for the determination and analysis of trace of mercury ion Hg^{2+} with high sensitivity and selectivity in environmental samples.

Experimental

Reagents

Aniline, ACS reagent 99.5% (Sigma-Aldrich), HCL, ACS reagent 37%, ethanol (99.8% Merck), 30% H_2O_2 (Sigma-Aldrich), 98% H_2SO_4 (Merck), 99% KCl (BioXtra 99%), 99.98% (Sigma) $\text{K}_3\text{Fe}(\text{CN})_6$ trace metals basis, 70% HClO_4 (Aldrich), 99.99% trace metals basis and for metal ion binding/assay using ultra-pure water (Millipore Milli-Q).

Preparation of 1000 ppm of mercury, cadmium, lead, zinc and arsenic ions

1000 ppm solution of cadmium ions was prepared by dissolving 2.744 g of cadmium nitrate (99.9%, Aldrich), 1.598 g of lead nitrate (99.9%, Aldrich) and 1.63 g of mercury nitrate (99.9%, Aldrich) in ultra-pure deionised water and diluted to 1000 ml. For As(III), we use the 1000 ppm (Fluka) stock solution immediately. From these stock solutions, you can prepare a range of concentrations of target metal ions (1nM to 10 mM). The measurements were first made for single metal ion solution (non-competitive binding) of Cd(II), Pb(II), Cu(II), Hg(II) and As(III) at the natural pH's of stock solutions (pH 6.3, 4.8, 5.4, 5.5 and 6.1 respectively). The effect of ligand protonation state was explored separately by adjusting the solution pH (with 0.01 M KOH or 0.01 M HNO_3). The pH was measured with a pH meter (WTW, Germany).

Apparatus

Electrochemical experiments were carried out on μStat 400 Bipotentiostat/Galvanostat from DropSens and CHI660E power Potentiostat from CH Instruments. A combined three electrode system (SPE) was employed with an Ag electrode as a reference electrode, Pt electrode as counter electrode and carbon electrode as working electrode. High purity argon gas was used to displace oxygen from the electrochemical cell before results were collected. Morphology of polyaniline nanoparticles was determined using high resolution Hitachi model, S4700 Scanning Electron Microscope. XRD patterns were obtained using Philips PW 1800 X-Ray Diffractometer by Cu K_α radiation at 30 KV and 30 mA. The FTIR spectra of the polyaniline nanoparticles modified electrode were recorded in the spectra range of 4000 to 400 cm^{-1} using Ventax 70 spectrometer. Mercury analyzer AA-6800 Shimadzu was also used.

Preparation of electrode surfaces

The screen printed electrodes were cleaned in a fresh Piranha solution (30% H_2O_2 :98% H_2SO_4 , 1:3 by volume) at room temperature for 1-3 minutes, rinsed thoroughly by Millipore Milli-Q deionised water, dried under a stream of argon, rinsed again with absolute ethanol to remove any remaining water and dried under a stream of argon. Cleaned electrodes were cycled in 5 mM $\text{K}_3\text{Fe}(\text{CN})_6$ +1M KCl aqueous solution from -0.4 to +0.6 V vs. SCE until a stable voltammogram was obtained.

After the cycling, the electrodes were rinsed with absolute ethanol and dried under argon to prepare the clean surface. In order to allow the modified electrodes formation.

Preparation of screen printed carbon electrode modified with PANI nanoparticles

The aniline monomer from Sigma-Aldrich was distilled under reduced pressure and hydrochloric acid 1M, aniline was dissolved in absolute ethanol at different mole ratios: (1:1), (1:2), (1:3), (1:4), (1:5) and (1:6).

