

RESEARCH PAPER

Hierarchical NiCo₂S₄@rGO Hybrid Nanocomposites as Advanced Non-Enzymatic Electrocatalysts for Ultra-Sensitive Urea Detection

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ABSTRACT

Developing highly selective, sensitive, and stable non-enzymatic biosensors for urea detection is highly significant for clinical characterization and environmental monitoring. This study successfully synthesized NiCo₂S₄@rGO by hydrothermal and thermal methods and coated it onto a glassy carbon electrode (GCE). Overall structural and surface characterization analyses, including XRD, XPS, Raman, BET, FESEM, TEM, and EDX mapping, revealed well-dispersed NiCo₂S₄ NPs on rGO nanosheets, providing numerous redox-active sites, high surface area, and good electronic conductivity. Electrochemical analyses demonstrated excellent sensing performance for urea oxidation. The cyclic voltammetry exhibited a broad linear range (0.5-10 mM), R² up to 0.9673, and a low detection limit. Electrochemical impedance spectroscopy (EIS) revealed a significant reduction in charge-transport resistance (R_s) and an increase in double-layer capacitance (C_{dl}) for the hybrid material, directly related to enhanced, rapid urea adsorption-oxidation kinetics and electron transport. Chronoamperometry (CA) further confirmed the fast response (3s), high reproducibility, and the strong concentration dependence of the current steps. The fabricated sensor showed excellent selectivity against common interferents, excellent stability (> 90% current retention over 40 days), and high precision in real samples, with RSD < 4% and recovery rates of 95-104%. The results show that NiCo₂S₄@rGO is a promising next-generation nanocomposite for robust non-enzymatic urea biosensing, with strong potential for biomedical and clinical applications.

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INTRODUCTION

Urea is one of the most sensitive indicators for the assessment of renal function and total nitrogen balance in human body [1]. Abnormal changes in urea concentration are directly related to serious pathophysiological conditions such as hepatic dysfunction, dehydration, and chronic

kidney disease [2]. Thus, real-time and accurate monitoring of urea levels is essential for patient management and clinical characterization [3]. Conventional colorimetric, enzymatic urease-based assays and chromatographic tests are widely used but suffer from serious drawbacks such as sensitivity to environmental factors, low

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operational stability, high reagent consumption and long assay times [4,5]. Enzymatic systems can be denatured as well. Spectral and optical techniques require sophisticated instrumentation which limits their use in point of care testing [6]. Electrochemical biosensing has emerged as a great alternative due to its fast response, compatibility with miniaturized systems, low cost and high sensitivity [7,8]. Ag, Pt and Au electrodes appear to be very good for urea oxidation. However, their scarcity, high cost and susceptibility to surface poisoning limit their practical application [9]. A more practical way is to be catalyzed by transition metal-based catalysts with multiple redox states, better catalytic kinetics and reduced cost [10-12]. Among them Ni-Co based nanostructures have attracted increasing attention. Mangrio et al. (2023) [13] Binary NiCo₂O₄ synthesized by hydrothermal method Low detection limit of 0.006 mM Linear detection range Real sample performance (urine and milk) is reproducible. Promising results but compromised long term stability due to lack of conductive propping. Conversely, the thermally reduced graphene oxide (TRGO) biosensor showed high recovery and long shelf-life for urine and blood samples. However, it still had a dependence on urease and therefore was sensitive to changes in temperature and pH [14]. These studies show the trade-offs between the oxide-based systems (rigid but limited by particle agglomeration and lower conductivity) and enzyme-based systems (good sensitivity but poor stability). A more and more attractive strategy to overcome these limitations is the construction of hybrid materials where transition metals are incorporated in conductive carbon frameworks [15]. NiCo₂S₄ is a spinel with unique properties thanks to its large number of redox states, abundance of sites for electro-oxidation and high intrinsic conductivity for oxides [16]. However, it is prone to structural instability

and agglomeration during continuous operation. rGO can be an ideal scaffold for the remediation of these defects due to the high electron mobility, large surface area and many defect sites for NPs anchoring [17]. The remarkable synergistic effects of fast charge transfer, enhanced electrochemical stability, uniform distribution of NPs and plentiful catalytic hotspots for urea adsorption could be obtained by the combination of NiCo₂S₄ with rGO and the formation of the nanohybrid structure. They are also non-enzymatic and are unaffected by changes in pH or temperature as are enzyme-based materials, thus providing reliable performance in complex biological matrices. In this work, NiCo₂S₄@rGO nanohybrids are prepared and designed as advanced non-enzymatic urea sensing catalysts. The current work is directed to the development of a cost-effective, robust and high-performance sensing platform by systematically correlating its structure-electrochemical relationships, comparing with oxide- and enzyme-based sensors and investigating its detection limit, sensitivity, stability and selectivity in real samples.

MATERIALS AND METHODS

Materials

Graphene oxide (GO) was prepared using a modified Hummer method [18]. Thiourea (CH₄N₂S), nickel nitrate hexahydrate (Ni(NO₃)₂·6H₂O), and cobalt nitrate hexahydrate (Co(NO₃)₂·6H₂O) were supplied from Sigma-Aldrich Co. At the same time, potassium hydroxide (KOH) and a Nafion solution (5 wt.%) were obtained from Merck Co. and used as received without further purification.

Synthesis of NiCo₂S₄@rGO Hybrid Nanocomposites

The NiCo₂S₄@rGO nanohybrid was prepared by a mixed hydrothermal and thermal method. Initially, 50 mg of GO (prepared by the Hummers method) was dispersed in 50 mL of deionized



Fig. 1. Synthesis process of NiCo₂S₄@rGO.

water for 30 min to obtain a homogeneous suspension. Separately, in a 150 ml conical flask, 1 and 2 mmol of Ni and Co salt precursor were dissolved in 40 ml of deionized water. Then, 10 mmol of thiourea was added to the Ni: Co solution under continuous stirring for 30 min. After that, GO dispersion was added to the mixed solution under constant stirring for 15 min, forming a black suspension. Subsequently, the mixture was transferred to a stainless-steel autoclave and subjected to hydrothermal treatment at 150 °C for 14 h. Thereafter, the precipitate was isolated by centrifugation, washed with ethanol, acetone, and deionized water, and dried at 70 °C for 4 h. The powder was then annealed at 300 °C under N₂ to improve conductivity and crystallinity, yielding the NiCo₂S₄@rGO nanohybrid. The preparation process of NiCo₂S₄@rGO nanohybrid is shown in Fig. 1.

