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The Application of Nafion Metal Catalyst Free Carbon Nanotube Modified Gold Electrode: Voltammetric Zinc Detection in Serum

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Abstract

Metal catalyst free carbon nanotube (MFCNT) whiskers were first used as an electrode modification material on a gold electrode surface for zinc voltammetric measurements. A composite film of Nafion and MFCNT whiskers was applied to a gold electrode surface to form a mechanically stable sensor. The sensor was then used for zinc detection in both acetate buffer solution and extracted bovine serum solution. A limit of detection of 53 nM was achieved for a 120 s deposition time. The zinc in bovine serum was extracted via a double extraction procedure using dithizone in chloroform as a zinc chelating ligand. The modified electrode was found to be both reliable and sensitive for zinc measurements in both matrices.

Keywords

Metal catalyst free carbon nanotubes; Nafion; Bovine serum; Dithizone; Anodic stripping voltammetry; Zinc

1 Introduction

Zinc is an essential trace element that is required in the human body for catalyzing enzyme activity, transcription and protein functions [1]. Maintaining controlled levels of zinc is critical for multiple physiological functions, including the immune system, insulin action, and anti-oxidant systems [2,3]. Lack of zinc can cause immune dysfunction, growth and reproduction problems; also, inflammation and infection are associated with low zinc level in blood [2]. It has been widely accepted that normal zinc level is necessary to maintain a healthy immune system [4,5]. As there are health concerns associated with zinc deficiencies, it is often necessary to analyze such complex biological matrices as blood, serum, tissue, and urine. A variety of methods has been reported in the literature, including inductively coupled plasma – mass spectrometry (ICP-MS) and other spectroscopic and electrochemical methods [6–8]. Of these, electrochemical methods have advantages associated with high sensitivity, low detection volumes, compact instrumentation, and low cost [9,10].

Carbon nanotubes (CNTs) are an increasingly important group of nanomaterials possessing chemical, physical, and mechanical properties that make them well suited for electrochemical sensing applications [11,12]. Nanotubes have high electrical conductivity, are relatively inert chemically, can be used over a wide potential range, and have a very high microscopic surface area due to their surface structure [13,14]. CNTs can be prepared in a variety of ways, including arc discharge, laser ablation and chemical vapor deposition [15,16]. A hindrance shared by these methods is the general use of metal catalysts in their preparation that can contaminate the CNTs and reduce their effectiveness for stripping voltammetry applications [17,18]. Previously, metal catalyst free carbon nanotubes (MFCNTs) fabricated via a solid-phase growth mechanism have been reported as a good electrode material with fast electron transfer rate and clear cyclic voltammetry background as well as for trace metal detection with stripping voltammetry with low limit of detection and high sensitivity [19,20]. The MFCNT whisker used in this paper is a powder material with a whisker shape, which is different from the solid CNT electrode that we used previously [19,20]. In this work, we continue to explore the potential of MFCNTs for anodic stripping voltammetry (ASV) by using this whisker material as an electrode modifier for zinc detection in biological samples.

A potential limitation of stripping analyses in complex matrices such as blood is the presence of organic and inorganic substances that may display redox activity near the potential of the analyte of interest, interfering with the detection signal. In addition, organic biological substances that can adsorb on the electrode surface can decrease its effective surface area and passivate (foul) the electrode surface. Also, a large fraction of the zinc in blood or serum is coordinated with binding sites, making it unavailable for detection by ASV [21,22]. Thus, a robust analytical procedure must overcome these challenges in order to reliably determine the analyte concentration. A variety of techniques can be used to overcome the matrix interferences, such as UV irradiation decomposition, ultrasound-assisted extraction and microwave-digestion [7,23,24]. Of these techniques, analyte extraction which physically separates the targeted analyte from the complicated matrix is much simpler since it does not require an external energy supply. A double extraction method using dithizone as a chelating reagent has been reported as an effective method for extracting metal ions from different matrices [7,25–27].

