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Investigating the Impact of Copper-PEDOT:PSS Matrix towards Nonenzymatic Electrochemical Creatinine Detection

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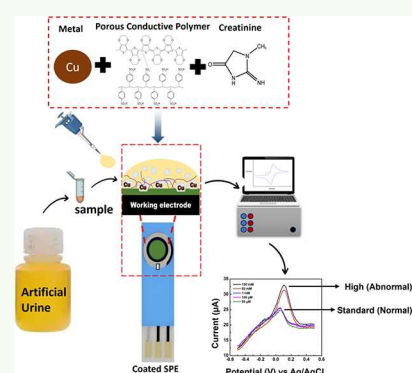
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ABSTRACT: Electrochemical creatinine sensors offer great promise toward rapid, reagent-free, and point-of-care (POC) kidney-function monitoring. However, challenges related to analyte binding, data reproducibility, sensitivity, fouling, and device degradation deter their widespread implementation. Here, we show how a carbon electrode modified with a combination of poly(3,4-ethylene dioxithiophene):poly(styrene sulfonate) (PEDOT:PSS) and copper nanoparticles (CuNPs) can rapidly and accurately detect creatinine (CT) in artificial urine media employing electrochemical techniques. Applying redox potential sweeps (vs Ag/AgCl) using copper sulfate (CuSO_4) solution on such a sensor facilitates the CuNP embedding process inside the conjugated polymer matrix, which has been further validated by supporting techniques. We predicted and validated the formation and contribution of two Cu–CT ion-exchange matrix interactions corresponding to Cu(I) and Cu(II) states, which are responsible for CT detection. The fabricated CT sensor exhibits high selectivity against major artificial urine interferents and is stable for a month, showing minimal degradation (1.87%) in peak current value. Such sensors can be utilized to detect and monitor different stages of renal failure in real-time patient samples.

KEYWORDS: creatinine, copper nanoparticle, conductive polymer, potentiodynamic electrodeposition, sensor fabrication, nonenzymatic electrochemical detection, artificial urine



1. INTRODUCTION

Chronic kidney disease (CKD) is characterized by a persistent alteration in the kidney structure and function, or both, with serious implications on the health of an individual.^{1–4} Individuals affected by kidney diseases encompassing acute kidney injury (AKI), CKD, and post-renal treatment kidney failure exceed 850 million globally.^{5–7} CKD has undeniably emerged as a critical global public priority that is spreading at an alarming rate and therefore requires early diagnosis and intervention to control. From a clinical perspective, the classification and risk stratification of CKD heavily hinge on the estimation of the glomerular filtration rate (GFR) and evaluation of the urine albumin-to-CT ratio (uACR).^{8,9} CT (2-amino-1-methyl-5H-imidazol-4-one) is the final product of creatine phosphate metabolism occurring in muscles, which is responsible for providing energy to muscle tissues and is the most important indicator of different stages of kidney diseases.^{10,11} Quantitative determination of CT in blood serum,^{12,13} urine,^{13,14} and, in recent times, sweat¹⁵ has been a significant clinical measurement factor for the diagnosis of renal dysfunction, muscle damage, and thyroid malfunction.¹⁶ For a healthy individual, the normal GFR ranges 90–100 mL/min/1.73 m², and the overall blood serum (sCr) and urine CT concentrations are within the range of 60–110 $\mu\text{mol/L}$ and 7–16 mmol/L.⁸

Abnormalities reflected in such ranges depict the onset of degradation in the bodily waste product filtration process through kidneys. Therefore, quantification of CT levels is a significant issue for clinical diagnosis that directly reflects on the quality of kidney function.

Historically, the quantification of CT began in 1886 when Max Jaffe discovered the reaction of CT with picric acid in an alkaline environment. This discovery laid the foundation for the Jaffe method, which clinicians and researchers have relied on due to its simplicity and cost-effective CT estimation.^{11,16–17} Apart from that, other conventional methods for CT detection involve high-performance liquid chromatography–mass spectrometry (HPLC–MS),^{18,19} liquid chromatography–tandem mass spectrometry (LC–MS/MS),^{20,21} and electrochemical enzyme-based sensing.^{22–24} HPLC separates CT from other

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metabolites before spectrometric analysis, while LC–MS/MS uses stable isotope dilution for CT measurement.

In Jaffe methods, CT forms a yellow-orange complex after reacting with picric acid in alkaline conditions. The dye concentration is a proportional indication of the CT concentration. However, this technique suffers from major limitations due to the interference of other substances in the sample, lack of specificity, and its being reagent-intensive, driving further costs and complexity.²⁵ Furthermore, the presence of comorbidities such as diabetes and the use of antibiotics or other medications apart from renal failure influence the measured sCr levels, causing delays in therapeutic solutions. In addition, blood collection necessitates a skilled person within a clinical facility or hospital, and the analysis can take several hours to days, impeding diagnosis, especially when the kidney function significantly deteriorates within a short span. Also, in cases of aged patients, clinical complications arise due to blood drawing, which include difficulty finding veins, diminished blood flow due to dehydration, fragile veins that are prone to injury, and reduced blood volume.²⁶ Further, HPLC and LC–MS/MS methods are often time-consuming and require removal of interfering molecules, such as using sodium dodecyl sulfate to eliminate bilirubin interference.^{10,11} Thus, there is an inherent need to develop cost-effective, portable, rapid, and specific methods to quantify CT noninvasively.

In recent times, enzymatic biosensors have offered excellent specificity and selectivity, incorporating enzymes such as CT deiminase (CD), creatine amidohydrolase (CA), and sarcosine oxidase (SO) to catalyze the reaction for CT detection, but have also suffered from constraints such as the complexity in immobilization onto the electrode surface, pH-dependent stability, storage, and high costs.^{10,11,24,27–33} In this context, non-enzymatic sensors offer several advantages such as lower fabrication costs, robust operation conditions, high selectivity and specificity owing to the target material-specific modification of the electrode, and understanding the ion-exchange matrix interactions occurring at the electrode–solution interface.^{34–48} Such reports show targeted employment of hybrid materials, developed from polymer-associated metals or metallic oxides,^{34,35,37,41} metal imprinted polymers (MIPs),^{37,38,42,44} multiwalled carbon nanotubes (MWCNTs),^{39,40,46} metal nanoparticles (Au, Ni, Cu, and Ag),^{35,40,41,45} and quantum dots (CdS),^{47,48} in various forms including thin films, micro/nanofibers, and wires, which have been found to be effective in addressing challenges faced by conventional chemical sensors. In recent times, various research works have incorporated conductive polymers with metal nanoparticles as transducers in electrochemical detection of targeted analytes.^{49–54} However, electrochemical potential sweep modulated metal particle embedding inside the conjugated polymer matrix for electrode modification has recently emerged to be a rapid process of physisorption of targeted materials,⁵⁵ which can be a potential method in fabricating electrochemical CT sensors for CKD monitoring.

