

Chitosan-Covered Pd@Pt Core–Shell Nanocubes for Direct Electron Transfer in Electrochemical Enzymatic Glucose Biosensor

Siva Kumar Krishnan,^{*,†} Evgen Prokhorov,[‡] Daniel Bahena,[§] Rodrigo Esparza,^{*,†} and M. Meyyappan^{||}

[†]Centro de Física Aplicada y Tecnología Avanzada, Universidad Nacional Autónoma de México, Boulevard Juriquilla 3001, Santiago de Querétaro, Querétaro 76230, Mexico

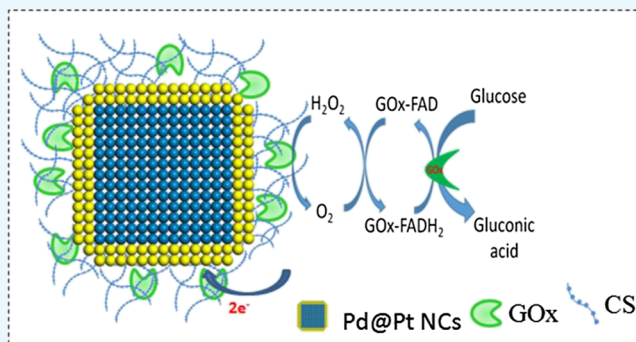
[‡]Centro de Investigación y Estudios Avanzados, Unidad Querétaro, Santiago de Querétaro, Querétaro 76230, Mexico

[§]Advanced Laboratory of Electron Nanoscopy, Cinvestav, Av. Instituto Politécnico Nacional, 2508, Col. San Pedro Zacatenco, Delegación Gustavo A. Madero, Mexico D.F. CP 07360, Mexico

^{||}Center for Nanotechnology, NASA Ames Research Center, Moffett Field, Mountain View, California 94035, United States

S Supporting Information

ABSTRACT: Development of biosensors with high sensitivity, high spatial resolution, and low cost has received significant attention for their applications in medical diagnosis, diabetes management, and environment-monitoring. However, achieving a direct electrical contact between redox enzymes and electrode surfaces and enhancing the operational stability still remain as challenges. Inorganic metal nanocrystals (NCs) with precisely controlled shape and surface structure engineered with an appropriate organic coating can help overcome the challenges associated with their stability and aggregation for practical biosensor applications. Herein, we describe a facile, room-temperature, seed-mediated solution-phase route to synthesize monodisperse Pd@Pt core–shell nanocubes with subnanometer-thick platinum (Pt) shells. The enzyme electrode consisting of Pd@Pt core–shell NCs was first covered with a chitosan (CS) polymer and then glucose oxidase (GOx) immobilized by a covalent linkage to the CS. This polymer permits covalent immobilization through active amino (–NH) side groups to improve the stability and preserve the biocatalytic functions while the Pd@Pt NCs facilitate enhanced direct electron transfer (DET) in the biosensor. The resultant biosensor promotes DET and exhibits excellent performance for the detection of glucose, with a sensitivity of $6.82 \mu\text{A cm}^{-2} \text{mM}^{-1}$ and a wide linear range of 1–6 mM. Our results demonstrate that sensitive electrochemical glucose detection based on Pd@Pt core–shell NCs provides remarkable opportunities for designing low-cost and sensitive biosensors.



1. INTRODUCTION

Metal nanocrystals (NCs) with distinct shapes and controllable facets have received substantial attention because of their attractive properties such as enhanced electrocatalytic activity and biosensing properties.^{1,2} Recently, development of platinum (Pt)-based bimetallic nanostructures with tailored geometries (e.g., core–shell nanostructures with ultrathin Pt shell thickness) has received attention toward enabling enhanced electrocatalytic activity and reducing cost.^{1,3} In particular, Pd@Pt nanostructures have been extensively reported for various applications such as fuel cell electrodes,³ hydrogen peroxide (H_2O_2) and glucose biosensing,^{4–6} and gas sensors.⁷ Also, Pd has been widely used as a substrate to deposit Pt layers because of its very similar lattice constant and chemical stability.⁸ Ultrathin Pt layers on single-crystal substrates have been prepared by vacuum deposition.⁹ The seed-mediated solution-phase technique has been widely used recently for the deposition of Pt-on-Pd NCs for the Pd@Pt core–shell nanostructures, wherein thickness of the Pt shell can

be controlled at 1–6 atomic layers.^{10,11} However, precise control of the thickness of the Pt layers down to the subnanometer level is difficult because of the galvanic replacement reaction between the two metals in aqueous solutions, which leads to the formation of voids (small holes) or concave structures at room temperature.³ In addition, the high intrinsic surface energy and interatomic bond energy (307 kJ/mol) of Pt lead to selective deposition at the corner sites typically adopts an island growth mode (Volmer–Weber mode)¹² and followed by the diffusion onto the other faces of the NCs. Thus, uniform deposition of Pt can be achieved only at a higher reaction temperature by altering the diffusion rates.¹⁰ More recently, Yang et al.¹³ have reported the galvanic replacement-free deposition of an ultrathin shell of 0.6 nm thick Au on Ag nanocubes. The galvanic reaction was avoided by

