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Flexible copper-biopolymer nanocomposite sensors for trace level lead detection in water

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ABSTRACT

In this work, a novel copper-chitosan (Cu-chitosan) nanocomposite-based flexible electrochemical sensor with an integrated Ag/AgCl reference electrode is fabricated. The electrochemical sensor is fabricated using low-cost screen-printing technology on a flexible substrate, followed by the electrochlorination of silver to form an integrated Ag/AgCl reference electrode and electrochemical deposition of a Cu-chitosan nanocomposite film to produce a working electrode. The surface chemical analysis of the working and reference electrodes using XPS (X-ray photoelectron spectroscopy) shows the formation of functional layers. The fabricated Cu-chitosan nanocomposite-based sensors are used to determine the trace level lead (Pb²⁺) ions in real-world water samples (i.e., tap water, mining wastewater, and soil leachate) using square wave anotic stripping voltammetry (SWSAV). A noticeable peak for lead ions is observed at ~0.46 V vs. integrated Ag/AgCl reference electrode. The limit of detection (LOD) of the developed flexible electrochemical sensor in tap water is 0.72 ppb with the relative standard deviations (n = 10) of 0.65 %. The fabricated electrochemical sensor. These results show that chitosan's presence on the composite material enhances the sensitivity and mechanical flexibility.

1. Introduction

Heavy metal pollution in the water and soil environment is a global problem because of the adverse effect on humans and other organisms [1,2]. Heavy metal ions are non-biodegradable and have a very long half-life time [3]. The presence of lead in the human bloodstream can cause kidney injury, behavior and learning problems, lower intelligence quotient (IQ) and hyperactivity, growth disorder, hearing problems, and anemia [4,5]. Although the United States (US) Environmental Protection Agency (EPA) restricted the permissible upper limit of lead to 15 ppb in drinking water, there is no safe blood lead level that has been established in young children [6-8]. Thus, the agency for toxic substances and disease registry (Lead Poisoning Prevention Subcommittee) has recommended lowering the permissible upper limit of lead in drinking water [9]. The recent lead poisoning cases in the US, such as Flint (2014) and Newark (2019) incidents, where lead levels were over 100 ppb and 50 ppb, respectively, drew the public attention due to their severity and long-term impact on human health [10,11]. CDC has recently introduced the Childhood Lead Poisoning Prevention Program to mitigate the effect of lead poisoning [12]. Thus, the presence of lead ions in drinking water is a serious problem, and the contamination needs to be detected at the trace levels at the drinking water facilities. Conventional methods for heavy-metal detection such as inductively coupled plasma atomic emission spectroscopy (ICP-AES), atomic adsorption spectroscopy (AAS), X-ray fluorescence (XRF), and inductively coupled plasma mass spectroscopy (ICP-MS) are highly sensitive, specific, and precise [13]. However, most techniques mentioned above are not suitable for real-time on-site monitoring of heavy metal ions because of bulky instruments, high cost, and trained personnel requirements to operate them. Over the last few decades, electrochemical sensors have emerged as a cost-effective, rapid, and portable alternative to such instruments for monitoring heavy metal ions [14-17]. A variety of sensing materials have been used for those sensors, for example, mercury (Hg), gold (Au), and platinum (Pt) [14]. The energy and cost-intensive synthesis of Au and Pt and the high cost of the materials themselves affect the low-cost mass production of the disposable sensors

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Received 17 December 2020; Received in revised form 28 May 2021; Accepted 5 June 2021 Available online 8 June 2021 0925-4005/© 2021 Elsevier B.V. All rights reserved. [18]. Mercury has been used as the electrode material due to its unique ability to pre-concentrate heavy metals. In spite of that, it has been replaced because of the severe toxicity associated with it [19,20]. Bismuth (Bi) film electrodes have been used as a replacement for the Hg electrodes due to its environmentally benign nature. Bismuth can form alloys with various heavy metals and has a wide potential window that is advantageous for electrochemical detection of heavy metal ions [21]. However, regardless of these benefits, when fabricating flexible microsensors using Bi, the real-world applicability is limited due to the brittleness and detachment problem associated with it [22]. This study seeks to integrate sensing layer into a flexible substrate which can be applied to a place that needs endurance against deformation events [23]. In addition, fabricating sensors directly on flexible substrate opens the possibility of a low-cost roll-to-roll mass fabrication of the sensors [24, 25].