Electrode Preparation

The working electrodes were prepared by modifying the GCE surface with the NiCo₂S₄@rGO nanohybrid. Before modification, the GCE surface was polished with Al slurry on a polishing pad, then washed with deionized water. Subsequently, the electrodes were ultrasonicated in a mixture of ethanol: water (1:1) for 10 min to remove impurities and confirm a clean and active surface. Finally, it was dried under a N₂ stream, and it was ready for modification. After electrode cleaning, the coating suspension was prepared by dispersing 5 mg of NiCo₂S₄@rGO/rGO nanohybrid in 950 mL of ethanol: water (1:1) and 50 mL of Nafion solution (5 wt.%) as a binder. Thereafter, the suspension was ultrasonicated for 20 min to produce a homogenous colloidal suspension. Then, 20 mL of the suspension was carefully coated onto the GCE surface and dried at room temperature for 8 h, resulting in a stable, uniform film. The loading of the catalyst on the electrode surface [19] was calculated as follows:

$$\text{loading} = m/A \quad (1)$$

Where *m* is the mass of NiCo₂S₄@rGO/rGO and *A* is the electrode area. The modified electrode was activated by cycling in 0.2 M KOH solution for 5 cycles between 0.0 and 0.6 V (vs. Ag/AgCl) before urea sensing. This process confirmed the stabilization of the catalyst layer and the detection of active redox sites for Ni/Co, thus improving the

reproducibility of subsequent measurements.

RESULTS AND DISCUSSION

The structure properties of pristine NiCo₂S₄ and synthesized NiCo₂S₄@rGO nanohybrid were examined via XRD, as exhibited in Fig. 2a. The results shown diffraction peaks located at $2\theta = 16.31^\circ, 26.82^\circ, 31.32^\circ, 38.51^\circ, 47.54^\circ, 50.91^\circ, 65.23^\circ, \text{ and } 73.61^\circ$ which assign to the planes of the (111), (220), (311), (400), (422), (511), (440), (533) and (444) for cubic phase and the results are in good agreement of (JCPDS no. 24-0334) [20]. On the other hand, the pristine sample showed intense, sharp diffraction peaks, indicating high crystallinity. However, after doping with rGO, the XRD pattern shows decreased intensities and peak broadening, indicating a considerable reduction in crystallite size and increased lattice strain [21]. The crystallite size was calculated using the Scherrer equation [22], and it decreased from 20 nm in pristine NiCo₂S₄ to 13 nm in NiCo₂S₄@rGO/rGO nanohybrid due to the anchoring effect of the rGO sheet, which delimits NiCo₂S₄ growth domains and enhances dispersion. Moreover, a smaller crystallite size is beneficial for sensing applications, as it increases the electroactive surface area available for the oxidation and adsorption of urea. Further insight into the distortions of microstructure can be obtained from the appreciation of micro strain, estimated from the following equation [23]:

$$\varepsilon = \beta/4\tan \quad (2)$$

The results showed that ε increased from 0.002 to 0.0035 upon incorporating rGO, indicating that lattice distortion occurs during interfacial interaction between NiCo₂S₄ and the rGO sheet. These distortions create additional defect sites that can act as catalytic hotspots during urea oxidation. Furthermore, the dislocation density appears to increase with the incorporation of rGO, further highlighting rGO's role in forming a defect-rich structure. The increase increases the number of adsorption sites and accelerates charge-transport processes, both of which are essential for active electrochemical sensing. On the other side, the characteristic (002) diffraction peak of rGO, usually noted at $\sim 25\text{--}26^\circ$, does not obviously appear in the nanohybrid pattern, which can be attributed to either complete exfoliation of rGO sheets through nanohybrid production or

overlapping with the (220) diffraction peak of NiCo₂S₄. The disappearance of this diffraction peak strongly supports the successful inhibition of rGO restacking and confirms that rGO layers are well dispersed and intimately mixed with NiCo₂S₄ NPs. Additionally, the diffraction peak positions of the nanohybrid sample were slightly shifted indicating the lattice strain and interfacial coupling between the S and GO frameworks. These interfacial interactions are synergistic, NiCo₂S₄ has rich redox chemistry (Co²⁺/Co³⁺ and Ni²⁺/Ni³⁺) and rGO provides continuous conductive pathways for fast electron transport [24-26]. The electronic states and chemical composition of NiCo₂S₄@rGO were estimated by XPS, as shown in Fig. 2(b-e). The survey spectrum (Fig. 2b) shows the presence of Ni, Co and S from the spinel structure of NiCo₂S₄, and O and C signals from the rGO framework and surface-adsorbed O species. The successful incorporation of rGO is evident from the pronounced C 1s binding energy peak which enhances the electrical conductivity and prevents particle aggregation. Furthermore, the O 1s binding energy signal indicates the presence of OH and O species, which are responsible for the surface hydrophilicity and help in efficient electrolyte penetration [27]. The Co 2p spectrum (Fig. 2c) revealed two binding energies at 781 and 796 eV, corresponding to the Co 2p_{3/2} and Co 2p_{1/2}, as well as the characteristic satellite peaks. Deconvolution indicated the presence of Co²⁺ and Co³⁺ states supporting the mixed valence nature of Co in the crystal lattice which is primary for electrocatalysis as the redox cycling between the two states is easy and provides multiple electron transport pathways thereby accelerating urea oxidation [28]. The Ni 2p spectrum in Fig. 2d exhibits two binding energies at 855 and 837 eV corresponding to 2p_{3/2} and 2p_{1/2}, respectively, thus confirming the presence of Ni²⁺ and Ni³⁺ [29]. The strong interfacial electronic conjugation between the 3+ states of the hybrid nanocomposite and rGO gives rise to enhanced conductivity and charge mobility. The presence of 2+ and 3+ oxidation states for Ni and Co respectively leads to a large network of electroactive sites which allows fast electron transfer thus improving the sensitivity and lowering the detection limit of urea. The S 2p spectrum gave more information about the chemical environment of S. Results (Fig. 2e) showed two peaks of binding energy at 162 and 163.5 eV representing the S 2p_{3/2} and 2p_{1/2} of S

