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Electrochemical Sensor for As(III) Utilizing CNTs/ Leucine/Nafion Modified Electrode

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The development of practical sensor for the detection and quantification of metal ions in environmental samples is the subject of considerable research. A CNTs/Leucine/Nafion modified Pt electrode was proposed to determine heavy metal at trace levels by using cyclic voltammetry. Maximum current was obtained at potential range of 0.36-0.75 V with an optimum response occurs at pH 5 (R.S.D. = 7.6%). The electrocatalytic redox peak current of As(III) showed a linear response towards different concentration of As(III) and linear calibration curves were obtained in the range of 0.005μM to 2 μM. The limit of detection was determined as 1.67 nM and the sensitivity response is 20.6 μA/μM. The developed method has been validated against ICP-OES. Interferences studies of several metal ions were carried out and it was observed that Fe(II), Zn(II) and NH_3 ion group gave the highest interference.

Keywords: Amino acids, sensor, cyclic voltammetry, nanotransducer

1. INTRODUCTION

Arsenic is a common compound found in drinking water, especially in some Asian countries. The toxicity of arsenic is greatly depends on its oxidation state since As(III) is 50 times more toxic than arsenate due to its reactions with enzymes in human metabolism. The World Health Organization's arsenic guideline value for drinking water is 10 ppb [1]. Exposure to arsenic can cause a variety of adverse health effects, including dermal changes, respiratory, cardiovascular, gastrointestinal, genotoxic, mutagenic and carcinogenic effects [2]. Arsenic does not generally impart colour, taste or smell to water; therefore, testing for arsenic by water sampling is the only way to determine its presence.

Many detection methods have been developed for determination of such levels of arsenic, as summarized in recent reviews [3-7]. Electrochemical methods provide accurate measurements of low concentrations of metal ions at ppb levels with rapid analysis times and low cost instrumentation.

Since the discovery of carbon nanotubes (CNTs) by Iijima in 1991 using transmission electron microscopy [8], CNTs have been the subject of numerous investigations in chemical, physical and material areas due to their novel structural, mechanical, electronic and chemical properties [9-11]. The subtle electronic properties confirmed that CNTs have the ability to promote electron-transfer reactions when used as an electrode in chemical reactions [12-14]. There have been many researches that prove the ability of CNT to promote electron transfer reactions when it was used to modify electrodes for oxidation of biomolecules including dopamine [15], protein [16], [17] and NADH [18]. Excellent electrocatalytic activities were observed from carbon nanotubes (CNTs) modified electrodes toward hydrogen peroxide and NADH due to fast electron transfer ability of CNTs [19], [20]. The CNTs modified electrode has been found to perform much better than other carbon electrodes in terms of reaction rate, reversibility and detection limit [21].

The application of electrochemistry in the investigation of metal-peptide binding is for the detection of amino acids and peptides where the metal ion is used as an electroactive label. The studies from previous researches in which the electrochemistry of different metal ions are used to detect many types of amino acids and peptides, implies that amino acid and peptides could be used as selective ligands for the detection of metal ions. Histidine has been detected in the presence of Cu(II) using differential-pulse adsorptive stripping voltammetry at a hanging mercury drop electrode [22]. Similarly Gly-His-Gly has been detected down to 10 nM in the presence of Cu(II) using cathodic stripping voltammetry with adsorptive accumulation [23]. An interesting development in using metal ions in the analysis of peptides is in the detection of toxic and tumour promoting peptides such as microcystins [24]. The complexation of metal ions by even simple amino acids has produced remarkably low detection limits. Tanaka and Yoshida [25] have reported detection limits of 0.6 nM Cu^{2+} with relative standard deviations at 10 nM of 4% for the anodic stripping of copper at a mercury electrode in the presence of cysteine. However, several analytical studies by mass spectroscopy have revealed that arsenate and arsenite do not complex readily with glutathione or cysteine A [26].

Leucine have good ability to coordinate to metal ions because it contains $–NH_3^+$ and –COOH groups which often involve in forming hydrogen bond networks [27]. There are several ways for metal ion to bind with peptide which are depending on the amino acid residues, pH and binding stoichiometry [28]. Several species coexist in solution and the involvement of oxygen from carboxylate groups have been demonstrated where the coordination often completed with oxygen atoms from water molecules [28]. A report from [29] mention a several study of electrochemical behaviours of some amino acids and transition metal ions studied on Pt electrode. Reference [30] studied the effect of several heavy metal compounds on the membrane transport of essential nutrients on the intestinal absorption of the essential amino acid, L-leucine, in the toadfish.