Copper has been used in flexible electronics because of its good formability, excellent electrical and thermal conductivity, and low cost [26]. The additional advantage of using a copper-based material is its compatibility with numerous microfabrication techniques [27]. These unique properties make copper-based material an attractive candidate as a sensing element in the electrochemical sensors. They have been used to detect various analytes (e.g., heavy metal ions, hydroquinone, catechol, etc.) [27-30]. Kang et al. (2017) have demonstrated the use of copper as a low-cost sensing material with an integrated reference and counter electrode to detect heavy metal ions in an aqueous solution [30]. The sensors were fabricated on the rigid silicon substrate using a high-cost cleanroom fabrication process and had relatively low sensitivity (0.016 μ A/ppb) and a high limit of detection (LOD) (4.4 ppb). Although copper is a low-cost material, it gets easily oxidized and consequently affects the conductivity and reproducibility of the sensor [27,31]. Organic/inorganic composite materials have demonstrated improved performance in many applications such as catalysts, sensors, drug delivery, water purifications, etc. [32-34]. In this context, chitosan biopolymer is a promising organic material because of its excellent film-forming ability, good biocompatibility, and adhesion [35,36]. Webster et al. (2007) have studied the physicochemical properties of various metal/chitosan composite materials. The result shows that Cu-chitosan composite material has the highest metal cation-to-polymer ratio among cobalt, nickel, zinc, iron, chromium, and manganese [37]. This implicates that enough copper cation can be loaded on the chitosan to form an electrically conductive composite material. Further, the composite material will be chemically stable compared to bare copper due to the copper cation complex formation with chitosan. Chitosan also contributed to the enhanced mechanical integrity of the sensing films as reported in our previous study [38-40].

In this work, a novel copper-chitosan (Cu-chitosan) composite nanomaterial has been used as a working electrode along with an integrated Ag/AgCl reference electrode and a carbon counter electrode. The biopolymer, chitosan, add benefits to the copper in terms of mechanical stability and sensitivity. Chitosan is a polycationic biopolymer because of the presence of amino groups, while most of the polysaccharides are either neutral or anionic [41,42]. Thus, chitosan can be electrochemically co-deposited with copper by applying a cathodic current, which simplifies the fabrication step. The morphology and the composition of the deposited Cu-chitosan composite were evaluated using a scanning electron microscope (SEM) and X-ray photoelectron spectroscopy (XPS). Parameters of sensor performance, including the limit of detection (LOD), repeatability, and sensor lifetime, were evaluated and optimized using SWASV for the Pb²⁺ ion detection in various water matrix samples.

2. Experimental section

2.1. Chemicals

Silver, carbon, and insulating polymer pastes were obtained from

Daejoo Electronic Materials Co. Ltd (South Korea) and used to screenprint electrochemical sensors. Chitosan was purchased from MP biomedicals (USA). Chemicals required for the electrochemical analysis were obtained from Sigma-Aldrich (USA). All the chemicals used in the experiments were analytical grade without further purification.

2.2. Water sample preparation and analysis

Acetic acid was used to make a 0.1 M acetate solution (AcB) at a pH of 4.5 as a buffer for the electrochemical methodology. In addition, lead (II) nitrate dissolved in deionized (DI) water was used to make 0.1 M Pb²⁺ stock solutions. To validate Pb²⁺ concentrations, a Thermo Fisher Scientific iCAP-Qc ICP-MS with QCell technology was utilized. The trace element analysis was done in kinetic energy discrimination (KED) mode with helium as the collision gas. Calibration, internal and quality control standards (Inorganic Ventures) were prepared in a 2% (v/v) HNO₃ solution. Further, calibration standards were prepared at concentrations of 10–50 mg/L¹ (ppb).

Environmental water samples utilized to test sensor performance in varying conditions include tap water, mining wastewater, and soil leachate. Tap water samples were obtained in Orlando, FL, USA and spiked using appropriate amounts of Pb^{2+} to achieve different concentrations of lead ions. The mining wastewater and soil leachate samples were obtained from a mining site (Il-gwang mine) and a soil contaminated site (Jukcheong mine) in South Korea, respectively. Both on-site samples have the multiple heavy metal ions such as Zn^{2+} , Pb^{2+} , Cd^{2+} , Cu^{2+} , and As^{3+} and similar pH values (~4.5). The concentration of heavy metal ions were 11.5 ppm of Zn^{2+} , 0.1 ppm of Cd^{2+} , 0.07 ppm of Pb^{2+} , 10.5 ppm of Cu^{2+} , and 0.3 ppm of As^{3+} in mining wastewater and 439.2 ppm of Zn^{2+} , 8.4 of Cd^{2+} , 394.4 of Pb^{2+} , 38.1 ppm of Cu^{2+} , and 300 ppm of As^{3+} in soil leachate, respectively.