bonds indicating the stability of the framework. Furthermore, the broad features at about 168-170 eV are related to SO_x²⁻, originated from partial surface oxidation [30]. These species enhance the surface polarity, electrolyte adsorption, and formation of a defect-rich active site, which is of great importance in the electrocatalytic process. The presence of defects and well dispersed NPs on rGO sheets confirm effective adsorption of OH ions and urea, which is favorable for their subsequent electro-oxidation. The XPS results indicate that NiCo₂S₄@rGO is a compound with unique properties, such as a mixed valence of Ni and Co for rapid redox transitions, stable S bonds with abundant sulfur species to produce catalytic defects, conductive rGO frameworks as fast electron pathways, and O functionalities for better electrolyte penetration and wettability. These synergistic properties are directly translated into enhanced electrocatalytic activity, so the hybrid electrode can reach high level of sensitivity, good stability and low detection limits [31]. The Raman spectra of GO and NiCo₂S₄@rGO, with the characteristic C-related bands, are shown in Fig. 2f. Two prominent peaks are located, namely D band at 1340 cm⁻¹, attributed to the mode of sp³-hybridized C atoms representing the existence of structural defects and lattice disorder and the G band near 1580 cm⁻¹, caused by the E_{2g} phonon vibration of sp² bonded C atoms in graphitic fields. The D and G bands in the GO spectrum are of moderate intensity and broad width indicating the presence of an O-containing group blocking the conjugated sp² structure and partial graphitization [32]. The incorporation with NiCo₂S₄ NPs, the results seems to be noteworthy changes. The D and G bands intensity increases and becomes more pointed, and the entire profile shows a higher ratio of I_D/I_G (generally improving from 0.9 in GO to 1.2 in NiCo₂S₄@rGO). The increase in I_D/I_G ratio leads to the generation of more structural defects and edge sites by anchoring of NPs on the rGO sheets. This disrupts the long-range order of sp² C atoms and at the same time creates defect-induced catalytic hotspots [33]. Additionally, the FWHM of the D and G bands decrease slightly in the NiCo₂S₄@rGO nanohybrid, showing improved crystallinity of the local graphitic domains, due to the partial renovation of rGO through the hybridization process. This defect-rich and conductive framework simultaneously has higher disorder and sharper graphitic properties, which is

more suitable for electrochemical catalysis. The relative increase in D-band intensity is a sign of the higher amount of adsorption centers for analyte molecules like urea. At the same time, the strong G-band reverses the effective electron delocalization and charge transfer through the sp² network [34]. Moreover, the interaction between NPs and rGO sheets slightly shifts the position of D and G bands reversing electronic coupling and charge transport between S and C frameworks, enhancing conductivity and allowing for redox transitions. The results directly translate into superior electrochemical urea-sensing performance in a functional context. The increased I_D/I_G ratio indicates the existence of abundant defects as active adsorption sites and reaction centers for urea molecules. The sharp G band

confirms fast electron transport through the conductive matrix reducing the charge-transport resistance through electro-oxidation. The balance between defects and disorder leads to the optimal density of catalytic hotspots and the conductivity of the electrode. These properties result in a defect-rich, highly conductive and structurally integrated electrode, affording enhanced sensitivity and stability for non-enzymatic urea detection [35]. The N₂ adsorption-desorption isotherms of pristine and rGO embedded NiCo₂S₄ are shown in Fig. 2g. The results indicate that the two samples exhibit type-IV isotherms with special H3-type hysteresis loops at P/Po = 0-1, which is characteristic of mesoporous structure. The introduction of rGO causes a remarkable increase in the adsorption capacity, which is confirmed by

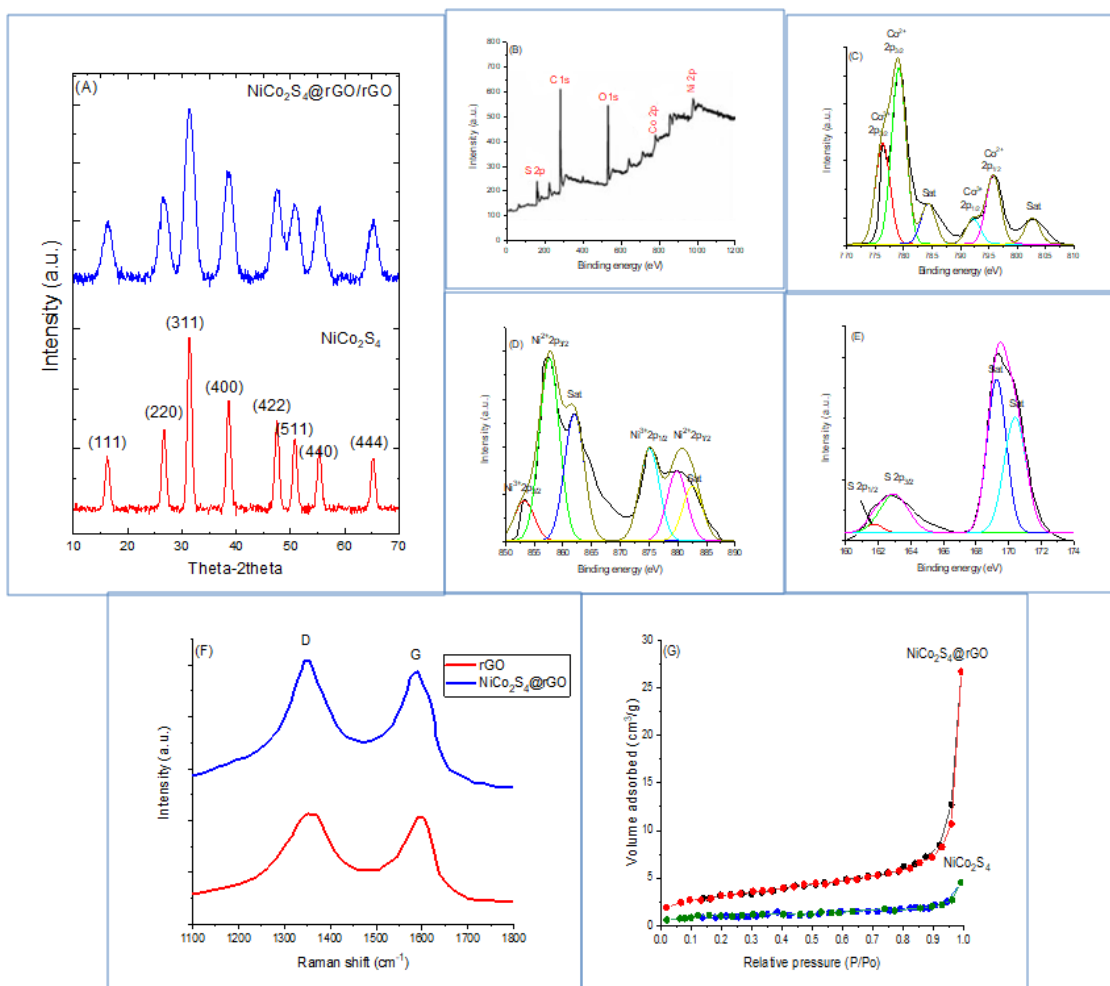


Fig. 2. (A) XRD of NiCo₂S₄ and NiCo₂S₄@rGO, XPS of NiCo₂S₄@rGO (B) survey spectrum, (C) Co, (D) Ni, (E) S, (F) Raman spectrum of rGO and NiCo₂S₄@rGO, (G) BET of NiCo₂S₄ and NiCo₂S₄@rGO.