In this study, we present an electrochemical surface modification process on a commercially available carbon-based screen-printed electrode (SPE) for the detection of CT in artificial urine media. Initially, a carbon working electrode is modified with PEDOT:PSS conductive polymer to provide a highly conductive network with an abundance of pores/sites on the surface. This is followed by electrochemical doping of such a polymer matrix with CuNPs using potential sweeps. Post the Cu embedding process, we observed different Cu species to

be physically adsorbed in the interstitial sites or pores of the conjugated polymer matrix. We further investigated the embedding process of Cu by employing typical characterization tools. The electrochemical behavior of Cu–CT interactions is also studied in detail using cyclic voltammetry (CV) and differential pulse voltammetry (DPV) techniques. In such voltammograms, two opposite trends have been observed with increasing CT concentration that are correlated to the formation of two Cu–CT interactions. A comprehensive understanding of such interactions could be a potential factor for noninvasive CT detection, which might be utilized to monitor different stages of renal abnormalities, thereby facilitating clinical diagnostic processes.

2. METHODS

2.1. Material and Methods

2.1.1. Reagents. Deionized (DI) water with a verified resistivity of 18.2 MΩ·cm was obtained using a purification system from Millipore, US. Phosphate-buffered saline (PBS) was made from NaCl, KCl, Na₂HPO₄, and KH₂PO₄ and adjusted to pH 7.4 with HCl. CT concentrations were prepared in PBS prior to the experiments. A copper sulfate pentahydrate (CuSO₄·5H₂O) solution of 10 mM was prepared in PBS. Interfering chemicals such as urea, uric acid, NaCl, and L-ascorbic acid were obtained from Merck Sigma Aldrich (Poland). Components for developing in-house artificial urine media (AUM) are summarized in Table S1. Monomer 3,4-ethylenedioxythiophene (EDOT), poly(sodium styrene-4-sulfonate) PSS, and benzoyl peroxide (dibenzoyl peroxide) were also obtained from Merck Sigma Aldrich (Poland).

2.1.2. Sensor Platform. For the experimental platform, carbon SPEs (model: 11L) were obtained from DropSens, MetroOhm (Warsaw, Poland). Each SPE consists of one carbon working electrode (diameter ~ 0.4 cm), one carbon counter electrode, and one silver/silver chloride (Ag/AgCl) pseudo-reference electrode, each connected to individual contact pads through Ag interconnecting lines.

2.1.3. Electrode Modification. **2.1.3.1. PEDOT:PSS Synthesis and Solution Preparation.** For the synthesis of PEDOT:PSS, 3.8 mL of PSS (poly(styrene sulfonate)) was dissolved in 50 mL of deionized water. The resulting solution was divided into two equal parts. In one part, 0.6 mL of EDOT was added. In the other part, 3 g of benzoyl peroxide was added. The two solutions were then combined and stirred together for 24 h inside an incubator with a constant temperature of 37 °C. After 24 h, the resulting mixture was removed from the incubator and allowed to cool down to room temperature.⁵⁶

2.1.3.2. PEDOT:PSS Functionalization and Cu Embedding. For the surface modification of carbon electrodes, 25 μL of as-polymerized PEDOT:PSS was drop-casted on the working electrode of the screen-printed electrodes (Metrohm 11L, DropSens), as shown in Figure S1. Post drop-casting, the SPEs were oven baked at 75 °C for 24 h. Sixty microliters of 10 mM CuSO₄ solution was pipetted on the sensor region followed by five potentiodynamic electrodeposition cycles (cyclic voltammetry) between –1 and +1 V at a scan rate of 100 mV/s. After electrodeposition, the sensor was rinsed thoroughly three times with pure PBS solutions to remove any residual CuSO₄. This procedure ensured the successful embedding of CuNPs into the conductive PEDOT:PSS matrix, resulting in the final fabricated sensor as shown in Figure S2. Prior to analyte testing, the sensors were stored at room temperature under dry conditions. Subsequently, the sensors were connected to a radiofrequency shielded connector attached to a potentiostat to ensure low-noise measurements (Figure S2).

2.1.4. Electrochemical Characterization. Cyclic voltammograms for different samples were acquired using a Bio-Logic SAS (model: VSP) potentiostat by sweeping the potential from –1 to +1 V vs Ag/AgCl at a scan rate of 100 mV s^{–1}. For each sample, five continuous cyclic scans were performed to ensure the data stability. Similarly, differential pulse voltammograms were recorded between

−0.5 and +0.5 V vs Ag/AgCl using a 5 mV step size, pulse width ~100 ms, and pulse height of 2.5 mV. All measurements were performed in three different devices ($n = 3$) to validate device reproducibility.

2.1.5. FESEM and EDX Measurements. For the morphological characterization of the samples, examinations were performed under a high vacuum (pressure 10^{-7} mbar) with a scanning electron microscope (FEI Nova NanoSEM 450). Images were obtained at a long scan acquisition time (20 μ s) of typically 30 s per frame for all the samples after carefully selecting the modification region. The FESEM was equipped with an EDX detector that was used to perform measurements on individual samples to observe any nature of variation in their chemical composition.

2.1.6. XPS Measurements. XPS measurements were performed with a Kratos Axis Supra spectrometer (Kratos Analytical Ltd, UK) equipped with a monochromatic Al K α radiation source (1486.7 eV). All data were collected with an analyzer with pass energy of 80 eV for the survey scan and 20 eV for region spectra. The instrument work function was calibrated to give a BE of 84.0 ± 0.1 eV for the 4f $_{7/2}$ line of metallic gold, and the spectrometer dispersion was adjusted to give a BE of 932.6 eV for the Cu 2p $_{3/2}$ line of metallic copper. The effect of sample charging was reduced by a coaxial neutralization system. The occurring shift of the energy scale was corrected by setting the main component of C1s at the literature value of 284.8 eV for adventitious carbon.⁵⁷

2.1.7. FTIR Measurements. FTIR spectra (in the middle IR spectral range) were measured with a Vertex 80 V (Bruker Inc., USA) Fourier transform infrared spectrometer. The spectrometer was equipped with the specular reflectance accessory GS19650 (SPECAC, UK). To obtain good signal-to-noise ratio, apparatus spectral resolution was maintained to 2 cm^{-1} , the number of scans performed were 1024, and pressure inside the spectrometer was restricted to <6 hPa. To limit the spot size of infrared radiation to the active dimension of the electrode, the angle of incidence / reflection was set to 30°.

2.1.8. AFM Measurements. In this section, experiments were performed by employing a MultiMode 8 atomic force microscope equipped with a J scanner under the control of a NanoScope V

controller from Bruker (Bruker Corporation, Santa Barbara, CA) with PeakForce Quantitative Nanomechanical Mapping (PF-QNM) mode used for sample topography imaging. RTESPA 300 (Bruker) cantilevers of 40 N m^{-1} spring constant and resonance frequency of 300 kHz were used for AFM imaging in air. The shape of the cantilever was rectangular, with a nominal radius <10 nm. Data analysis was performed using NanoScope Analysis 1.8 from Bruker.