Screen printed carbon electrode surfaces were polished using alumina slurry on a soft cloth were sonicated in first ethanol and then doubly distilled water for 5 min each to remove possible contaminants. Electropolymerization of polyaniline nanoparticles was performed by the pulsed potentiostatic method. A freshly cleaved screen printed carbon electrode was immersed into a solution of 1 mM aniline (pH=5.0) and 1.0 M HClO_4 at a potential of -0.2 V versus Ag electrode, then the potential was stepped from this initial value to +0.9 V for polymerization. Following a polymerization pulse for 10-100 ms, the electrode potential was returned to -0.2 V, and the screen printed carbon electrode was removed from the solution and rinsed with ultrapure water. The sample of polyaniline nanoparticles was prepared by repeating the potential step process three times. After the polymerization of PAN, the fabricated PAN/SPEs were dipped into doubly distilled water for 5 min to remove unpolymerized aniline monomers remaining in the PAN coatings if any. The electrode was sonicated in ethanol and doubly distilled water for 5 min, successively, to remove any adsorbed substances on the electrode surface. Finally, it was dried under nitrogen atmosphere and putted in small tube for characterization measurements.

Results and Discussion

Characterization of screen printed carbon electrode modified with PANI nanoparticles

X-Ray diffraction: Powders X-ray diffraction observation were made using computer controlled XRD system, RTNT 1800 wide angle goniometer at an operating rate of 49 KN and 30 mA, Cu Ka radiation $\lambda=0.154186$ nm in the 2θ range of 2-80 degree, the step of scanning rate was 0.02 degree of a scan speed to deg/min. Figures 1 and 2 show the XRD patterns for PANI nanoparticles prepared by electrochemical method at different mole ratio between aniline and ethanol.

The PANI nanoparticles exhibits are amorphous reflection at $2\theta=19^\circ$, there is another peak in $2\theta=6^\circ$ which reveal the local crystallinity of PANI nanoparticles. The peak at $2\theta=6^\circ$ may be caused by

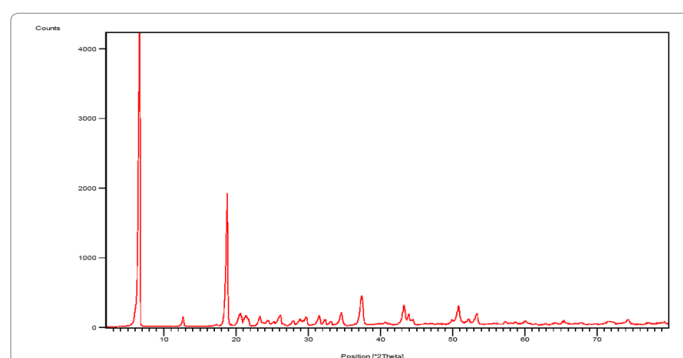


Figure 1: XRD patterns for PANI nanoparticles prepared by electrochemical method, mole ratio (1:2) between aniline and ethanol.

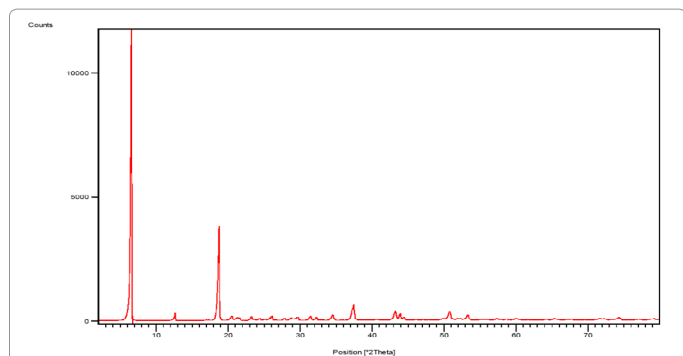


Figure 2: XRD patterns for PANI nanoparticles prepared by electrochemical method, mole ratio (1:3) between aniline and ethanol.

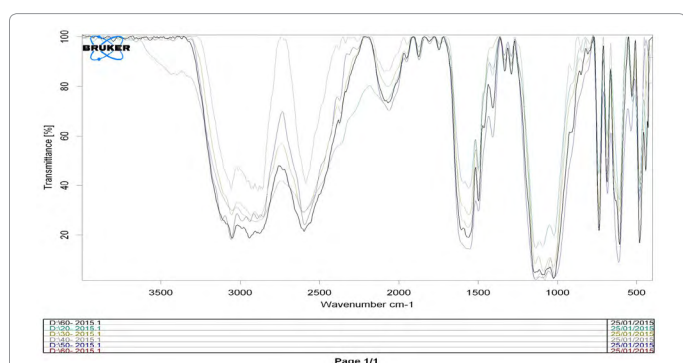


Figure 3: FTIR spectrum of PANI nanoparticles prepared by electrochemical method at different mole ratios between aniline and ethanol (1:2), (1:3), (1:4), (1:5) and (1:6).