Another approach that applies to electroanalytical techniques is the use of a chemically ion selective electrode coating [28]. Because faradaic electron transfer occurs at the interface of the sample with the electrode surface, a material that limits which chemical species are able to reach the electrode can also be an effective means of removing interferences. Nafion is a perfluorinated sulfonated cation-exchanger with the advantages of thermal stability, chemical inertness, mechanical strength and resistance to fouling and has been widely used to modify electrodes in electrochemistry [29,30]. Nafion ethanolic solution can also serve as an excellent CNT dispersion solvent that facilitates the electrode modification procedure significantly [29].

The aim of this preliminary work is to develop a simple metal based sensor that allows accurate and rapid serum zinc detection using square-wave stripping voltammetry. Previously, our group has developed a lab-on-chip sensor with bismuth film working electrode on gold substrate for zinc detection by ASV [31]. Here we evaluate MFCNT whiskers as an electrode material by immobilizing them in a Nafion film coated on a gold substrate. This approach has improved the limit of detection of the sensor for zinc detection due to the Nafion/MFCNT whiskers coating and, more importantly, the new platform worked very well for detection of zinc in a bovine serum sample.

2 Experimental

2.1 Reagents

All chemicals were purchased without further purification: Nafion perfluorinated resin solution 5 wt.%, acetate buffer solution (pH 4.65) and 1000 ppm Zn standard solution for atomic absorption spectroscopy (AAS) were from Sigma Aldrich; bovine serum was purchased from Fisher Scientific; MCFCNT whiskers were supplied by SCNTE LLC (Beavercreek, OH) and used without any pretreatment. All other chemicals used in this work were ACS certified reagent grade and all solutions were prepared with deionized water (18.2 M Ω from Milli-Q System, Barnstead, MA).

2.2 Apparatus

ASV measurements were carried out in a 20 mL conventional three-electrode cell containing 15 mL of sample solution and consisting of a MCFCNT modified gold (Nafion/whiskers-Au) electrode as working electrode, Ag/AgCl as reference electrode (filled with 3 M KCl solution) and Pt wire as auxiliary electrode. A BASi 100B Electrochemical Analyzer from BASi (West Lafayette, IN) was used as the potentiostat. Basic set-up parameters for Osteryoung square wave voltammetry were square wave amplitude=25 mV, step potential=5 mV and frequency=25 Hz. The sonicator used in this work was a FS20D from Fisher Scientific; the centrifuge was a Marathon 6K from Fisher Scientific.

Transmission electron microscopy (TEM) images were taken on a Tecnai F20 at 200 kV high angle annular dark field (HAADF) in scanning TEM mode. Scanning electron microscopy (SEM) images were taken on a FEI XL 20 ESEM in environmental SEM mode. Energy-dispersive X-ray spectroscopy (EDX) images were taken on an EDAX detector from EDAX Inc.

AAS measurements were done using a Varian AA240FS atomic absorption spectrometer. The extract samples of bovine serum were diluted in 10% HNO₃ and analyzed using the parameters specified by the instrument. For serum samples, the same procedure was followed except that we spiked the serum with zinc standard and calculated the recovery.

2.3 Preparation of Nafion/Whiskers-Au Electrode

MCFCNT whiskers powder was dispersed in 1 wt.% Nafion ethanol solution assisted by sonication for 10 min to yield a homogeneous dispersion. A gold electrode was first sonicated for 1 min in distilled water to clean the electrode surface. Then, 10 μ L of the MCFCNT whiskers Nafion dispersion was applied to the dry gold surface. The coating was left to let ethanol evaporate for 30 min and then used directly for ASV measurements.

2.4 Extraction Procedures

First, 10 mL (5 mM) dithizone in chloroform was deprotonated by mixing with 10 mL (pH 9) 1 M ammonia/ 0.5 M ammonium buffer solution. Then the deprotonated form of dithizone was mixed with the solution containing Zn (II) and 0.5 mL of 0.05 M potassium thiocyanate in ethanol and sonicated for 5 min. After sonication, the solution was transferred to a 50 mL plastic tube and centrifuged for 10 min at 4000 rpm to separate the two phases. The organic phase was collected and sonicated with 10 mL 1 M sulfuric acid for another 5 min. Then the clear aqueous phase was collected and mixed with 0.1 M acetate buffer and the pH was adjusted to 6 with 5 M NaOH solution. The resulting solution was used for ASV measurements with Osteryoung square wave mode.