3. RESULTS AND DISCUSSION

3.1. Electrode Surface Modification and Cu Nanoparticle Embedding

To develop a CT detection system, we prepared a method whereby CuNPs can be embedded inside the PEDOT:PSS matrix coated on top of carbon working electrodes by employing the potentiodynamic electrodeposition technique. Compared to conventional nonenzymatic CT detectable biosensors (Table 1), this method enables direct and rapid embedding of CuNPs onto the PEDOT:PSS interstitial sites, allowing targeted CT complex formation. Figure 1 depicts the schematic of the present research work initiated by the process of oxidative polymerization of the EDOT monomer to the PEDOT:PSS polymer (Figure 1a and see Methods), which is subsequently drop-casted on top of the carbon working electrode as shown in Figure 1b. After baking the coated devices at 75 °C for 24 h, we performed an electrochemical sweep from −1 to +1 V vs Ag/AgCl at a scan rate of 100 mV/s with phosphate buffer saline (PBS) droplet placed on top of the three electrodes. Such voltammogram is then compared with the reference carbon-PBS voltammogram (Figure S3) that shows an increase in current values for the PEDOT:PSS modified device. PEDOT:PSS functions as a conductive polymeric matrix that, under applied electrochemical bias,

Table 1. Summary of Methods Used, Transducer Material, Nanomaterials For Electrode Modification, Linear Range of Detection, Limit of Detection, Response Time of the Measurements, and Samples Considered for Nonenzymatic Electrochemical Creatinine Sensing

methods used	transducer	nanomaterial	linear range (μM)	sensitivity ($\mu\text{A mM}^{-1}\text{cm}^{-2}$)	LOD (μM)	response time	sample	references
CV/DPV	PAA gel–Cu(II)/Cu ₂ O NPs/SPCE	Cu ₂ O NPs	200–100,000		6.5		urine	34
SWV	rGO/Ag NPs/GCE	Ag NPs, rGO	1–12	0.04	74.3		urine	35
amperometry	CNT–ABTS/Nafion/SPCE	CNTs	0–21,300	27.3	11	50 s	urine	36
amperometry	CuO@MIP/CPE	CuO NPs	0.5–200	0.265	0.083		urine	37
EIS	MIP/carbon paste electrode		0.18–5.92		0.18		human serum and artificial urine	38
DPV	CP/MWCNT–Inu–TiO ₂	TiO ₂ NPs, MWCNTs	0.2–1 50–12,000	0.2	0.06 90	20 s	human serum urine	39
DPV	Ag NPs/MWCNTs/FA/CPE	Ag NPs, MWCNTs	0.01–200		0.008	1.5 s	human serum and urine	40
CV	Cu NPs/PDA–rGO–NB/GCE	Cu NPs, rGO	0.01–100		0.002		human serum and urine	41
DPV/EIS	MIP/Au–SCPE		0.00088–0.00884		0.00014		urine	42
CV	phosphotungstic acid/poly(ethyleneimine)/ITO	C	0.125–62.5		0.06	20 s	urine	43
DPV	MIP/Ni@PANI NPs/MCGE	Ni@PANI NPs	0.004–0.8		0.0002		urine	44
DPV	Fe ³⁺ /CB NPs/SPCE	CB NPs	100–6500		43		urine	45
DPV	Pectin–MWCNT/CPE	MWCNTs	0.016–3.3	3.5	0.6241		urine	46
DPV	CdS quantum dots/PGE	CdS quantum dots	0.442–8840		0.229		human serum and urine	47
DPV	CDs/WO ₃ @GO/GCE	CDs	0.0002–0.112		0.0002		human blood and urine	48
DPV, CV	Cu@PEDOT:PSS	porous polymer matrix	1–100,000	0.13	6	10 s	artificial urine	this work

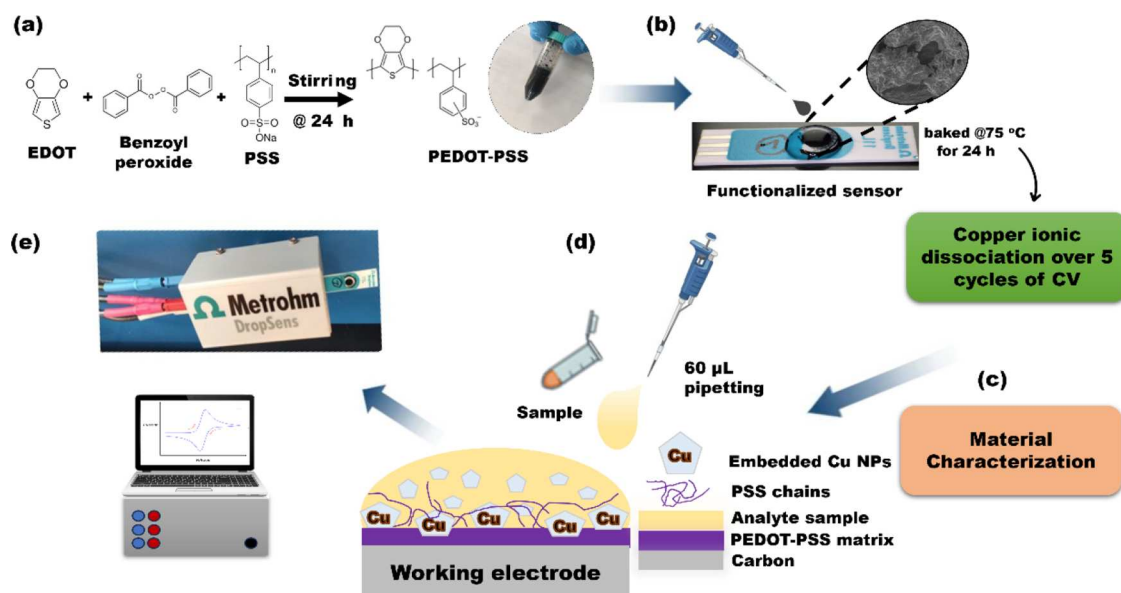


Figure 1. Scheme of the workflow. (a) Synthesis of PEDOT:PSS, (b) modification of SPE with PEDOT:PSS and Cu embedding via electrochemical potential sweeping, (c) characterization of the SPE to confirm embedding of Cu in the conductive polymer matrix, (d) schematic of the sensor region, and (e) electrochemical measurements and output differential pulse voltammogram for CT detection.

enables the electrochemical deposition of metal nanoparticles (e.g., CuNPs) onto its surface and within its porous structure.⁵¹ This incorporation of nanoparticles substantially increases the electroactive surface area, thereby enhancing the electrode's current response observed in voltammetry.

Post PEDOT:PSS modification, the first step is to assess whether Cu can be embedded inside the pores of the polymer matrix. A recent literature survey reports that Cu in various phases (nanoparticles, cuprites, and oxides) can be incorporated in polymers and participates in ion-exchange interactions with CT;^{17,58} however, such processes are quite complicated and expensive. Further, the nature of such interactions is still not clarified yet, which results in discrepancies related to real-time CT detection for CKD monitoring. We addressed this issue by directly embedding Cu inside the PEDOT:PSS matrix where copper sulfate (CuSO_4) mixed with PBS solution is drop-casted on top of the PEDOT:PSS modified sensor and subjected to five electrochemical sweeps (cyclic) from potential (E) -1 to $+1$ V vs Ag/AgCl with a scan rate of 100 mV/s. The next step involves removing CuSO_4 remnants from the electrode's surface through PBS rinsing followed by the acquisition of CV and DPV data in the presence of a bare PBS sample. Further, to test the embedding of Cu inside the PEDOT:PSS matrix, we performed validity field-emission scanning electron microscopy (FESEM), energy-dispersive X-ray spectroscopy (EDX), X-ray photoelectron spectroscopy (XPS), and atomic force microscopy (AFM) experiments, shown under "Material Characterization" in Figure 1c. Following the fabrication of the Cu embedded PEDOT:PSS sensor, 60 μL samples containing different concentrations of CT are placed on top of the three-electrode setup as shown in Figure 1d, and their respective CV and DPV data were acquired (Figure 1e) to assess the variations in output peak current values. From such difference in peak current values for a variety of CT concentrations corresponding to normal (2 – 20 mM) and chronic (>20 mM) renal failure stages, the final CT concentrations in test samples can be validated and recorded to form a clinical database.