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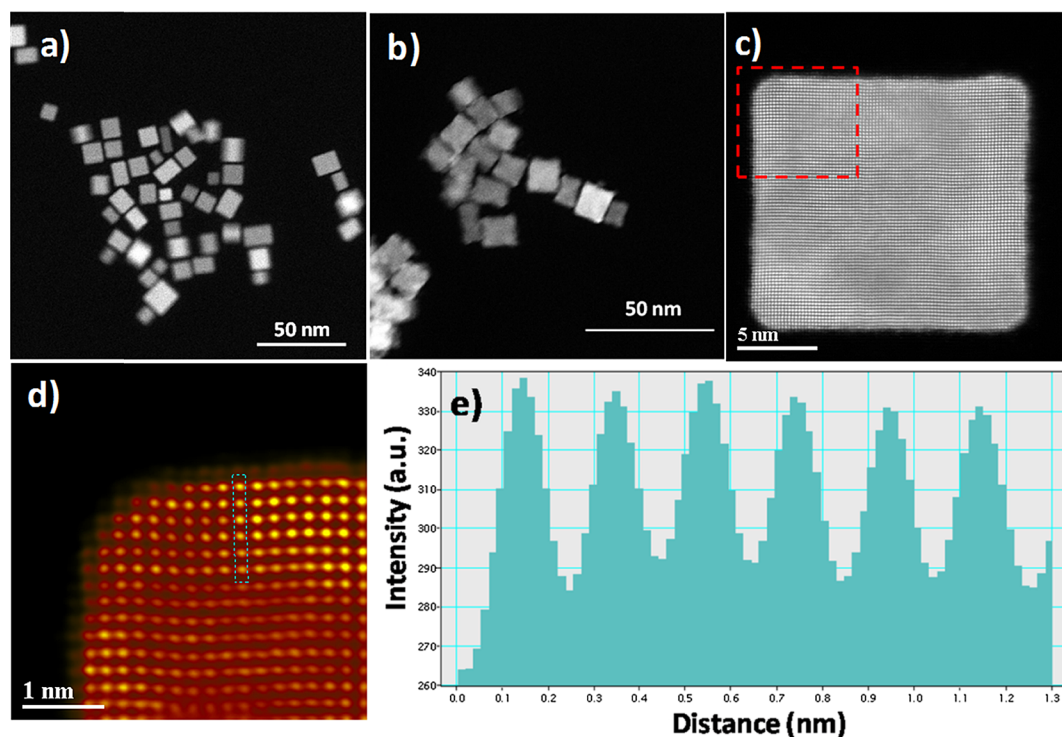


Figure 1. (a,b) STEM images of Pd and Pd@Pt core-shell nanocubes. (c) HAADF-STEM image of the individual Pd@Pt NCs. (d) High-resolution HAADF-STEM image taken from the region marked by a box in (c), revealing a Pt shell thickness of four atomic layers. (e) Intensity profile along the region marked in (d) showing six atomic layers.

merely increasing the pH to induce the reduction power of ascorbic acid (AA), thereby blocking the galvanic reaction between Ag and Au^{3+} , and to achieve conformal deposition of three to six atomic layers of Au shell on Ag nanocubes. However, such a method has not been investigated for other types of nanoparticles (NPs).

Several strategies have been reported to overcome the limitation for the enzyme immobilization and facilitate enhanced direct electron transfer (DET) process for metal NP-based biosensors.¹⁴ Pt NPs have been highly exploited for developing glucose sensors¹⁵ and human metabolite detection.¹⁶ Nevertheless, the high cost of Pt significantly limits its practical applications in biosensors. Therefore, many groups have attempted to create Pt-based bimetallic NCs because of the possibility to tune the Pt d-band structure, which correlates with the adsorption strength of the catalyst surface, that is, Pt (100) surface through the strain and ligand effects.⁶ Moreover, bimetallic Pd@Pt core-shell nanocubes with sharp corners and edges can significantly enhance the glucose oxidation activities.¹⁷ Pt/Pd alloy NPs exhibit enzyme-mimic activity that can actively catalyze the H_2O_2 reduction to detect H_2O_2 in various environments.¹⁸ Dumbbell-like $\text{PtPd}-\text{Fe}_3\text{O}_4$ NPs exhibit an enhanced sensitivity relative to individual components for the continuous monitoring of H_2O_2 released from RAW264.7 cells.^{4,5} The enhancement in the activity is not only due to the alloy structure and composition but also due to the NP interface, which acts as a tuning factor to improve the catalytic activity of the biosensor.¹⁹ So far, there have been limited studies on glucose monitoring and the detection mechanism using Pd and Pt-based NCs as electrocatalytic materials.

Engineering the metal NP surface by organic coatings remarkably improves the biocatalytic activity and preserves the

structure of glucose oxidase (GOx).^{20,21} Polymeric capping agents can facilitate the incorporation of enzymes on the NP surface without losing their activity, and the charge transfer between the electron donor [flavin adenine dinucleotide (FAD)] and the surface of the electrode is more efficient in amperometric enzyme-based glucose biosensors.^{22,23} The immobilization of the enzymes on the solid support is the critical issue that strongly affects the biocatalytic activity and thus the sensitivity of the biosensor.^{14,24} Numerous materials have been developed such as block copolymers,²⁵ conducting hydrogels,²⁶ mesoporous silica,²⁷ DNA scaffolds,²⁸ bacteriophage,²⁹ ionic liquids,³⁰ metal-organic frameworks,³¹ carbon nanotubes (CNTs),³² halloysite nanotubes,³³ and graphene oxide (GO)³⁴ as efficient templates for effective immobilization, preserving the biocatalytic activity of enzymes and facilitating DET. Among all, chitosan (CS) biopolymers hold promise for the immobilization of enzymes not only due to their active $-\text{NH}_3^+/\text{NH}_2$ and $-\text{OH}$ functional groups but also due to their high biocompatibility and low cost.^{35,36} In our previous study, we have shown that CS-stabilized silver nanowires show enhanced charge transfer and stability for electrochemical detection of glucose.²⁰ The Pd@Pt core-shell nanocubes covered with CS provide synergizing activity of highly biocompatible CS and excellent electrocatalytic activity, high surface area, and superior binding affinity to biomolecules and, thus, are well-suited for the design of biosensors with high sensitivity and stability.