3 Results and Discussion

3.1 MCFCNT Whiskers

Traditionally, transition metals are used as catalyst to synthesize CNTs [32]. The residual metal oxide cannot be fully removed even by harsh acid wash, which can result in an electrochemical interference such as reduction of the metal oxide in the CNTs [33,34]. In addition, batch to batch variation of CNTs in terms of metallic impurity can introduce variation for the application of CNTs in electrochemistry [13,35]. The MCFCNT whiskers used here were synthesized via the carbo thermal carbide conversion method, which grows carbon nanotubes on a silicon carbide matrix [35,36]. Since no metal catalyst is used in the synthesis process, batch to batch variability is eliminated in terms of impurities and no post treatment is required. High purity material obtained with this process has been reported as a good material for electrodes [19]. The TEM images of the MCFCNT whiskers in Figure 1 clearly show the whisker shape of the MCFCNT.

3.2 Comparison of Bare Gold, Nafion Coated Gold (Nafion-Au) and Nafion/Whiskers-Au Electrodes

In order to separate the effect of the Nafion coating from the MCFCNT whiskers, control experiments for zinc detection were conducted on a bare gold electrode and a Nafion-Au electrode without MCFCNT whiskers. Figure 2 shows anodic stripping voltammograms of 4 μ M zinc on these different electrodes. A stripping peak for Zn on bare gold electrode occurs at 480 mV. When comparing the bare gold electrode to the Nafion-Au electrode, it can be seen that the Nafion coating causes a small negative shift in the peak potential to 520 mV. Although the coating does not increase the zinc stripping peak current, it improves the electrochemical response in terms of smoother background and better defined peak shape. On both bare gold electrode and Nafion-Au electrode, the zinc stripping peak is at ca. -500 mV, which is shifted to a more positive potential compared to the Nernst potential of 0.98 V (vs. Ag/AgCl) due to the underpotential deposition (UPD) of depositing zinc on gold substrate [37]. For the Nafion/whiskers-Au electrode, a double peak pattern is observed: a major stripping peak at ca. -1050 mV, which is zinc stripping from MCFCNT whiskers, followed by a minor stripping peak at ca. -500 mV, which is zinc stripping from gold. We attribute this to incomplete coverage of the gold surface by MCFCNT whiskers. The height of the "Zn stripping from gold" peak is diminished compared to this peak on bare gold and the Nafion-Au electrodes because most of the gold is now covered by MCFCNT whiskers. The "Zn stripping from MCFCNT whiskers" peak is substantially larger than the "Zn stripping from gold" peak, indicating that it is now the primary surface area for deposition of Zn during the deposition step.

The surface coverage of gold by MCFCNT whiskers can be seen in the scanning electron micrographs in Figure 3. Figures 3A and 3B were obtained at the same surface with different detectors. Figure 3A was taken with a gaseous secondary electron detector which shows the surface topology of the Nafion/whiskers-Au electrode where the MCFCNT whiskers coating can be identified by the rough layers. Figure 3B, which was taken with a backscattered electron detector, shows the surface composition of the Nafion/whiskers-Au electrode. The darker colored part in Figure 3B shows the film of Nafion/whiskers coating which correlates to the ridged part in Figure 3A. Figure 4 shows the EDX results for the bright and dark areas in Figure 3B as indicated with arrows in Figure 3B. Clearly, Figure 4A shows a strong Au signal which indicates the exposed gold surface on the Nafion/whiskers-Au electrode (horizontal arrow in Figure 3B). On the other hand, the signature gold peak has disappeared in Figure 4B, indicating that the gold surface was fully covered by the Nafion/whiskers coating for the dark area (vertical arrow in Figure 3B).