the larger volume of adsorbed gas for the hybrid sample compared with NiCo_2S_4 . The enhancement shows that rGO can prevent the agglomeration of NiCo_2S_4 NPs and provide more surface area and porosity. Besides, the pore size distribution is mainly mesopores with an average pore size in the range of 3-10 nm which can act as effective channels for diffusion and electrolyte transport. In addition, the BET method is used to get the specific surface area of pure and rGO-incorporated NiCo_2S_4 . It increases with rGO incorporation, thereby enhancing the total pore volume,

suggesting the presence of a hierarchical porous network [36]. These structural properties are directly beneficial for urea sensing. A higher surface area indicates a higher density of active sites, while mesopores facilitate rapid mass transfer of urea molecules and OH ions to the electro-oxidation sites. The hierarchical pore structure reduces diffusion resistance and enhances the accessibility of redox-active centers, thereby accelerating electron-transport kinetics. Moreover, the presence of rGO in the hybrid system enhances both electronic conductivity and

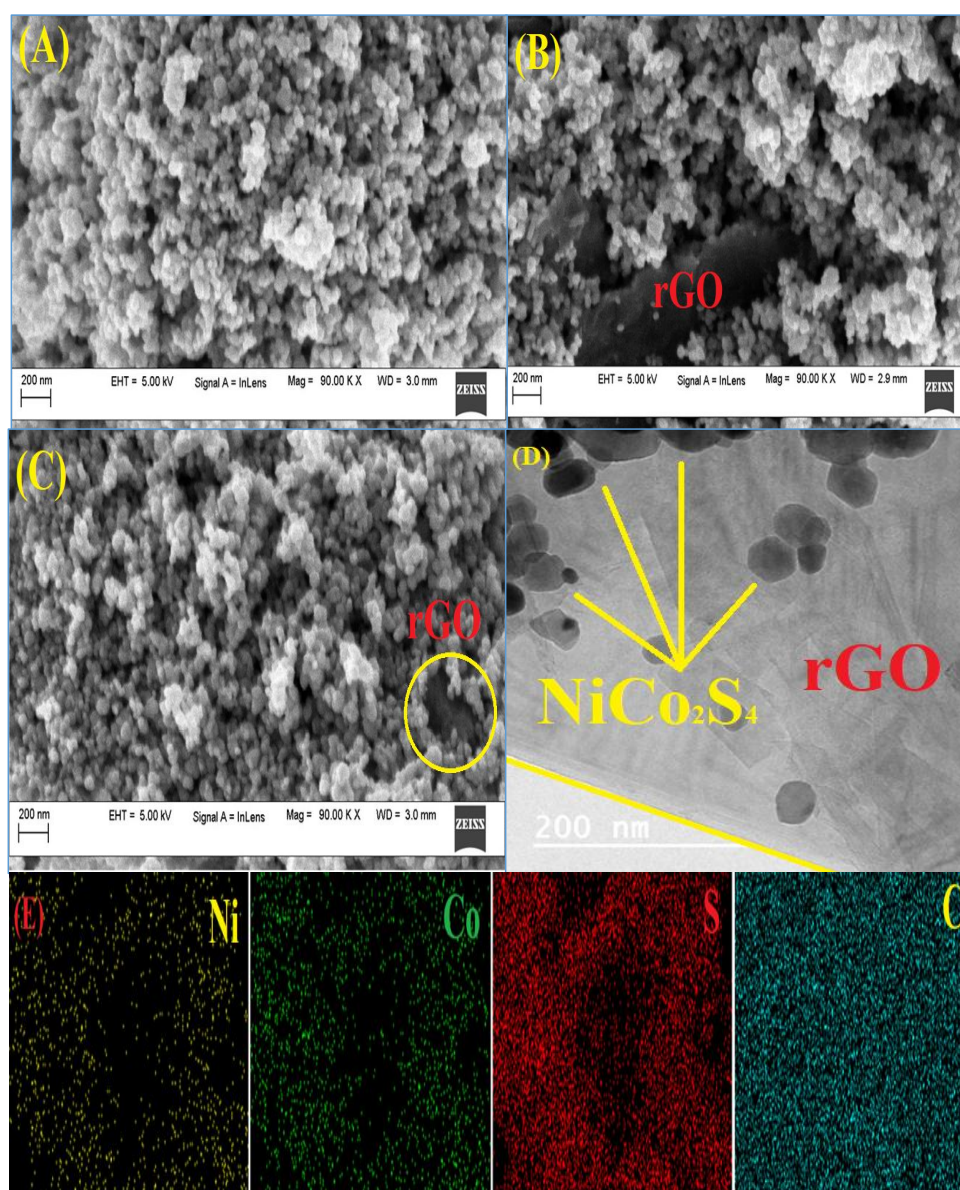


Fig. 3. FESEM of (A) NiCo_2S_4 , (B,C) $\text{NiCo}_2\text{S}_4@\text{rGO}$, (D) TEM of $\text{NiCo}_2\text{S}_4@\text{rGO}$, (E) EDX-mapping of $\text{NiCo}_2\text{S}_4@\text{rGO}$.

porosity, thereby exerting a synergistic effect on charge transfer and structural accessibility [37]. Consequently, the BET results indicate that the hybrid compound has a highly porous surface, an improved architecture, and a defect-rich structure, suggesting superior electrocatalytic performance for urea sensing.

The morphological properties of pristine and rGO-incorporated NiCo₂S₄ were systematically investigated employing FESEM and TEM, and elemental mapping as displayed in Fig. 3a-f. The FESEM image of NiCo₂S₄ (Fig. 3a) appears as agglomerated clusters of irregular NPs, suggesting limited surface exposition and an inclination for particle aggregation. In contrast, the FESEM images (Fig. 3b,c) of NiCo₂S₄@rGO show that NiCo₂S₄ is uniformly distributed and anchored to the rGO sheets, which act as a conductive scaffold that inhibits agglomeration and provides a large accessible surface area. The strong interfacial conjugation and structural stability is confirmed through the intimate contact between the NPs and rGO sheets. Further investigation was carried out by TEM. The TEM image (Fig. 3d) gives additional evidence for the hybrid architecture. The results showed that NiCo₂S₄ was well dispersed and distributed on rGO sheets to form a hierarchical nanostructure consisting of a two-dimensional conductive support and nanoscale particles. This result benefits the electrochemical sensing, as it reduces active-site exposure, promotes electrolyte

penetration, and shortens ion-transfer pathways. Elemental mapping analysis (Fig. 3e) indicates that the elements are uniformly distributed in the hybrid compound, demonstrating the successful incorporation of the pristine NiCo₂S₄ into the rGO framework. The compositional purity of NiCo₂S₄@rGO nanohybrid is guaranty by the regular elemental dispersion and the synergistic benefits of Ni, Co and S are demonstrated. Co and Ni provide redox-active sites, S provides stability to the spinel framework and introduces defect states. In contrast, rGO provides mechanical robustness and high conductivity. Such compositional and structural properties directly translate to improve urea sensing performance. The uniform dispersion of NiCo₂S₄ NPs increases the density of electroactive sites for urea adsorption and oxidation, while rGO accelerates charge transport and reduces resistance during electrochemical reactions. The inhibition of particle agglomeration confirms the sensor's reproducibility and long-term stability, while its hierarchical morphology with ample macro- and meso-channels enhances efficient electrolyte transfer. Collectively, TEM, FESEM, and EDX measurements confirm that the NiCo₂S₄@rGO nanohybrid is a highly conductive, defect-rich, structurally robust material that demonstrates excellent electrocatalytic activity, improved sensitivity, and stability.