In this study, a modified CNTs/Leucine/Nafion platinum (Pt) electrode is proposed to determine As(III) at trace levels by means of the voltammetry method. The ratio of CNTs/Leucine/Nafion is optimized and identified to be used in the modification of platinum electrode. It was found that the modified CNTs/Leucine/Nafion electrode is comparable in the detection of

As(III) when validated against ICP-OES in a real sample application. The detailed characterizations for developing the electrochemical sensor for As(III) are also discussed.

2. EXPERIMENTAL

2.1 Apparatus

Cyclic voltammetry experiments were performed with μAutolab (Type III) (Microchemie) interfaced to a PC using GPES (version 4.9) software for windows. The experiments were carried out in a three-electrode cell at room temperature (25 °C). The three-electrode system consists of platinum as working electrode, an Ag/AgCl reference electrode (3 M KCl) and platinum auxiliary electrode. Prior to any measurements, the solutions were deoxygenated by bubbling N_2 gas through the solution for 15 min and N_2 gas was allowed to flow freely over the surface of the solution during measurements. The signal of the accumulated modified and unmodified Pt electrode were measured using cyclic voltammetry (CV) by scanning the potential from −1.0 to +1.2V with amplitude of 100mV/s performed in each solution. The pH was adjusted using a Toledo pH meter. The TEM images were obtained on a transmission electron microscope (TEM, JEOL JEM-2010F).

Prior to the surface modification, the platinum electrode was polished on a polishing cloth with wet alumina powders, starting with 0.3 μm particle size and then with finer grades down to 0.05 μm. It was then rinsed with deionised water and sonicated for 2 min.

2.2 Reagents

All chemicals used were of analytical reagent grade. Aqueous solutions were prepared with deionised water. A 1 mM As(III) stock solution was prepared from As_2O_3 Merck (Germany). Multiwall carbon nanotubes, MWNTs (90%) and potassium phosphate monobasic (SigmaUltra, 99.0%) were purchased from Sigma-Aldrich (Sydney, Australia). L-leucine (98.5-100%) was from Fisher (Japan) while Nafion (117 solution, ~5%) from Fluka (Switzerland). Sodium citrate and sodium hydroxide were purchased from Merck (Germany). Nitric acid, boric acid, acetic acid, sodium chloride and potassium chloride were obtained from R&M Chemicals (United Kingdom).

Wastewater samples were adjusted to pH 5 with concentrated nitric acid and filtered through membrane filters of 0.45 μm pore size. The sample tested was diluted 10 times with citrate buffer pH 5. All experiments were carried out at room temperature (25 ± 1 °C). Each individual experiment was performed at least three times, and then the results were averaged.

2.3 Preparation of Modified Electrode

Prior to the surface modification, the platinum electrode is polished with 0.3 and 0.05 μm alumina slurry on polishing cloth, then rinsed thoroughly with distilled water and ultrasonically agitated in order to remove adsorbed particles. CNTs/Leucine/Nafion film was casted on the surface of platinum electrode. Films formed from Nafion-solubilised MWNTs are more uniform and stable than those cast from organic solvents. Nafion assists the dispersion of MWNTs, whereby the MWNTs remain well dispersed on prolonged standing [16]. Modified electrode was eventually washed with double distilled water and stored at ambient temperature (25 °C) before being used in experiments.

3. RESULTS AND DISCUSSION

3.1 Electrochemical Characterization of the CNTs/Leucine/Nafion Modified Pt Electrode

The bare platinum electrode was immersed in a cell containing 15 ml of 1 mM As(III) with citrate buffer solution pH 5. In Fig. 1, the cyclic voltammetry pattern for CNTs/Leucine/Nafion and CNTs/Nafion modified platinum electrode were compared with an unmodified Pt electrode in the presence of 0.15 M citrate buffer solution (pH 5.0) at scan rate of 100 mVs^{-1}. The ratio for CNTs and leucine were studied (not shown) where ratio 1 to 4 is the best mixture. In Fig. 1a, small peaks at 0.2 V suggested a reduction of the oxide layer. A peak at –0.357 V and -0.022 correspond most likely to the reduction of As(III) to As(0) and oxidation of As(0) to As(III) whereas a peak at 0.697 V suggest the formation of leucine-OH corresponds to the oxidation of As(III) to As(V) [31].

