Development of Electrochemical Sensor for Simultaneous Determination of Cd(II) and Hg(II) Ion by Exploiting Newly Synthesized Cyclic Dipeptide

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An electrochemical sensor for simultaneous detection of Cd(II) and Hg(II) were developed by using MWCNT/peptide modified Au electrode with cyclic voltammetry (CV). Peptides were synthesized by cyclooligomerisation of cysteine monomers in the presence of diphenylphosphoryl azide (DPPA). The synthesized peptides were attached to the Au electrode and the experimental conditions such as electrochemical characterization, supporting electrolyte, pH and scan rates were optimized. The developed electrochemical sensor was found sensitive toward simultaneous determination of Cd(II) and Hg(II). The modified Au electrode were reproducible with R.S.D value of 2.25% and had significantly superior analytical performance compared to the unmodified electrode. The slope value of $\log I_p$ versus $\log v$ is 0.61–0.85 demonstrate that the modified Au electrode undergoes diffusion-adsorption controlled process. The sensitivity expressed as the slope of linear region of calibration curve was 6×10^{-6} Appm^{-1} in borate buffer (pH 3) and 8×10^{-7} Appm^{-1} in acetate buffer (pH 2). The limit of detection (L.O.D) of Cd(II) is 2.749×10^{-8} M whereas the limit of detection of Hg(II) is 9.068×10^{-10} M. The developed electrochemical sensors were applied in real industrial waste water sample of electroplating industry and wood industry for simultaneous determination of Cd(II) and Hg(II). The results of the waste water sample analysis from the developed method showed a comparable result with the ICP-OES method.

1. INTRODUCTION

The synthesis of peptides and studies of their metal binding have recently been demonstrated as a fast moving and increasingly exciting field. Several cyclooligomerisation or self assembly reaction

have been studied and carried out under dilute concentrations (between 10–50 mM), which were found to be optimum in producing peptides with good yield [1]. Cyclooligomerisation when presented with appropriate reaction conditions can result in simple peptides to undergo spontaneous assembly to form linear or cyclic peptide [2]. Cysteine is a good precursor because ligands or complexing agents containing sulphur (S-H) bearing group, amino compound (-NH_2-), or carboxyl compound (-COOH-) can strongly form complexes species with a number of metal ions through coordinate covalent bond. Such complexes can be studied using classical voltammetric technique. Our goal was to explore further this cyclooligomerisation approach with amino acid cysteine, and then use it to develop the electrochemical sensor for metal detection. Cadmium and mercury are poisonous metals that cause short-term and long-term effect on humans and environment. The effect involves lung cancer, acrodynia, skin discoloration, stomach pain, nausea, vomiting, diarrhea, hypertension, hypersalivation, hypercardia and numbness. These effects make the monitoring of toxic metal extremely important.

The addition of nanoparticles together with the recognition element has proven to increase the sensitivity of the detection system developed [3]. The nanoparticles, especially carbon nanotubes have high electrical conductivity, high mechanical strength, chemical stability, large surface area [4], which lead to high sensitivity with good detection limit. The CNTs modified electrode has been found to perform much better than other carbon electrodes in terms of reaction rate, reversibility, and detection limit [5]. This paper discussed the characterization of the developed chemical sensor of MWCNT/peptide modified Au electrode for simultaneous detection of Cd(II) and Hg(II) using cyclic voltammetry (CV) technique.

2. EXPERIMENTAL

2.2 Materials

All chemicals used were analytical grade and used without further purification. Multiwalled carbon nanotubes (90%), L-cysteine (≥97%), 4-methylmorpholine (≥99.5%), diphenyl phosphoryl azide (≥97%), *N,N*-Dimethylformamide (≥99%), were purchased from Sigma-Aldrich (Sidney, Australia). Nafion (117 solution, 5%) was from Fluka (Switzerland) while mercury nitrate hydrate (≥95%), cadmium bromide tetrahydrate (98%) were from Merck (Germany). Boric acid, nitric acid, acetic acid, phosphate acid, citric acid, ethyl acetate, magnesium sulphate, sodium chloride were obtained from R&M Chemicals (United Kingdom).