Herein, we report a facile, room-temperature route to synthesize Pd@Pt core-shell nanocubes with conformal deposition of ultrathin Pt shell (six atomic layers). The thickness of the Pt shells on the Pd nanocubes could be precisely tuned by simply varying the concentration of the Pt precursor. The key success of our synthesis strategy mainly

relies on using AA for fast reduction of Pt atoms by increasing the pH of the reaction, which allows the control of the reduction kinetics. Moreover, the resultant Pd@Pt NCs are covered with biocompatible CS as an efficient covalent immobilization matrix for the enzymatic electrochemical detection of glucose. We demonstrate remarkably enhanced sensitivity and selectivity to detect glucose based on CS/Pd@Pt NC/GOx enzyme electrodes.

2. RESULTS AND DISCUSSION

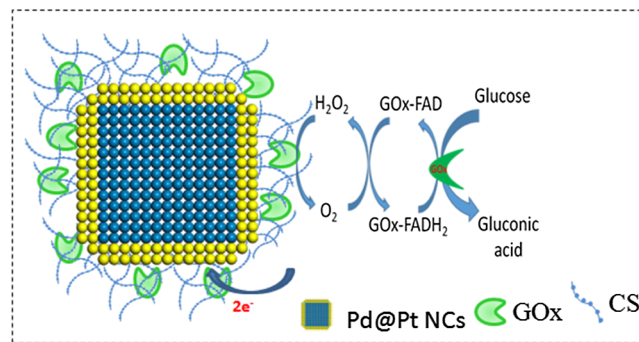
A typical scanning transmission electron microscopy (STEM) image of the as-prepared Pd nanocubes and Pd@Pt nanocubes has an average size of 13 nm, as shown in the STEM images, and a corresponding size distribution histogram, as shown in Figure S1. Figure 1a,b shows the STEM analysis of bare Pd nanocubes and Pd@Pt NCs revealing the conformal deposition of Pt layers of a few-atomic-layer thickness onto the surface of each Pd nanocube. The high-angle annular dark-field STEM (HAADF-STEM) image of the individual Pd@Pt NCs shows a clear contrast between the Pd core and the subnanometer-thick Pt shell that exhibits a periodic lattice plane extending across the entire surface, suggesting the single-crystalline nature of the Pt shell (Figure 1c). These contrast variations between the Pd core and Pt shell can be due to the large difference in atomic numbers between Pd and Pt elements.³⁷ Furthermore, the initial cubic shape of the Pd NCs remains the same, indicating the controlled layer-by-layer deposition of Pt. The atomic-resolution HAADF-STEM measurements for the resultant Pd@Pt NCs reveal six atomic layers of Pt shell (Figure 1d). Varying from 6 atomic layers to 10 and 20 layers is feasible by altering the amount of the Pt precursor solution from 0.1 to 0.25 mL and 0.5 mL, respectively (Figure S2). The intensity-profiling analysis (Figure 1e) along the region shown in Figure 1d further confirms the Pd core and the six atomic layers of Pt shell thickness. In addition, the energy-dispersive X-ray (EDX) elemental analysis and mapping (Figure S3) show the presence of Pd and Pt elements, and the color difference between the Pd core and Pt shell further confirms the homogeneous deposition of Pt on the surfaces of Pd nanocubes. The Pt atomic ratio was obtained using the inductively coupled plasma-atomic emission spectroscopy (ICP-AES) analysis as 7.05% of Pt in the resultant Pd@Pt NCs. Furthermore, X-ray photoelectron spectroscopy (XPS) measurements indicate a presence of both Pd and Pt elements with a Pt content of 7.15 wt % (Figure S4), which is consistent with the ICP result.

Previous reports have shown that because of the relatively higher standard reduction potential of $\text{PtCl}_6^{2-}/\text{Pt}$ [0.74 V vs rotation reversible hydrogen electrode (RHE)] than PdCl_4/Pd (0.62 V vs RHE), the deposition of Pt on the Pd nanocubes undergoes a galvanic replacement reaction, leading to the formation of concave Pd@Pt core-shell NCs.³⁸ Pt atoms can be uniformly deposited by heterogeneous nucleation on the Pd cubes at higher temperatures (180 °C), in which the homogeneous deposition with a well-defined shell thickness is largely determined by the difference in the Pt deposition rate (R_{dep}) and the surface diffusion rate (R_{dif}).^{10,39} In our present synthesis strategy, the deposition was done using a relatively strong reducing agent (AA) at room temperature, allowing the reduction of Pt immediately and selectively at the corner sites of the Pd nanocubes because of the high-energy Pd {100} facets and then diffusing onto the side faces. Therefore, the homogeneous deposition largely depends on the R_{dif} . The pH of the reaction system is found to be critical for accelerating the

surface diffusion of reduced Pt atoms for uniform deposition. The reduction power of reducing agents such as AA can be controlled by simply adjusting the pH value of the reaction solution, and thus, the reaction kinetics can be easily altered.¹³ When the pH of the reaction solution is adjusted to pH = 11, the surface diffusion of Pt atom is significantly enhanced, thereby creating the condition of $R_{\text{dep}} < R_{\text{dif}}$. Consequently, the reduced Pt atoms quickly diffuse onto the side faces to achieve a uniform Pt shell with controlled thickness. By contrast, relatively lower pH (pH = 4) under equivalent reaction conditions fails to produce uniform deposition. Interestingly, the resultant products are concave Pd@Pt core-shell NCs (Figure S5). These results clearly demonstrate that the pH of the reaction system plays a key role in modulating the reduction kinetics.

