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Assessment of Urea Concentrations in Human Blood Utilizing a Graphite Electrode modified with copper/4,4-Nitrobenzylpyridine Nanoparticles

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Abstract:

This research introduces an innovative electrochemical sensor made of copper nanoparticles and 4,4-nitrobenzylpyridine electroplated onto bare pencil graphite electrode (PGE) for the detection of urea in human blood.The electrochemical films were decorated onto the graphite electrode by cyclic voltammetry technique at low scan rates, and films exhibiting both chemical and mechanical stability were obtained. The existence of nanostructured layers in the structural texture of the sensor was confirmed by scanning electron microscope (SEM). Using the square wave voltammetry (SWV) In monitoring mode, a linear electrochemical response to urea was observed over a concentration range of 30 to 300 nanomolar (nM), and a detection limit of 9.79 nM was determined. Furthermore, this innovative electrochemical sensor with graphite/cu nanoparticles/4,4-nitrobenzylpyridine structure was successfully validated for the quantitative determination of urea concentration in human blood serum samples, showing high sensitivity and accuracy without significant interference.

Keywords: Cyclic voltammetry, square wave voltammetry, Modified electrodes, Electrodeposition, Electro polymerization, Copper nanoparticles, 4,4-nitrobenzylpyridine, Urea.

Introduction

Urea ($CO(NH_2)_2$) It is a critical compound widely used in the chemical and agricultural fertilizer industries, and annual production is projected to reach 332 million tons by 2030 [1]. The industrial production of urea involves two main processes: (1) the Haber-Bosch process, which operates at temperatures of 350–550 °C and pressures of 150–350 bar for the synthesis of ammonia (NH3) [2], and (2) the Bosch-Messer process, which operates at 150–200 °C and 150–250 bar for the reaction between NH3 and carbon dioxide [3]. High urea levels can result from high-protein diets, the use of anti-anabolic drugs, and gastrointestinal bleeding, Conditions such as starvation, malnutrition, liver disease resulting in reduced liver metabolic activity, or genetic defects in urea cycle enzymes can lead to low blood urea concentrations. Currently, the most common and reliable method for determining urea levels is laboratory testing in a hospital or pathology diagnostic center. Although this method has an accuracy rate of more than 2%, it is time-consuming, relatively expensive, and requires qualified personnel and modern equipment [4]. Traditional techniques such as high-performance liquid chromatography (HPLC) and gas chromatography (GC) are combined with spectroscopic methods such as infrared spectroscopy. (IR) and Raman spectroscopy and para-dimethylaminobenzaldehyde (DMAB)-based wet chemistry tests are wellestablished approaches to detect milk urea. However, their disadvantages, such as high costs, long procedures, and environmental concerns related to chemical use, have encouraged the search for more efficient, user-friendly, and rapid detection methods [5]. In contrast to other detection methods such as spectrophotometry, gas chromatography, and HPLC, electrochemical sensors have gained interest in various fields from healthcare to environmental monitoring due to their desirable qualities such as portability, cost-effectiveness, fast response times, and high sensitivity [6,7]. These sensors convert chemical reactions of target substances into electrical signals through changes in potential, current, impedance, and conductivity [8]. Graphite pencil electrodes (GPEs) are carbon-based electrodes known for their affordability, simplicity, widespread availability, ease of modification, and disposable nature. GPEs serve as attractive substrates for electrochemical sensing due to their unique disposable nature, which distinguishes them from other commonly used carbon-based electrodes. Their mechanically rigid structure allows for simple modification and miniaturization. The sensitivity and selectivity of GPEs towards specific analytes can be improved by applying various modification materials. This review article primarily aims to highlight the applications of GPEs in analyzing inorganic and organic pollutants in different environmental matrices. It provides a concise overview of various inorganic and organic pollutants and their environmental impacts. Additionally, the review critically evaluates the key features of modified GPEs that enhance their electrochemical activity to detect specific target analytes [9] .