2.3 Instrumentation

Cyclic voltammetry (CV) of each measurement was collected with µAutolab (Ecochemie). The analyzer was equipped with three-electrode cell and experiments were carried out at room temperature (25°C). Three-electrode cell involved platinum wire as a counter electrode, Ag/AgCl, 3 M KCl as reference electrode, and modified gold electrode as a working electrode. Prior to any measurements, the solutions were deoxygenated by bubbling N_2 gas purged through the solution for 15 min and was

allowed to flow freely throughout the whole experiment to remove the interfering oxygen gas during measurements.

2.4 Cyclooligomerisation of amino acid

4-methylmorpholine and DPPA were added to a suspension of amino acid cysteine in dry DMF at room temperature under a nitrogen atmosphere. The mixture was stirred at room temperature for three days. The DMF was evaporated to dryness in vacuo. The residue was diluted with ethyl acetate (60 ml) and the organic solutions were combined and washed successively with aqueous NaOH (2 M, 2×50 ml) and brine (2×40 ml). The solution was dried ($MgSO_4$) and the solvent was then removed under reduced pressure to leave mixtures of cyclic peptide products. The crude products are then purified by column chromatography giving a colourless solid. The product was elucidated as cyclic dimer [6].

2.5 Electrode preparation

Prior to the surface modification, the electrode is polished with 0.05 μm alumina slurry, then rinsed thoroughly with distilled water, and ultrasonically agitated. 2.0 mg of purified MWCNT and 2.0 mg of synthesized peptide mix with 0.5 ml Nafion is dispersed with the aid of ultrasonic agitation that results in black dispersion. A 20.0 μL of this black dispersion is cast on the Au electrode surface with a microsyringe and the casting is evaporated to dry at room temperature in the air overnight.


3. RESULT AND DISCUSSION

*3.1 Electrochemical characterization of the MWCNT/peptide modified*

Electrochemical characteristic of the MWCNT/peptide modified and unmodified gold electrode was studied and the results are shown in Figure 1 and Figure 2. The MWCNT/peptide modified Au electrode proven to have significant superior analytical performance compared to the unmodified electrode. The addition of nanoparticles MWCNT together with the recognition element (peptides) managed to increase the sensitivity of the detection system developed. Carbon nanotubes have unique electrical properties with high electrical conductivity which is useful for enhancement of peak current [7, 8]. Furthermore the polymeric character of peptides permits polydentate chelation which provides strong binding with the metal ion.

A study was carried out to evaluate the ability of the modified Au electrode in simultaneous detection of Cd(II) and Hg(II) ion. The CV of simultaneous detection of Cd(II) and Hg(II) ion is shown in Figure 3. Tan *et al.,* (2001), in his studies has reported the voltammetric behaviour of Cd(II) ion in the presence of L-cysteine at mercury electrode [9]. The detection was only for Cd(II) by using modified mercury electrode. Whereas Jian-Hui Jiang *et al.,* (2009), has developed a novel electrochemical sensor that is sensitive and selective towards Hg(II) ion [10]. Both researcher manage