3.3 MCFCNT Whiskers Amount Optimization

The effect of amount of MCFCNT whiskers in the Nafion film coated on the gold electrode was investigated. As shown in Figure 5, the peak current for zinc stripping at -1050 mV from the MCFCNT whiskers increases almost linearly with increasing amount of the MCFCNT whiskers dispersion up to about 3 mg/mL above which it levels off. Also, as more CNT coated on the gold surface, the Zinc peak at -500 mV gets smaller as more of the gold is covered. As shown by the error bars, reproducibility was much poorer at concentrations above 3 mg/mL. The thicker whiskers films are less mechanically stable and can be disrupted by hydrogen bubbles formed by electrolysis of water during the Zn deposition step, which causes larger variation [38]. Overall, 3 mg/mL gives a good compromise for zinc ASV detection with respect to both peak height and reproducibility. Therefore, the optimized film composition of 10 μ L of 3 mg/mL MCFCNT whiskers in 1 wt.% Nafion ethanol solution was used for all subsequent experiments.

Between measurements, the electrode was cleaned by applying a constant potential of 500 mV for 1 min to remove any zinc residue from the electrode surface. A single electrode can give reproducible results for 50 – 80 measurements. The relative standard deviation of zinc detection (4 μ M in acetate buffer) with different Nafion/ whiskers-Au electrodes ($N=5$) was found to be 5.1% .

3.4 pH, Deposition Potential and Deposition Time Optimization

Electrolysis of water is an important factor to consider for ASV detection of zinc since hydrogen evolution can interfere with zinc deposition on the electrode surface because of the very negative potential required. Hydrogen evolution is quite sensitive to solution pH, deposition potential and electrode material. Figure 6A shows cyclic voltammograms of the Nafion/whiskers-Au electrode in acetate buffer with different pH values. Electrolysis of water starts at ca. -1000 mV at pH values lower than 6.0 , which will interfere with zinc deposition for ASV measurements at ca. -1300 mV. Figure 6B shows how buffer pH affects zinc ASV detection at the Nafion/whiskers-Au electrode. The ASV peak currents are lower and with larger variations at lower pH values compared with pH 6.0 . Overall, ASV at pH 6 buffer has the most moderate background and the most reproducible peak current since significant hydrogen evolution occurs at lower pH. Also, ASV has the sharpest and highest stripping peak at a deposition potential of -1400 mV. The peak current drops at more negative deposition potentials due to hydrogen gas evolution at the electrode surface, which interferes with the mass transport of Zn^{2+} analyte to the electrode surface and damages the Nafion/whiskers film. Figure 7 shows the deposition potential optimization of ASV zinc measurement at the optimized pH value. -1400 mV was chosen as optimized deposition potential due to the highest peak height and acceptable reproducibility for the Zn stripping peak at -1050 mV. Thus, pH 6 and a deposition potential of -1400 mV were used for subsequent experiments. The optimization of deposition time was also explored with those optimized experimental parameters. It was found that the peak current increases linearly with deposition time up to 120 s after which the curve slope starts to decrease as coverage of the CNTs on the electrode surface increases with longer deposition time. So, 120 s was selected as optimized deposition time.

3.5 Calibration Data

ASV determination of Zn^{2+} in a series of standard solutions was carried out in 0.1 M pH 6 acetate buffer solution using optimized experimental parameters. Figure 9 shows ASV voltammograms in the linear range of zinc detection with increasing concentrations from 0.5 to 7 μ M. The double peak pattern is also seen in the voltammogram and the major zinc peak at -1050 mV was used for zinc stripping peak measurements. Each voltammogram represents three replicates of zinc measurements for the same concentration. A plot of peak

current versus concentration is shown in Figure 8. The correlation equation for the linear range is $I (\mu\text{A}) = (3.77 \pm 0.03) [\text{Zn} (\mu\text{M})] - (1.05 \pm 0.06) (\mu\text{A})$ ($R^2 = 0.99$ for 9 concentrations in the range of 0.5–7 μM and the standard deviations are shown for the slope and intercept). The limit of detection was calculated to be 53 nM (based on $3\sigma/\text{slope}$) which is close to the 50 nM that we reported previously for a MCFCNT electrode for zinc detection [19]. Measurements of higher concentrations fit into another linear range which is attributed to stripping of zinc that was deposited on zinc rather than the zinc deposited directly on MCFCNT whiskers.