Fabrication of the enzyme electrode based on CS/Pd@Pt NC/GOx-glassy carbon electrode (GCE) comprises of three steps: preparation of the Pd@Pt core-shell NCs, surface functionalization with CS, and covalent immobilization of GOx enzyme (Scheme 1). Initially, the NCs were prepared and then

Scheme 1. Schematic Displaying the Surface Modification of Pd@Pt NCs Using CS Biopolymer and the Covalent Immobilization of GOx to the CS by Reacting with GA to Cross-Link the Amino Group of CS and the FAD Site of GOx



the CS biopolymer was used to functionalize through electrostatic interactions. After coating the NCs with the CS polymer, small CS layers encapsulated onto the NCs are formed, as can be seen in the transmission electron microscope (TEM) image shown in Figure 2a. The presence of amino groups in the highly biocompatible and hydrophilic CS permits covalent immobilization of GOx enzymes by reacting with the bifunctional cross-linker glutaraldehyde (GA).^{35,40} The glucose-sensing principle of GOx-functionalized NCs is based on the catalytic oxidation of glucose into gluconic acid and H_2O_2 in the presence of oxygen via an enzymatic reaction (Scheme 1). The GOx enzyme contains its active cofactor FAD bound to its two identical 80 kDa subunits. The efficient covalent immobilization of the GOx, the FAD cofactors being electrically contacted properly, and minimizing the electron-tunneling distance by bringing down the deeply buried FAD are all key factors.^{41,42} As a result, DET between the FAD center and the electrode surface is promoted and protection from losing the biocatalytic function improves the lifetime.^{25,43} Homogeneous dispersion of Pd@Pt NCs in CS polymer catalyzes the electrochemical oxidation of the enzymatically liberated H_2O_2 and promotes the enhanced charge transfer, thereby resulting in

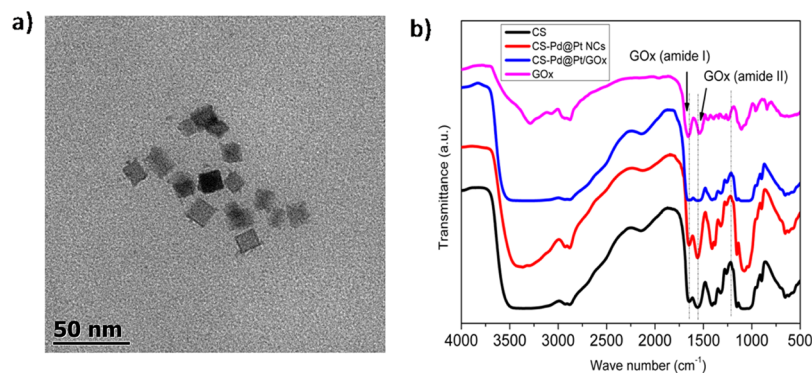


Figure 2. (a) TEM image of the CS-covered Pd@Pt NCs. (b) FT-IR spectra of the bare CS, CS/Pd@Pt NCs, CS/Pd@Pt NC/GOx, and native GOx.

the remarkable improvement in the sensitivity of the resultant biosensor.

The strategy for enzyme immobilization is the key factor that affects the biosensor performance by altering the charge transfer between the FAD center and the electrode surface, modulating the electron-tunneling distance and controlling the leaching effects.^{24,44} The CS contains an abundance of $-\text{HN}_3^+/\text{NH}_2$ and $-\text{OH}$ functional groups.²⁰ The $-\text{NH}$ -terminated surfaces were reacted with the bifunctional compound GA to cross-link the $-\text{NH}_2$ groups of the GOx by covalent linkage.^{16,35} The NH_2 -terminated surface of the CS was covalently linked by forming C–N bonds to the amino groups on the GOx by reacting with the two aldehyde groups on the GA.⁴⁵ We confirmed the structural interaction and immobilization of GOx enzymes with the CS/Pd@Pt NCs using Fourier transform infrared (FT-IR) spectroscopy (Figure 2b). The GOx shows characteristic transmittance bands at 1658, 1542, and 1103 cm^{-1} associated with the amide I and amide II absorption bands of the proteins and the C–O stretching vibration of GOx, respectively.⁴⁶ From the comparison of the spectra, the following are evident: appearance of new peaks at 1643 cm^{-1} band for amide I (C=O stretching vibrations), at 1556 cm^{-1} for amide II (N–H bending vibrations), and bands at 1410 cm^{-1} for $-\text{OH}$ bending vibrations upon GOx conjugation, suggesting the covalent binding through the C–N bonds and retaining the secondary structure of GOx.⁴⁷ In addition, dynamic light scattering (DLS) and zeta potential measurements were recorded to characterize the functionalization process. Table S1 presents the results for the hydrodynamic diameter determined from DLS and the corresponding surface charge obtained from the zeta potential analysis. From the DLS, the bare Pd@Pt NCs show a diameter of 13 nm, and this value increases to 17.3 nm after the CS coverage. At the same time, the surface charge of the bare Pd@Pt NCs inverts from a negative -1.02 mV to a positive value of 36.23 mV, suggesting the increase in the size and stability upon CS coverage on the NCs. After the immobilization of GOx, the diameter further increases to 37.1 nm, with a decrease in the zeta potential to 7.25 mV. The decrease in the surface charge is due to the interaction of $-\text{NH}$ groups with the GOx enzyme through the GA molecules.⁴⁰