to detect toxic metal Cd(II) and Hg(II) ion but in separate batch. We have manage to develop peptide modified electrode that can differentiate between Hg(II) and Cd(II) that exist in the same solution. The values of peak separation, ΔE_p of Hg(II) using the separate batch is 0.618 whereas the ΔE_p of Hg(II) in simultaneous detection is 0.599. For Cd(II) the ΔE_p using the separate batch 1.25 compared to the ΔE_p peak separation in simultaneous detection which is 1.19.
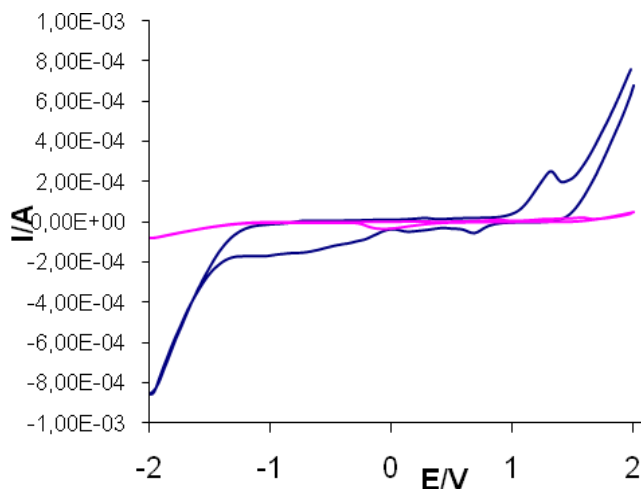
Figure 1. Cyclic voltammetric renspose of MWCNT/peptide modified (---) and unmodified (---) Au electrode in 10 ppm Hg(II) solution
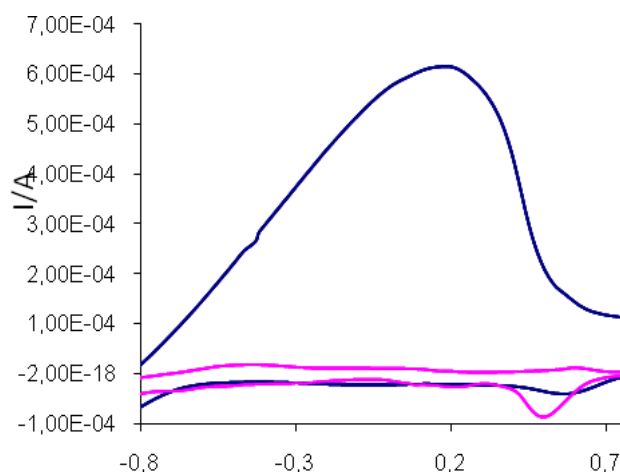
Figure 2. Cyclic voltammetric renspose of MWCNT/peptide modified (---) and unmodified (---) Au electrode in 10 ppm Cd(II) solution.

In comparison the ΔE_p for both Hg(II) and Cd(II) in simultaneous detection are much lower compared to the ΔE_p of Hg(II) and Cd(II) in separate batch detection. The lower ΔE_p indicate that the redox peak for both Cd(II) and Hg(II) are more reversible.
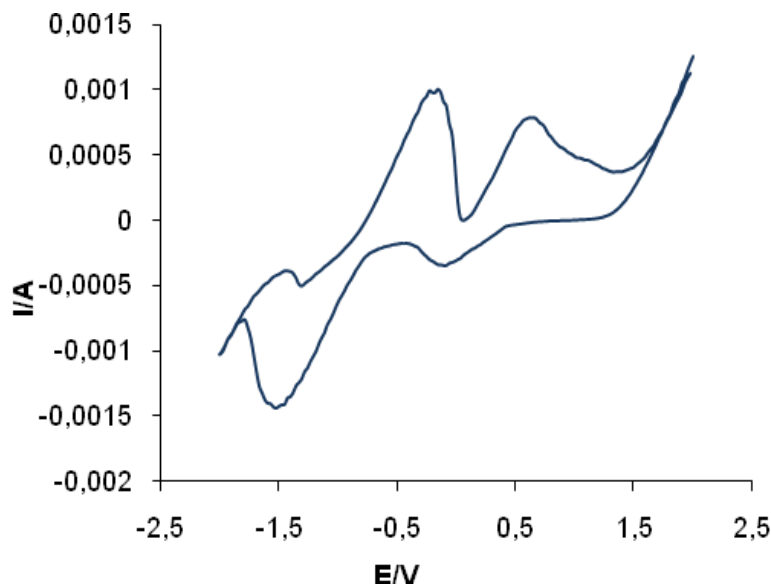
Figure 3. Cyclic voltammogram for simultaneous detection of Cd(II) and Hg(II) ion using MWCNT/peptide modified Au electrode immersed in in 100 ppm Cd(II) and Hg(II).