2.3. Fabrication of sensor

The carbon electrodes with a well-defined counter electrode, a working electrode, and a reference electrode were fabricated using a screen-printing technique. For this purpose, three different patterns were designed using AutoCAD. Metal mesh screens (mesh size 200) with distinct patterns were obtained from NBC Meshtec Americas Inc. (USA). The sensors were fabricated on the low-cost flexible polyvinyl chloride (PVC) film and were obtained from Grafix, USA. Fig. 1 illustrates the screen-printing process. First, silver tracks were printed on the PVC substrate and cured at ambient conditions for 4 h. The carbon layer was then printed over the silver tracks to form a counter and a working electrode and cured at ambient conditions for 4 h. Finally, the insulating layer was printed with openings on a counter electrode, a reference electrode, a working electrode, and electrical contacts. The polymer insulating layer was cured under 365 nm centered ultra-violet (UV) radiation for 2 h. Prior to further modification, the electrodes were polished using 1 μ m alumina particles dispersed in DI water (1 mg/mL), rinsed with DI water, and dried at ambient conditions. This step was taken to ensures the effective removal of surface contaminants [43].

There are several methods to fabricate reference electrodes on flexible substrates [44,45]. The integrated reference electrode was fabricated by *in-situ* electrochemical chlorination of the silver to form silver chloride. The *in-situ* electrochemical chlorination was done using the three-electrode system, which consists of a silver layer as a working electrode, an integrated carbon electrode as a counter electrode, and a separate Ag/AgCl external reference electrode (MI-401, Microelectrodes, Inc, NH, USA). A solution of 0.1 M KCl was used as an electrolyte, and electro-chlorination was conducted at a constant supply of 5 mA/cm² DC for 30 s. The electrochlorination process was optimized with respect to the applied current.

The electrodeposition of biopolymer metal composite material was carried out using an integrated three-electrode system (integrated Ag/ AgCl reference electrode, carbon counter electrode, and carbon working



Fig. 1. A screen-printing process for sensor fabrication: (a) top view, (b) cross-sectional view, and (c) photograph showing a fabricated flexible sensor.

electrode). Chitosan is water-soluble in acidic solution and is cationic, and these unique properties of chitosan enabled it to co-deposit with metal ions to form a composite film. The electrolyte for electrodeposition consists of 50 mg of chitosan and 0.1 M copper nitrate trihydrate in 40 mL of a diluted acidic aqueous solution (acetic acid, pH 4.5); the parameters are based on our previous work [39]. The solution was thoroughly mixed for 24 h using a magnetic stirrer. The schematic illustration of the electrodeposition of the composite material is presented in Fig. SI 1. The electrodeposition was carried out under a constant supply of -0.5 mA/cm² DC for 30 s. Before testing, the sensor was cleaned with DI water and dried at room temperature. The photograph of the fabricated flexible sensor is shown in Fig. 1C.

2.4. Characterization of the fabricated Cu-chitosan nanostructure

The surface morphology of the composite films was investigated using the Zeiss Ultra-55 SEM operated at an accelerating voltage of 5 kV. An ESCALABTM XI⁺ X-ray Photoelectron Spectrometer Microprobe (Thermo Scientific, Waltham, Massachusetts, USA) with a monochromatic, micro-focused Al K α source was used to collect X-ray photoelectron spectroscopy (XPS) spectra. The XPS analyzer consists of a 180° double-focusing hemispherical analyzer with a dual detector system. The pressure in the XPS analysis chamber during measurement was not higher than 1.0×10^{-7} torr. Electrochemical impedance spectroscopy (EIS) spectra were measured using a PalmSens 3 EIS potentiostat under the three electrode system in 10 mM potassium ferricyanide (K₃[Fe(CN)₆]) solution at the frequency range 50 mHz to 50 kHz.

