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Electrochemically Engineered Gold Nanostructures for Selective Detection of Glucose and Ascorbic Acid

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Abstract

This study reports the development and characterization of modified screen-printed carbon electrodes (SPCE) for the non-enzymatic simultaneous detection of glucose and ascorbic acid. The electrodes were modified with gold nanoparticles (AuNPs) using a cyclic voltammetry electrodeposition process. The morphological and topographical evolution of the AuNPs layer was systematically investigated for 5, 10, and 15 deposition cycles using scanning electron microscopy and confocal microscopy. The key contribution of this work lies in correlating the electrodeposition-driven morphological evolution of gold nanostructures with analyte-specific oxidation behavior and electrochemical signal discrimination under mutual interference conditions. The electrochemical performance of the SPCE/15AuNPs electrode was evaluated showing an enhanced electrochemically active surface area of 1.154 mm². The optimized platform demonstrated excellent electrocatalytic activity towards the oxidation of glucose and ascorbic acid in individual and mixed solutions. Analytical validation in a 0.1 M NaOH/PBS matrix showed that the sensor maintains high selectivity and sensitivity even in the presence of significant interference. For glucose detection in a 10 mM ascorbic acid background, the sensor exhibited a linear range of 0.1–10 mM with a sensitivity of 0.03 mA/mM and a limit of detection (LOD) of 1.46 mM. Conversely, for ascorbic acid detection in a 10 mM glucose background, a linear response was obtained from 0.1 to 7 mM with a sensitivity of 0.02 mA/mM and an LOD of 1.02 mM. Interference studies with urea, sucrose, and histidine further confirmed the robust selectivity of the hierarchical AuNP surface. These results suggest that the developed SPCE/15AuNPs electrode is a promising, low-cost tool for multi-analyte sensing in complex biological fluids.

Keywords: Gold nanoparticles; Screen-printed electrodes; Non-enzymatic sensing; Glucose; Ascorbic acid; Hierarchical nanostructures.

Introduction

Diabetes mellitus has evolved into a critical global health challenge, characterized by chronic hyperglycemia that leads to severe long-term complications, including cardiovascular diseases, stroke, and chronic renal failure [1–4]. The management of this metabolic disorder necessitates the precise and frequent monitoring of blood glucose, which in a healthy physiological state is strictly regulated within the fasting range of 3.9 – 6.1 mM [5]. Simultaneously, ascorbic acid (AA), or Vitamin C, serves as a vital micronutrient and a potent antioxidant that maintains cellular integrity by neutralizing free radicals [6–8]. Beyond its metabolic role, abnormal concentrations of AA are significant clinical indicators for conditions such as ischemic injury, Alzheimer’s disease, and various immune deficiencies [9,10]. Given that glucose and AA often coexist in biological matrices and can mutually interfere during electrochemical detection, there is an urgent demand for a diagnostic platform capable of their simultaneous and highly accurate determination in both clinical and food safety sectors [11].

Traditionally, electrochemical biosensors have relied on enzymatic recognition units to achieve high selectivity [12]. However, these bio-macromolecules are inherently limited by their fragile nature; their catalytic activity is highly susceptible to environmental fluctuations in pH, temperature, and humidity [13,14]. Furthermore, the high cost of enzymes, combined with tedious immobilization procedures and issues regarding denaturation, significantly hinders the shelf-life and reproducibility of such sensors [15]. To overcome these bottlenecks, the scientific community has pivoted toward non-enzymatic electrochemical sensing. These systems utilize direct electrocatalysis on inorganic surfaces, offering substantially higher chemical stability, lower manufacturing costs, and the robust reproducibility required for commercial mass production [14,16,17].

The performance of non-enzymatic sensors is fundamentally dictated by the architecture and composition of the electrode materials [18,19]. Transition metal-based catalysts have demonstrated unique advantages, including rapid electron transfer kinetics and high sensitivity [20,21]. Among these, gold nanoparticles (AuNPs) are particularly effective [14,22]. AuNPs not only provide a high effective surface area but also exhibit distinct electrocatalytic activity toward the oxidation of glucose and AA under neutral and alkaline conditions [23]. The incorporation of these nanostructures facilitates a higher adsorption capacity and creates a synergistic environment that enhances the sensing signal [24]. This increased roughness and

the presence of numerous inner cavities within the nanostructure allow for a higher Faraday current, effectively accelerating slow redox reactions and reducing both the required sample volume and the total response time [12].

The choice of substrate is equally critical for the transition from laboratory prototypes to point-of-care (POC) devices [25]. Carbon screen-printed electrodes (SPCEs) represent a transformative technology in this regard, offering a portable, inexpensive, and disposable alternative to conventional bulky electrodes. SPCEs are characterized by their versatile surface chemistry, which can be easily modified to tailor the sensor's sensitivity and selectivity [26]. Their porous carbon framework provides an ideal support for the deposition of AuNPs, resulting in a robust heterostructure with a large specific surface area and enhanced electronic properties [27].

In this study, we investigate the relationship between electrodeposition-controlled gold nanostructure morphology and the electrochemical discrimination of glucose and ascorbic acid on disposable screen-printed carbon electrodes. Rather than focusing exclusively on signal enhancement, the work examines how the evolution of hierarchical gold deposits influences electrochemical surface characteristics, analyte oxidation kinetics, and signal resolution in mixed-analyte environments. By combining morphological characterization, scan-rate-dependent kinetic analysis, and calibration measurements under mutual interference conditions, this study provides insights into the potential of structurally engineered gold electrodes for non-enzymatic dual-analyte sensing.

Experimental part

Chemicals and reagents

$K_4[Fe(CN)_6]$ ($\geq 99.5\%$), $K_3[Fe(CN)_6]$ ($\geq 99.5\%$), Phosphate Buffered Saline tablets, $AuCl_3$ were purchased from Sigma Aldrich. Ethanol (99.9%), HCl, KCl (more than 99.5%), and NaOH (99%) were obtained from CentralChem and microCHEM companies. All chemicals were used without further purification. Solutions and mixtures were prepared with deionized water (18.2 M Ω). All experiments were carried out at room temperature (22 °C) and atmospheric pressure.