3.2 Effect of different type of supporting electrolyte

The effects of different type of supporting electrolyte on the MWCNT/peptide modified Au electrode are investigated. The types of supporting electrolyte used in electrochemical measurement may influence the working potential range, capacitive current and faraday current of the system [11]. For Cd(II), the peak current is enhance when borate buffer ($NaH_2BO_{3)}$ is used as the supporting electrolyte. Whereas for Hg(II), the peak is enhance when acetate buffer (CH_3COONa) is used as supporting electrolyte. The different in enhancement effect with regards to different type of supporting electrolyte will increase the selectivity of the developed detection system.
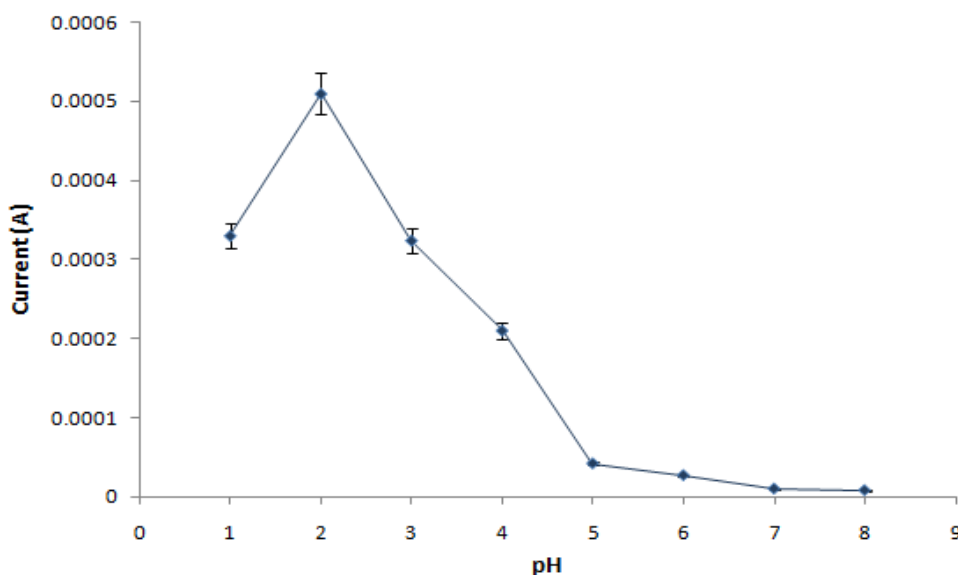
3.3 Effect of varying pH

The effect of different pH are carried out in order to assess the optimum condition under which Cd(II) and Hg(II) ion oxidation peak were enhanced. The enhancement of the cathodic peak of Cd(II) and Hg(II) ions by the complexing agent (peptides) is largely dependent on the pH of the solution. Since most of the complexing agents are weak acids or bases, control of pH is very important. In such cases the pH of the solution will control the stability of the complex and in turn affect the resultant current and the peak potential.

The optimum pH conditions for both metal ions were in acidic range (Figure 4 and 5). In the presence of borate buffer the optimum pH is at pH 3 whereas in the presence of acetate buffer the optimum pH is at pH 2. This is beneficial since selective detection can be achieved in acidic media as most metal detections were optimized in basic medium [12].
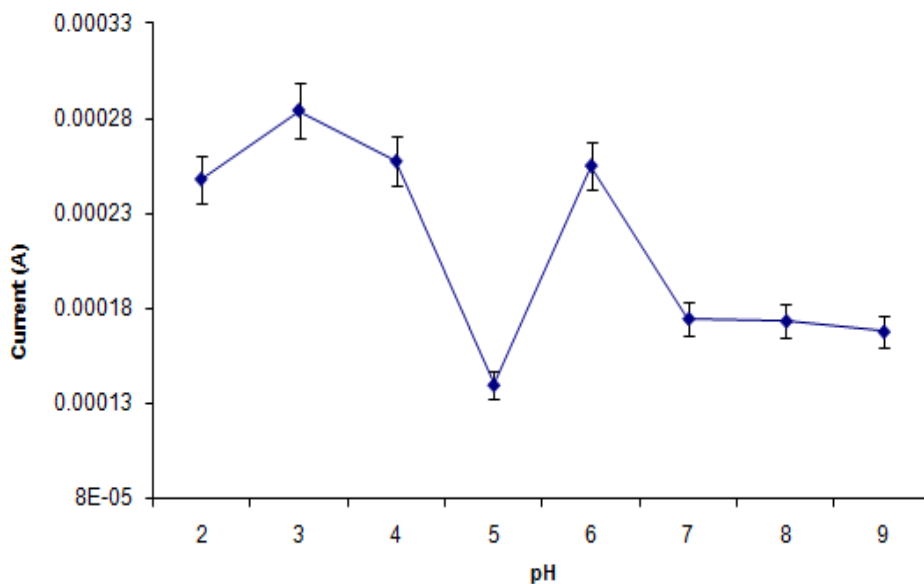
Figure 4. Effect of pH for Cd(II) detection using MWCNT/peptides modified Au electrode in 100 ppm Cd(II) and Hg(II) with 0.1M borate buffer at scan rate 100 mVs^{-1}.