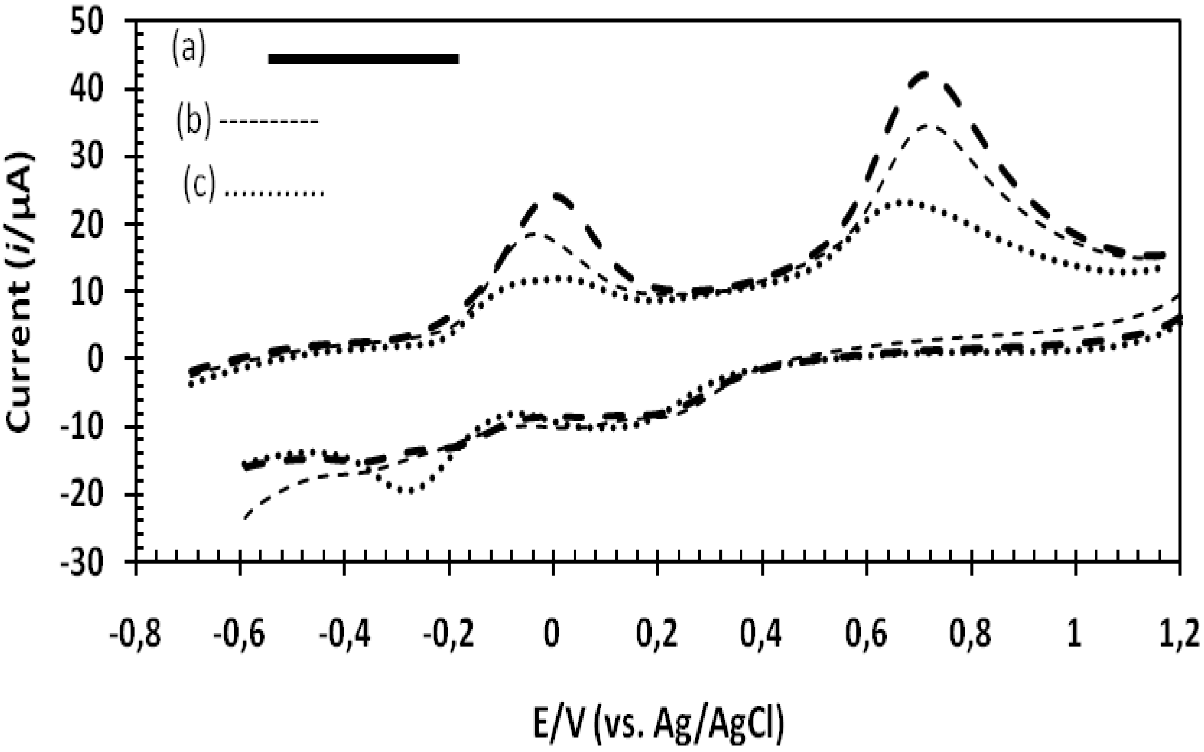


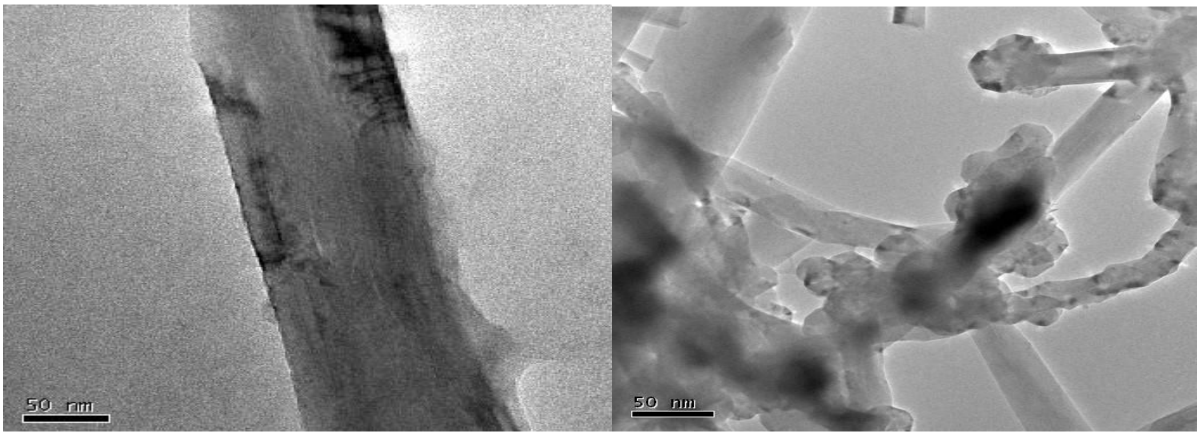
Figure 1. Cyclic voltammograms of 1 mM As(III) solution using a) CNTs/Leucine/Nafion nanoparticles modified Pt electrode b) CNTs/Nafion nanoparticles modified Pt electrode and c) bare Pt electrode in the presence of 0.15 M citrate buffer solution (pH 5) and at a scan rate of 100 mV s−1 .