2.5. Heavy metal detection using SWSAV

The electrochemical methodology utilized for sensor performance evaluation and heavy metal determination was developed from a threeelectrode system paired with SWASV. Electrochemical measurements were carried on a commercial electrochemical cell (10 mL, Compact Voltammetry Cell-Starter Kit, Pine instrument, Grove City, PA, USA) using a three-electrode system which consists of a Cu-chitosan codeposited surface working electrode, carbon counter electrode, and an integrated Ag/AgCl reference electrode. The system was connected to a PalmSens 3 EIS potentiostat to run the experiments and extrapolate data. For the evaluation of the developed sensor performance, optimal operational conditions of SWASV (e.g., applied potential, deposition time, amplitude, and frequency) were determined in a 15 ppb of Pb²⁺ solution (0.1 M AcB, pH 4.5). The initial evaluation was conducted using the 0.1 M AcB (pH 4.5) buffer and Pb²⁺ spiked solutions deposited at -1.2 V vs. Ag/AgCl for 300 s under continuous stirring. Then, 4 mV step potential, 100 mV amplitude, and 20 Hz frequency was used to strip the Pb²⁺ ions from the working electrode. Residual Pb²⁺ ions on the working electrode were removed before each successive measurement by applying 0.0 V for 300 s. The applied voltage from this cleaning step was monitored not to cause any structural damages to the Cu-chitosan film on the working electrode surface. Since each experiment was conducted more than once, the data is presented in terms of the mean \pm standard deviation (SD). In addition, the limit of detection (LOD) was calculated using the equation: $C_L = kS_B/b$ (Equation (1)) [46], where C_L signifies the detection limit, S_B represents the standard deviation of blank signals, k is a parameter with a value of 3, and b is the value of the calibration curve slope.

3. Results and discussions

3.1. Chemical and morphological analysis

The elemental and morphological analysis of the Cu-chitosan film was characterized using XPS and SEM analysis. A survey spectrum of the nanocomposite film over a wide range of binding energies of 1100 to 0 eV is shown in Fig. 2a. The survey spectrum clearly shows the presence of the C, O, N, and Cu peaks in the composite film. Fig. 2b shows the core line Cu 2p high resolution peak of the composite material. The presence of asymmetric peaks indicates that copper is present in the mixed oxidation states. The Cu 3p_{3/2} and Cu 3p_{1/2} spin orbital splitting for Cu (I) oxidation state (chitosan-Cu(I)) is at binding energies of 932.7 eV and 952.5 eV respectively, these values are comparable with data from published literature [47,48]. Similarly, The Cu 3p_{3/2} and Cu 3p_{1/2} spin orbital splitting for Cu(II) oxidation state (chitosan-Cu(II)) is at binding energies of 934.9 eV and 955.1 eV, respectively [47,48]. The weak peaks at 942 eV and 946 eV correspond to satellite peaks of Cu (II) 3p3/2 and Cu (I) 3p_{3/2}, respectively. Fig. 2c shows the N 1s spectra of the composite material. Peaks at 399.3 eV and 400.1 eV are associated with the chelation of the amino group of chitosan with the copper and N-H bonding, respectively. The XPS O1s high resolution spectrum in Fig. 2d shows the peaks 531.9 eV and 533.1 eV due to C=O and OH- [49]. The C 1s high-resolution signal is presented in Fig. 2e. On the basis of the literature, three peaks at 284.4 eV, 285.7 eV, and 288.1 eV can be assigned to C-C, C-O, and C=O, respectively [50]. The XPS analysis shows the presence of Cu-chitosan composite material on the carbon electrode. Fig. 3 presents the Cu-chitosan SEM images taken ona



Fig. 2. XPS analysis of Cu-chitosan nanocomposite film. (a) Survey spectra (b) Cu 2p spectra (c) N 1s spectra, (d) O 1s spectra, and (e) C 1s spectra.



Fig. 3. SEM images of the (a,b) Cu-chitosan nanocomposite deposited on carbon electrode at 40k magnification and 30k magnification (c) bare carbon electrode at 30k magnification.

composite film deposited on the carbon electrode (Fig. 3a, b) and a bare carbon electrode (Fig. 3c). The Cu-chitosan SEM images show the nanoparticle structure with an average size of 86 ± 12 nm in diameter. The XPS analysis and SEM affirms the Cu-chitosan nanoparticle deposited on the carbon electrode. Similarly, the SEM and XPS results of the silver chloride (Fig. SI 2) show the successful fabrication of the integrated reference electrode. The DFT (Density-functional theory) study by Lu et al. shows that -NH₂ functional group has a high tendency to coordinate with the Cu ions [51]. Copper ions are bound to amine functional group to form the composite material. The possible sensing mechanism of the composite material would be the reaction between hydroxyl functional group and lead ions (Fig. SI 3)."