EIS was used to further investigate the charge transport dynamics and ion diffusion processes

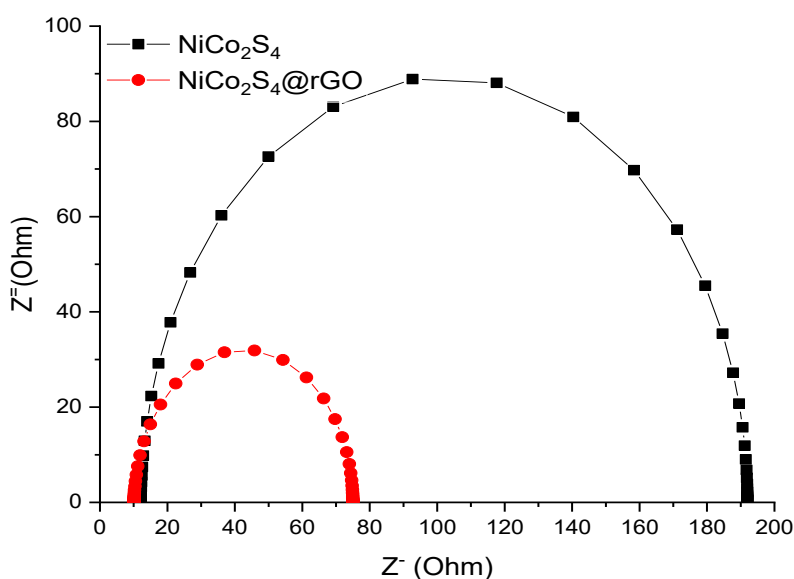


Fig. 4. EIS of NiCo₂S₄ and NiCo₂S₄@rGO.

happening at the NiCo₂S₄@rGO electrode surface compared to the NiCo₂S₄ electrode, and the results are shown in Fig. 4. The Nyquist curves showed apparent differences in the linear regions and semicircular, which are directly related to the electrochemical kinetics of the electrode. The radius of the semicircle was found to be much larger for NiCo₂S₄ with a value of R_{ct} equal to 180 Ω . This indicates slow electron transfer and lack of electroactive sites. In contrast, the NiCo₂S₄@rGO electrode showed a much smaller semicircle ($R_{ct} = 65 \Omega$) with faster charge transport, thanks to the highly conductive scaffold of the rGO that connects the active NPs and prevents them from aggregating. This reduction in R_{ct} is significant, since it directly correlates with lower overpotential, and enhanced sensitivity for oxidation of urea. Moreover, the R_s were almost constant for both electrodes, which indicates that the enhancement is due to interfacial processes at the surface of the electrode and not changes taking place in the bulk electrolyte. However, the results show that the Cdl of NiCo₂S₄@rGO (60 $\mu\text{F}/\text{cm}^2$) is higher than that of bare electrode (20 $\mu\text{F}/\text{cm}^2$). This improvement in Cdl is very crucial for increasing the electroactive surface area and wettability, both needed for the effective adsorption of urea molecules for oxidation. Moreover, the Nyquist plot of the hybrid electrode exhibited steeper slope at the low frequency end, which corresponds to reduced Warburg impedance and thus faster ion diffusion pathways in the porous NiCo₂S₄@rGO. Such behavior is especially useful for sensing applications where the response time and sensitivity are dictated by the fast mass and electron transport.

The electrocatalytic activity of NiCo₂S₄-rGO/GCE for urea oxidation was confirmed by cyclic voltammetry measurements using 5 mM KOH as the standard redox probe at urea concentrations of 0-10 mM (Fig. 5a-c). The CV measurement at 50 mV/s reveals a systematic and negligible increase in the anodic current with the increase in the concentration of urea, which indicates the effective catalytic urea oxidation at NiCo₂S₄-rGO/GCE. From this direction it becomes apparent that the addition of NiCo₂S₄ into rGO leads to high electroactive surface area to oxidize and adsorb urea. At scan rates of 75 and 100 mV/s, the anodic current increased further and the oxidation peak positions were shifted remarkably. This behavior is typical for a diffusion controlled electrochemical

process, where the rate of electron transfer is greatly enhanced at higher scan rates. According to the diffusion-dominance model, the urea molecules are adsorbed on the active sites of the electrode provided by the Ni²⁺/Ni³⁺ and Co²⁺/Co³⁺ redox couples, and then rapidly oxidized by the high conductivity and defect-rich nature of rGO. Furthermore, the corresponding calibration plots (Fig. 5d-f) showed a linear relationship between urea concentration and oxidation current density at all scan rates with correlation coefficients of 0.885, 0.906, and 0.967 at 50, 75, and 100 mV/s, respectively. The increased linearity at higher scan rates indicates a better use of electroactive sites and more reproducible electron-transfer kinetics. The steep slopes of these calibration curves are remarkable for increasing the sensitivity, which is directly proportional to the quick adsorption-desorption dynamics of OH ions and urea molecules at the NiCo₂S₄-rGO/GCE interface. This correlation not only demonstrates that NiCo₂S₄-rGO can sustain a stable current restraint in a wide range of concentration, but also emphasizes the structural advantage of the integration of NiCo₂S₄ NPs with rGO. Standard equations were used for the analytical implementation. The linear regression can be calculated as:

$$I = S \cdot C + I_0 \quad (3)$$

where I is the measured density of oxidation current ($\text{A}\cdot\text{cm}^{-2}$), C is the concentration of urea (mM) and S is the slope of calibration curve. Indicating the response of the fabricated electrode to the increasing concentration of urea efficiently. Also, the detection limit was calculated according to the IUPAC criterion:

$$\text{LOD} = 3\sigma/S \quad (4)$$

Where σ is the blank standard deviation response, the LOD results (Table 1) revealed that NiCo₂S₄@rGO has a significantly low detection threshold especially at higher scan rates, showing its applicability for ultra-sensitive non-enzymatic urea detection. Such excellent electrochemical performance can be ascribed to the following synergetic effects. First, the presence of +2 and +3 oxidation states in NiCo₂S₄ matrix provides abundant redox-active sites which facilitate electron transport during urea oxidation. Second, the rGO scaffold can prevent the agglomeration

of NiCo₂S₄ and acts as a highly conductive matrix to reduce the charge transport resistance and guaranty the efficient transfer of electrons between the electrolyte and the electrode. Third, the NiCo₂S₄@rGO architecture facilitates the rapid diffusion of electrolyte and effective utilization of active sites by providing access to both mesoporous channels and surface defects. All these compositional and structural properties have collectively resulted in the observed improvements of current density, linearity and sensitivity. Generally, the results of the CV supply compelling evidence that the NiCo₂S₄@rGO/GCE is a highly effective platform for non-enzymatic sensing of urea. The wide linear detection range, strong concentration-dependent current response, high sensitivity, and low detection limit

are direct results of the synergistic interactions between the components of the hybrid structure.