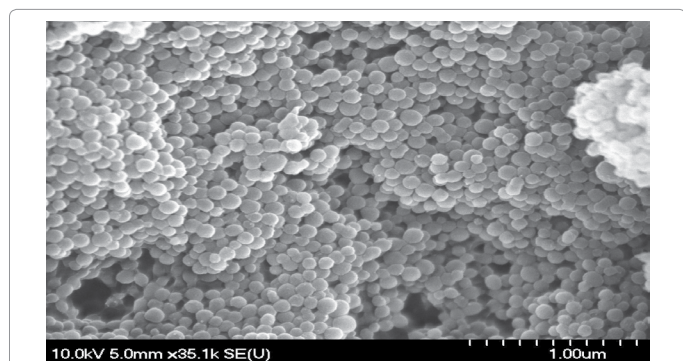


Figure 4: SEM image of PANI nanospheres electrodeposited in solution 1mM aniline solution (pH=5.0).

the scattering along the orientation parallel to the polymer chains [18]. The peak at $2\theta=19^\circ$ also represents the characteristic distance between the ring planes of benzene rings in the adjacent chains or close-contact interaction distance. These results indicate that the PANI subchains become more rigid and ordered in nanoparticle scale (19-40 nm). The degree of crystallinity for PANI nanoparticles prepared by this method is higher than that for PANI/HCl prepared by chemical method [19].

FTIR measurements: The FTIR spectra examination of PANI nanoparticles prepared at different mole ratios between aniline and ethanol are shown in Figure 3. The characteristic band in the IR spectrum occurs at 3054.69, 2878, 1558, 1498, 1291, 1141 and 740. The band observed at 3054.69 cm^{-1} represents the N-H stretching modes, the one at 2878.58 cm^{-1} could be attributed to the C-N stretching of

secondary aromatic amine (-N - benzenoid -N -) and the ones at 1558 and 1498 cm^{-1} are attributed to C=N and C=C stretching modes for the Quinoid and benzenoid rings, the peaks at 1291 and 1141 cm^{-1} correspond to C-N stretching (-N - benzenoid -N) and C=N stretching (-N =quinoid=N) respectively. The band at 740 cm^{-1} is attributed to aromatic C-H bending out of the plane of the 1:4 substituted aromatic ring. The presence of all these vibrational modes clearly indicates that polyaniline nanoparticles electrochemically prepared by a potential pulse are in the emeraldine form [20].

Scanning Electron Microscope (SEM): The SEM micrograph for PANI nanoparticles electrodeposited in solution 1mM aniline (pH=5) are shown in Figure 4. The PANI nanoparticles have a medium globular (rice grain) morphology similar to that previously reported [21]. However, the bulk powder rice grain features are fused together rather than being discrete particles. The results show the PANI nanospheres were successfully prepared (Figure 3). The average width of PANI nanospheres formed on screen printed carbon electrode is 20 μm and the average thickness is between 40 to 100 nm.

Cyclic voltammetry of screen printed carbon electrode: Cleaned screen printed carbon electrodes were cycled in 5 mM $\text{K}_3\text{Fe}(\text{CN})_6$ + 1M KCl aqueous solution from 0.0 to +0.4V vs. Ag reference electrode until a stable voltammogram was obtained (Figure 5). After the cycling, the screen printed carbon electrodes were rinsed with absolute ethanol and dried under argon to prepare the clean surface.