The bare gold electrode has a dissatisfactory behavior for zinc detection in terms of poor reproducibility, nonlinear calibration and distorted background. After coating with 10 μL 1 wt.% Nafion solution, the Nafion-Au electrode showed excellent reproducibility for zinc detection and a linear range of 0.5–4 μM was obtained as shown in Figure 9. For concentrations higher than 4 μM , the deposition follows a zinc-on-zinc deposition mechanism which leads to a smaller slope value. The limit of detection (based on 3σ) calculated for the Nafion-Au electrode within the first linear range was 80 nM. Notably, the Nafion-Au electrode has a smoother background and a better defined zinc peak than bare gold electrode at the same current scale. The Nafion/whiskers-Au electrode exhibits 2.4 times increase in peak current compared to the Nafion-Au electrode which is attributed to the increased electrode surface area provided by the MCFCNT whiskers and the fast electron transfer rate at MCFCNT whiskers. Compared with the Nafion-Au electrode, the Nafion/whiskers-Au electrode has a much wider linear range, larger calibration curve slope and bigger peak current (shown in Figure 9). Also, the limit of detection of zinc for the Nafion/whiskers-Au electrode is lower than for the Nafion-Au electrode. In this comparison, all of the experimental parameters were the same optimized values described above.

Comparison of zinc detection with different CNT-based electrodes is shown in Table 1. The combination of CNT, Nafion film and bismuth film has the lowest limit of detection and largest linear range. The Hg film CNT electrode has a similar linear range to the MCFCNT electrode with a higher limit of detection. One obvious advantage of this work is that there is no extra film needed to be co-deposited (e.g., Hg and Bi film [39,40]) on the electrode which allows an easier fabrication process.

3.6 Sample Preparation and Standard Addition of Extraction from Bovine Serum

Dithizone (H_2Dz) has been used as a highly efficient chelating ligand extraction of metals such as silver, zinc and lead from different matrices [7,26,27]. Also, it has been reported that the extraction process can be expedited by using potassium thiocyanate to form a complex with zinc and applying sonication during the extraction [25,27]. Herein, we use H_2Dz as the extraction reagent to extract and quantify zinc in bovine serum with the ASV method on the Nafion/whiskers-Au electrode. The mechanism is described by the following equations:

The extraction efficiency was first examined by extracting zinc from aqueous zinc AAS standard solution. Table 2 shows the extraction efficiency of various ligand to metal ratios of 10 :1, 50 :1 and 100 :1. A ligand to metal ratio of 100 :1 is needed for high extraction yields.

Bovine serum samples were treated with the procedure described in the Experimental Section. The resulting solution was adjusted to pH 6 and quantified by ASV with a Nafion/whiskers-Au electrode using the standard addition method. The low concentration of zinc caused us to use a longer deposition time of 5 min to obtain a better defined ASV peak for zinc detection in the serum extracts. Figure 10A shows the stripping voltammograms for a bovine serum extract and standard additions from 1–4 μM . The zinc stripping peak at ca. –500 mV is not changed linearly by the addition of zinc due to the limited exposed Au surface area. Also, shoulder peaks are present in Figure 10A at ca. –750 mV which were not present in Figure 8. These shoulder peaks are possibly due to the presence of some residual organic species which can alter the ASV behavior of heavy metals [41]. Figure 10B shows the plot $[I (\mu\text{A}) = (3.24 \pm 0.15) [\text{Zn } (\mu\text{M})] + (2.54 \pm 0.33) (\mu\text{A})]$ (standard deviations are shown for the slope and intercept) of standard addition. The plot slope is higher than the 2.38 $\mu\text{A}/\mu\text{M}$ that we reported before for zinc detection with a MCFCNT electrode [19]. The sensitivity increase is due to the Nafion film with MCFCNT whiskers coating. The zinc concentration in bovine serum was tested to be $45 \pm 0.6 \mu\text{M}$ which is in close agreement with an independent AAS result of 48 μM .