The distinctive chemical and physical characteristics of nanoparticles render them highly effective for the development of innovative and enhanced sensing devices, particularly in the realm of electrochemical sensors and biosensors. Various types of nanoparticles, including metallic, oxide, and semiconductor nanoparticles, have been utilized in the fabrication of electrochemical sensors and biosensors, each serving different functions within diverse sensing frameworks. Key roles of nanoparticles encompass the immobilization of biomolecules, facilitation of electrochemical reactions, improvement of electron transfer between electrode surfaces and proteins, biomolecule labeling, and even functioning as reactants

In this research, we aimed to develop a novel electrochemical sensor made of copper nanoparticles (CuNPs) and 4,4-nitrobenzylpyridine, which were electrodeposited onto a bare pencil graphite electrode (PGE). The beneficial synergistic effect between the copper nanoparticles and 4,4-nitrobenzylpyridine resulted in the formation of chemically and mechanically stable Nano films at low scan rates, utilizing the cyclic voltammetry technique on the surface of the pencil graphite electrode. This newly developed sensor, designed as PGE/CuNPs/4,4-nitrobenzylpyridine, accurately quantifies urea concentrations at Nano molar levels in human blood serum samples, demonstrating high precision and minimal interference.

2 .Experimental

2.1 . Instrumentals and Chemicals

Voltammetry experiments were conducted using a potentiostat model N302PGSTAT manufactured by Metrohem. Measurements were recorded using NOVA 1.10 software on a Windows operating system. This software facilitates automatic control of the potentiostat. All graphs and calculations were performed using Excel software. The electrochemical experiments were carried out in a three-electrode system comprising a calomel reference electrode (Ag/AgCl) (diameter 50 mm), a platinum wire (diameter 2 mm) serving as the carrier and sensor electrode, and a graphite electrode modified with copper nanoparticles and 4,4-nitrobenzylpyridine, which functioned as the working electrode. A pH meter model 710 manufactured by Metrohem was employed to adjust the required pH and to prepare identical solutions in an ultrasonic bath. Nitrate salts of metal ions, copper nitrate, vitamins, sugars, 4,4-nitrobenzylpyridine as a surface modifier, and urea powder were procured from Merck or Sigma-Aldrich. All preparation and solubilization steps were conducted using deionized water. To prepare the Brayton-Robinson (B-R) buffer, a precise amount of 0.2 M sodium hydroxide solution was added to 0.04 M solutions containing a mixture of boric, acetic, and phosphoric acids. It is noteworthy that 500 milimolar (mM) solutions of each of these substances were prepared separately in double distilled deionized water in 50 mL flasks and subsequently utilized to create more dilute solutions.

2.2 . Sensor Construction

In this study, the cyclic voltammetry (CV) method was employed to fabricate the sensor. The procedure is as follows: To prepare the electrode surface for modification, the bare surface of the electrode was meticulously cleaned on regular white paper, and a flat surface of pencil lead graphite was created by rubbing it with the paper to enhance the contact surface area between the electrode and the analyte. The electrode was then completely covered with appropriate Teflon tape to prevent any solution from contacting the side surface of the electrode. One of the free ends of the graphite electrode was connected to the Autolab device using a secure copper wire connection, while the top surface of the electrode was left exposed for deposition. Subsequently, the bare graphite electrode was immersed in a Brayton-Robinson buffer solution with a pH of 11, and a potential sweep in the range of -1.2 to +1.2 volts was performed using the cyclic voltammetry technique to clean the electrode surface. Afterward, the electrode was removed from the solution, thoroughly washed with distilled water, and dried. To fabricate the sensor, 15 mL of a 50 mM solution of copper nanoparticles was transferred into the cell of the Autolab device. By applying 14 cyclic voltammetry sweeps in the range of -1.2 to +0.4 volts at a scan rate of 30 mV/s, the graphite electrode surface was modified to form a composite of graphite and copper nanoparticles. Following the completion of the modification layer, the modified electrode surface was washed with distilled water and dried. In the subsequent step, the graphite/copper nanoparticle electrode was immersed in a 30 mM 50% (v/v) water-alcohol solution. By applying 10 consecutive cycles of cyclic voltammetry in the range of -1.2 to +1.2 V at a scan rate of 50 mV/s, the target electrode with the structure of graphite/copper nanoparticles/4,4-nitrobenzylpyridine was produced for optimal chemical conditions for urea measurement.

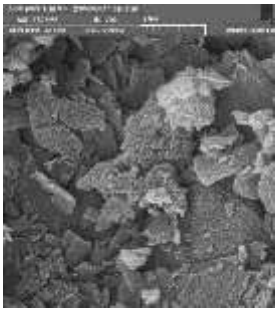
2.3 .Preparing the Real Sample

A 1.5 mL sample of human blood was collected and mixed with 2 mL of concentrated ethanol, which facilitated the precipitation of proteins in the blood sample through shaking. To further separate the proteins, the solution was centrifuged for 3 minutes at 6000 rpm. The blood serum was then passed through a filter with a pore size of 0.45 μm to ensure complete separation of the precipitated proteins. The filtered volume of blood serum was diluted with 25 mL of distilled deionized water, and 0.5 mL of the diluted blood serum was transferred into the cell of the Autolab device. This setup was optimized under mechanical and chemical conditions, and using the square wave voltammetry technique, the urea concentrations in human blood were measured in the presence of a urea-sensitive sensor. The external standard addition method was employed at pH 7, optimized using the Britton-Robinson buffer, to validate the response of the urea-sensitive sensor.