Amperometric glucose detection using the fabricated electrode was performed using cyclic voltammetry (CV) and chronoamperometry measurements (CA). The CVs comparing the bare GCE, CS/Pd@Pt NC-GCE, and CS/Pd@Pt NC/GOx-GCE in N_2 -saturated phosphate-buffered saline (PBS, pH 7.4) at a scan rate of $50 \text{ mV}\cdot\text{s}^{-1}$ are shown in Figure 3. The

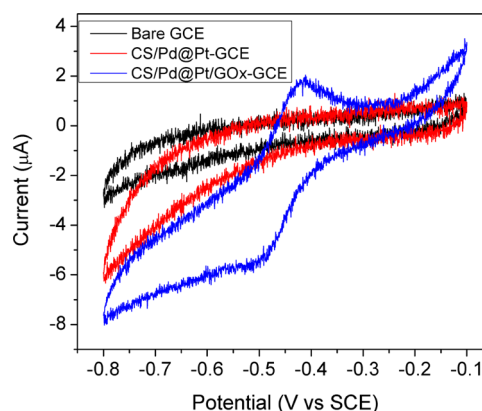


Figure 3. CVs of a bare GCE (black), CS/Pd@Pt NC-GCE (red), and CS/Pd@Pt NC/GOx-GCE (blue) in a N_2 -saturated PBS (pH = 7) at a scan rate of $50 \text{ mV}\cdot\text{s}^{-1}$.

results show that the bare GCE and CS/Pd@Pt NC-GCE exhibit no redox peaks in the potential range of interest. Nevertheless, the CV of the CS/Pd@Pt NC/GOx-GCE shows two well-defined redox peaks at cathodic (E_{pc}) and anodic (E_{pa}) peak potentials of -0.49 and -0.42 V, respectively. These redox peak potentials are close to the standard oxidation and reduction potentials of FAD/FADH_2 of GOx.^{43,46} This result indicates that our modified electrode can promote the DET between the electron donor (FAD) and the electrode surface.

The enzyme coverage density (Γ , mol/cm^2) of the immobilized GOx onto the CS/Pd@Pt NC/GOx-modified electrode can be estimated by integrating the cathodic peak according to the following equation.⁴⁸

$$\Gamma = Q/nFA \quad (1)$$

where Q is the charge consumed in the redox reaction (obtained by integrating the anodic peak and dividing by the scan rate of $50 \text{ mV}\cdot\text{s}^{-1}$), n is the number of electrons transferred (in this case, $n = 2$), F is the Faraday constant, and A is the geometric area of the GCE (0.07 cm^2). The estimated electroactive immobilized enzyme coverage on the electrode is $4.2 \times 10^{-8} \text{ mol}/\text{cm}^2$. This value is comparable to the previous report for covalent conjugation with AuNPs, which supported the M13 bacteriophage ($4.74 \times 10^{-8} \text{ mol}/\text{cm}^2$),²⁹ and is higher than those for GOx immobilized onto CS/carbon nanodots ($8.78 \times 10^{-11} \text{ mol}/\text{cm}^2$),⁴⁹ graphene oxide ($1.22 \times 10^{-10} \text{ mol}/\text{cm}^2$),⁵⁰ TiO_2 nanostructures ($2.57 \times 10^{-10} \text{ mol}/\text{cm}^2$),⁵¹ and boron-doped CNT ($1.94 \times 10^{-9} \text{ mol}/\text{cm}^2$).⁵²