The reduction peak of the surface oxide disappeared in the cathodic scan. The suggested mechanism of the electrochemical oxidation of As(III) as in (1):

$$H_3AsO_3 + H_2O \rightarrow H_3AsO_4 + 2H^+ + 2e^- \qquad (1)$$

The increase peak current of As(III) oxidation suggest that CNTs/Leucine/Nafion nanohybrids has high electrocatalytic ability for arsenic oxidation. Therefore, CNTs/Leucine/Nafion nanohybrids are suitable as mediator to shuttle electron between arsenic and working electrode and facilitate electrochemical regeneration following electron exchange with arsenic.

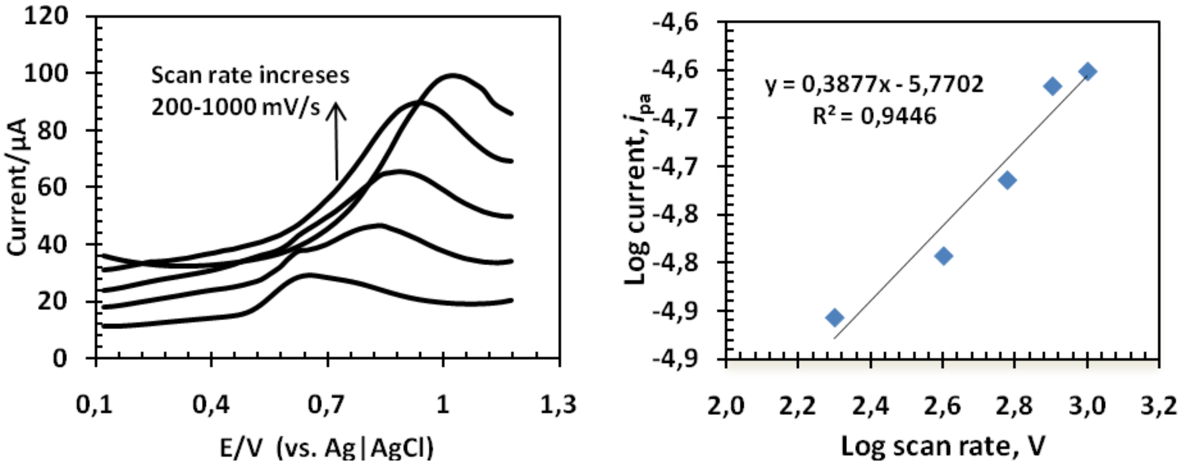
The prepared electrodes were characterized by TEM. In Fig. 2(a), the needle thread line of nanotubes was crossing to each other and more uniform in size before modification with amino acid whereas in Fig. 2(b), we can see that the attachment of leucine onto individual carbon nanotubes was successful. The attachment of leucine on Nafion-CNTs film indicates that the CNTs sidewalls are not entirely masked by the Nafion. It has been suggested that the solubilisation action of Nafion is very similar to the other polymeric materials bearing polar side chains, which ‘wrap’ around the CNTs, and the electrocatalytic properties of CNTs are not impaired by Nafion under optimal conditions [16]. Therefore, dispersing CNTs in 1% Nafion ensures that the amount of CNTs is controllably casted onto platinum electrode and the CNTs still retains its electrocatalytic carbon surface.



(a) (b)

Figure 2. TEM micrograph of MWCNTs (a) before and (b) after attachment of the leucine molecules.