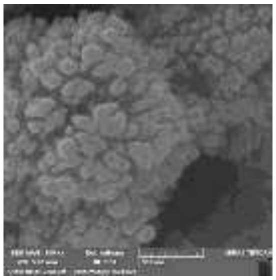
3. Results and Discussion

3.1. Electrochemical Characterization

Scanning electron microscopy (SEM) serves as a powerful technique for examining surface morphology and confirming the successful deposition of electrode structures. In this context, the surfaces of the bare graphite electrodes (Figure 1 A), the graphite electrode coated with copper nanoparticles (Figure 1 B), and the graphite/copper nanoparticles/4,4-nitrobenzylpyridine electrode (Figure 1 C) were imaged using a scanning electron microscope. For the bare graphite electrode, an unmodified surface characterized by graphite layers was observed. However, upon modification with copper nanoparticles, distinct clusters of copper nanoparticles were detected, which were subsequently confirmed following the electro polymerization of 4,4-nitrobenzylpyridine on the surface of the copper nanoparticles (Figure 1 C). Therefore, the successful fabrication of a graphite electrode featuring a composite film of copper nanoparticles and 4,4-nitrobenzylpyridine, which exhibits an enhanced effective surface area for electrochemical reactions, has been conclusively demonstrated.



(A)



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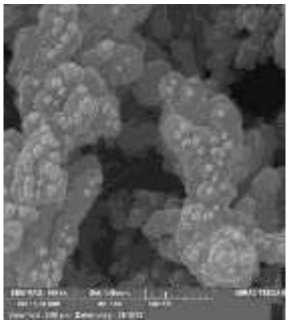


(C)

Fig. 1 SEM images of the electrode surfaces of A) bare PGE B) PGE/ CuNPs and C) PGE/CuNPs/4,4-nitrobenzylpyridine.

3-2 Investigation of the Electrochemical Behavior of Urea on the Electrode Surface

To elucidate the electrochemical response of the modified bare graphite electrode incorporating copper nanoparticles and 4,4-nitrobenzylpyridine, the cyclic voltammetry technique was employed. The voltammogram for these electrodes was recorded in the presence of 120 micro molar (μM) urea, with the results depicted in Figure 2. Notably, the bare graphite electrode exhibited the lowest response intensity, while the maximum current response due to urea oxidation was observed on the graphite electrode modified with copper nanoparticles and 4,4-nitrobenzylpyridine at a potential of 0.01 V vs the Ag/AgCl electrode. This enhancement in signal intensity can be attributed to the high electronic conductivity of copper nanoparticles and their synergistic interaction with both 4,4-nitrobenzylpyridine and urea. Copper, as an electrode material, is particularly advantageous for the analysis of carbohydrates and amino acids [11-14], as it facilitates amperometric detection at a constant potential under high pH conditions. When compared to noble metals such as platinum (Pt), gold (Au), nickel (Ni), silver (Ag), and cobalt (Co), copper has been reported to exhibit superior performance in terms of response range, detection limit, and stability [11]. The oxidation of carbohydrates using Au or Pt electrodes in direct amperometric detection modes often encounters significant electrode poisoning, necessitating the use of pulsed amperometric detection to mitigate this issue [15]. Furthermore, copper-based microelectrodes have been effectively employed in capillary electrophoresis for carbohydrate analysis [16]. Additionally, 4,4-Nitrobenzylpyridine was initially developed as a colorimetric probe for monitoring alkylation reactions, particularly in the context of the toxicology of alkylators within the human body [17]. The reactive site in 4,4-Nitrobenzylpyridine demonstrates nucleophilicity akin to that of guanine, which is recognized as the most reactive site in DNA with respect to alkylating toxins. The 4,4-Nitrobenzylpyridine structure has also been integrated into various sensor designs. For instance, Zhao et al. reported a strategy for the colorimetric detection of chromium (III) in wastewater utilizing 4,4-Nitrobenzylpyridine structures modified on gold nanoparticles [18]. Given the observed electrochemical signals, the electrode featuring the graphite/copper nanoparticles/4,4-nitrobenzylpyridine configuration was identified as the optimal electrode for subsequent electrochemical measurements and urea quantification.