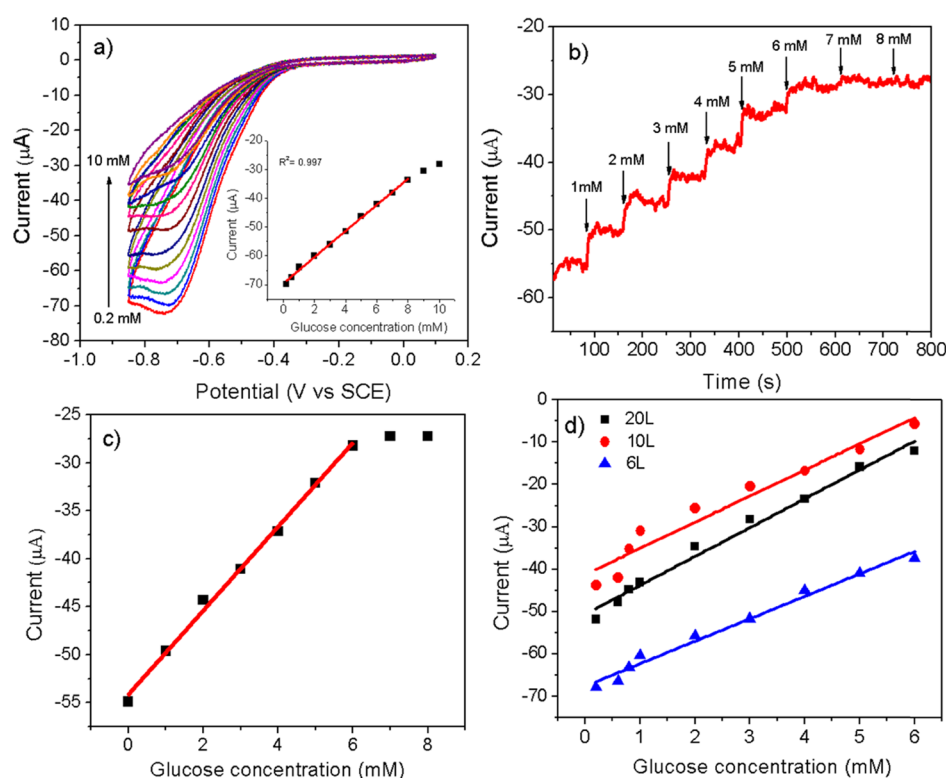


Figure 4. (a) CVs corresponding to the electrocatalyzed oxidation of different concentrations of glucose by the GOx-immobilized CS/Pd@Pt NC/GOx-GCE. CVs were recorded in PBS (0.1 M, pH 7.4) at a scan rate of $50 \text{ mV}\cdot\text{s}^{-1}$. (b) Current–time response curves for the successive addition of glucose (1–8 mM) at a fixed potential of -0.65 V . (c) Linear calibration curve corresponding to the amperometric response of the CS/Pd@Pt NC/GOx-GCE in the presence of variable concentration of glucose. (d) Linear calibration curves corresponding to varying Pt shell thicknesses in the presence of different glucose concentrations.

Table 1. Comparison of the Analytical Performance of Different Nanomaterial-Based DETs for Electrochemical Sensing of Glucose^a

biosensor	sensitivity ($\mu\text{A cm}^{-2} \text{ mM}^{-1}$)	response time (s)	detection limit (μM)	linear range (mM)	K_m (mM)	ref
PtNW/GOx	4.21		83.3	0.083–182	17.6	57
DMIm/Au ₂₅ /GOx	0.21			1–6	3.4	30
1-D TiO ₂ /GOx	9.9	<5		0.2–1	1.54	46
PMA/diamond/GOx	0.006		1	0–3		23
GR/CNT/ZnO NPs/GOx	5.36		4.5	0.01–6.5		58
CNT/GOx	0.47		4	1–30		59
PtNPs/GR/CS/GOx			0.6	0.2–5		53
CS/Pd@Pt NC/GOx	6.82	<5	0.2	1–6	0.857	this work

^aPtNW: platinum nanowires; DMI: 1-decyl-3-methylimidazolium; TiO₂: titanium dioxide; PMA: poly(methacrylic acid); CNT: carbon nanotubes; ZnO: zinc oxide; PtNPs: platinum nanoparticles; GR: graphene.

To investigate the DET characteristics for the electrochemical detection of glucose, we recorded CV measurements in oxygen (O₂)-saturated 0.1 M PBS solution (pH 7.4) at a scan rate of $50 \text{ mV}\cdot\text{s}^{-1}$ in the presence of different concentrations of glucose. Figure 4a shows the CVs along with the respective calibration plots (inset) corresponding to the bio-electrocatalytic reduction of successive addition of varying glucose concentrations. The reduction current gradually decreases upon the addition of glucose, exhibits a linear range between 1 and 6 mM, and saturates after 6 mM. It should be pointed out that without covering with the CS, the bare Pd@Pt/GOx electrode exhibits a rapid reduction in the low glucose concentration range and reaches saturation quickly after 4 mM (Figure S6). This result indicates that the CS polymer confers stability to the modified electrode and prevents inactivation of biocatalytic functions, which enables an enhanced performance

for glucose detection. The sensitivity of the biosensor was measured using current–time (*i*–*t*) curves at a constant potential of -0.5 V versus saturated calomel electrode (SCE), which is shown in Figure 4b. The electrocatalytic current decreases upon successive addition of glucose and reaches a steady state with an average response time of 5 s. Notably, we can see that the reduction current decreases and saturates above 6 mM.

A typical steady-state current as a function of glucose concentration (Figure 4c) shows a linear relationship in the 1–6 mM range, thus suggesting that the sensor can be used in this concentration range for the continuous monitoring of glucose. The observed low linear range can be attributed to the combined effect of intrinsic peroxide activity catalyzed by the enzymatic release of H₂O₂ and the competitive oxygen consumption by glucose and FADH₂.⁴⁶ The linear range here

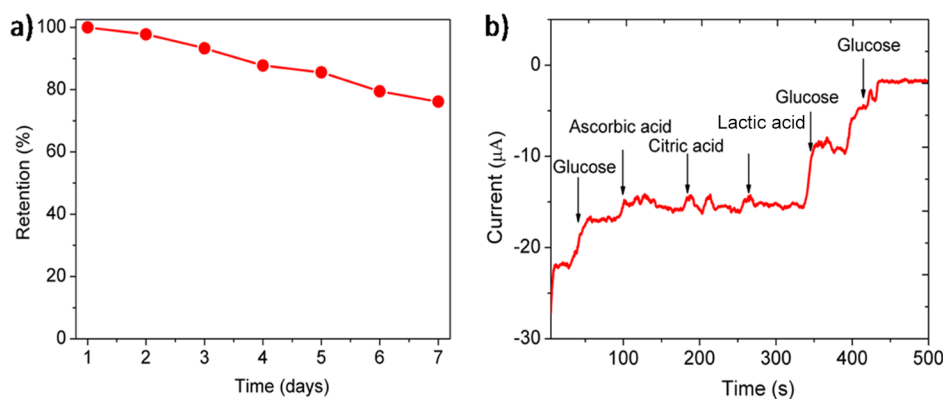


Figure 5. (a) Stability of the CS/Pd@Pt NC/GOx-GCE-modified electrode over a week-long storage period. (b) Amperometric response showing the effect of interfering substances (1 mM AA, CA, LA, and glucose).