2.2. Modification of Screen-Printed Carbon Electrodes (SPCEs)

Commercial screen-printed carbon electrodes (SPCE, type 11L, Metrohm DropSens), featuring a carbon working electrode, a carbon counter electrode, and an Ag/AgCl pseudoreference electrode, were used as the base transducers. The electrodes were modified by gold

nanoparticles via cyclic voltammetry from 0 V to -1 V at 100 mV/s during 5, 10, and 15 cycles. The used solution composed from 5mM HAuCl₄ and 0.1 M KCl.

2.3. Morphological and Electrochemical Measurements

Surface morphology and topographical evolution were characterized using a PLuneox 3D Optical Profiler (Sensofar) and scanning electron microscopy (SEM/FIB ZEISS AURIGA) for the calculation the Gwyddion software was used. All electrochemical experiments were performed using an Solartron Analytical Modulab (Model 2100 A, CA). The electrochemical properties and the electrochemically active surface area were evaluated using a 5 mM [Fe(CN)₆]^{3-/4-} redox probe in 1 M KCl. Further characterization was conducted in a 10 mM glucose or/and ascorbic acid solution in phosphate-buffered saline (PBS) with and addition 0.1 M NaOH. Cyclic voltammetry (CV) was employed for these preliminary assessments within a potential window of – 1.5 V to +1 V by varying the scan rates (10, 20, 40, 60, 80, 100, 200, and 300 mV/s). Selectivity was evaluated through interference studies in PBS containing 68 μM histidine, 7 mM urea, and 0.1 mM sucrose in the PBS solution with 0.1 M NaOH.

Results and discussion

The surface morphology of the electrodeposited AuNPs as a function of the number of cyclic voltammetry (CV) deposition cycles was investigated using scanning electron microscopy (SEM). Figure 1 A, B, and C illustrate the evolution of the AuNPs layer after 5, 10, and 15 cycles, respectively.

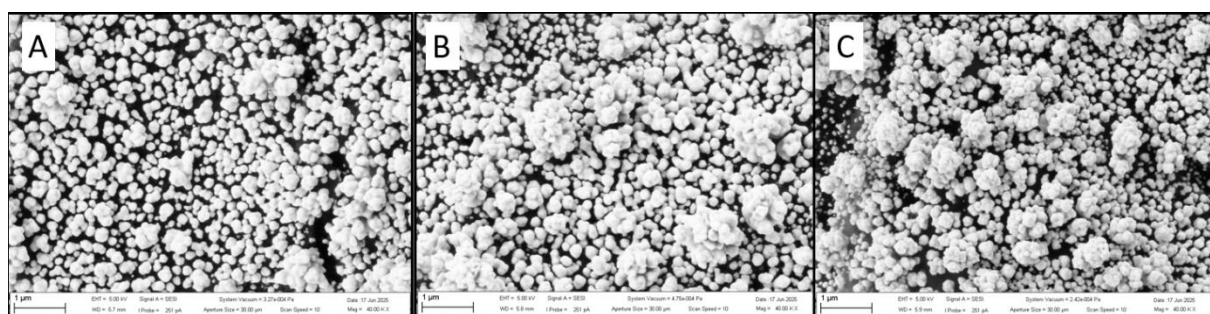


Figure 1 Scanning electron microscopy images of SPCE after the AuNPs electrodeposition during 5 (A), 10 (B), and 15 (C) cycles with magnification 40x – scale 1 μm.

At 5 cycles (Figure 1 A), the substrate surface is predominantly covered by a high density of primary, quasi-spherical gold nuclei. The distribution appears relatively uniform, with

individual nanoparticles exhibiting diameters in the sub-100 nm range. At this initial stage, the nucleation process dominates, resulting in a discrete particulate morphology with visible gaps between the nascent clusters. As the number of deposition cycles increases to 10 (Figure 1 B), a transition from independent nucleation to particle growth and localized coalescence is observed. The average grain size increases noticeably compared to the 5-cycle sample. The particles begin to form larger, secondary cauliflower-like aggregates, which is a characteristic feature of diffusion-limited growth during repetitive potential cycling. While the individual spherical nature of the primary particles remains discernible, the inter-particle spacing decreases as the clusters expand. Upon reaching 15 cycles (Figure 1 C), the SEM micrographs reveal a highly developed, three-dimensional porous network of gold nanostructures. The morphology is characterized by significant vertical and lateral growth, leading to the formation of prominent hierarchical clusters. The high-surface-area architecture observed in Figure C suggests that the increased number of CV cycles promotes the continuous deposition of gold onto pre-existing sites, favouring the growth of complex, multi-layered nanocluster formations over the formation of new nuclei.

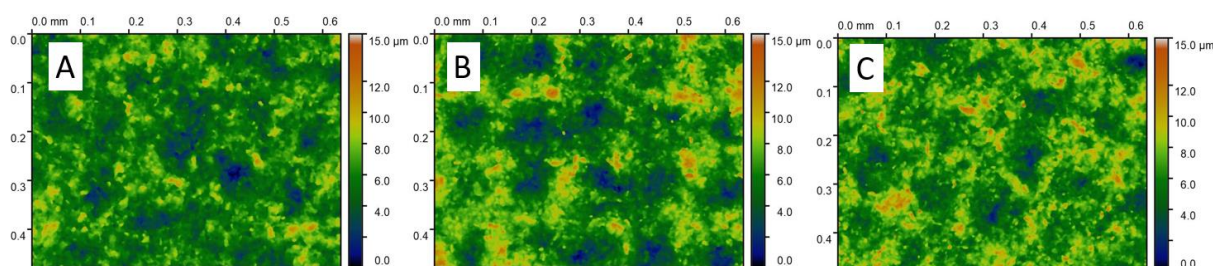


Figure 2 Confocal microscopy images of SPCE after the AuNPs electrodeposition during 5 (A), 10 (B), and 15 cycles (C).

To further quantify the morphological evolution of the electrodeposited gold nanostructures, confocal microscopy was employed. The surface topography maps (Figures 2 A–C) and the corresponding numerical data (Table 1) provide insight into the vertical growth and surface complexity as a function of the deposition cycles.