The effect of varying the scan rate on the oxidation current of As(III) in 0.15 M citrate buffer solution (pH 5) is shown in Fig. 3(a). The increase of oxidation current with the increase of scan rate could be explained by the fact that there is less time available for the Nernst diffusion layer to relax into the solution by diffusion [39]. Thus, as the scan rate increase, the rate of change of the concentration of arsenic at the electrode surface increase. The oxidation potential/anodic peak is shifted positively as the scan rate increased because the reaction rate is much faster than the rate of resolution of product to bulk solution. The flux to the electrode surface is considerably smaller at slow scan rates than it is at faster rates [32]. A graph of log i_{pa} versus log scan rate is plotted as illustrated in Figure 3(b). The slope value is 0.39, which is below 0.50 (for surface-complex process) [33].



(a) (b)

Figure 3. (a) Effect of varying scan rate on the cyclic voltammograms of 1 mM As(III) solution, 0.15 M citrate buffer (pH 5) using CNTs/Leucine/Nafion nanoparticles modified Pt electrode (b) plot of log oxidation current of arsenic versus log scan rate.

3.2 pH Study

The pH value of the supporting electrolyte has a significant influence on the current and potential of the oxidation peak.

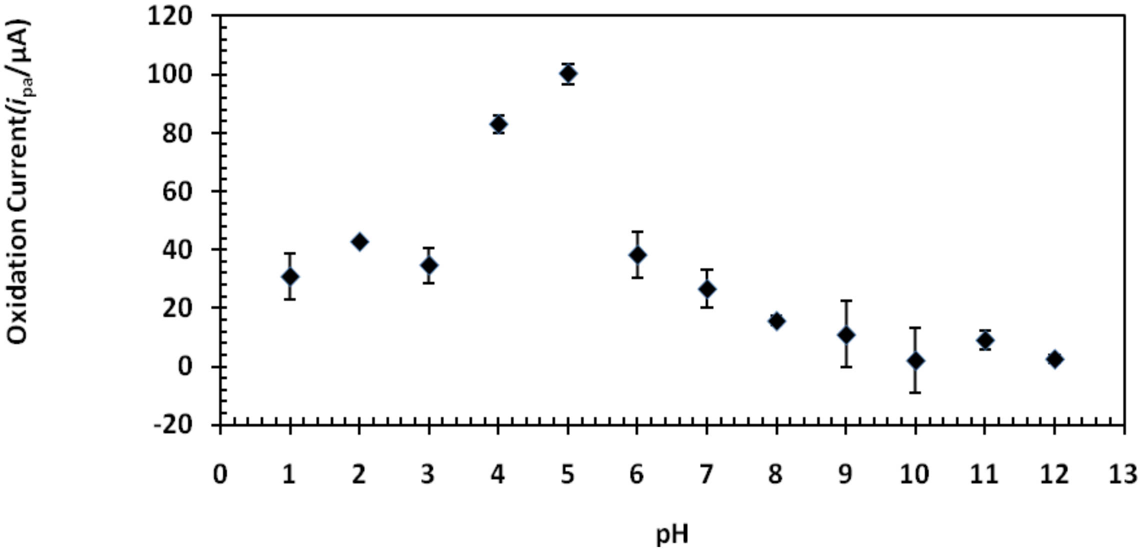


Figure 4. Oxidative current at room temperature of 1 mM As(III) in 0.15 M citrate buffer as a function of pH using a (40 µL dispersed CNTs/Leucine/Nafion) modified Pt electrode with start potential 1250 mV at scan rate 100 mV s-1.

The effect of pH on the electrochemical behaviour of the modified electrode was investigated. In Fig. 4, the CVs of the modified Pt electrode were recorded in 0.15 M citrate buffer solutions at pH ranging from 1 to 12. The pH was adjusted using 0.1 M HNO_3 and 0.2 M NaOH. The peak current decreased with increasing pH until pH 5 and no significant signal was detected after pH 12, it is appeared to be less prominent when the solutions become more alkaline. At all pH, the formal potential of the surface redox couple, E°, remains unchanged. The most stable and sensitive signals was observed at pH 5 and this value was chosen as optimum pH in this study.