is slightly broader compared with that of Pt NPs supported on graphene and CS (0–5 mM).⁵³ The sensitivity of the biosensor was calculated to be $6.82 \mu\text{A mM}^{-1} \text{cm}^{-2}$, which is relatively higher than those of other reported glucose biosensors (Table 1). The sensitivity of our biosensor is less compared with the Pt NP-based enzyme electrode; the CS coating alters the reactivity of the Pd@Pt NCs and the conductivity, which significantly decreases the charge transport. The glucose concentration in the standard blood sample is approximately 4.89 mM.⁵⁴ Our biosensor represents the linear relationship between the steady-state current as a function of the concentration of glucose (0.2–6 mM), which is higher than the average glucose level in the blood. We also carried out additional experiments to establish the detection limit of the biosensor, which was $0.2 \mu\text{M}$ (Figure S7). This detection limit is also significantly better than those of the other recently reported biosensors (Table 1). The apparent Michaelis–Menten constant (K_m) was determined to evaluate the biological activity of the immobilized enzyme, which is estimated using Lineweaver–Burk equation as follows⁴⁶

$$\frac{1}{i_{ss}} = \frac{K_m^{\text{app}}}{I_{\text{max}}} \times \frac{1}{C} + \frac{1}{i_{\text{max}}} \quad (2)$$

where i_{ss} is the steady-state current after the addition of glucose, i_{max} is the maximum current under the saturated conditions, and C is the bulk concentration of glucose. For a given glucose concentration, the calculated K_m is 0.58, which is very small compared with those of the previously reported different nanostructure-based enzymatic glucose sensors presented in Table 1. The relatively low value of K_m suggests a higher binding affinity of the immobilized GOx to the CS/Pd@Pt core–shell NC-based enzyme electrode and enzymatic activity. The excellent immobilized GOx affinity for glucose can be attributed to the high biocompatibility of CS, which preserves the biocatalytic function and the structure of GOx.

Recent studies have shown that the catalytic activity of the Pd@Pt core–shell NCs is dramatically altered by varying Pt shell thickness.^{8,10} This effect is attributed to the changes in the adsorption strength of the NCs through surface strain arising from the lattice mismatch between Pd and Pt and ligand effects.⁵⁵ Therefore, we have examined the glucose-sensing performance with different Pt shell thicknesses. Figure 4d shows the calibration plots for the detection of glucose with three different Pt shell thicknesses. The amperometric currents of the modified electrode are linearly dependent upon glucose concentration (0–6 mM), which yields the sensitivity values of

6.82, 6.172, and $5.303 \mu\text{A cm}^{-2} \text{mM}^{-1}$ for 6, 10, and 20 Pt atomic layers, respectively. These results demonstrate that the Pd@Pt NCs with a few atomic layers of Pt show a higher sensitivity to detect glucose, ascribed to the significantly higher catalytic activity of the Pt shells with fewer atomic layers.

To evaluate the stability of the biosensor, the electrode was stored at 4°C in a 0.1 M PBS after use and tested every day for the current response for 1 mM glucose for a 1 week period (Figure 5a). Our biosensor retained approximately 80% of its original response over 7 days, indicating excellent stability. The biosensor exhibits almost the same current with identical glucose concentrations, thus pointing out a good stability for practical applications. The biosensor is highly selective for the detection of glucose. As shown in Figure 5b, addition of different interference substances such as 1 mM AA, citric acid (CA), uric acid (UA), and lactic acid (LA) results in negligible changes in the reduction current, whereas an apparent response in the current is observed for the subsequent addition of 1 mM glucose, suggesting an excellent anti-interference ability of the Pd@Pt NC/GOx-based biosensor.

3. CONCLUSIONS

We have demonstrated a platform based on CS-covered Pd@Pt core–shell NCs for the sensitive electrochemical detection of glucose. The new room-temperature synthesis methodology is exploited for depositing ultrathin shells of Pt on the Pd nanocubes to obtain uniform Pd@Pt core–shell nanocubes. The covering of CS on the nanocubes allows the covalent linkage of GOx enzyme, which not only promotes DET but also confers stability and preserves biocatalytic functions of GOx. The biosensor here is capable of glucose detection with a high sensitivity of $6.82 \mu\text{A cm}^{-2} \text{mM}^{-1}$, a linear range of 1–6 mM, and a fast response time (approximately 5 s). Moreover, the sensor shows high stability and specificity toward different interfering compounds. This biosensor platform offers many advantages such as low cost, superior electrocatalytic activity, significant enhancement in charge transport, and high sensitivity in detecting glucose.