Figure 5. Effect of pH for Hg(II) detection using MWCNT/peptides modified Au electrode in 100ppm Cd(II) and Hg(II) with 0.1 M acetate buffer a scan rate 100mVs^{-1}.

3.4 Effect of varying scan rates

Voltammetric response on the effect of varying scan rates ranging from 10 until 1000 mVs^{-1} was studied. A plot of log I_p (peak current) versus log υ (scan rate) with experimental slope of 0.61–0.85 for Cd(II) and 0.81–0.85 for Hg(II) as shown in Figure 6 and 7 fit the theory of diffusion-adsorption-controlled process [13].
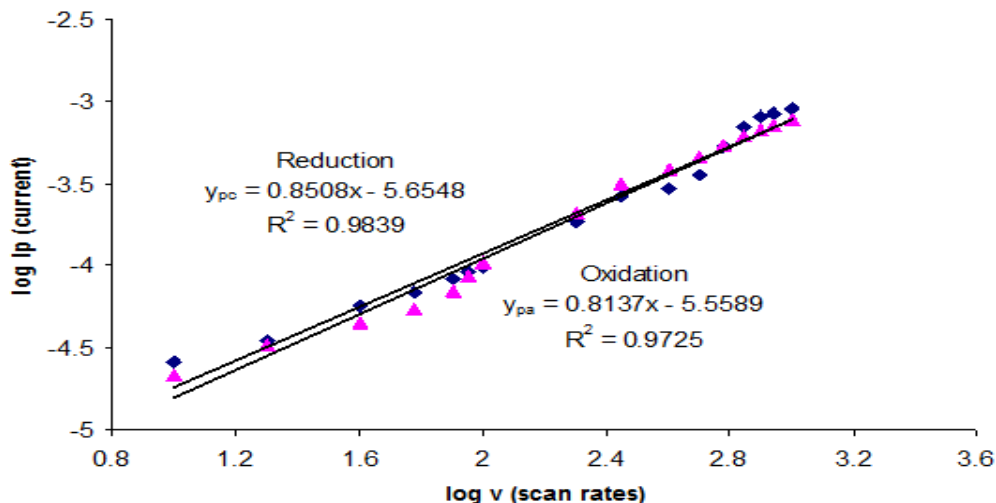
Figure 6. The slope of peak current log I_{pa}/log I_{pc} versus log v (scan rates) for Hg(II) detection using modified MWCNT/peptides nanohybrid modified Au electrode immersed in 100 ppm Cd(II) and Hg(II) + 0.1 M acetate buffer at pH 2 at various scan rate.
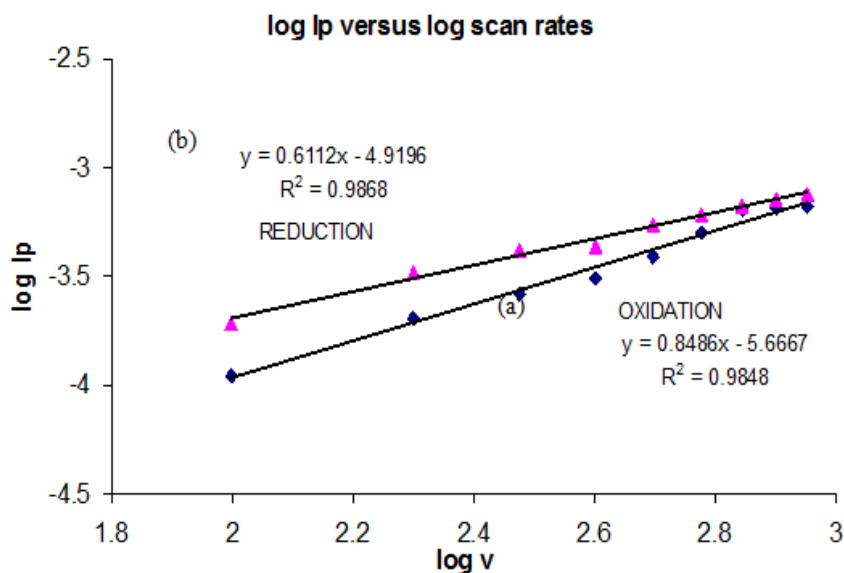
Figure 7. The slope of peak current log I_{pa}/log I_{pc} versus log *v* (scan rates) for Cd(II) detection using MWCNT/peptides nanohybrid modified Au electrode immersed in 100 ppm Cd(II) and Hg(II) + 0.1M borate buffer at pH 3 at various scan rate.