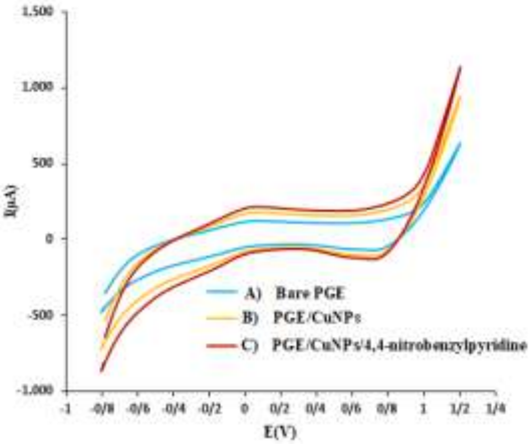


Fig 2. Cyclic voltammogram related to the response of 120 μM urea in Britton-Robinson buffer pH 7 (a) bare graphite electrode: (PGE) (b) graphite electrode / copper nanoparticles: (PGE/CuNPs) (c) graphite electrode/copper nanoparticles/4,4-nitrobenzylpyridine: PGE/CuNPs/4,4-nitrobenzylpyridine.

3.3 The Impact of Variations in Urea Concentration on Cyclic Voltammogram Behavior

In this investigation, the concentration of urea in the Britton-Robinson buffered solution at pH 7 was systematically varied from 0 (no urea) to 120 μM, and the corresponding cyclic voltammograms were recorded. The results, illustrated in Figure 3, confirm the electrocatalytic behavior of the PGE/CuNPs/4,4-nitrobenzylpyridine composite in its reaction with urea, thereby facilitating effective electrochemical measurement.

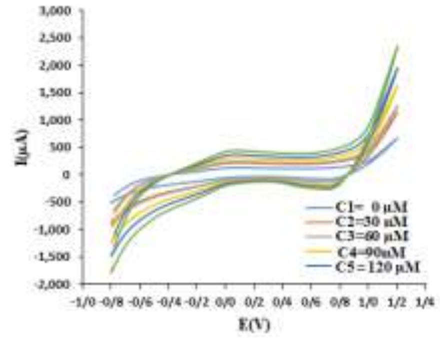


Fig 3. Cyclic voltammograms recorded at different urea concentrations on the surface of the PGE/CuNPs/4,4-nitrobenzylpyridine.

3-4 pH Effect

The electrochemical behavior of the system under study is significantly influenced by the properties of the solvent, including proton retention and buffer capacity. Consequently, the selection of an appropriate solvent is crucial. Buffers are routinely employed in various electrochemical methods, serving both as suitable solvents and as carrier electrolytes. In this study, the Brayton-Robinson buffer was utilized, and the cyclic voltammograms corresponding to the response of the PGE/CuNPs/4,4-nitrobenzylpyridine to 120 μM urea at varying pH values from 4 to 11 were thoroughly investigated. The maximum response signal was recorded at pH 7 (Figure 4A). Thus, pH 7 was established as the optimal pH within the Brayton-Robinson buffer medium for subsequent experiments. Furthermore, the linear relationship between pH variations and the applied potential was quantified (Figure 4B). The slope of 0.037 indicates the non-equilibrium state of electrons and protons exchanged during electrochemical reactions.of urea on the PGE/CuNPs/4,4-nitrobenzylpyridine platform [19].

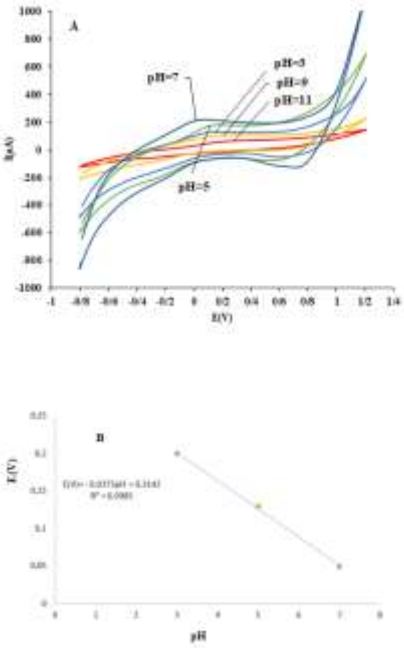


Fig 4 A) Cyclic voltammogram of the response of PGE/CuNPs/4,4-nitrobenzylpyridine towards 120 µM urea at different pH values B) Linear changes of potential versus pH.