4. EXPERIMENTAL SECTION

4.1. Materials. CS (medium molecular weight $M_w = 300$ kDa, 82% degree of deacetylation), sodium tetrachloropalladate (II) (Na_2PdCl_4), potassium tetrachloroplatinate (II) (K_2PtCl_4), potassium bromide (KBr, 99%), poly(vinyl pyrrolidone) (PVP, M_w of 55 000), L-ascorbic acid (AA, 99%), GOx (from *Aspergillus niger*), D (+) glucose, CA ($\geq 99\%$), UA ($\geq 99\%$),

LA ($\geq 99\%$), sucrose ($\geq 99\%$), and GA (50%) were purchased from Sigma-Aldrich. Acetic acid (glacial, 99–100%) was purchased from Merck. All reagents were of analytical grade and used as received. Ultrapure deionized (DI) water (resistivity of 18 M Ω -cm) was used throughout all experiments.

4.2. Synthesis of Pd@Pt_{nl} Core–Shell NCs. The Pd nanocube seeds with an average edge length of 13 nm were synthesized using a previously described method.⁵⁶ In a typical synthesis, 15 mL of aqueous solution containing PVP, L-ascorbic acid (60 mg), KBr (300 mg), and Na₂PdCl₄ (57 mg) was prepared and heated for 3 h at 80 °C under magnetic stirring. After that, the reaction mixture was cooled to room temperature, and then the resultant products were centrifuged at 12 000 rpm for 10 min and were washed with DI water to obtain Pd nanocubes. For the synthesis of Pd@Pt core–shell NCs, the Pd nanocubes were used as seeds for conformal deposition of Pt. In a typical procedure, 100 mg of AA and 66.6 mg of PVP were dissolved in 15 mL of DI water in a vial, followed by adding 2.5 mL of aqueous suspension of Pd nanocubes, and the mixture was stirred well. After that, 0.5 mL of sodium hydroxide (0.2 M) was added to increase the pH of the reaction to 11. The mixture was magnetically stirred for 15 min, and then, another aqueous solution (0.1 mL) containing K₂PtCl₄ (0.1 M) was added slowly to the vial to obtain Pd@Pt core–shell NCs. Varying the Pt atomic layers on the Pd cubes was done by simply changing the concentration of K₂PtCl₄ from 0.1 to 0.25 mL and 0.5 mL.

4.3. Covering of Pd@Pt NCs with CS Biopolymer and Covalent Immobilization of GOx. The CS solution was obtained by dissolving 1 g of CS powder in a 1% acetic acid solution, as described in our previous work.²⁰ The CS coating on the resultant NCs was performed by stirring 5 mg of Pd@Pt nanocubes in 1 mL of CS solution (1 wt %) for 30 min. The covalent immobilization of GOx on the CS-covered Pd@Pt NCs was accomplished by reacting with 1 mL of GA (50%), and the suspension was magnetically stirred for 2 h, followed by the addition of 0.2 mL of GOx enzyme (40 mg/mL in PBS); the resultant solution was allowed to react overnight. The GOx-immobilized CS/Pd@Pt NCs were then stored for the preparation of the enzyme electrode.

4.4. Preparation of the Enzyme Electrode. GCEs ($d = 3$ mm, homemade) were polished with 1.0, 0.3, and 0.05 μ m alumina powders to obtain a mirror surface. The GCE was rinsed thoroughly with DI water between each polishing step and sequential ultrasonication in a DI/acetone mixture and then dried with a nitrogen stream. After drying, 30 μ L of covalently immobilized GOx on the CS/Pd@Pt NCs was deposited on the surface of the cleaned GCE and left to dry at 4 °C for at least 4 h. The fabricated modified enzyme electrodes were stored at 4 °C in a refrigerator under dry conditions when not in use.

4.5. Material Characterization. The morphology of the Pd nanocubes and Pd@Pt NCs was analyzed by TEM using a JEOL JEM-1010 instrument operated at 80 kV by drop-casting the resultant NCs on the Cu grids and drying at room temperature. HAADF-STEM analyses were obtained using JEM ARM 200F equipment operated at an accelerating voltage of 200 kV. The covalent immobilization and the interaction of GOx with the CS/Pd@Pt NCs were identified using FT-IR spectroscopy using a Perkin Elmer spectrophotometer with an attenuated total reflection accessory in the range of 4000–650 cm⁻¹. Electrochemical measurements were recorded on an electrochemical workstation (VoltaLab 40 PGZ 301). The DLS

and zeta potential measurements were recorded using Nano ZS (Malvern).

4.6. Electrochemical Measurements. All electrochemical experiments were carried out using a VoltaLab 40 PGZ 301 electrochemical workstation with a conventional standard three-electrode cell. The homemade GCE used as the working electrode was cleaned well before and after each experiment. A platinum foil and an SCE were used as the auxiliary and the reference electrodes, respectively. The electrochemical glucose-sensing measurements of the modified electrodes were recorded using CV and recorded in 0.1 M PBS (pH 7.4) at room temperature. The PBS buffer solution was purged with oxygen for at least 20 min before the measurements. The chronoamperometric measurements were recorded at a fixed potential of -0.5 V.

■ ASSOCIATED CONTENT

● Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsomega.7b00060.

STEM images, TEM images of different Pt shell thicknesses, EDX-elemental mapping analysis, XPS results, DLS and zeta potential analysis, and electrochemical response for low detection limits (PDF)

■ AUTHOR INFORMATION

Corresponding Authors

*E-mail: skumar@fata.unam.mx (S.K.K.).

*E-mail: resparza@fata.unam.mx (R.E.).

ORCID

Siva Kumar Krishnan: 0000-0002-9672-9335

Notes

The authors declare no competing financial interest.

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