As shown in the Table. 1, boosting the scan rate from 50 to 100 mV/s considerably enhances both the linearity and sensitivity, while decreasing the detection limit. The findings suggest that the prepared hybrid electrode exhibits rapid electron-transport kinetics, abundant active surface sites, and effective adsorption/desorption dynamics, making it a strong candidate for practical urea sensing.

The selectivity and stability of NiCo₂S₄@rGO-GCE for urea detection were evaluated to assess its applicability to biological samples. Fig. 6A shows the sensor current response to urea (0.1 mM) and several interfering species, all measured at a scan rate of 100 mV/s. The findings clearly indicate that

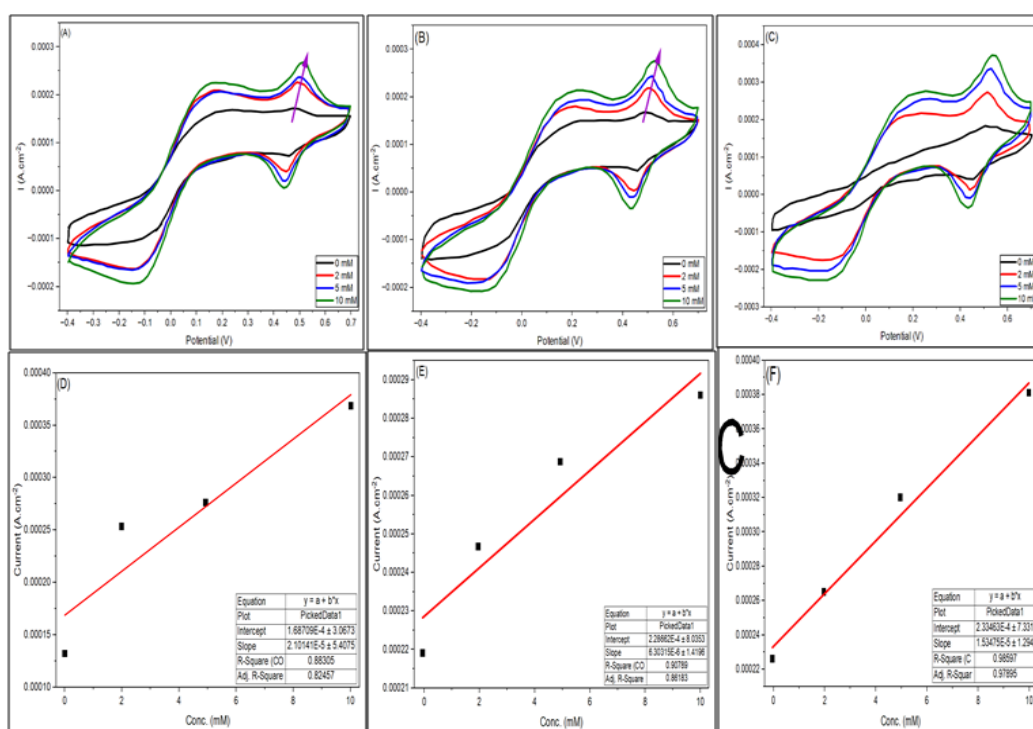


Fig. 5. CV response of NiCo₂S₄@rGO-GCE with urea conc. (0-10 mM) at (A) 50mV/s, (B) 75mV/s, (C) 100mV/s; calibration curve of urea conc. for scan rate (D) 50mV/s, (E) 75mV/s and (F) 100mV/s.

Table 1. Effect of scan rate on the analytical performance parameters of the modified electrode for urea sensing.

Scan rate (mV/s)	Linear range (mM)	LOD (mM)	R ²
50	0.5-10	43	0.88
75	=	58	0.90
100	=	74	0.98

urea produced a considerably higher oxidation current ($2 \times 10^{-4} \text{ A.cm}^{-2}$) than other species, which showed much lower responses. This is entirely different from the robust preference for a prepared electrode for urea oxidation, which can be attributed to its defect-rich structure, profuse redox-active $\text{Co}^{2+}/\text{Co}^{3+}$ and $\text{Ni}^{2+}/\text{Ni}^{3+}$ sites, and a porous morphology that catalyzes and adsorbs urea molecules from coexisting biomolecules. The minimal interference from sugar and metabolites underscores the sensor's exceptional anti-interference performance, confirming its ability to detect with precision in complex matrices such as urine or serum. Further, the long-term stability of

the $\text{NiCo}_2\text{S}_4@\text{rGO}$ sensor was studied by measuring the current response to 5 mM urea for 40 days, as displayed in Fig. 6B. The $\text{NiCo}_2\text{S}_4@\text{rGO}$ still keeps above 90% of the initial current response after 30 days storage with a little decrease, revealing its great toughness. The contribution for stability is attributed to the excellent interfacial bonding between NPs and rGO, which prevents the agglomeration and structural collapse of NPs during the longtime use. In addition, the conductive scaffold of rGO offers mechanical stability and prevents active material leaching, whereas the synergistic redox chemistry of Co and Ni maintains sustained catalytic activity. Overall,

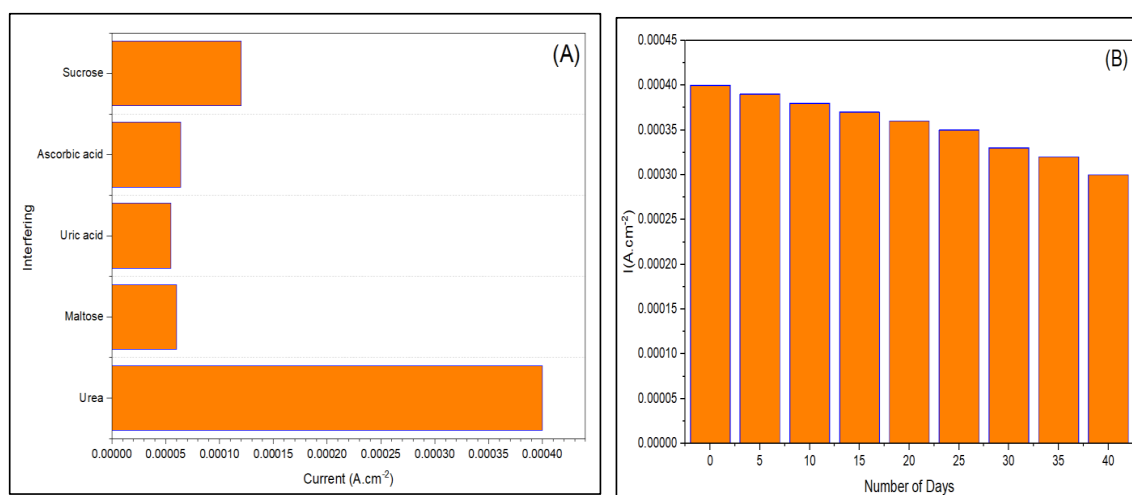


Fig. 6. (A) Selectivity of various interferents in human blood; (B) Stability of the electrode for detection of urea.