3.5 Effect of Scan Rate

In the context of a PGE/CuNPs/4,4-nitrobenzylpyridine electrode, the influence of potential sweep rates ranging from 25 to 250 mV/s on the electrochemical response of 120 µM urea was systematically investigated under optimized chemical and instrumental conditions. Cyclic voltammograms were recorded at the specified sweep rates, with the results illustrated in Figure 5(A). A notable observation was that as the scan rate increased, the intensity of the recorded current signal correspondingly increased. Moreover, the relationship between current changes and scan rate within the range of 25 to 250 mV/s exhibited linear behavior, suggesting that the electrochemical behavior of urea is governed by an adsorption-controlled mechanism on the electrode surface (Fig 5B). Consequently, utilizing the Laviron equation [20], the electron transfers coefficient (α) was calculated to be 0.78, derived from the slope of the potential change curve in relation to the logarithm of the scan rate (Fig5C). Additionally, the logarithm of the rate constant (LogKs = 3.34) and the concentration of the electroactive layer deposited on the electrode surface (3.2×10^{-8}) were also determined from the slope of the current curve concerning the scan rate (Equations 1 and 2):

$$\text{Log } k_s = \alpha \log (1-\alpha) + (1-\alpha) \log \alpha - \log (RT/nFn) - \alpha (1-\alpha) nFE/2.3RT$$

(Eq.1)

$$I_p = \frac{n^2 F^2 v A \Gamma_c}{4RT}$$

(Eq.2)

The electrochemical parameters obtained indicate a favorable electron transfer rate, a high kinetic rate constant, and exceptional electrocatalytic properties of this innovative sensor for urea measurement.

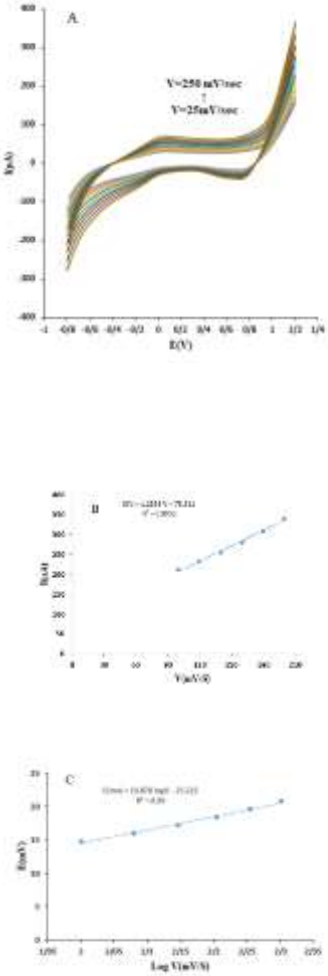
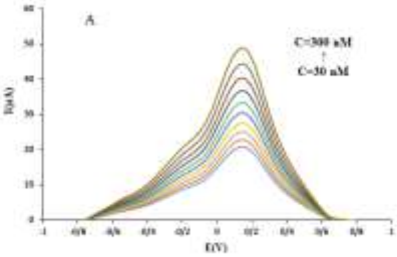


Fig 5 A) Cyclic voltammogram of sensor towards 120 µM urea at different scan rates B) Linear behavior of current as a function of scan rate C) Linear behavior of potential as a function of the logarithm of scan rate.

3.6 Calibration Curve, Repeatability, and Reproducibility of the Method for Urea Measurement

The optimized chemical conditions were established at pH 7, with optimal device parameters set to a 55-mV potential step, a 50-mV potential intensity, and a 60 Hz frequency for recording square wave voltammograms. Square wave voltammetry spectra for urea solutions with varying concentrations from 10 to 400 Nano molar were recorded by applying potential sweeps in the range of -0.1 to +0.1 volts. Ultimately, a calibration curve for urea in the presence of PGE/CuNPs/4,4-nitrobenzylpyridine sensor was constructed, revealing a linear current response in the concentration range of 30 to 300 nanomolar. The results are depicted in Figures 6 A and B, with a limit of detection LOD of 9.97 Nano molar and a limit of quantification LOQ of 29.98 nanomolar. Furthermore, the repeatability of the method was assessed by evaluating the response of the modified electrode with PGE/CuNPs/4,4-nitrobenzylpyridine structure to 120 Nano molar urea through 10 consecutive measurements using square wave voltammetry, yielding a relative deviation error of 4.73%. The reproducibility of three separate electrodes responding to 120 Nano molar urea was also measured, resulting in an error value of 4.56%. These findings affirm that the proposed method is both repeatable and reproducible