The topographic maps reveal a progressive increase in surface heterogeneity. Sample prepared during 5 cycles of electrodeposition exhibits a relatively uniform distribution of nucleation sites with a maximum height of 11.57 μm . As the deposition proceeds to 10 cycles (Figure 2 B), the formation of larger aggregates is reflected in the increase of both the maximum height to

13.5 μm and the surface roughness to 1.526 μm . This trend correlates with the SEM observations of particle coalescence and the development of three-dimensional clusters.

Table 1 List of calculated characteristics based on the confocal microscopy measurements.

Number of electrodeposition cycles	Roughness	Maximum high	Surface area
5 cycles	1.19 μm	11.57 μm	0.3202 mm^2
10 cycles	1.53 μm	13.5 μm	0.3216 mm^2
15 cycles	1.29 μm	14.24 μm	0.3256 mm^2

The quantitative analysis highlights a continuous increase in the effective surface area, expanding from 0.3202 mm^2 (5 cycles) to 0.3256 mm^2 (15 cycles). This steady growth is critical for applications such as electrochemical sensing or catalysis, where a higher active surface area typically enhances performance. Interestingly, the roughness values show a non-linear progression. An initial increase is observed between 5 and 10 cycles (1.185 μm to 1.526 μm), corresponding to the rapid growth of isolated hierarchical structures. A slight decrease in roughness to 1.285 μm is noted at 15 cycles. This phenomenon can be attributed to the "filling-in" effect, where secondary deposition occurs within the valleys of the initial clusters, leading to a partial topographical levelling despite the increase in the overall maximum height (14.24 μm). This suggests that at 15 cycles, the film transitions from a collection of discrete tall peaks toward a more continuous, albeit highly structured, porous layer.

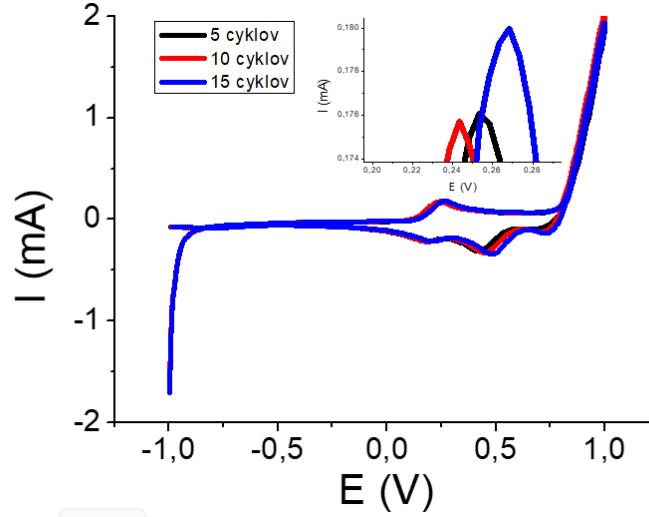


Figure 3 Cyclic voltammograms for SPCE modified by AuNPs during 5, 10, and 15 cycles in the 5 mM $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ in 1M KCl solution.

The electrochemical performance and the electrochemically active surface area of the SPCE/AuNP electrodes were evaluated using cyclic voltammetry (CV) in a 5 mM $[Fe(CN)_6]^{3-/4-}$ redox probe solution. Figure 3 displays the cyclic voltammograms for the electrodes modified with 5, 10, and 15 deposition cycles. All modified electrodes exhibit the characteristic reversible redox peaks of the $[Fe(CN)_6]^{3-/4-}$ couple. As illustrated in the inset of Figure 3, an increase in the number of deposition cycles leads to an enhancement of the anodic peak current. Specifically, the sample prepared with 15 cycles shows the highest current response, which correlates with the increased mass of deposited gold and the formation of the complex hierarchical structures observed in the SEM and topographical analyses. Furthermore, the potential of the anodic peak shifts slightly toward more positive values with increasing cycle numbers, and the peak-to-peak separation remains relatively stable. This indicates that the AuNP modification successfully facilitates electron transfer kinetics while maintaining the electrochemical stability of the screen-printed carbon substrate. The active surface area was calculated using the Randles-Sevcik equation:

$$I_p = 0,4463nFAC\sqrt{\frac{nFvD}{RT}} \quad (1)$$

where A represents the electrochemically active area, c is concentration of electroactive species, v is scan rate, D is diffusion coefficient, and n , F , R , and T have usual meaning. For 5 and 10 cycles, the active surface area remains constant at 1.150 mm², suggesting that although the particle size increased (as shown by SEM), the number of accessible electroactive sites did not change significantly at this stage. However, upon reaching 15 cycles, an increase in the active

area to 1.154 mm² is observed. This slight enhancement is attributed to the development of the 3D porous network and hierarchical nanoclusters identified in the morphological study, which provide additional surface sites for the redox probe interaction. The discrepancy between the geometrical surface area (from confocal microscopy) and the ECSA highlights the importance of the hierarchical nanostructure in improving the overall electrochemical sensitivity of the sensor. Due to highest active surface area electrode prepared by 15 cycles of gold electrodeposition (SPCE/15AuNPs) was used for further experiments.