Cyclic voltammetry of screen printed carbon electrode modified with polyaniline nanoparticles: Figure 6 shows the repetitive cyclic voltammograms of screen printed carbon electrode modified with PANI nanoparticles from -0.2 V to +0.2 V against Ag reference electrode, scan rate 50 mV/s, 10 cycles. During the cyclic voltammograms of PANI nanoparticles modified screen printed carbon electrode, oxidation and reduction peaks were observed between +0.070 V and +0.150 V, thus presenting the electron transfer between PANI nanoparticles. The best cathodic and anodic peaks were observed at $+0.9 \pm 0.07$ V and $+0.105 \pm 0.02$ V, respectively for PANI nanoparticles prepared from (1:3) mole ratio between aniline and ethanol.

Square wave anodic stripping voltammetry of Hg(II) at screen printed carbon electrode modified with polyaniline nanoparticles

Square wave anodic stripping voltammetry was used for the detection of Hg(II) under optimized conditions. Hg(II) was deposited

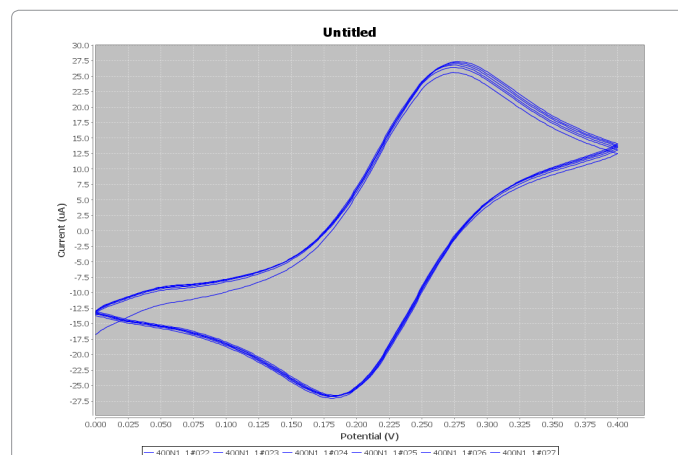


Figure 5: Repetitive cycling voltammograms of screen printed carbon electrode in 5 mM $\text{K}_3\text{Fe}(\text{CN})_6$ + 1M KCl, scan rate 50 mV/s.

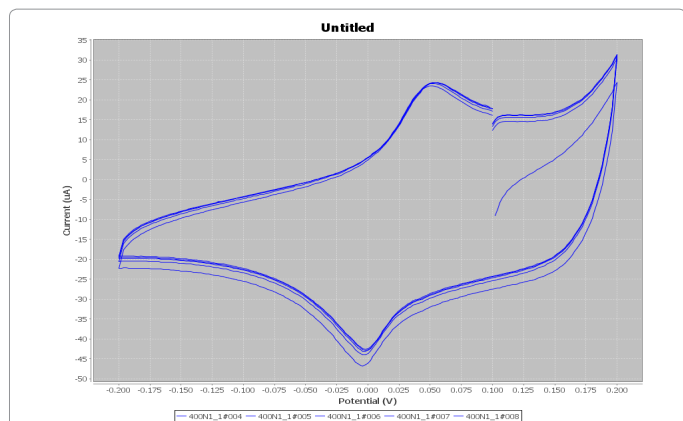


Figure 6: Repetitive cyclic voltammograms of screen printed carbon electrode modified with PANI nanoparticle (1:3) in 5 mM $K_3Fe(CN)_6$ + 1M KCl, scan rate 50 mV/s, 5 cycles.

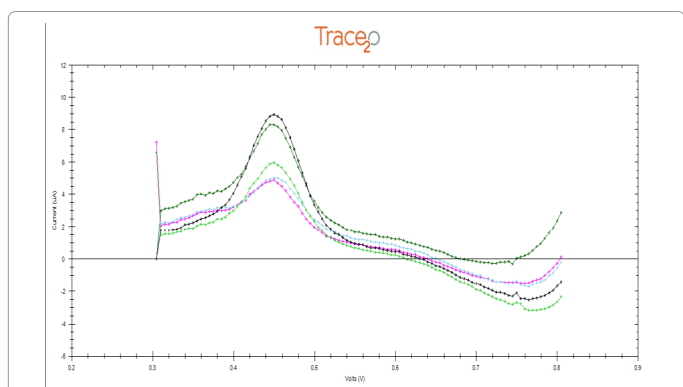


Figure 7: Square wave anodic stripping voltammograms with PANI nanoparticles (1:6) modified screen printed carbon electrode in solution containing 10, 20, 30, 40, 60 ppb Hg(II) at pH 2.30, deposition Time 60 seconds.