Interferer	Tolerated Ratio [Interference] ∕[Urea]	Interferer	Tolerated Ratio [Interference] ∕[Urea]
Cu^{2+}	36	Mn^{2+}	48
Zn^{2+}	65	Fe^{3+}	32
Co^{2+}	51	Fe^{2+}	34
K^{+}	97	Glucose	57
Vitamin A	128	Ammonia	62
Vitamin B12, B6, B5	≥ 42	Vitamin C	29

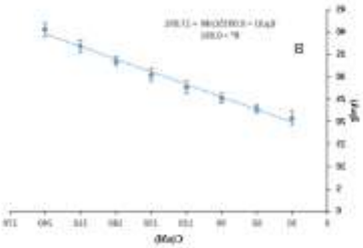


Figure 6 A) Square wave voltammogram in presence of urea different concentrations on the surface of PGE/CuNPs/4,4-nitrobenzylpyridine sensor B) Linear calibration curve of current vs concentration.

3.7 Interference effects

This study examined the interference effects of various concentrations of organic and inorganic materials, including cations, anions, vitamins, and amino acids, on the electrochemical response behavior of PGE/CuNPs/4,4-nitrobenzylpyridine sensor towards 120 Nanomolar urea, utilizing square wave voltammetry (Table 1). A permissible interference limit of 5% was established. The results indicate that the materials listed in Table 1 did not exhibit any significant interference effects on the sensor's behavior towards urea. Consequently, this study confirms that the modified PGE/CuNPs/4,4-nitrobenzylpyridine electrode is capable of accurately detecting urea in real samples.

Table 1. Effect of interference from various organic and inorganic species on the response of the new sensor to a concentration of 120 Nano molar urea.

3-8 sensor validation

To assess the efficacy of the proposed method for the electroanalytical determination and quantification of urea concentration in real human blood samples, the modified electrode featuring a PGE/CuNPs/4,4-nitrobenzylpyridine structure was successfully employed using the square wave voltammetry technique. The human serum samples were validated by systematically injecting varying amounts of urea under optimal chemical and instrumental conditions, utilizing the standard addition method in conjunction with the introduced sensor. The results, as detailed in Table 2, revealed a relative standard deviation of less than 5.5%, indicating satisfactory precision and reliability. The developed sensor demonstrated a robust capability to accurately measure urea concentrations in real samples, such as human blood serum, with commendable analytical accuracy and precision.

Sample	Spiked value (nM)	Found value (nM)	Recovery %
Sample 1	60	63.21±3.37 (RSD%=5.33)	105.35
Sample 2	90	94.63±4.96 (RSD%=5.24)	105.14
Sample 3	120	114.21±5.52 (RSD%=4.83)	95.17
Sample 4	150	156.98±7.23 (RSD%=4.60)	104.65

Table 2. Investigation of the efficiency of the new electrochemical sensor in determining urea concentration in human blood serum samples by square wave voltammetry (five repetitions).

4.Conclusion

In this study, the square wave voltammetry technique has been established as a highly sensitive analytical method for measuring urea concentrations in human serum samples. By coating a bare graphite pen electrode with copper nanoparticles followed by 4,4-nitrobenzylpyridine, a novel sensor with a PGE/CuNPs/4,4-nitrobenzylpyridine architecture was developed. This electrode exhibits an exceptionally low detection limit, making it suitable for precise urea measurement in human serum samples. A significant advantage of this method lies in the creation of a new electrochemically active surface specifically designed for urea detection. The modification of the graphite pen electrode's surface markedly enhanced its conductivity and electrochemical properties, thereby improving its sensitivity to urea measurement. The raw materials used in constructing the modified electrode—copper nanoparticles and 4,4-nitrobenzylpyridine—were readily dissolved in a water-ethanol mixture and deposited onto the graphite electrode surface through electrochemical scanning techniques, including electrodeposition and electro polymerization. The square wave voltammetry method was employed at a specific potential for urea measurement, which is particularly recommended due to its high sensitivity and capability to detect very low concentrations of the analyte. Additionally, the nature of the electrode surface minimizes potential side disturbances, ensuring high accuracy and precision in measuring urea levels in human blood serum samples. The results obtained from cyclic voltammetry studies, square wave voltammetry, and scanning electron microscopy imaging collectively confirm the successful modification of the electrode surface, which has been rigorously evaluated and investigated throughout this research.

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