3.5 Reproducibility study

Reproducibility refers to the successive runs made by using the develop method to evaluate the discrepancy in its responses. The reproducibility was evaluated by using 5 similarly constructed MWCNT/peptides modified Au electrodes in 5 similarly prepared analytes solutions. The variation in current is due to constructional variation which could arise from different thickness of the immobilized

peptide on the surface of the electrode. The relative standard deviation (R.S.D) for reproducibility of the developed method was calculated to be 2.25 %. Low R.S.D value indicates that the precision of the develop method is good.

3.6 Calibration Curve

The effect of different concentration of Cd(II) at constant Hg(II) concentration or vice versa are investigated in order to obtained the calibration plot.
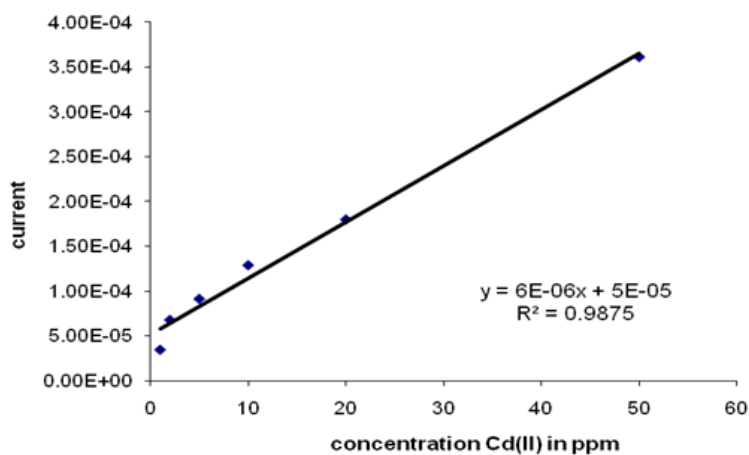
Figure 8. Calibration curve plot of Cd(II) in 0.1 ppm Hg(II) (0.1 M borate buffer at pH 3) using MWCNT/peptide modified Au electrode
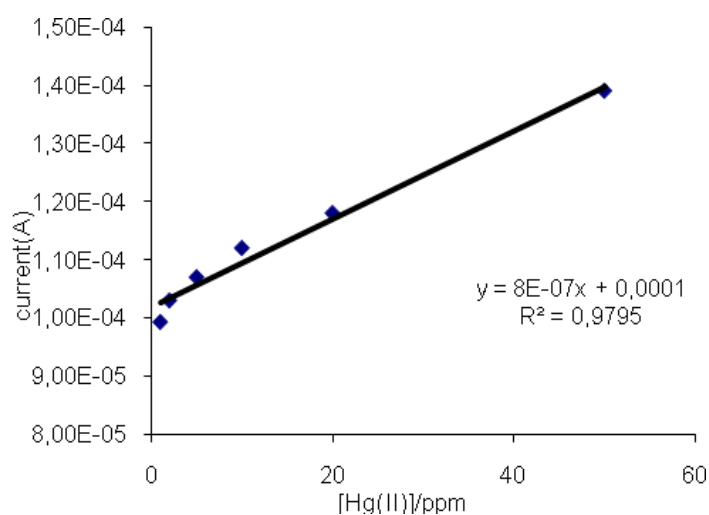
Figure 9. Calibration curve plot of Hg(II) in 0.1ppm Cd(II) (0.1M acetate buffer at pH 2) using MWCNT/peptide modified Au electrode