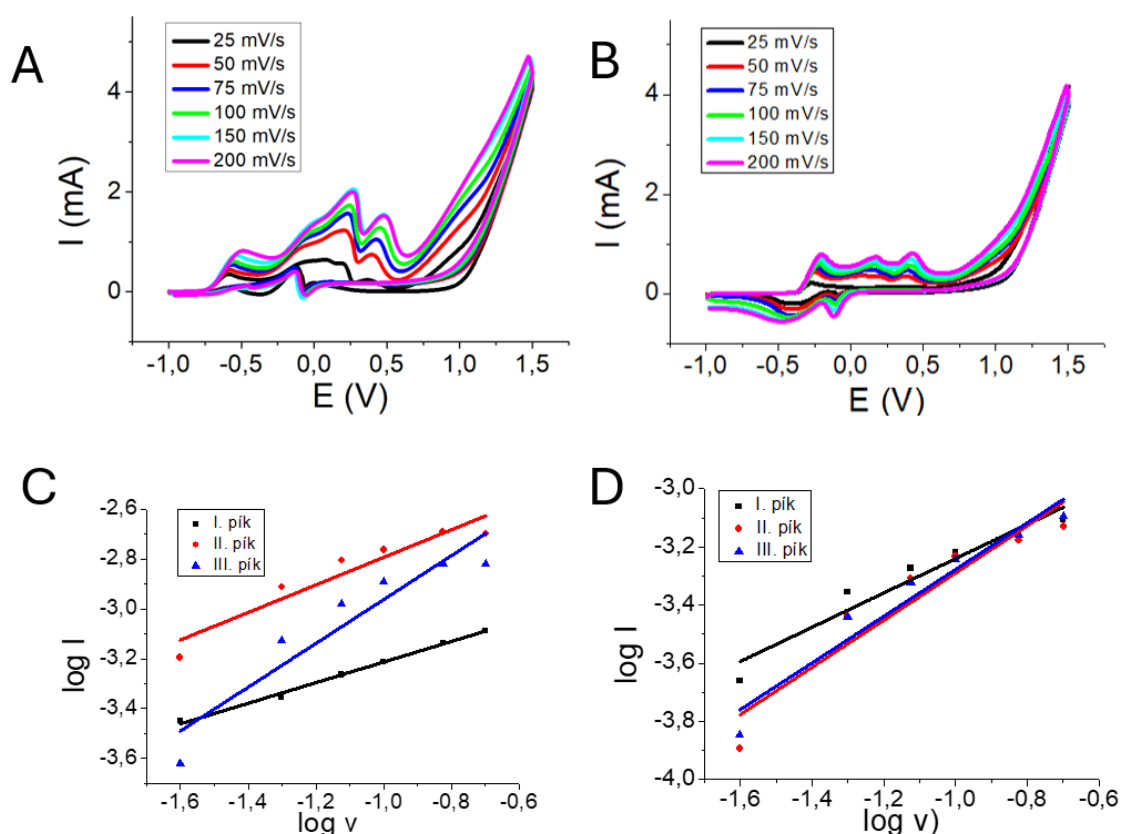


Figure 4 Cyclic voltammograms and $\log I$ vs. $\log v$ for SPCE/15AuNPs electrode in 30 mM glucose (A, C) and 10 mM ascorbic acid (B, D) in 0.1 M NaOH/PBS at various scan rates (25, 50, 75, 100, 150, and 200 mV/s).

The electrocatalytic activity of the SPCE/AuNP electrodes was further evaluated towards the oxidation of glucose and ascorbic acid. Kinetic investigations were performed by varying the scan rate from 25 to 200 mV/s to determine the nature of the mass transport and electron transfer processes. Figure 4 A illustrates the cyclic voltammograms of glucose oxidation on SPCE/15AuNPs electrode. Multiple anodic peaks are observed at potential, corresponding to

the complex multi-step oxidation mechanism of glucose on gold surfaces, typically involving the formation of gluconolactone. First peak was occurred at potential - 0.5 V, second broad peak at 0.2 V, and third at 0.4 V. The mentioned peaks pertain to glucose adsorption and electrooxidation and gold oxidation to AuOH followed by second oxidation to Au₂O₃.

The peak current increases consistently with the scan rate for all oxidation peaks. To elucidate the reaction kinetics, the logarithm of the peak current (log) was plotted against the logarithm of the scan rate (log ν), as shown in Figure 4 C. According to the slope of linear regression first process is diffusion limited (slope 0.41), second process is diffusion limited (slope 0.55), and third process is adsorption limited (slope 0.87). Slopes suggest a mixed kinetic regime, where diffusion of glucose from the bulk solution is the primary rate-limiting step, potentially influenced by weak adsorption of intermediate species on the hierarchical AuNPs surface.

The electrochemical response towards ascorbic acid is presented in Figure 4 B. Compared to glucose, the oxidation of ascorbic acid exhibits distinct, well-defined anodic peaks at lower potentials (1. at - 0.26 V, 2. at 0.15 V, and 3. at 0.4 V). The kinetic analysis in Figure 4 D (log I vs. log ν) shows a high degree of linearity for all three peaks. The slopes derived from these plots are significantly steeper than those observed for glucose (1. peak 0.59, 2. peak 0.81, and 3. peak 0.80). This trend indicates a strong contribution from adsorptive phenomena or a more robust electrocatalytic facilitation by the AuNPs clusters. The convergence of the log I values at higher scan rates suggests that the SPCE/15AuNPs platform provides excellent electronic coupling for the ascorbic acid redox reaction, making it a promising candidate for sensitive amperometric sensing.

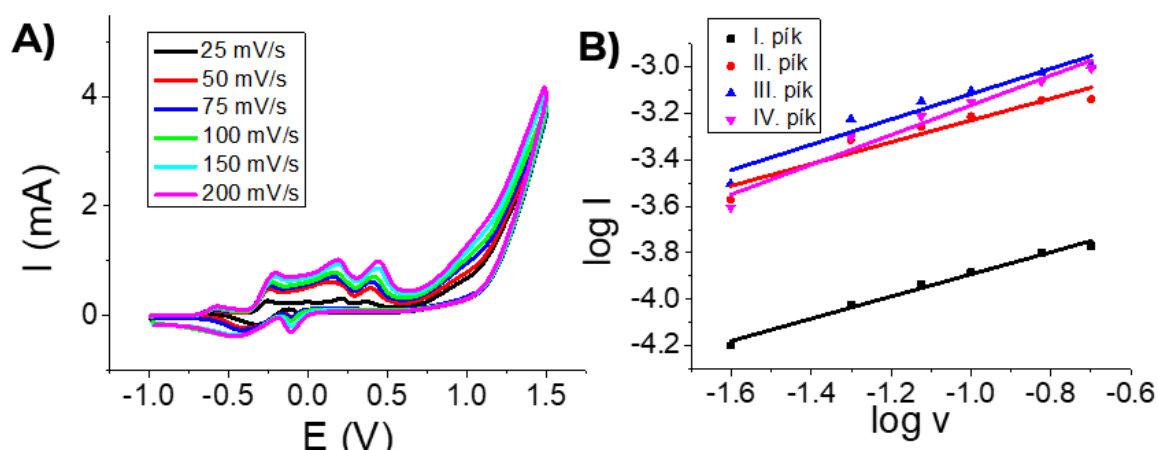


Figure 5 Cyclic voltammograms for SPCE/15AuNPs electrode in 10 mM ascorbic acid, 5 mM glucose, 0.1 M NaOH/PBS solution at various scan rates (25, 50, 75, 100, 150, and 200 mV/s) (A), log I vs. log ν (B).