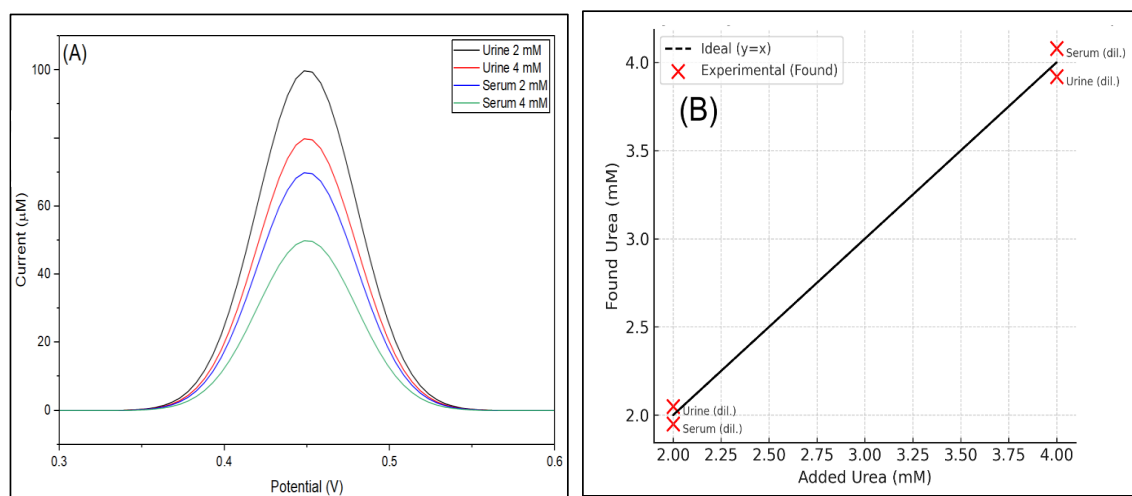


Fig. 7. (A) CV plot of $\text{NiCo}_2\text{S}_4@\text{rGO}$ -GCE in real samples (B) recovery performance of $\text{NiCo}_2\text{S}_4@\text{rGO}$ for urea detection in real serum and urine.

the stability and selectivity studies confirm that the prepared electrode is specific not only for urea in the presence of biological interferents but also retains good electrochemical activity over long periods. These results strongly affirm its promise as a credible, long-term, stable and non-enzymatic platform for urea detection in clinical and real-world biological applications.

The practical applicability of the prepared electrode was checked with diluted serum and spiked human samples with 0.2 mM urea for the real samples. CV (Fig. 7a) responses show evident oxidation currents depending on the concentration, in accordance with the calibration curve obtained in the standard electrolyte solution. However, the results showed that the current density decreased with increasing concentration because of the cumulative saturation of the electroactive sites on the prepared electrode surface and the aggregation of oxidation products which hinder electron transport and mass diffusion at higher concentrations. This behavior is indicative of a diffusion controlled electrochemical process and emphasizes the strong surface dependence of the catalytic activity. The recovery rates (Fig. 7b) ranged from 95-104% showing high accuracy and low matrix effects. The RSD across $n = 3$ was less than 4% indicating high reproducibility. Moreover,

the high selectivity of the interference studies confirmed that the coexisting molecules such as ascorbic acid, uric acid and glucose did not interfere in the electrochemical response of urea. The results presented here, along with a long-term stability study that showed that the current response remains over 90% after 40 days, suggest that the prepared electrode has the sensitivity and robustness necessary for credible clinical use. The synergism between the rGO scaffold and the redox-active couples of Ni and Co enhances the electrochemical signal and enables accurate determinations in physiologically relevant samples. Also, the efficient detection of urea in serum and urine highlights the great potential of the fabricated electrode as a future non-enzymatic biosensor.

The dynamic sensing performance of the prepared electrodes was investigated by CA measurements. The results (Fig. 8) revealed that NiCo₂S₄@rGO/ GCE showed rapid current jumps and reached the steady state within 3 sec. Such rapid stabilization is significant for biosensing applications, as it guaranties that the electrode can reliably and consistently monitor small changes in the urea concentration in real time. Conversely, the conventional NiCo₂S₄ electrode shows longer equilibrium time (~7 s) and smaller

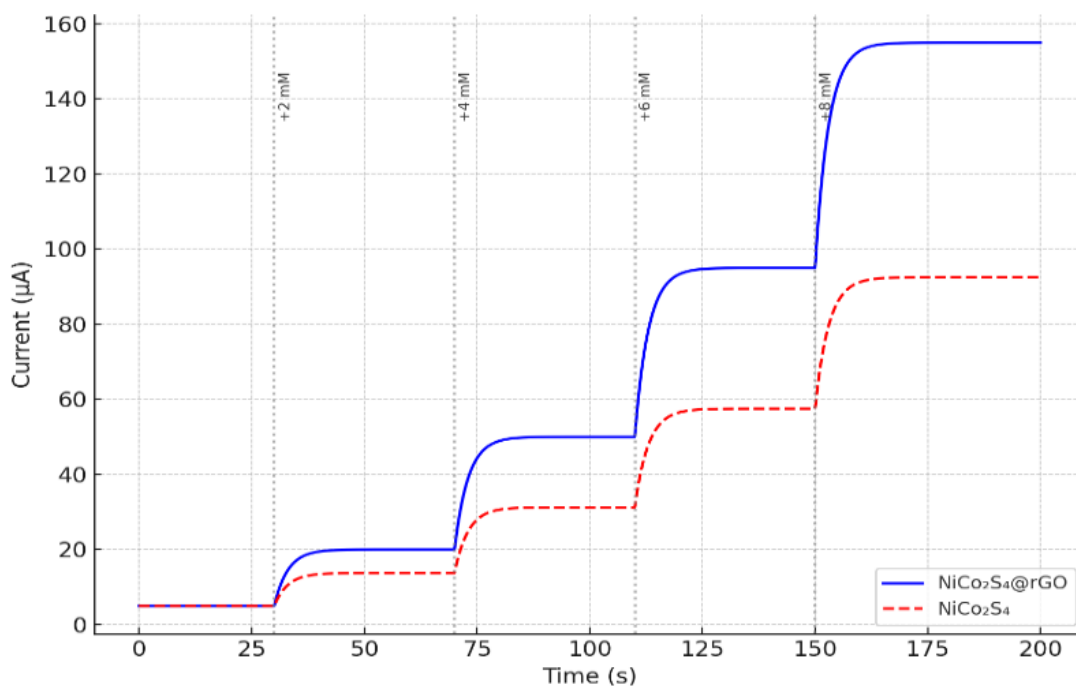
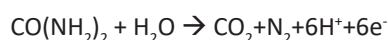