o Min	1 nA	o Min	1 mA
E begin	0.30 V	E end	0.81 V
E step	0.005 V	E amp	0.025 V
E cond	0.60 V	E dep	-0.20 V
Frequency	100 Hz	T cond	20 seconds
T dep	60 seconds	T eq	15 seconds

Table 1: Summary of optimum conditions for Hg(II) determination in aqueous samples using the polyaniline nanoparticles modified screen printed carbon electrode constructed in this study.

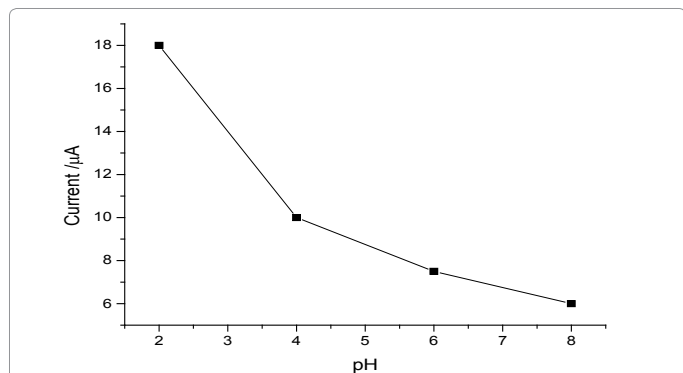


Figure 8: The effect of pH on the peak height for PANI nanoparticles (1:3) modified screen printed carbon electrode in solution containing 40 ppb Hg(II).

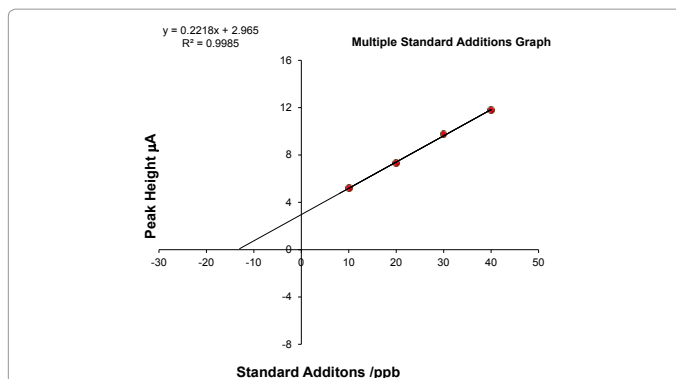


Figure 9: Multi point standard addition method for PANI nanoparticles (1:6) modified screen printed carbon electrode in solution containing 10, 20, 40, 60 ppb Hg(II) at pH 2.30, deposition Time 60 seconds.

for 60 seconds by the reduction of Hg(II) in buffer solution: (citric acid and sodium chloride pH 2.30), followed by Hg stripping between 0.3 and 0.8 V at the following parameters: scan rate: 100 mV s⁻¹, frequency: 60 Hz, amplitude: 0.025 V and increment: 0.004 V. The characteristic peak for Hg(II) on screen printed carbon modified PANI nanoparticles electrode was observed at 0.45 V as shown in Figure 7. The Hg(II) sample (5.0 ppm = 5000 ppb) was prepared fresh daily from a 1000 mg/L standard solution (Fluka). The optimum conditions for determination of Hg(II) using SWASV on polyaniline nanoparticles modified screen print carbon electrodes is shown in Table 1.

The effect of pH on the peak height (Ip): The PANI nanoparticles modified screen printed carbon electrode (1:3), (1:6) were immersed in a 40 ppb solution of Hg(II) solution at different pH (2, 4, 6 and 8). Solutions of different pH were adjusted to the appropriate pH with 0.01 M HNO₃ and 0.01 M KOH. Figure 8 shows the influence of electrolyte solution pH on the electrochemistry of PANI nanoparticles modified screen printed carbon electrode. Clearly, at pH 6 and 8 a poor response peaks were obtained, as seen by low currents and a large separation in peak potential and the peaks exhibited irregular shape indicating that the pH had a strong effect on the preconcentration processes and the pH 2 in both PANI nanoparticles modified screen printed carbon electrode (1:3) and (1:6) have a very good response, peak height at (18.0 ± 2.05 µA).