To evaluate the practical applicability and selectivity of the SPCE/15AuNPs platform, cyclic voltammetry measurement was performed in a mixed solution containing both glucose and ascorbic acid. Figure 5 A illustrates the voltametric response at various scan rates ranging from 25 to 200 mV/s. The modified electrode exhibits excellent electrocatalytic resolution, as evidenced by the appearance of four distinct and well-separated anodic peaks. The peak I at -0.6 V is attributed to the oxidation of glucose because the peak corresponds to the peak at -0.5 V for glucose sample. While the subsequent peaks at -0.25 V corresponds to the ascorbic acid oxidation process on the gold nanostructures. Peak at 0.2 V, and 0.4 V are common for both glucose and ascorbic acid oxidation mechanism. It could be expected that the adsorption of glucose and ascorbic acid take a place at different potential while the oxidation of both molecules and gold surface occurs at the same potential. The clear potential separation between these species indicates that the hierarchical 3D architecture of the AuNPs effectively mitigates the overlapping of redox signals, which is a common challenge in non-enzymatic sensing. As the scan rate increases, a consistent enhancement in the peak current for all four oxidation processes is observed, alongside a typical slight shift in peak potentials toward more positive values, characteristic of quasi-reversible electron transfer kinetics.

To further elucidate the mass transport and electron transfer mechanisms in this competitive environment, a kinetic study was conducted. Figure 5 B shows the linear relationship between the logarithm of the peak current ($\log I$) and the logarithm of the scan rate ($\log \nu$) for each of the four identified peaks.

The calculated slopes, represented by the gradients in Figure 5 B, provide insight into the rate-limiting steps:

- Peaks I (glucose): The slopes are 0.48, indicating that the oxidation of glucose is primarily governed by a diffusion-controlled process.
- Peaks II (ascorbic acid): The slopes for the ascorbic acid oxidation peaks also maintain high linearity, suggesting that despite the presence of glucose, the ascorbic acid oxidation remains stable. The slope for this peak was calculated 0.47 which indicates diffusion-controlled process, too.
- Peaks III., IV.: The slopes were 0.55 and 0.64 for peak III. and peak IV., respectively. The values indicate the diffusion-controlled process, which changed in comparison to slopes for single molecules.

The fact that all four signals remain resolvable and follow a predictable kinetic trend across the entire range of scan rates demonstrates the high stability and sensitivity of the SPCE/15AuNPs

electrode. This performance highlights the potential of the developed sensor for the simultaneous determination of multiple analytes in complex biological matrices.

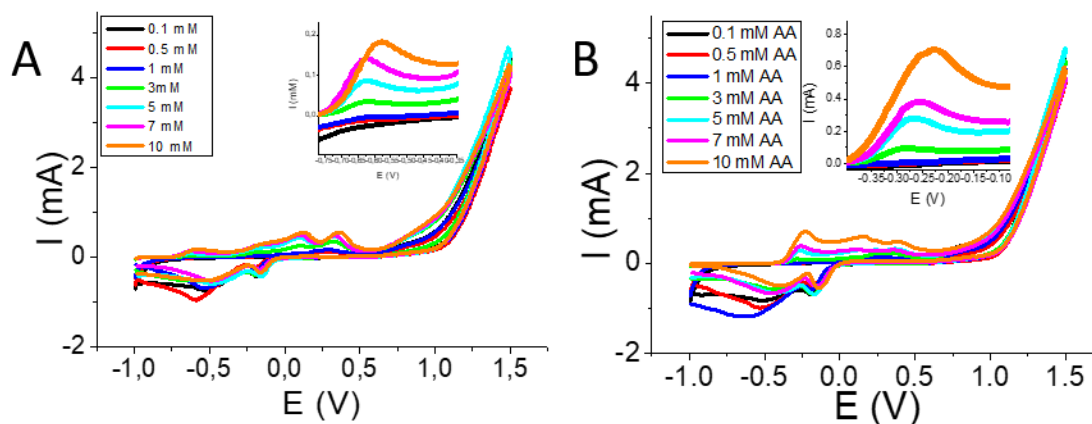


Figure 6 Cyclic voltammograms for SPCE/15AuNPs electrode for various concentration of glucose (A) and ascorbic acid (B) 0.1 M NaOH/PBS solution at 100 mV/s (A).

The analytical capability of the SPCE/15AuNPs electrode for the individual quantification of glucose and ascorbic acid was evaluated using cyclic voltammetry at varying concentrations (0.1–10 mM). The resulting voltammograms are presented in Figure 6 (A for glucose, B for ascorbic acid). As shown in Figure 6 A, the anodic peak current at potential increases proportionally with the addition of glucose. In this case, the attention was paid mainly anodic current at -0.6 V. The inset highlights the well-defined oxidation peaks even at lower concentrations, indicating the strong electrocatalytic influence of the gold nanostructures. The calibration plot (Current I vs. Concentration c , Figure 7) exhibits excellent linearity over the entire investigated range (0.1 to 10 mM). The sensitivity, derived from the slope of the linear regression, was calculated to be $20 \mu\text{A}/\text{mM}$. Based on the signal-to-noise ratio, the limit of detection (LOD) for glucose was determined to be 0.8 mM. These values suggest that the modified electrode is highly suitable for monitoring glucose levels within a physiologically relevant range.