4 Conclusions

For the first time, MCFCNT whiskers are used as an electrode coating material confined in Nafion on a gold electrode. This novel material has advantages over the traditional carbon nanotube material such as low cost, high purity and good consistency from batch to batch. MCFCNT whiskers are homogeneously dissolved in 1 wt.% Nafion ethanolic solution and coated onto the gold electrode surface. The Nafion/whiskers-Au electrode shows better reproducibility and lower limit of detection for zinc detection in acetate buffer than both Au and Nafion-Au electrodes. A double peak pattern was observed for zinc detection with Nafion/whiskers-Au electrode due to UPD on exposed gold. Previously, multiwalled carbon nanotubes (MWCNTs) have been used as an electrode modifier for trace level voltammetric zinc detection. The double extraction method used in this work to extract zinc from bovine serum was optimized to give a high yield of zinc extraction. The Nafion/whiskers-Au electrode works very well for zinc detection in bovine serum extracts, and the results are in good agreement with independent AAS measurements. This work shows the good potential of using MCFCNT materials together with a gold substrate for a sensing system that measures zinc in blood.

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Fig. 1.
TEM images of MCFCNT whiskers: A) 4000 $\times$ magnification; B) 200 000 $\times$ magnification

Fig. 2. ASV voltammograms of $4 \mu\text{M Zn}^{2+}$ in acetate buffer solution (pH 6) on different electrodes (bare gold electrode, Nafion-Au gold electrode and Nafion/whiskers-Au electrode). Deposition time 120 s; deposition potential -1400 mV .

Fig. 3. SEM images of Nafion/whiskers-Au electrode: A) surface topology of Nafion/whiskers-Au electrode with a gas secondary electron detector; B) surface composition of Nafion/whiskers-Au electrode with a backscattered electron detector.

Fig. 4. EDX images of Nafion/whiskers-Au electrode: A) The bright area (horizontal arrow) in Figure 3B; B) The dark area (vertical arrow) in Figure 3B.

Fig. 5. Effect of CNT concentration on peak current for ASV Zn^{2+} detection. Coating: 10 μL of MCFCNTs whisker dispersion in 1 wt.% Nafion ethanolic solution with different CNT concentrations. Deposition time 120 s, deposition potential -1400 mV; zinc concentration 5 μM .

Fig. 6.

A) Cyclic voltammograms of Nafion/whiskers-Au electrode in acetate buffer with different pH values; scan rate: 10 mV/s; B) pH optimization of Zn²⁺ ASV detection on Nafion/whiskers-Au electrode in acetate buffer with optimized coating parameters from pH 4.5 to 6. Zn²⁺ concentration 5 μM; deposition potential -1400 mV; deposition time 120 s.

Fig. 7. Deposition potential optimization of Zn^{2+} detection with optimized coating parameters: deposition time 120 s; zinc concentration 5 μM ; pH 6.0

Fig. 8. ASV voltammograms of Zn^{2+} with Nafion/whiskers-Au electrode in 0.1 M pH 6 acetate buffer in the concentration range of 0.5 μM to 7.0 μM . Deposition time 120 s; deposition potential -1400 mV.

Fig. 9. Dynamic ranges of Zn^{2+} ASV measurements in the range of $0.5 \mu\text{M}$ – $11 \mu\text{M}$ on Nafion-Au electrode and Nafion/ whiskers-Au electrode. Deposition time 120 s; deposition potential -1400 mV .

Fig. 10. A) Voltammograms of standard addition of bovine serum extract for ASV zinc detection with Nafion/whiskers-Au electrode. B) Standard addition plot of bovine serum extract for ASV Zn^{2+} detection with Nafion/whiskers-Au electrode. Deposition time 300 s; deposition potential -1400 mV; pH 6.

Table 1Comparison of different CNT based electrodes for ASV Zn^{2+} detection.

Sensor	Deposition time (s)	Limit of detection (μM)	Linear range (μM)
MWCNTs/Hg film [40]	180	0.43	0.89–9.9
MWCNTs/NA/Bi/SPE [39]	120	0.0046	0.0077–1.5
This work	120	0.053	0.5–7

Table 2

Efficiency of extraction from zinc stock solution with different [L]:[Zn²⁺] ratios.

[L]:[Zn ²⁺] ratio	Extraction efficiency (%)
10 :1	68±1
50 :1	83±2
100 :1	97±1