The multi point standard addition method was shown in Figure 9. The results show that the PANI nanoparticles modified screen printed carbon electrode prepared (1:6) has a very good response to Hg(II) in aqueous solution than the one prepared by (1:3).

The polyaniline nanoparticles modified screen printed carbon electrode towards mercury ions results were further evaluated to determine the limit of detection for the modified nanoelectrode. Using the results in Figure 9, the limit of detection (LOD) was calculated as the standard deviation multiplied by three and that value divided by the slope of the calibration plot in the linear range. The LOD value was found to be 2.50 ± 0.03 ppb. The LOD value for the poly aniline nanoparticles modified screen printed electrode sensor compares favourably to the values obtained for other sensors constructed for the determination of Hg(II). In the work done by Guo et al. [22], it was shown that a thin-film sol-gel-modified glassy carbon electrode functionalized with tetrasulfide has a detection limit of 100 ppb. Similarly, Walcarius et al. [23] have shown that a carbon paste electrode modified with dispersed pure silica particles can detect Hg(II) at 10 ppb. A very low detection limit of 1.4 ppb for Hg(II) determination was obtained by Piro et al. [24], with the use of a carbon paste electrode containing sonogel and

modified with poly(3-methylthiophene). The results of this study therefore demonstrate the possibility of applying a modified polyaniline nanoelectrode to analysis Hg(II) in aqueous solutions.

Application of polyaniline nanoparticles modified SPCE using square wave anodic stripping voltammetry to water reference material sample

With the purpose to evaluate the potential application of the developed method, reference material water sample (NIST-1641E Natural water-101.6 ppb mercury) was used for analytical assay. Multi point standard addition protocol has been used for detection, and satisfactory data of recoveries were obtained (present in Table 2).

	(NIST-1641E)
Certified Hg Conc. (ppb)	101.6 ppb
Experimental % recovery	98.5
	78
	96
	99
	103
Average % recovery	94.90
Standard Deviation	9.77

Table 2: Certified Hg concentrations and the % recoveries for the amount of Hg as determined by square wave anodic stripping voltammetry. % recovery is the experimental concentration of mercury in the sample divided by the certified mercury concentration in the reference material.

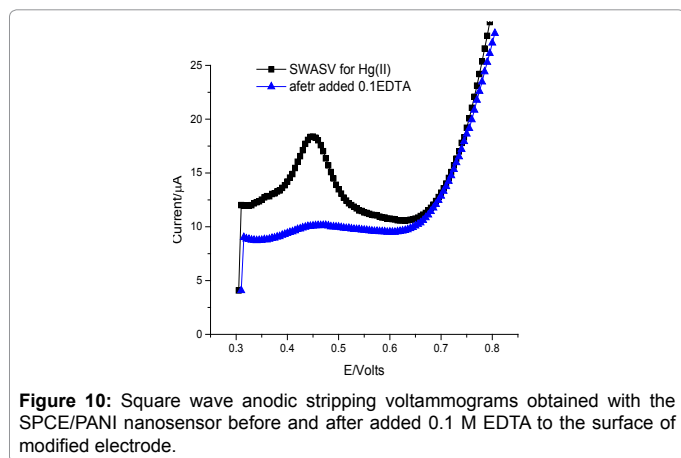


Figure 10: Square wave anodic stripping voltammograms obtained with the SPCE/PANI nanosensor before and after added 0.1 M EDTA to the surface of modified electrode.