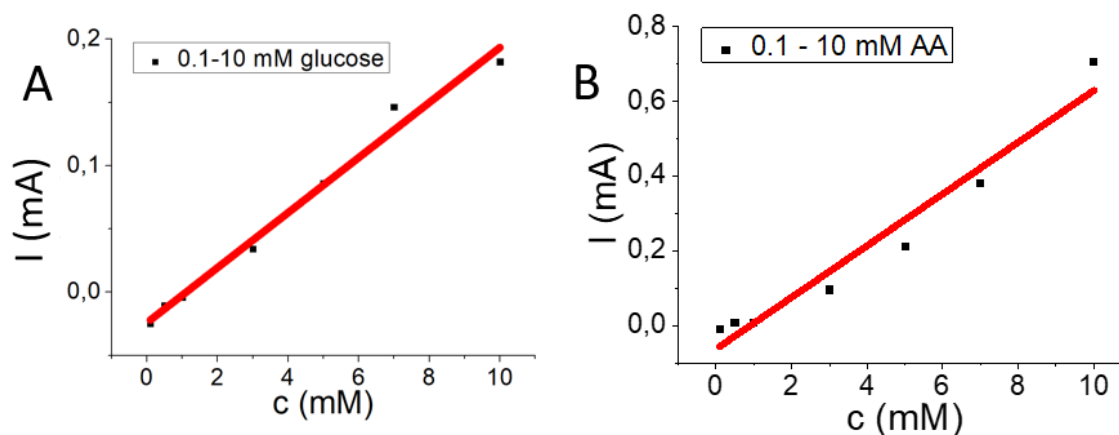


Figure 7 Dependences of current on concentration for SPCE/15AuNPs electrode for various concentration of glucose (A) and ascorbic acid (B) 0.1 M NaOH/PBS solution fitted by linear function.

The sensor's response toward ascorbic acid is depicted in Figure 6 B. Similar to glucose, ascorbic acid exhibits a concentration-dependent increase in the oxidation peak current at potential, the main attention was paid to anodic peak at -0.25 V. The calibration curve demonstrates a linear response from 0.1 to 10 mM. The sensitivity for ascorbic acid was found to be 0.07 mA/mM, which is significantly higher (more than triple) than that of glucose. This results from the greater ability of ascorbic acid to oxidize, which is why this molecule is well known. The calculated LOD for ascorbic acid is 1.5 mM. Although the LOD is slightly higher than that of glucose, the superior sensitivity indicates that the sensor can detect minute changes in AA concentration with high precision. The combination of a broad linear range, reproducible sensitivity, and low detection limits confirms that the SPCE/15AuNPs platform is a robust candidate for non-enzymatic sensing applications. The ability to detect glucose and ascorbic acid at different potential represents prerequisite to simultaneous detection of both analytes.

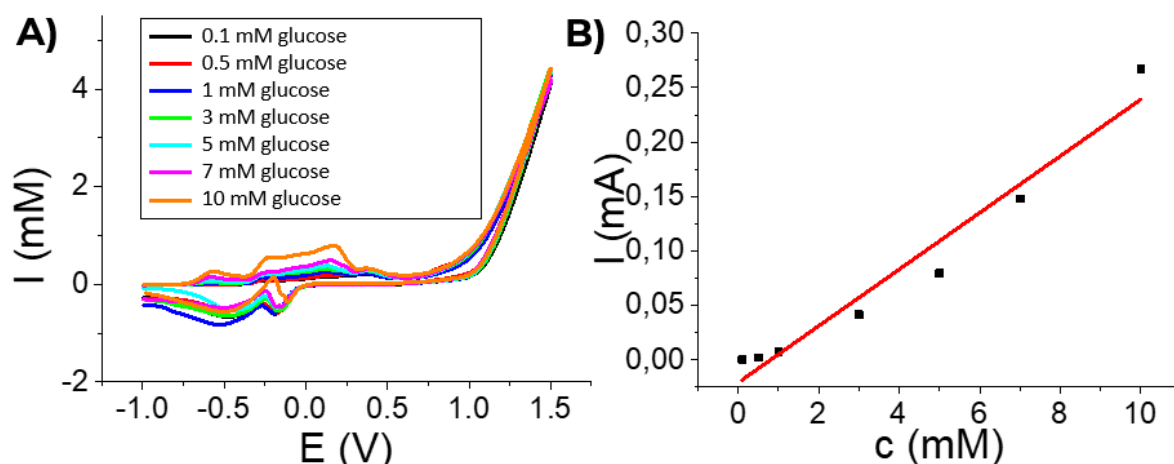


Figure 8 Cyclic voltammograms (A) and dependences of current on concentration fitted by linear function (B) for SPCE/15AuNPs electrode for various concentration of glucose in 10 mM ascorbic acid, 0.1 M NaOH/PBS solution at 100 mV/s.

The analytical capability of the optimized SPCE/15AuNPs electrode was evaluated through the quantification of glucose in a complex matrix containing 10 mM ascorbic acid. As illustrated in Figure 8A, the cyclic voltammograms exhibit a clear concentration-dependent increase in the anodic peak current as glucose levels rise from 0.1 to 10 mM. Despite the high background concentration of ascorbic acid, the gold hierarchical nanostructures maintain significant electrocatalytic activity, allowing for the clear resolution of glucose oxidation signals. The well-defined peaks, even at low concentrations, suggest that the 3D porous morphology of the 15-cycle AuNPs layer provides a high density of active sites that are not easily saturated by interfering species. The corresponding calibration curve, depicted in Figure 8B, demonstrates a robust linear relationship between the peak current and glucose concentration across the investigated range. The quantitative analytical parameters derived from this linear regression are sensitivity (0.03 mA/mM) and limit of detection 1.46 mM. The electrode maintains a stable and linear response from 0.1 to 10 mM, covering both physiological and pathological glucose concentrations. While the presence of 10 mM ascorbic acid introduces a competitive environment at the electrode interface, the SPCE/15AuNPs platform demonstrates remarkable selectivity and stability. These results indicate that the developed hierarchical gold nanostructure is a promising candidate for the development of non-enzymatic sensors capable of operating in real-world biological samples where interferents are typically present.