A simple amino acid exists in neutral aqueous solution as a dipolar ion (Zwitterions). The net charge of an amino acid can be altered using the isoelectric point, pI. Isoelectric point for leucine is 5.95 and at a pH lower than the pI value, leucine will carry a net positive charge whereas at pH above their pI, leucine will carry a net negative charge [34]. The highest peak current of As(III) oxidation was observed at pH 5 and it can be inferred from the observation that the modification of platinum electrode with CNTs/Leucine/Nafion nanohybrids has high electrocatalytic ability when leucine is in net positive charge.

An acceptable reproducibility with 7.6% relative standard deviation (R.S.D) was obtained for determination of 1 mM As(III). By resurfacing the electrode once after each run, the voltammogram obtained are about the same for five trials as shown in Table 1.

Table 1. Reproducibility and repeatability study of the determination of As(III) using CNTs/Leucine/Nafion modified Pt electrode in the presence of 0.15 M citrate buffer solution (pH 5) at scan rate 100 mVs^{-1} .

1. **Repeatability**		2. **Reproducibility**	
3. Number of replicates	4. Current, $\times 10^{-5}$ A	5. Number of replicates	6. Current, $\times 10^{-5}$ A
7. 1	8. 2.85	9. 1	10. 2.92
11. 2	12. 2.25	13. 2	14. 2.99
15. 3	16. 2.63	17. 3	18. 2.84
19. 4	20. 2.76	21. 4	22. 2.58
23. 5	24. 2.46	25. 5	26. 2.50

Data obtained from repeatability study of the modified electrode is also summarized in Table I. The repetitive experiments with the same modified electrode lead to current decay with five replicates because the process has not achieved the steady state in the beginning of the reaction. Therefore, a freshly prepared electrode needs to be employed after four replicates on each analytical application.

3.3 Interferences Study

Using the developed modified electrode, the effect of various cations and anions for determination of 0.1 mM of As(III) was studied in the presence of 0.15 M citrate buffer solution (pH 5) at scan rate 100 mV s^{-1}. Ten foreign ions containing cations and anions were examined for effects on the determination of 1 mM of As(III) at 1:1 mole ratio of As(III) : foreign ion. Comparisons of anodic peak currents were evaluated. Zn^{2+} and Fe^{2+} could be tolerated up to at least 0.05 mM whereas commonly encountered matrix components such as Cd^{2+}, Co^{2+}, Mg^{2+}, Ni^{2+} and Cu^{+} did not show high percentage of interference. The anions investigated, did not possess high interference on detection of As(III), except for NH_3. Thus, it could be concluded that the proposed method tolerates the presence of ions at concentration to the level of at least 0.05 mM. The peak potential for arsenic detection at modified electrode is at less positive potential, which is great advantage since the number of interferences in electrochemical detection increases exponentially with increasing of potentials.

3.4 Analytical Application of the Developed Modified Electrode

The effect of varying As(III) ion concentration at a constant buffer condition is illustrated in Figure 5.

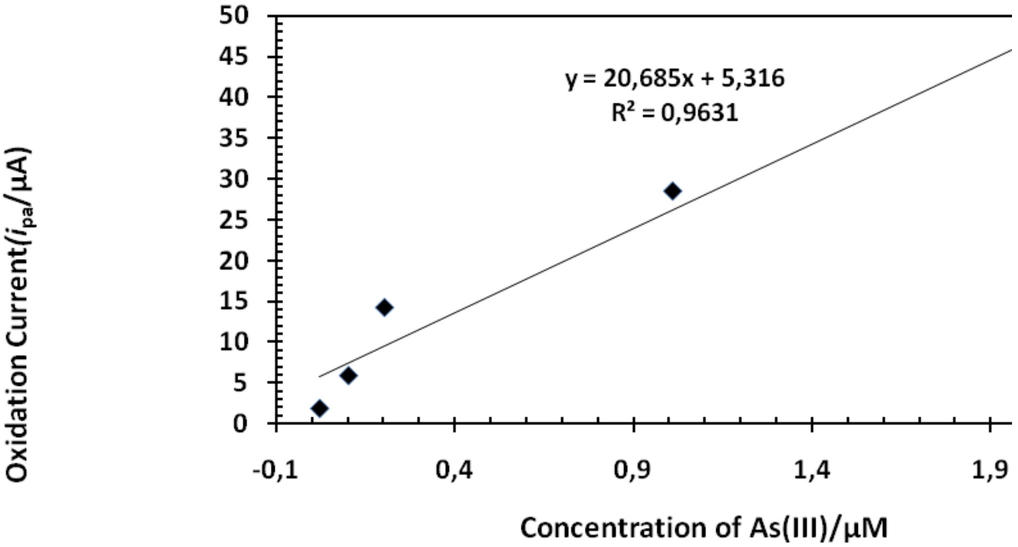


Figure 5. Calibration curve of anodic As(III) solution using CNTs/Leucine/Nafion nanoparticles modified Pt electrode in the presence of 0.15 M citrate buffer solution (pH 5) at scan rate 100 mVs^{-1}.