Fig. 8. CA response of electrodes for urea detection.

increase in steady state current, indicating the sluggish electron transport and reduced density of active catalytic sites. The current of NiCo₂S₄@rGO-GCE increased stepwise with the increase of urea concentration which clearly suggests the concentration dependent electrocatalytic urea oxidation. This linearity is critically important for quantitative biosensing applications as it demonstrates that the electrode can faithfully transduce molecular binding events (adsorption and oxidation of urea) into measurable electrical signals. Additionally, the high signal to noise ratio and reproducible current steps upon repeated injections indicate the hybrid robustness, which is an important quality for clinical characterization, where matrix complexity often results in drift and instability. Mechanistically, the CA response can be directly correlated to the synergistic properties of the composite NiCo₂S₄@rGO. The decreased Rct of NiCo₂S₄@rGO observed in EIS, compared to NiCo₂S₄, enables fast electron transport from urea-oxidized molecules at the NiCo₂S₄@rGO/GCE surface to the external circuit. The larger Cdl suggests a greater electroactive surface of area, giving more active sites for adsorption and

oxidation of urea. At the same time, the reduced Warburg impedance confirms fast mass transfer and effective ion diffusion, inhibiting retards in the level of current plateaus after each addition of urea. These electrochemical improvements translate to higher sensitivity and rapid response times in the CA urea sensing.

The overall process can be obtained via the following urea oxidation reaction in an alkaline medium:



In this reaction (Fig. 9), the molecules of urea are adsorbed at the NiCo₂S₄@rGO/GCE, where they succumb to oxidation facilitated by the redox-active couples of Co²⁺/Co³⁺ and Ni²⁺/Ni³⁺ in NiCo₂S₄, while rGO gives rapid electron transfer pathways. The released electrons are promptly collected through the external circuit, producing the measurable current observed in the CA measurements. The rapid kinetics of the oxidation reaction, together with the high active-site density and effective mass transfer in the NiCo₂S₄@rGO electrode, result in immediate,

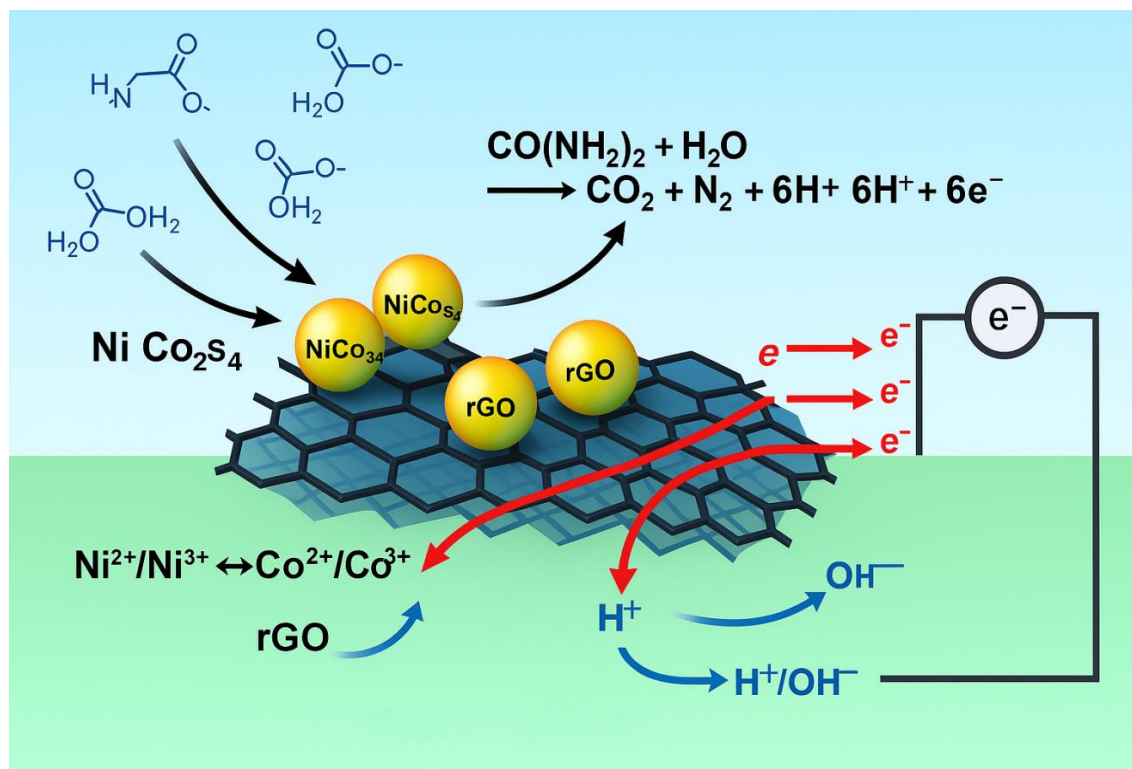


Fig. 9. electrochemical oxidation mechanism of urea at the electrode.

concentration-dependent current responses. The CA results support the conclusion that NiCo₂S₄@rGO is an eminent non-enzymatic urea sensor. The electrodes might provide instantaneous, concentration-dependent, and reproducible current responses, demonstrating their strong potential for measuring urea levels in serum and urine. These performance benefits, combined with selectivity, stability, and good recovery in a real matrix, position NiCo₂S₄@rGO as a next-generation biosensor for urea diagnostics.

CONCLUSION

This study successfully synthesized NiCo₂S₄@rGO by hydrothermal and thermal methods and coated it onto a glassy carbon electrode (GCE). The cyclic voltammetry exhibited a broad linear range (0.5–10 mM), R₂ up to 0.9673, and a low detection limit. Electrochemical impedance spectroscopy (EIS) revealed a significant reduction in charge-transport resistance (R_s) and an increase in double-layer capacitance (C_{dl}) for the hybrid material, directly related to enhanced, rapid urea adsorption-oxidation kinetics and electron transport. Chronoamperometry (CA) further confirmed the fast response (3s), high reproducibility, and the strong concentration dependence of the current steps. The results show that NiCo₂S₄@rGO is a promising next-generation nanocomposite for robust non-enzymatic urea biosensing, with strong potential for biomedical and clinical applications.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

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