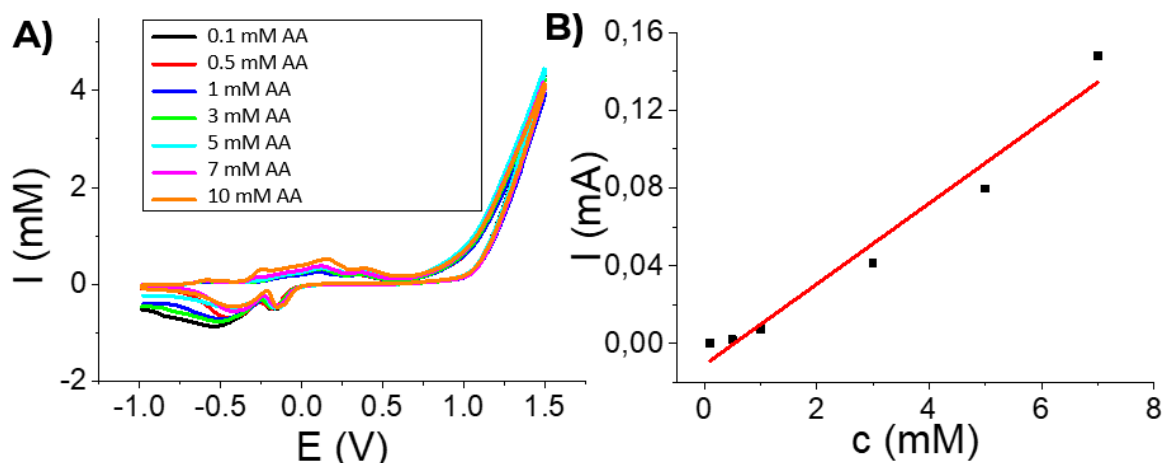


Figure 9 Cyclic voltammograms (A) and dependences of current on concentration fitted by linear function (B) for SPCE/15AuNPs electrode for various concentration of ascorbic acid in 10 mM glucose, 0.1 M NaOH/PBS solution at 100 mV/s.

To complement the glucose sensing studies, the analytical performance of the SPCE/15AuNPs electrode was further evaluated for the quantification of ascorbic acid in a competitive environment containing 10 mM glucose. Figure 9A presents the cyclic voltammograms obtained for ascorbic acid concentrations ranging from 0.1 to 10 mM. A clear, well-defined anodic oxidation peak for ascorbic acid is observed, with the peak current exhibiting a systematic increase as a function of analyte concentration. The stability of the baseline and the resolution of the ascorbic acid oxidation signal in the presence of a high glucose concentration (10 mM) demonstrate the effective electrocatalytic discrimination provided by the hierarchical gold nanoclusters. The calibration plot, shown in Figure 9B, reveals a linear relationship between the anodic peak current and ascorbic acid concentration within the range of 0.1 to 7 mM. At concentrations beyond 7 mM, a slight deviation from linearity is observed, likely due to the partial saturation of the active sites on the AuNP surface or competitive adsorption between the oxidation products of ascorbic acid and the background glucose molecules. The quantitative parameters derived from the linear portion of the calibration curve were calculated from linear regression. The sensitivity for ascorbic acid detection in this mixed matrix was calculated to be 0.02 mA/mM. This value confirms that the SPCE/15AuNPs electrode remains highly active for ascorbic acid oxidation, even when subjected to competitive intermediate species from glucose oxidation. The sensor exhibits a reliable linear response from 0.1 to 7 mM, which is suitable for the detection of AA in various biological and industrial applications. The LOD was determined to be 1.02 mM. Comparing these results with the glucose sensing performance (LOD of 1.46 mM), the lower LOD for AA (1.02 mM) suggests a higher affinity

of the hierarchical AuNP surface for ascorbic acid under these specific experimental conditions. The successful individual quantification of both analytes in the presence of a 10 mM concentration of the other (as shown in Figures 8 and 9) highlights the SPCE/15AuNPs as a versatile platform for non-enzymatic sensing with minimized cross-interference suitable to determine both glucose and ascorbic acid simultaneously.

To objectively assess the analytical standing of the developed SPCE/15AuNPs electrode, its performance was compared with various previously reported modified electrodes for glucose and ascorbic acid detection, as summarized in Table 2. The proposed sensor exhibits a competitive linear range of 0.1 – 10 mM, which is notably broader than several complex modifications. While specialized nanomaterials like Zn/Co₃O₄/rGO/GCE or Cu/Cu₂O/CDs/CC achieve significantly higher sensitivities and lower limits of detection (LOD) in the micromolar range, the SPCE/15AuNPs platform offers a balanced trade-off between sensitivity (0.03 mA/mM for GLU and 0.02 mA/mM for AA) and operational simplicity. Furthermore, unlike the Ni-Cu alloy [6] or Sonogel carbon [5] systems which focus primarily on narrow concentration windows, our developed hierarchical gold nanostructure provides reliable quantification across a wide range of concentrations relevant to both physiological and pathological monitoring. The robust performance in the presence of mutual interference, coupled with the inherent advantages of disposable screen-printed transducers, positions the SPCE/15AuNPs as a highly practical and cost-effective alternative for routine non-enzymatic multi-analyte sensing.

Table 2 List of electrode modifications and its electroanalytical characteristics.