CT is electrochemically inactive as suggested by literature reports,^{23,58} which also has been validated in this study by performing CV of pure CT in PBS solution placed on top of bare carbon electrodes (Figure S4). However, certain metals can form complexes with CT inside a solution, thereby turning an electrochemically inactive CT to "electrochemically active" as a complex, which can act as an indirect route to detect and monitor CT levels. Following such route, we performed CT detection in artificial urine media by studying the voltammograms of the tripartite complex using a three-electrode sensor chip. Figure 2a shows a comparative CV of a 60 μL droplet of 10 mM CuSO_4 mixed with PBS in bare and PEDOT:PSS modified electrodes. In addition to the amplification in base current values, both anodic and cathodic scans depict a substantial increment in the oxidation and reduction peaks of Cu in the PEDOT:PSS modified electrode as compared to the bare one. The two oxidation peaks at ~ 0.1 and ~ 0.25 V are attributed to the stepwise oxidation of embedded copper: the first corresponding to $\text{Cu}^0 \rightarrow \text{Cu}^+$ and the second to $\text{Cu}^+ \rightarrow \text{Cu}^{2+}$. These species are stabilized within the PEDOT:PSS matrix, likely via electrostatic interactions with sulfonate groups from PSS. It is also observed from Figure 2a that amplification of the second oxidation peak surpassed the first in contrast to the bare. This increment in the Cu^{2+} -PEDOT:PSS complex peak suggests an increase in the number of Cu^{2+} ions getting attached to the sulfonate chains of PEDOT:PSS. It is imperative to note that PEDOT acts as a conductive and ion-permeable matrix that can retain copper-containing species and facilitate their redox response. This validates the increment in the second oxidation peak in our PEDOT:PSS modified device. The DPV plot of 10 mM CuSO_4 in PBS (Figure S5) also shows two distinct peaks representing both Cu^{1+} and Cu^{2+} ions attached to the PEDOT:PSS matrix. Post the Cu embedding and PBS rinsing process, the acquired DPV spectra of PBS sample, as shown in Figure 2b, also categorically depict both the peaks corresponding to such Cu species, represented by Z I and Z II,

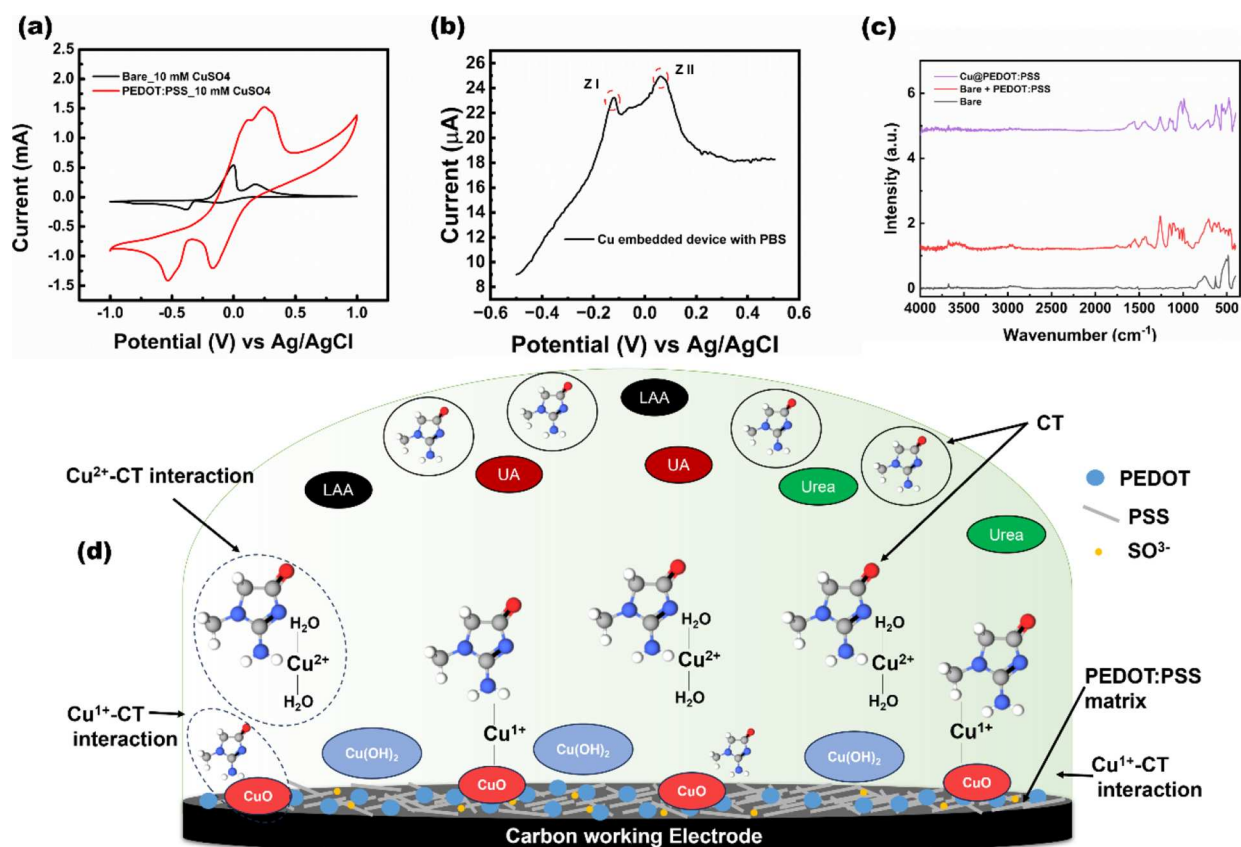


Figure 2. (a) Comparative CV of 10 mM CuSO₄ on bare and PEDOT:PSS modified SPE. (b) DPV voltammogram of a Cu embedded device in PBS. (c) FTIR spectra of the various stages of biosensor fabrication. (d) Schematic of the ion-exchange interactions occurring at the working electrode interface.

respectively. This is attributed to the Cu nanoparticles getting embedded inside the polymer matrix, which would not be washed away even after rinsing the electrode.