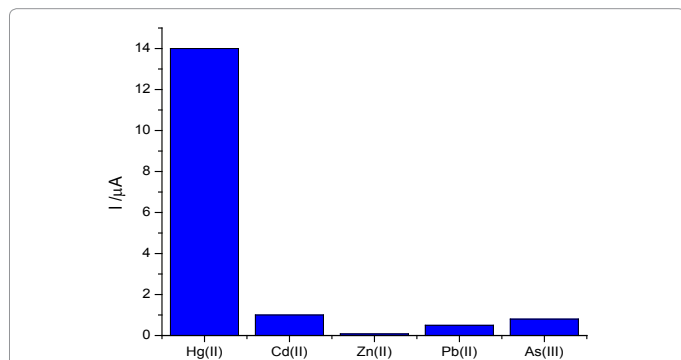


Figure 11: Effect of interference metal ions on Hg(II) 40 ppb determination in aqueous solutions pH=2.30, accumulation time 60 s, accumulation potential 0.45 V, scan rate 0.1 Vs⁻¹, frequency 100 Hz, amplitude 0.025 V and increment 0.005 V.

Sample	Concentration of Hg(II) ± SD* (mg.g-1)	
	Electrochemical Nanosensor	Mercury Analyzer
1	0.65 ± 13.5	0.38 ± 13.1
2	0.14 ± 21.0	0.62 ± 21.6
3	2.07 ± 55.8	1.07 ± 56.4
4	1.68 ± 47.9	0.87 ± 49.1
5	2.11 ± 80.6	0.93 ± 82.1
6	4.05 ± 105.7	2.1 ± 107
7	0.703 ± 38.7	1.65 ± 41.09
8	1.08 ± 98.05	0.65 ± 97.43

*Standard deviation calculated based on three replicate measurement

Table 3: Electrochemical nanosensor and mercury analyzer results for Hg(II) real samples.

Renewal of polyaniline nanoparticles modified SPCE: To utilize the electrode for multiple and long term operation, the regeneration of the polyaniline nanoparticles modified screen printed carbon electrode in a reproducible manner is very important. The incorporated mercury species were readily removed by exposing the modified electrode to 0.1 M EDTA solution for a few minutes. Moreover, there was no decrease in the electrode response after several successive runs. The polyaniline nanoparticles modified screen printed carbon electrode can be used for multiple analytical determinations for about two weeks. The electrode with a fresh polyaniline nanoparticles surface gave signals with approximately the same degree of precision (about 10%) as that obtained with the electrode with a “used” surface. The results of square wave anodic stripping voltammetry for Hg(II) on screen printed carbon modified polyaniline nanoparticles in solution of 30 ppb Hg(II) before and after added 0.1 M EDTA to the electrode is shown in Figure 10. The results show that no current peak appear after adding 0.1 M EDTA, the reason for that is all the mercury bonded to polyaniline nanoparticles is removed from the surface of electrode.

The effect of interfering metal ions: The effects of various possible interferences were evaluated at the screen-printed carbon modified polyaniline nanoparticles electrode at pH 2.30, and the anodic peak height currents were compared with that of 40 ppb of Hg(II). When the concentrations of Pb²⁺, Zn²⁺, Cd²⁺ and As⁺³, were more than 10 times of Hg(II) (300 ppb), no significant interference could be observed from Figure 11. Then, this modified nano electrode can be used successfully for detection of Hg(II) in real samples in the presence of different interferences.

Application of this method to real environmental samples: The applicability of this method was evaluated by analysis of Hg(II) in real honey samples. Samples of natural honey (100 mL) were collected in clean polyethylene (PE) containers. The honey samples (6 samples) were collected from different sites near to Garbooly city near to the many industrial factories. The samples of honey were digested as Bozena et al reported previously [25]. The results were also validated with Mercury Analyzer analysis, very good correlation was obtained as show in Table 3.

Conclusions

We have developed a novel electrochemical nanosensor based on screen printed carbon electrode modified with polyaniline nanoparticles to determine mercury ions in environmental aqueous samples. The novel nanosensor possessed nice sensitivity, selectivity and reproducibility for sensing of trace mercury ion in water and food samples with nice recovery, showing its promising application in environmental pollution monitoring. The detection limit obtained in this work was 2.50 ± 0.03 ppb and was better than previously reported

for other electrodes. The electrochemical nanosensor was demonstrated by testing certified reference material with good recoveries. Potential applicability to food honey samples has been demonstrated, with accurate results obtained for natural honey samples.

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