3.2. Electrochemical impedance spectroscopy (EIS) analysis

EIS was used to study the electrode's electron transfer properties before and after surface modifications. Nyquist plots of the bare carbon and two modified carbon electrodes (chitosan only and Cu-chitosan composite) were presented in Fig. 4. The semicircle portion at higher frequencies of the Nyquist plot corresponds to the electron transfer limited process [52,53]. The charge transfer resistance of bare carbon, chitosan modified carbon electrode, and Cu-chitosan modified electrode calculated using the EIS spectrum analyzer were 235 Ω , 398 Ω , and 147 Ω respectively. This results suggest that the chitosan, when



Fig. 4. Nyquist plot of carbon electrode and modified carbon electrodes (chitosan only vs. Cu-chitosan composite) obtained from EIS. Potassium ferricyanide used as an electrolyte.

deposited alone, is hindering the charge transfer from the redox probe of $[Fe(CN)_6]^{3-/4-}$ to the electrode surface. In contrast, charge transfer resistance of the Cu-chitosan modified carbon electrode is lower

compared to both a chitosan modified carbon electrode and a bare carbon electrode. This result illustrates the role of copper in the composite material in enhancing the charge transfer from the electrolyte to an electrode surface.

3.3. Optimization of sensor operating parameters for heavy metal detection by SWASV

To determine optimal operating conditions of sensor performance, the SWASV parameters (e.g., deposition potential, deposition time, frequency, and amplitude) on Pb^{2+} detection were characterized in a 15 ppb of Pb^{2+} solution (0.1 M AcB, pH 4.5). These factors can affect the efficacy of depositing heavy metal ions on the working electrode, which is considered a first step in the SWASV determination of heavy metals as the sensing area of the working electrode is related to surface availability during the deposition step. The area of the working electrode in the Cu-chitosan sensor system is 3 mm OD.

Deposition potential is a critical parameter in SWASV. Investigation of the optimal deposition potential was conducted within the range -1.4 to -0.6 V vs. Ag/AgCl, and the effects were observed by depositing a solution containing 15 ppb of Pb^{2+} (Fig. 5a). As the deposition potential increased from -1.4 to -0.6 V with deposition time of 300 s, step potential of 4 mV, 100 mV amplitude, and a frequency of 20 Hz, the peak currents produced by the stripping step were decreased. The relationship between deposition potential and peak current can be likely linked to preferential reduction as well as the effects of metal ion deposition on the working electrode surface. At deposition potential of -1.4 V, the Cuchitosan sensor surface became unstable, and consequently, the repeatability decreased. Hydrogen evolution was observed at this potential as well and was ascribed to the degradation of the sensor. Other studies have also reported damages of the metallic coated films on the working electrode at similar potentials and its correlation to the reduction of the sensor lifetime [54]. To avoid the effects of hydrogen

evolution while maximizing sharp peak currents, a deposition potential of -1.2 V was selected as the optimal operating condition for Pb^{2+} detection using the Cu-chitosan sensor.

The deposition of heavy metal ions on the working electrode area is also affected by the time allotted to this step. An optimal deposition time was investigated between the range 30-600 s with a deposition potential of -1.2 V in a standard solution of 15 ppb Pb²⁺ (Fig. 5b). An increase in deposition time tended to increase the residual ion concentration after each measurement, which then increased the required cleaning potential duration. Cleaning procedure was conducted for 300 s, time equivalent to the deposition time during the measurement. Cleaning was performed at 0 V to prevent stripping of Cu from the working electrode of the sensor. The cleaning step prepares the analyte-free surface for the subsequent measurement by removing the residual metals [55]. Hence, the sensor reliability could be maintained for repeated measurements. The increased of deposition time resulted in an increase of current from 1.24 ± 0.09 – 2.65 ± 0.11 μA as the deposition time increased from 60 to 600 s. Similarly, related studies have shown that longer deposition time (e.g., 300 and 400 s) could increase sensitivity and avoid interference from other ions [56]. However, the deposition time greater than 300 s showed no significant improvement in sensitivity (i.e., increase in peak currents), but increased the total measurement time (Fig. 5(b)). Thus, the 300 s deposition time was used considering reliability and the need for a rapid measurement. This condition promotes sufficient deposition of lead ions on the Cu-chitosan sensor, which supports sensitive and stable Pb^{2+} detection.