3.2. Characterization and Validation of Cu Embedding and Understanding of Different Cu–CT Interactions

Fourier transform infrared spectroscopy (FTIR) of bare carbon, PEDOT:PSS modified, and Cu@PEDOT:PSS devices was performed to assess the presence of any covalent bonds arising due to the formation of such complexes. Figure 2c shows comparative FTIR spectra of such modified layers that depict the formation of their respective bonds. The FTIR spectrum of PEDOT:PSS contains peaks from C–H, S=O, and S–C phenyl bonds in sulfonic acid at 1426, 1463, 1153, 1121, and 1019 cm⁻¹ and peaks from C=C, C–C, and C–S bonds in the thiophene backbone at 1550, 950, 855, and 709 cm⁻¹, respectively.⁵⁹ However, post assessing the detailed summary of all the bonds and their corresponding assignments (Table S2), no new covalent bonds are observed in the Cu@PEDOT:PSS device and after its interaction with CT (Figure S6). The most intriguing part of this CT detection scheme is to have a comprehensive understanding of the formation of different ion-exchange matrices at the working electrode and its interaction with different interfering complexes present in the urine sample. Herein, we focused on the complexities related to an artificial urine sample droplet placed on top of the working electrode of our fabricated sensor device. Figure 2d shows the schematic of such

droplet consisting of major interfering components like urea, uric acid (UA), and L-ascorbic acid (LAA) related to CT detection. The artificial urine medium used in the present work is developed in-house (see Table S1) following standard protocol.⁶⁰ In terms of selective sensing, Cu ions (Cu¹⁺ and Cu²⁺) pose an affinity toward binding with the CT molecule,⁶¹ and it was interesting to observe how Cu¹⁺ and Cu²⁺ ionic species acted in the presence of the CT molecules. We predicted that the most common sources of Cu after CuSO₄ dissociation are copper oxide (CuO), which is expected to be found on the surface of the PEDOT:PSS; copper hydroxide (Cu(OH)₂) molecules inside the solution; and Cu nanoparticles, which are embedded inside the polymer matrix crosslinked at the interstitial sites/pores of the conjugated polymer.^{62,63}

Field emission scanning electron microscopy (FESEM) was performed to understand the morphology of the Cu@PEDOT:PSS matrix, as shown in Figure 3a. Such micrograph depicts a clustered morphology of the conjugated polymer matrix that acts as potential sites containing embedded Cu. As compared to the SEM micrographs of bare and PEDOT:PSS (Figure S7a,b), it is quite evident that such clustered morphology arises due to the PEDOT:PSS matrix being treated with CuSO₄. Post CT addition, such clusters reorient themselves into a globular morphology (Figure S7c), which is attributed to the attachment of CT within the Cu@PEDOT:PSS matrix. Further, energy-dispersive X-ray spectroscopy (EDX) was carried out in situ under the FESEM chamber to verify the presence of Cu, with

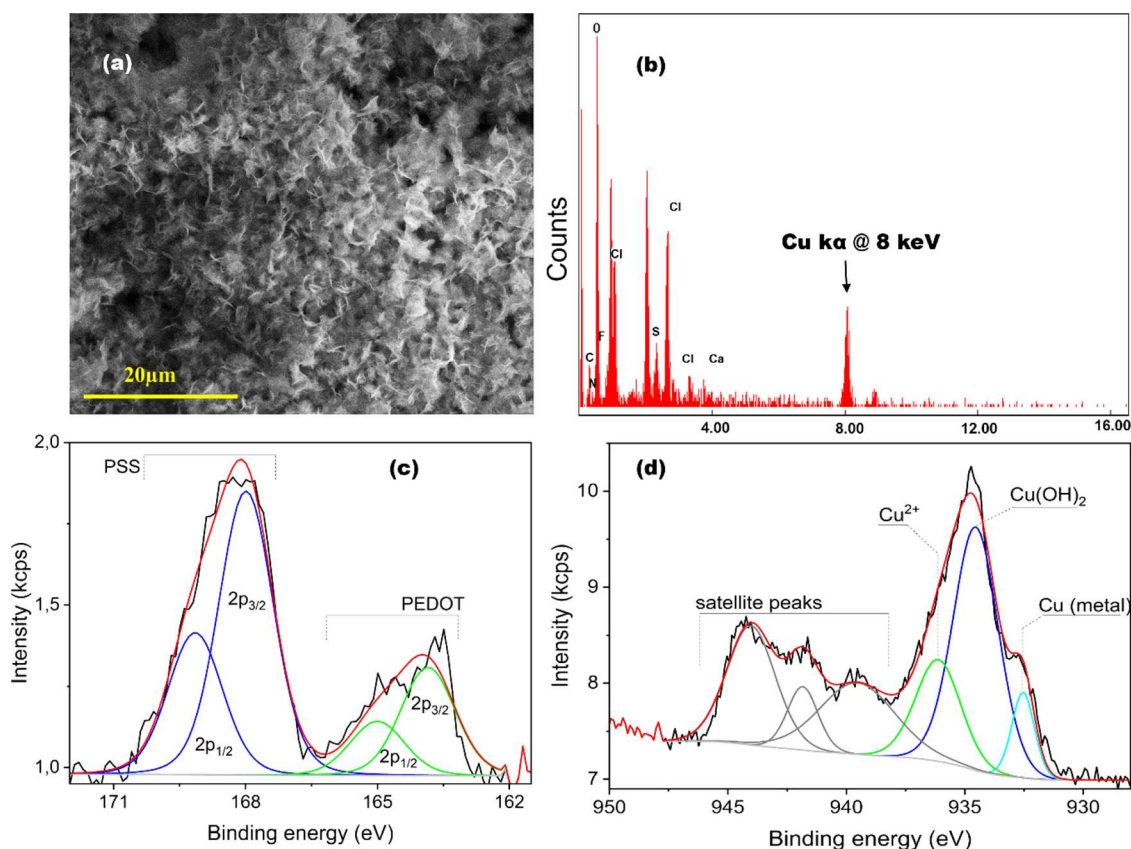


Figure 3. Characterization of the Cu@PEDOT:PSS modified electrode. (a) Field emission scanning electron micrograph of the electrode surface. (b) The EDX analysis shows distinct peaks for Cu at 8 keV. (c, d) XPS measurements and analysis of the modified working electrode.

particular focus on such potential sites. Figure 3b indicates the presence of metallic Cu corresponding to a sharp peak at 8 keV along with the presence of other elements such as Na, Cl, S, and C with their respective sources being PBS, PEDOT:PSS, and the carbon electrode itself. The EDX spectra of Cu@PEDOT:PSS are compared with both the bare and PEDOT:PSS modified surfaces (Figure S7d,e), which depict no such peak at 8 keV, with CT (Figure S7f) showing a slight degradation in such peak. Figure 3c,d shows the X-ray photoelectron spectroscopy (XPS) results for the Cu-embedded sample. In Figure 3c, the typical S 2p signatures of PEDOT at a binding energy of 164 eV and of PSS at 168 eV are observed.⁶⁴ The presence of Cu was analyzed in the Cu 2p_{3/2} region (950–928 eV), as shown in Figure 3d. Peak fitting of the spectra revealed multiple components. Three of them, at binding energies of 932.8, 934.8, and 935.8 eV, are assigned to metallic copper, Cu(OH)₂, and Cu²⁺ species, respectively.⁶⁵ It should be noted that the presence of CuO, which exhibits a similar binding energy, cannot be excluded. However, additional analysis of Auger-type transitions (Figure S8) indicates that the main signal appears at a kinetic energy of approximately 916 eV, which is expected for Cu(OH)₂ or CuSO₄. In contrast, the characteristic signal for CuO should dominate at 920 eV. A weaker but distinct peak at 922 eV is characteristic of metallic copper, confirming its correct assignment in the Cu 2p_{3/2} spectrum. The remaining group of components in the 938–948 eV range corresponds to satellite features that are typically observed for copper in the +2 oxidation state.⁶⁵ It is imperative to note that, although an abundance of

oxidized and free ionic copper species dominates the spectrum, given the experimental nature of the XPS technique and its information depth relations, we assume that these species represent the outer strata of the sample. The sample core remains metallic, as also suggested by the EDX results.