Electrode material	Linear range	Limit of detection	Sensitivity	References
AuNP-MIPs	1.25 – 3.2 μM (GLU)	1.25 nM (GLU)	-	[12]
Cu@PHEMA/SPE	0 – 2 mM (GLU), 0 – 220 μM (AA)	4.5 μM (glucose), 0.96 μM (AA)	122.24 $\mu\text{A}/\text{mMcm}^2$ (GLU), 799.85 $\mu\text{A}/\text{mMcm}^2$ (AA)	[28]
Zn/Co ₃ O ₄ /rGO/GCE	0.015 – 10 mM (GLU)	0.043 μM (GLU)	1551.38 $\mu\text{A}/\text{mMcm}^2$	[29]
Cu/Cu ₂ O/CDs/CC	0.2 – 100 μM , 0.1 – 1 mM (GLU)	0.66 μM	4510.89 $\mu\text{A}/\text{mMcm}^2$, 2385.94 $\mu\text{A}/\text{mMcm}^2$ (GLU)	[30]
Sonogel carbon	0.5 – 100 μM , 100 – 1000 μM (AA)	0.04 μM (AA)	0.468 $\mu\text{A}/\mu\text{M}$, 0.021 $\mu\text{A}/\mu\text{M}$ (AA)	[7]
Ni-Cu alloy	0.5 – 8 mM	19 μM (AA)	507.7 $\mu\text{A}/\text{mMcm}^2$ (AA)	[31]
SPCE/15AuNPs	0.1 – 10 mM	1.46 mM (GLU), 1.02 mM (AA)	0.03 mA/mM (GLU), 0.02 mA/mM (AA)	This work

AA – ascorbic acid, CC – carbon cloth, CDs – carbon dots, GCE – glassy carbon GLU- glucose, PHEMA – poly(2-hydroxyethyl methacrylate), rGO – reduced graphene oxide, SPE -screen-printed electrode

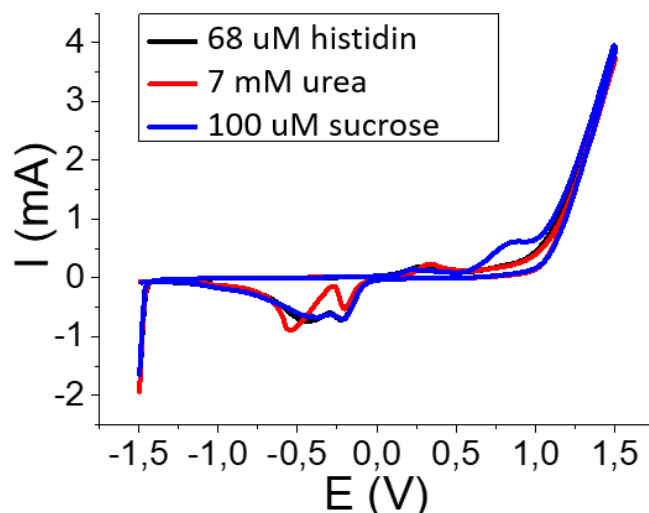


Figure 10 Cyclic voltammograms for SPCE/15AuNPs electrode 68 μ M histidine, 7 mM urea and 100 μ M sucrose in 0.1 M NaOH/PBS solution at 100 mV/s.

To further validate the robustness of the SPCE/15AuNPs electrode for real-world applications, its electrochemical response was tested in the presence of other common biological interferents, namely histidine (68 μ M), urea (7 mM), and sucrose (100 μ M). As shown in Figure 10, the cyclic voltammograms recorded for the three different interferents exhibit distinct profiles, yet the overall current magnitudes remain significantly lower or localized at potentials different from the primary analytes (glucose and ascorbic acid). The voltametric curve for sucrose shows a relatively stable baseline with minor anodic features at higher potentials, indicating that its oxidation kinetics on the hierarchical gold surface are slow and do not interfere significantly with the quantification of smaller sugars or acids at lower potentials. Despite its high concentration (7 mM), urea exhibits negligible electrochemical activity within the investigated potential window. The absence of prominent oxidation peaks confirms that the SPCE/15AuNPs electrode is highly resistant to fouling or signal interference from urea, which is critical for measurements in physiological fluids such as urine or serum. The response to histidine displays small anodic features, typical for the oxidation of amino acid groups on gold nanostructures; however, these signals are well-separated from the typical detection windows of glucose and ascorbic acid. The overlay of these voltammograms demonstrates that the SPCE/15AuNPs platform provides a high degree of discrimination against diverse classes of biomolecules (amino acids, amides, and disaccharides). The hierarchical 3D morphology of the gold nanoparticles appears to favour the specific electrocatalytic pathways of the target analytes while exhibiting minimal cross-reactivity with these interferents at their respective physiological concentrations. This selective behaviour confirms that the developed sensor is a

highly reliable tool for the non-enzymatic determination of glucose and ascorbic acid in complex biological matrices where histidine, urea, and sucrose are co-present.

Conclusion

In this study, we successfully developed a non-enzymatic electrochemical sensing platform based on screen-printed carbon electrodes modified with hierarchical gold nanostructures. The morphological evolution of the AuNPs was systematically controlled by the number of electrodeposition cycles using cyclic voltammetry. SEM and confocal microscopy confirmed that increasing the deposition to 15 cycles resulted in the formation of a high-surface-area, three-dimensional porous network of hierarchical nanoclusters. This specific architecture provided superior electrocatalytic properties compared to the lower cycle counts, as evidenced by an increased electrochemically active surface area of 1.154 mm². The analytical performance of the SPCE/15AuNPs electrode demonstrated remarkable efficiency for both individual and simultaneous detection of glucose and ascorbic acid. Kinetic studies revealed that the oxidation processes are predominantly diffusion-controlled, with high stability across various scan rates. The sensor exhibited a broad linear range (0.1–10 mM) and high sensitivity for both analytes, even when subjected to competitive environments. Specifically, the sensor maintained its quantitative accuracy for glucose in the presence of 10 mM ascorbic acid (sensitivity: 0.03 mA/mM; LOD: 1.46 mM) and for ascorbic acid in the presence of 10 mM glucose (sensitivity: 0.02 mA/mM; LOD: 1.02 mM). Furthermore, interference studies involving urea, sucrose, and histidine confirmed the high selectivity of the hierarchical AuNP surface, showing negligible cross-reactivity with these common biological molecules. The synergistic combination of the low-cost SPCE transducer and the tailored 3D gold nanostructure offers a robust, sensitive, and selective tool for multi-analyte sensing. These results suggest that the developed platform holds great potential for future applications in point-of-care diagnostics and the monitoring of complex biological matrices without the need for biological recognition elements. Beyond demonstrating the analytical response of a gold-modified screen-printed electrode, this study highlights the importance of deposition-controlled surface morphology and analyte-dependent oxidation kinetics in developing non-enzymatic platforms capable of distinguishing multiple electroactive compounds under competitive conditions.

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