Two other prominent parameters in SWASV include frequency and amplitude. These variables can have a significant impact on the stripping response of the targeted heavy metal ion (Fig. 5c and d). Optimal conditions for frequency were observed at lower values with the highest peaks observed at a frequency of 20 Hz. Increasing the amplitude ranging from 0.1 to 0.25 V resulted in a higher ambient noise with a larger SD at 0.15 and 0.25 V due to vibrations (Fig. SI 4d) [57,58]; thus,



Fig. 5. Optimization of SWASV operating parameters: (a) deposition potential, (b) deposition time, (c) frequency and (d) amplitude. SWASV was performed in a 15 ppb of Pb²⁺ solution (0.1 M AcB, pH 4.5).

the optimal condition for amplitude was determined to be 0.1 V, with the highest peak current occurring at 5.4 μ A. In conclusion, optimal performance conditions of the Cu-chitosan sensor system were determined, which are -1.2 V deposition potential, 300 s deposition time, 0.1 V pulse amplitude, and 20 Hz frequency. Further, these conditions were used to develop the calibration curves of the samples containing lead ions.

3.4. Calibration of the Cu-chitosan sensor

The preliminary evaluation of the Cu-chitosan sensor was conducted using SWASV, with conditions including a 300 s deposition time, -1.2 V deposition potential, 0.004 V potential step, 0.1 V amplitude, and 20 Hz frequency. The results show a well-defined lead ion peak located at -0.46 V vs. integrated Ag/AgCl reference electrode (Fig. 6a). As the lead ion concentration increased, the anodic peak potential shifted towards more positive potential due to the positive electrocatalytic activity of the lead ions with the electrode [59]. This phenomenon of peak shift is common as adsorption product tends to shift the peak potential positively. Using the Cu-chitosan sensor system, the sharp peaks produced in proportion to the concentration of Pb²⁺ indicate that this sensor measurement is appropriate for Pb²⁺ detection in aqueous solutions. Fig. 6 shows SWASV responses to concentrations from 2.5 ppb up to 60 ppb.

The baseline current was produced by conducting the experiment without any Pb²⁺ in the solution. Then, the peak currents produced in response to the presence of lead were corrected relative to the baseline current. Calibration plots are presented based on these peak adjustments (Fig. 6b). Results of the preliminary evaluation introduce a calibration plot that exhibits a linear fit described by the function y = 0.183 x + 0.37 (where x: concentration (ppb), y: current (µA)), with a correlation coefficient of $R^2 = 0.995$. Further, the produced calibration curves were used to calculate LODs based on three times of the signal to noise (S/N) according to the methodology [60] described by Lee et al. Based on Lee et al.'s method, the LOD of the fabricated Cu-chitosan sensor is 0.72 ppb for Pb²⁺.

The Cu-chitosan electrochemical sensor's performance was compared with other reported copper-based electrochemical sensors, as shown in Table 1. It could be observed that the Cu-chitosan sensor has a lower detection limit than other copper-based electrochemical sensors. The fabricated flexible electrochemical sensor provided a comparable sensitivity range while demonstrating better performances in the other metrics. The lower detection limit and excellent analytical performance of the fabricated Cu-chitosan sensor with an integrated reference electrode suggest strong absorbance of chitosan towards lead ions and higher surface area of the composite nanomaterial.

3.5. Interference of other heavy metals in Pb^{2+} detection

Metal ions that are commonly found in water containing Pb²⁺ are known to compromise the accuracy of electrochemical measurements [61]. Thus, commonly recognized interfering metal ions $(Zn^{2+}, Cd^{2+},$ As^{3+} , Fe^{2+} , Na^+ , K^+ , Mg^{2+} , and Ca^{2+}) were added to the standard solutions containing Pb²⁺ to observe changes in sensor signals (peak current) as a result of their presence. The additional heavy metals were spiked with the same concentration as the Pb^{2+} in solution (15 ppb). Na⁺, K⁺, Fe²⁺, Mg²⁺, and Ca²⁺ were added at different concentrations ranging from 0.2 mM to 11 mM based on literature that includes cation concentrations in municipal wastewater and drinking water [62-66]. Experiments were conducted by spiking Zn²⁺, Cd²⁺ and Cu²⁺ concentrations in the Pb²⁺ standard solution individually and simultaneously to study the interference [58,67]. Fig. 7 shows a similar range of sensor responses in the presence of these heavy metal ions. No decrease in peak current or interfering voltammetric peaks was observed with the general ions. From the individual and cumulative addition of these constituents. relatively insignificant signal changes were observed as small as 0.58% -2.42% at most (Table SI 1). These results indicate that their presence does not significantly alter the sensor performance. To further validate this, we performed the experiment by changing Zn^{2+} concentration from 100 to 300 ppb with a fixed concentration of lead at 15 ppb and a fixed concentration of Cd²⁺ ions at 100 ppb. The experiment shows (Fig. SI 5) that there is no interference in Pb^{2+} measurement. This signifies the validity of the developed Cu-chitosan sensor in detecting lower concentrations of lead ion species in the presence of other frequently found heavy metal ions found in polluted sites.