Figure 4a–f further shows atomic force microscopy (AFM) images of the carbon electrode modified with the PEDOT:PSS polymer (a) before and (b) after Cu embedding, while Figure 4c shows the AFM image of Cu@PEDOT:PSS after interaction with CT. Cu trapping in the PEDOT:PSS polymer matrix significantly alters the morphology of this polymer. The morphology of PEDOT:PSS before Cu trapping is a homogeneous porous structure with an average surface roughness (*r.m.s*) of *R_s* = 1.35 nm. The embedding of Cu ions into the polymer results in a significant decrease of the roughness value to *R_s* = 0.76 nm. This is clearly evident in the example cross-sectional profiles shown below each of the AFM images. The resulting Cu@PEDOT:PSS composite exhibits a more compact structure with small, circular pores of ~30 nm in diameter. Further treatment of the Cu@PEDOT:PSS sample with CT caused the polymer morphology to become more granular with a roughness of *R_s* = 0.78 nm. The morphology changes visible on these AFM images are accompanied by viscoelastic changes in the polymer, resulting in an increase in adhesion (Figure 4d–f), suggesting that the polymer became more viscous to the AFM probe after Cu addition. Thus, such analyses validate the successful embedding of Cu inside the polymer matrix, which can be further exploited to detect different CT levels in urine samples.

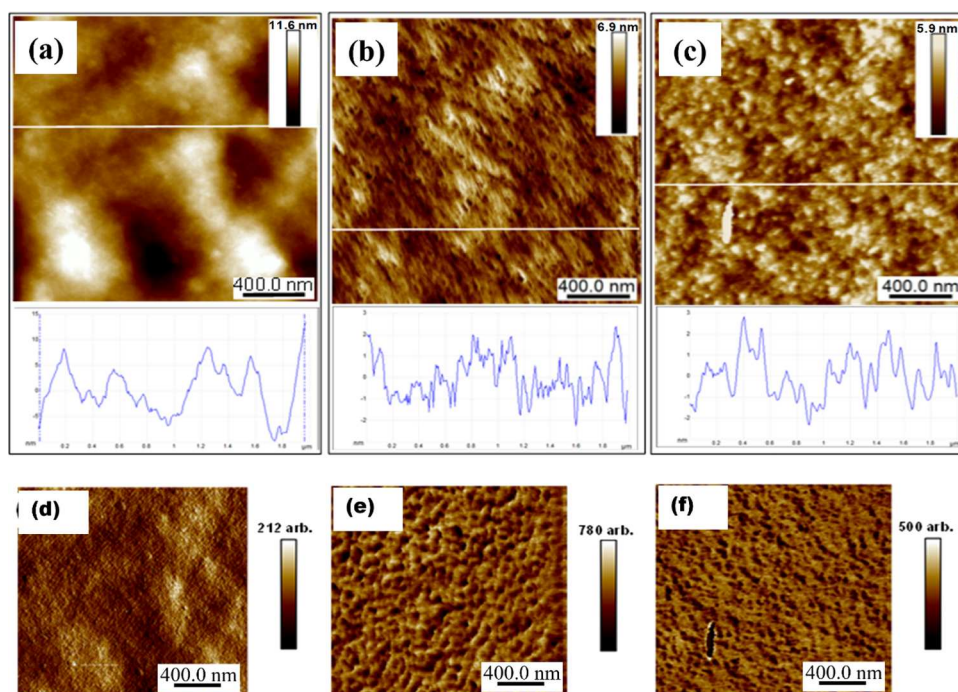


Figure 4. AFM images and corresponding cross-sectional profiles taken along white lines of (a) PEDOT:PSS, (b) Cu@PEDOT:PSS, and (c) Cu@PEDOT:PSS after interaction with CT. (d–f) Corresponding adhesion maps collected simultaneously with the AFM images.

3.3. Electrochemical Detection of Different CT Levels in PBS

We next tested different samples of spiked CT levels in PBS samples employing CV and DPV techniques on-chip. CV and DPV spectra of blank PBS were measured prior to the spiking of calibrated CT concentrations. Further, CT concentration levels such as 20, 40, 60, 80, and 100 mM associated to chronic stages of renal failures are subjected to CV and DPV measurements.

CV of PBS samples spiked from 1 μ M to 100 mM CT (Figure S9) shows distinct alterations in peak current values; however, it is not very specific toward CT level detection. Interestingly, DPV spectra showed distinct changes in output peak current values on addition of different CT levels in both Z I and Z II; however, the nature of change is observed to be opposite. Figure 5a depicts individual DPV plots corresponding to different CT levels, which indicates both the Cu^{1+} –CT and Cu^{2+} –CT interaction peaks for Z I (~ -0.2 V) and Z II (~ 0.15 V). It is also apparent from such figure that there is a gradual disappearance of the oxidation peak current in Z I as compared to Z II, where the increment is prominent with the increase in CT levels. To venture further into the spectra, we deconvoluted both the peaks for Z I and Z II (Figure 5b,c), which show a more clarified picture of the nature of changes occurring at such potentials with the increase of CT levels in PBS.

Deconvoluted Z I shows a slight increment in peak current values ranging from ~ 5 μ A for 20 mM CT to ~ 7 μ A for 100 mM CT in PBS. However, the broadening of the peaks in Figure 5b from 20 to 100 mM is only possible if the number of Cu^{1+} ions is exhausted inside the solution. The steady decrement demonstrates the fact that even if more CT molecules are included inside the solution, the formation of Cu^{1+} ions is restricted as their sources CuO and $\text{Cu}(\text{OH})_2$ were completely exhausted and, therefore, no Cu^{1+} –CT interactions are

occurring henceforth. This phenomenon is further validated by conducting comparative DPV experiments with 1 and 10 mM CT in PBS (see Figure S10), where Z I shows a distinct decrement in peak current for increasing CT level but Z II depicts a completely reversed trend. In Figure 5c, Z II deconvoluted peaks show an increment in current values from ~ 8 μ A for 20 mM CT to ~ 24 μ A for 100 mM CT in PBS with increase in CT concentration, which validates the continuous formation of Cu^{2+} –CT interactions as the source of Cu^{2+} ions originates from inside the polymer matrix. Also, the narrowing of the peaks suggests the availability of more Cu ions that are still embedded inside the polymer matrix that would take part in complex formation. We further investigated the alteration in peak width by calculating FWHMs of the peaks for both Z I and Z II to find a suitable sensor readout. It is observed from Figure 5d that Z I is following a quasi-oscillatory incremental trend with increasing CT concentration, whereas Z II shows an exponential decremental trend, which suggests that FWHM could be a potential to distinguish the respective zones; however, for Z I, it would not be possible to assign FWHM as the sensor readout. On the other hand, Z II makes it suitable for sensing and monitoring of CT levels in a sample that matches our prediction corresponding to Figure 2d. Apart from FWHM, we tried to evaluate the associated charges corresponding to both Z I and Z II complex formations (see Figure S11 and eq S1) to delve into the formation of different charges with CT concentration increment at every step. However, the results were inconclusive and did not shed enough light on the individual charge calculations. Calibration plots with error bars (Figure 5e) also suggest an upright linearity of Z II ($R^2 \sim 0.98$) as compared to Z I ($R^2 \sim 0.82$) for the chronic CT range, which makes Z II a reliable parameter to detect CT levels in PBS. However, careful observation on Z II anodic peaks suggests a slight shift in their potentials (see Figure S12), which could be due to the oxidation