The response of different Cd(II) concentration at constant 0.1 ppm Hg(II) concentration was carried out in 0.1 M borate buffer at pH 3 whereas the response of different Hg(II) concentration at constant 0.1 ppm Cd(II) concentration was carried out in 0.1 M acetate buffer at pH 2. Linear response was obtained when the concentration of both metal ions are within the range of 0.1 ppm to 50.5 ppm as shown in Figure 8 and 9. The calculated LOD for detection of Cd(II) and Hg(II) is 2.749×10^{-8} M and 9.068×10^{-10} M respectively. These values are below the EPA guideline level for Cd(II) and Hg(II) in drinking water and much lower compared to other developed sensors, [14–19]. The results obtained demonstrated that the developed MWCNT/peptide modified gold electrode are capable in simultaneous detection of Cd(II) and Hg(II) at low concentration level.

3.7 Analytical Application of the Developed Modified Electrode

The applicability of modified Au electrode for detection of cadmium and mercury was validated using wastewater sample from wood treatment and electroplating industry by studying the recoveries of spiked wastewater samples. The recoveries of Cd(II) and Hg(II) from the wood treatment industry and electroplating industry are shown in Table 1 and 2. The developed method was also validated against ICP-OES and the results obtained from the developed method were observed to be comparable with the ICP-OES method. These results proved that the developed method is reliable and has a potential to be developed as sensor for simultaneous detection of Cd(II) and Hg(II) in environmental monitoring.

Table 1. Cd(II) analysis for (a) wood treatment industry (b) electroplating treatment industry using the developed method and ICP-OES

Standard Spike (ppm)	Develop Method (ppm)	ICP-OES (ppm)	Recovery %		Mean recovery%		RSD%	
			CV	ICP-OES	CV	ICP-OES	CV	ICP-OES
50	51.83	51.66	101.66	100.94				
	52.41	51.62	102.48	100.89	101.85	100.9	0.6176	0.5253
	51.88	52.11	101.43	101.87				
(a)								

Standard Spike (ppm)	Develop Method (ppm)	ICP-OES (ppm)	Recovery %		Mean recovery%		RSD%	
			CV	ICP-OES	CV	ICP-OES	CV	ICP-OES
50	41.33	38.53	80.55	74.95				
	43.33	38.58	84.55	75.05	79.88	75.5	6.138	1.125
	38.33	39.31	74.55	76.51				
(b)								

Table 2. Hg(II) analysis for (a) wood treatment industry and (b) electroplating industry using developed method and ICP-OES.

Standard Spike (ppm)	Develop Method (ppm)	ICP-OES (ppm)	Recovery %		Mean recovery%		RSD%	
			CV	ICP-OES	CV	ICP	CV	ICP-OES
50	47.51	43.81	92.43	85.03				
	48.13	43.67	93.67	84.75	93.2	84.7	0.7088	0.4036
	48.06	43.46	93.53	84.33				
(a)								

Standard Spike (ppm)	Develop Method (ppm)	ICP-OES (ppm)	Recovery %		Mean recovery%		RSD%	
			CV	ICP-OES	CV	ICP	CV	ICP-OES
50	45.31	38.72	88.52	75.34				
	45.77	38.95	89.44	75.8	87.42	75.47	3.0488	0.3667
	43.21	38.69	84.32	75.28				
(b)								

4. CONCLUSION

The results demonstrated by the use of MWCNT/peptide nanohybrid modified Au electrode, was observed to be sensitive and stable in simultaneous determination of Cd(II) and Hg(II). Nafion was used to serve as peptide binder to the electrode and to solubilize the MWCNT. Through this procedure, the electrocatalytic activity of MWCNT/peptide modified Au electrode has been optimized in simultaneous determination of Cd(II) and Hg(II) and the performance of the modified Au electrode improved greatly compared to the unmodified electrode. The developed method was applied in spiked industrial wastewater sample of electroplating and wood industry for simultaneous determination of toxic metal Cd(II) and Hg(II) and the result obtained are comparable with the ICP-OES method.

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