3.6. Applications to contaminated real-world samples

The Cu-chitosan sensor performance in contaminated real-world samples was studied using mining wastewater and soil leachate. These samples were used to evaluate the challenges associated with the on-site electrochemistry-based measurements due to the presence of unknown interfering species during lead ion detection. To conduct this investigation, calibration curves were developed for each of the real wastewater samples. The mining and soil leachate samples extracted from the contaminated sites are known to contain very high lead concentrations. To construct a calibration surface in a real wastewater matrix, the original solutions (Table SI 3) were diluted to lower the heavy metal concentrations (below 1 ppb Pb^{2+}) using DI water. In this case, the diluted sample was used for the baseline curve. Then, a calculated amount of the Pb (II) stock solution was spiked at 7 different concentrations to construct a calibration curve over the range of 1 - 25 ppb.

Application of the Cu-chitosan sensor in this experiment resulted in



Fig. 6. Characterization and evaluation of the Cu-chitosan sensor for Pb^{2+} detection. (a) SWASV with various Pb^{2+} concentrations (2.5 – 60 ppb) and (b) a corresponding calibration curve.

Table 1

Comparison of the fabricated Cu-chitosan sensor with other reported copper based electrochemical sensor for lead ion detection.

Electrochemical sensor WE CE RE			LOD* (ppb)	Fabrication method	n*	Flexibility	Sensitivity (µA/ppb)	Ref.
Cu-chitosan	С	Integrated Ag/AgCl	0.72	Screen-printing electrodeposition	20	Yes	0.18	This work
Cu	Cu	Integrated Cu/CuCl ₂	4.4	Photolithography	7	No	0.016	[1]
Cu	Cu	Integrated Cu/CuCl ₂	9.3	Thermal transfer printing	N/A	Yes	0.0058	[2]
Cu-Bi	Pt	External Ag/AgCl	1.2	Electrodeposition	N/A	No	0.36	[3]
Cu	Pt	External Ag/AgCl	5	Electrodeposition	N/A	No	0.28	[4]

*LOD=Limit of detection, *n=Reproducibility.



Fig. 7. Sensor responses (μA) at a fixed Pb^{2+} concentration (15 ppb) in presence of Zn^{2+} .

 Cd^{2+} , As^{3+} , and various cations (Na⁺, K⁺, Fe²⁺, Mg⁺, and Ca²⁺) in 0.1 M AcB at pH 4.5. Mix indicates the co-presence of Zn²⁺, Cd^{2+} , $Cd^{$

well-defined peaks for a range of Pb^{2+} concentrations, 0–25 ppb for both mining wastewater and soil leachate. The Cu-chitosan sensor detected $0.123 \,\mu\text{A/ppb}$ for mining wastewater (R² = 0.985) and 0.175 $\mu\text{A/ppb}$ for soil leachate ($R^2 = 0.979$) (Fig. 8). The LOD were 0.72 ppb for tap water, 1.4 ppb for mining wastewater and 1.54 ppb for soil leachate.

pH is known to affect redox potential (Eh) and Pb^{2+} speciation [68]. These factors are significant and can alter the currents and potentials observed during the electrochemical measurements. Thus, pH characterization of the original samples was conducted. Between pH 4.0 and 5.5, Pb^{2+} is the predominant species (Fig. SI 6) and is mobilized in water samples at a lower pH (<5.5) [69]. The original wastewater samples and diluted wastewater (adjusted by 0.1 M HCl) had a pH of approximately 4.4, which is within this range.