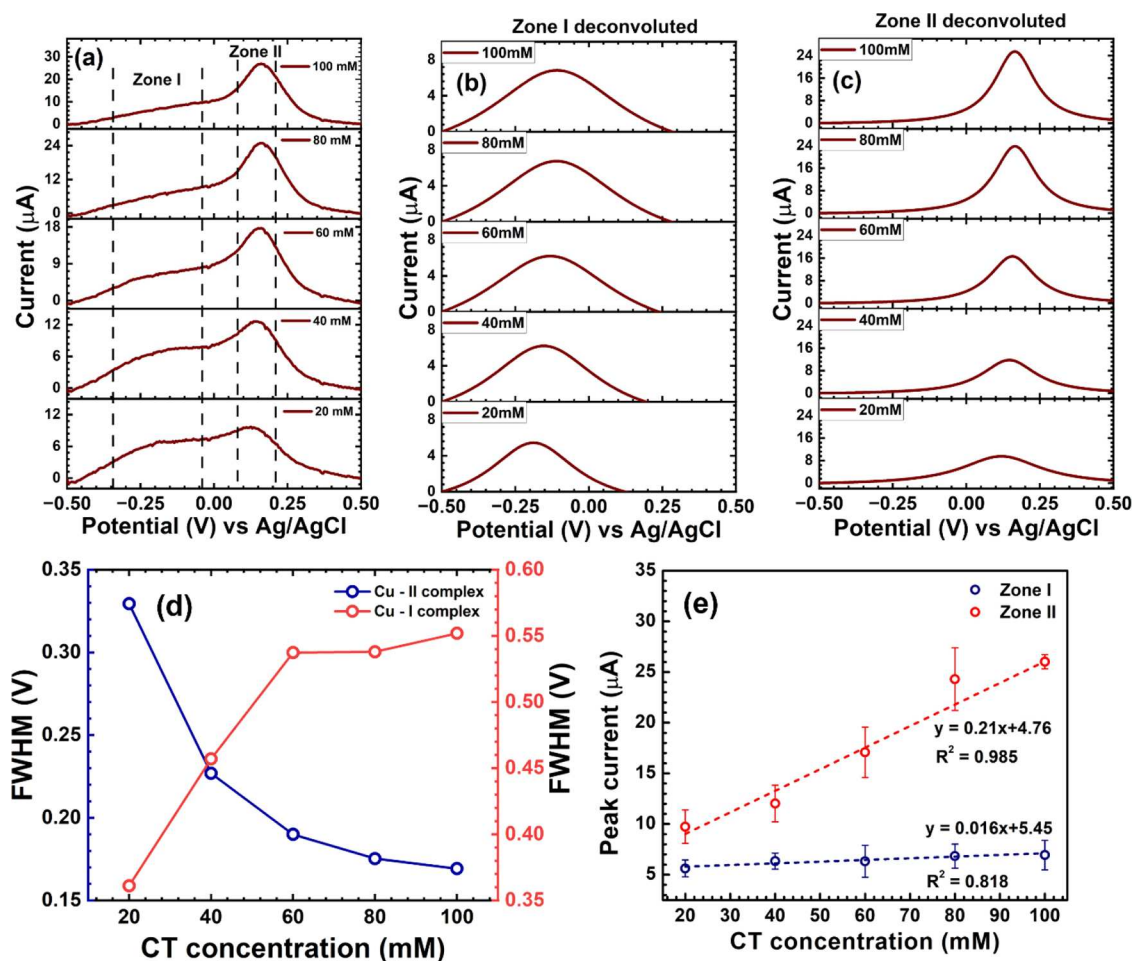


Figure 5. (a) DPV spectra showing zone I and zone II Cu-PEDOT:PSS-CT oxidation peaks for CT concentrations 20, 40, 60, 80, and 100 mM. (b) Deconvoluted DPV peaks in zone I for such concentrations. (c) Deconvoluted DPV peaks in zone I for such concentrations. (d) Full width at half-maximum (FWHM) plotted against CT concentration for zone I and zone II. (e) Calibration curves of zones I and II plotted with data obtained from three different devices ($n = 3$) with error bars and R^2 values showing linearity differences.

mechanism occurring as a result of one-electron transfer from Cu^{1+} to Cu^{2+} during the forward scan. Another potential sensor parameter based on the ratio of peak currents from Z II and Z I, i.e., $I_{Z\text{ II}/Z\text{ I}}$ plotted against CT concentrations could reveal a systematic incremental/decremental trend that can be further explored for a comprehensive understanding of such Cu-CT ion-exchange mechanisms. In this work, we plotted $I_{Z\text{ II}/Z\text{ I}}$ vs 20, 40, 60, 80, and 100 mM CT concentrations (see Figure S13), which shows an incremental trend with the increase of CT concentration, validating our earlier prediction on the increase in the formation of Cu^{2+} -CT interactions.

3.4. CT Detection in Artificial Urine Media with Interference and Stability Studies

Herein we investigated the effect of different reactive species normally coexisting in human urine such as uric acid, L-ascorbic acid, and urea that could act as potential interfering agents during CT detection and monitoring. Based on the previous experimental results and analyses, we selected Z II as the sole CT detection zone for the electrochemical experiments on artificial urine medium henceforth. DPV voltammograms of AUM in the presence of interfering components such as urea, uric acid, and L-ascorbic acid show its representative peak at ~ 0.14 V

(Figure 6a), which is attributed to the CT level that is already present in the AUM. However, when AUM is spiked with CT concentrations such as 25, 50, 75, and 100 mM, the potential shifts to ~ 0.16 V with an increase in peak currents. The increase in peak currents and shifting of the peak potential occur due to the repeated addition of CT along with the constant formation of Cu^{2+} -CT interactions due to the generation of more Cu^{2+} ions during forward anodic scans.