It was observed that the linearity existed below 2μM for the plot obtained with the differential pulse cathodic stripping voltammetry (DPCSV) technique on the modified electrode in the presence of different concentrations of arsenic. The anodic peak current increased with an increasing level of the As(III) concentration. The plot of i_{pa} vs. As(III) ion concentration was observed for the concentration range of 0.02 μM to 2 μM. The sensitivity, expressed as the slope of the linear region of the calibration curve, was observed as 20.7 μA/μM. The limit of detection (LOD) was calculated as the concentration of As(III) and the total arsenic, which was equal to three times the standard deviation of the background signal (3σ). The value was determined as 1.67 nM. This value was low in comparison to the LOD value found by Sim [35] which was $6.3x10^{-7}$ M for As(III) for a silver chloride using anodic stripping voltammetry (ASV). This value was almost same as the value found by Salimmi [36]. Here, the detection limit (S/N = 3) was 11 nM with linearity of up to 4 orders of magnitude and sensitivity of 111.3 nA/µM. This was achieved using a cobalt oxide nanoparticles based sensor for the detection of a trace amount of As^{3+} ion in an aqueous solution [36]. The low concentration regions corresponded to a linear adsorption process. Thus, it can be concluded that the surface concentration of the adsorbed species is directly proportional to the bulk concentration before the modified electrode attains full coverage. However, there is still sufficient space on the modified electrode surface for additional adsorbate in this region. For a higher concentration, the capacity of the electrode surface to accept additional adsorbing species limits an increase in adsorption. The LOD we report herein is well within the range defined by the WHO regulations [1].

To validate the accuracy of the method, we choose to study recoveries of As(III) spiked from wastewater sample. The applicability of modified electrode for detection of arsenic was tested on real wastewater sample from Wood Treatment Industry and Electroplating Industry. The recovery of As(III) from the mixtures prepared by spiking different amount of As(III) is summarized in Table II. The developed method was validated against Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES) method. Good agreement was obtained between the proposed method and ICP-OES based on the analysis of the waste water samples as shown in Table 2.

Table 2. Percentage recovery of As(III) from wastewater samples from Electroplating and Wood Treatment Industry.

27. **Waste water Samples**	28. **Electroplating Industry**	29. **Wood Treatment Industry**
30. Originally present (ppm)	31. 0.2431	32. 55.99
33. Added (ppm)	34. 10	35. 10
36. Obtain from ICP-OES (ppm)	37. 13.66±0.01	38. 63.62±0.01
39. R.S.D.	40. 0.1%	41. 1.50%
42. Recovery 43. (%)	44. 133±0.01	45. 96±0.01
46. Obtain from developed method (ppm)	47. 11.08±2	48. 57.65±4
49. R.S.D.	50. 1.8%	51. 1.70%
52. Recovery 53. (%)	54. 108±2	55. 87±3

4. CONCLUSIONS

A new method for detecting As(III) ion solution using the CNTs/Leucine/Nafion nanohybrids modified Pt electrode was developed. Nafion served as the electrode binder and was also used to solubilise the MWCNTs. The electrocatalytic activity of the CNTs/Leucine/Nafion modified Pt electrode was optimized for determination of As(III) solution. It was observed that the performance of the resulting modified electrode improved greatly in comparison to the unmodified electrode. Further, it was found to be free of any interference at pH 5 using a citrate buffer. An acceptable reproducibility with 7.6% relative standard deviation (R.S.D) was obtained for the determination of 1 mM As(III) which was linear at As(III) concentration of 0.02µM to 2 µM with a high sensitivity response of 20.6 µA/µM. The limit of detection was determined as 1.67 nM. The proposed method produced good results for the analysis of the inorganic arsenic species in waste water.

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