The Cu-chitosan sensor exhibits reproducible sensitivity towards $\ensuremath{\text{Pb}}^{2+}$ detection, which is evidenced by stable, sharp peak currents in repetitive measurements. The results recorded from the Cu-chitosan

sensor measurements were compared to the Pb²⁺ concentrations determined by ICP-MS (inductively coupled plasma mass spectrometry). The sensor data are in good agreement with the deterministic results from ICP-MS. The reproducibility experiments conducted using tap water spiked with Pb^{2+} (15 ppb) achieved 20 stable, successive measurements (Table 2) with a RSD of 0.65 % and a recovery of 98.1 %. The reproducibility experiments using the mining wastewater and soil leachate samples exhibited consistent sensitivity for 10 successive measurements with a RSD of 1.93 % and 8.75 %, and a recovery of 96.5 % and 94.8 %, respectively (Table 2 and Fig. SI 7). These values support the stability of the sensor in contaminated real-world samples.

4. Conclusion

A flexible electrochemical sensor based on a novel Cu-chitosan nanocomposite with an integrated reference electrode was fabricated.



Fig. 8. Heavy metal detection in wastewater environment using Cu-chitosan sensors: (a) mining wastewater and (b) soil leachate wastewater. Error bars are shown in the calibration curves.

Table 2

Sensor characteristics obtained in real-world environmental samples and comparative data obtained form ICP-MS.

Water matrix	LOD* (ppb)	n*	RSD* (%)	Cu-chitosan Sensor	Recovery (%)	ICP-MS
Tap water Mining wastewater Soil leachate	0.72 1.4 1.54	20 10 10	0.65 1.93 8.75	$\begin{array}{l} 15.2 \pm 0.1 \ \text{ppb} \\ 50.9 \pm 0.98 \ \text{ppb} \\ 12.8 \pm 1.12 \ \text{ppm} \end{array}$	98.1 96.5 94.8	$\begin{array}{c} 15.5 \pm 0.13 \text{ ppb} \\ 52.8 \pm 0.85 \text{ ppb} \\ 13.5 \pm 2.62 \text{ ppm} \end{array}$

*LOD=Limit of detection, *n=Reproducibility, *RSD=Relative standard deviation.

The proposed sensor was able to detect traces of lead ions in complicated water matrices with a good sensitivity. The surface elemental and morphological analysis of the composite nanomaterial using SEM and XPS shows the formation of the Cu-chitosan nanocomposite. Also, the EIS result indicates that the Cu-chitosan modified electrode has lower electron transfer resistance than the bare carbon and chitosan modified electrode. As a result, the fabricated sensor has a lower LOD compared to previously reported copper-based electrochemical sensors while providing the mechanical flexibility. The LOD results of the fabricated sensor in drinking water, mining wastewater, and soil leachate samples were 0.72 ppb, 1.12 ppb, and 1.01 ppb, respectively. Similarly, the RSD for 10 consecutive measurements of the fabricated sensor in drinking water, mining wastewater, and soil leachate samples were 0.65 %, 1.93 %, and 8.75 %, respectively. The minimal interference in the presence of other heavy metal ions was observed in the detection of lead ion traces using the fabricated sensor. In conclusion, the newly proposed sensor based on a Cu-chitosan nanocomposite has demonstrated the reliable sensing characteristics for monitoring lead ions in drinking water, mining wastewater, and leachate water samples.

A schematic illustration of deposition of a Cu-chitosan composite film, XPS survey peak and SEM image of an AgCl film of Ag/AgCl pseudo reference electrode, effect of deposition potential, deposition time, frequency, and amplitude on the anodic stripping peak current of Pb²⁺ using a Cu-chitosan sensor, chemical speciation of lead complex ions as a function of pH, validation of Pb²⁺ concentration measurements using ICP MS, the reproducibility of Pb²⁺ detection using a Cu-chitosan sensor, and evaluation of Pb²⁺ detection using a Cu-chitosan sensor against possible interfering heavy metal ions.

Author contributions

H.J.C., P.P., and W.H.L. conceived the perspective idea. P.P. and J.-H.H. designed the experiments. P.P., J.-H.H., H.T.L., K.L.R., and M.M.R., performed the experiments and analyzed the data. P.P., J.-H.H., H.J.C., and W.H.L. wrote the manuscript. H.J.C and W.H.L. supervised the project. All authors discussed the results and contributed to the manuscript. H.J.C. and W.H.L. acquired funding.

Declaration of Competing Interest

The authors report no declarations of interest.

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Appendix A. Supplementary data

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