It is imperative to note that the addition of CT in artificial urine media is altering the peak corresponding to CT and that no other new peaks are observed in the selected potential range. Further, the calibration plot of the current values (Figure 6b) shows linearity ($R^2 = 0.98$) with the increase of CT concentration, which validates the efficiency of the detection process. The sensitivity and limit of detection (LOD) of the experimental process are obtained to be $0.13 \mu\text{A mM}^{-1} \text{ cm}^{-2}$ and $6 \mu\text{M}$, respectively, which fit well within the scope of previously reported results. The LOD is calculated using the standard formula $\text{LOD} = 3\sigma/\beta$, where σ is the standard deviation of the response at the lowest concentration of CT (1 and 5 μM) and β is the sensitivity factor of the calibration curve. The sensitivity factor represents dy/dx , i.e., the change in current (I)/change in CT concentration as extracted from the experimental

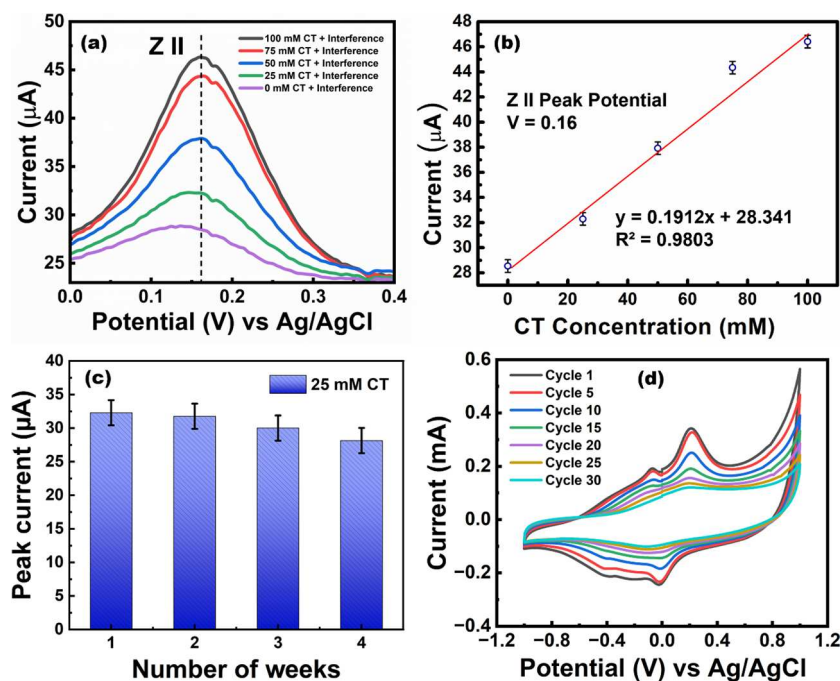


Figure 6. Interference and stability of the fabricated biosensor. (a) DPV spectra of AUM along with potential interfering components: urea, L-ascorbic acid, and uric acid, spiked with 25, 50, 75, and 100 mM CT. (b) Calibration plot with error bars showing linearity of the measured current values for such CT concentrations at Z II peak potential ~ 0.16 V. (c) Electrode stability plot showing peak current variation with time measured for 25 mM CT in three devices ($n = 3$). (d) Cyclic voltammograms of PBS on Cu@PEDOT:PSS sample showing 1th, 5th, 10th, 15th, 20th, 25th, and 30th cycles.

data for different CT concentrations. Further, to obtain the necessary data repeatability results, one device is subjected to DPV measurement every week for 4 weeks (Figure 6c). The resulting variation in peak current is observed to be 1.87% after 1 month, which shows excellent electrode stability toward repeated usage. Another study was conducted to virtually peek into the possible quantity of Cu ions embedded inside the conjugated polymer matrix where the 1st, 5th, 10th, 15th, 25th, and 30th cycles out of a total of 30 cycles of redox CV spectra were shown for PBS sample drop-casted on a Cu embedded device (Figure 6d). Such plots reveal a gradual decrement in peak current for both Z I and Z II corresponding to a proportional exhaustion in both Cu^{1+} and Cu^{2+} ions with every passing cycle. This degradation is attributed to the constant formation of both Cu^{1+} and Cu^{2+} matrices inside the system with the increase in cycles. However, the device still seems to work up to 20 cycles, showing the corresponding peaks and thereby depicting the stability of the electrode modification as well as the amount of Cu ions embedded inside the polymer matrix to sustain these electrochemical cycles. The results from this CV can propel further research to determine the exact number of Cu ions embedded in the polymer matrix for further strengthening the reliability factor on the detection and monitoring of CT levels. Based on our findings, we have summarized several recent works toward the noninvasive detection of CT, and their key features are shown^{34–48} in Table 1 for comprehensive state-of-the-art comparison.

4. CONCLUSIONS

In conclusion, we have successfully fabricated a Cu-embedded PEDOT:PSS modified carbon sensor for the detection of CT

in artificial urine media employing electrochemical techniques. A simple five-cycle potentiodynamic electrodeposition using CuSO_4 solution on top of PEDOT:PSS modified carbon electrode facilitates the Cu embedding process inside the polymer matrix. Evidence of Cu embedding at the interstitial sites/pores of the conjugated polymer is obtained from EDX, XPS, AFM, and electrochemical experimental data. We predicted the formation of two Cu^{1+} –CT and Cu^{2+} –CT ion-exchange interactions inside the sensor zone, which is validated from the electrochemical measured data. PEDOT:PSS modification of the carbon electrode provides a highly conductive network with an increase in the number of pores/sites on the surface to accommodate different Cu ionic species. Cu^{1+} –CT is observed to gradually decrease with increasing CT concentration, whereas Cu^{2+} –CT increases, thereby proving the latter to be a perfect candidate for CT detection in real-time CT detection and monitoring systems. The ratio of peak currents from Z II and Z I ($I_{Z\text{ II}/Z\text{ I}}$) depicts a steady incremental trend, which also validates our zone of choice. The FWHM study of the oxidation peaks for different CT concentrations shows significant potential toward CT detection as compared to the determination of associated ion charge for Z II (Cu^{2+} –CT). Interference experiments show the detection of inherent CT in artificial urine media with no unwanted interference signals from the interfering components. Further spiking the artificial urine media with CT concentrations increases the DPV peak current corresponding to the Cu–CT complexes. Device stability and repeatability studies also confirm that such device can be used for at least a month or more with minimal degradation (1.87%) in experimental peak current value. Therefore, such novel yet simple concept of a metal-embedded conjugated polymer modified electrode can be further used to fabricate biomedical sensor devices for CKD monitoring in patients.

■ ASSOCIATED CONTENT

Data Availability Statement

The data that support the findings of this study are available from the corresponding authors upon request.

SI Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acsaelm.6c00572>.

Pictures of consumables, sensor and experimental setup, additional electrochemical CV and DPV plots, FTIR plot and table, FESEM and EDX plots, artificial urine constituents table, Cu L₃M_{4,5}M_{4,5} Auger spectra, associated charge plot, and combined readout plot (DOCX)

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Notes

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CV	cyclic voltammetry
DPV	differential pulse voltammetry
SWV	square wave voltammetry
EIS	electrochemical impedance spectroscopy
PAA	polyacrylic acid
NPs	nanoparticles
SPCE	screen-printed carbon electrode
rGO	reduced graphene oxide
GCE	glassy carbon electrode
CNT	carbon nanotube
ABTS	2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)
MIP	molecularly imprinted polymer
CPE	carbon paste electrode
CP	carbon paste
MWCNT	multiwalled carbon nanotube
Inu	inulin
FA	folic acid
PDA	polydopamine
NB	Nile blue
SCPE	screen-printed carbon paste electrode
ITO	indium tin oxide
Ni@PANI	nickel nanoparticles embedded in polyaniline
MCGE	modified carbon graphite electrode
CB	carbon black
CdS	cadmium sulfide
PGE	pencil graphite electrode
CDs	carbon dots
PEDOT:PSS	poly(3,4-ethylenedioxythiophene):polystyrene sulfonate

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