Original Article	*Curr. Appl. Sci.*, **2024**, *2*(2):67-78 DOI:10.22034/cas.2022.343533.1019	Current Applied Sciences

Nonenzymatic Electrochemical Detection of Glucose Using Screen-Printed Electrode Modified with Pd–Au Nanoparticles Encapsulated on Dendrimer Grafted Multi-Wall Carbon Nanotubes

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Article Information

Received: 21 May 2023
Revised: 14 September 2023
Accepted: 18 September 2023
Published online: 25 July 2024

Keywords

Palladium–gold nanoparticles
Dendrimer-encapsulated NPs
Glucose
Electrochemical sensor
Carbon nanotubes
Screen-printed electrode

Abstract

A novel nonenzymatic glucose sensor based on palladium–gold nanoparticles encapsulated on polypropylene amine dendrimer–grafted multi-wall carbon nanotubes (PdAu/PPI–MWCNTs) has been successfully fabricated and applied to glucose detection. PdAu/PPI–MWCNTs was prepared in a three–step process. First, polypropylene amine (PPI) dendrimers were grown on the surface of functionalized multi-wall carbon nanotubes (MWCNTs–NH_2) by a divergent method; second, metal ions were trapped within the dendrimers; and third, the ions were chemically reduced. Therefore, the well-distributed nanoparticles with an average size range of 1.6-3.2 nm were obtained on the surface of PPI–MWCNTs. The prepared PdAu/PPI–MWCNTs nanocomposite was immobilized on screen-printed carbon electrode and its electrocatalytic activity for glucose oxidation reaction was studied. Under the optimized conditions, the glucose oxidation current was linear to its concentration within the range of 0.03 – 3.0 mM, and the detection limit was found to be 0.01 mM (S/N = 3). Finally, the prepared sensor has been successfully applied to determine the glucose content in human blood serum samples.

1. Introduction

Producing new electrocatalysts for the accurate determination of glucose is a growing interest in electroanalytical chemistry because of the great importance of glucose sensing in clinical and fuel cell applications [1–5].

The enzymatic biosensors of glucose that are usually based on glucose oxidase [6–9] or glucose dehydrogenase [10] showed good selectivity and sensitivity for the detection of glucose. However, high cost, complicated

immobilization procedure, and chemical and thermal instabilities of enzymes are the main drawbacks of enzymatic biosensors.

The development of nonenzymatic biosensors for glucose to overcome these drawbacks is of great significance. This kind of biosensor is based on the direct oxidation of glucose at the surface of electrodes modified with appropriate electrocatalysts. Therefore, the development of new electrode materials to provide sensitive responses for glucose and minimal responses towards typical interfering substances, such as ascorbic acid (AA) or uric acid (UA), has been attracting more and more attention [11–13].

Metal nanoparticles are known to be one of the most effective catalysts for the fabrication of nonenzymatic glucose biosensors. Previously, some electrocatalytic systems based on mono- and bimetallic nanoparticles have been successfully applied in the construction of nonenzymatic glucose sensors [14–18]. Using stabilizers or protective agents in preparing metal nanoparticles is necessary in order to control the size and shape of the nanoparticles and also to prevent their agglomeration. Dendrimers, a new type of macromolecules, characterized by a highly branched treelike morphology, regular structure, high density of surface active groups, good structural homogeneity, and high internal porosity, could be used as templates for the synthesis of nanoparticles. Using dendrimers as a stabilizer for metal nanoparticles provides us with many advantages, such as obtaining high dispersion, high stability against aggregation, good solubility, and very small particle size of metal nanoparticles. As a result, dendrimers have been successfully used as templates for preparing mono- and bimetallic nanoparticles [19–21].

Also, dendrimers can be immobilized on surfaces and used as templates for nanoparticles with the aim of heterogeneous catalysis [22–23] or electrocatalysis [24–25]. Using suitable support materials could lead to obtaining high surface area catalysts and high efficiencies. The basic properties of these heterogeneous composite catalysts are strongly affected by the properties of support materials.

Unique properties of carbon nanotubes (CNTs), such as high surface area, high chemical stability, good conductivity, and electrocatalytic activity, suggest them as a suitable support material for electrocatalytic applications [26–29]. However, there are difficulties in dispersing and stabilizing metal nanoparticles on the CNTs surfaces, and to overcome this problem, the surface of CNTs must be modified via proper functionalization. In general, the functionalization of CNTs with dendrimers provides an excellent platform for electrochemical sensing applications.

In this article, the third generation of polypropylene amine (PPI) dendrimer was covalently conjugated with the multi-wall carbon nanotubes to prepare PPI–MWCNTs. Covalent grafting can make the composites more stable and controllable. Then, PPI–MWCNTs have been used as a template for bimetallic PdAu nanoparticles to prepare PdAu/PPI–MWCNTs hybrid material [22, 30–31]. Finally, the prepared PdAu/PPI–MWCNTs nanocomposite was immobilized on screen-printed carbon electrode and its electrocatalytic activity for glucose oxidation was studied. It was found that PdAu/PPI–MWCNTs exhibited a good promotion in the electrochemical oxidation of glucose, and the fabricated glucose sensor showed advantages such as excellent selectivity and reproducibility, long-term stability, rapid response time, and acceptable detection limit.

2. Materials and Methods

2.1 *Chemicals*

The used MWCNTs were prepared by chemical vapor deposition procedure in the presence of Co/Mo/MgO as the catalyst at 900 ºC [32]. The outer diameter of MWCNTs was between 20 and 40 nm. PPI–MWCNTs and PdAu/PPI–MWCNTs were synthesized according to the reference [22, 30, 31]. All solvents and reagents were purchased from Aldrich or Merck and used without further purification. Blood serum samples were kindly provided by the Taleghani Hospital (Tehran, I. R. Iran). All solutions were prepared with double distilled water.

2.2 *Apparatus*

All electrochemical measurements were performed using a computer-controlled μAutolab potentiostat/galvanostat type III with general-purpose electrochemical software operating system GPES version 4.7 (Eco Chemie B.V., 3508 AD Utrecht the Netherlands). Voltammetric experiments were performed on three-electrode screen-printed electrochemical strips (DropSens, Spain). Each strip consists of a 4 mm diameter disk screen-printed carbon electrode (SPCE) as a modified and unmodified working electrode and a carbon counter electrode, both printed on an alumina substrate using heat curing carbon composite inks, and a silver pseudoreference electrode. Ink formulation and production characteristics of commercial SPCEs are regarded by manufacturers as proprietary information. An ultrasonic bath (EUROSONIC® 4D) was used to disperse materials in solvents.

2.3 *Preparation of PdAu/PPI–MWCNTs modified screen-printed carbon electrode (PdAu/PPI–MWCNTs/SPCE)*

Suspension of the catalyst was prepared by dispersing 5 mg of PdAu/PPI–MWCNTs in 10 mL DMF using ultrasonic agitation to obtain a relatively stable suspension. Then, the screen-printed carbon electrode was coated by casting 5 μL of the black suspension of PdAu/PPI–MWCNTs, and then it was dried at room temperature. Also, the suspensions of MWCNTs and PPI–MWCNTs were prepared and used for the fabrication of MWCNTs/SPCE and PPI–MWCNTs/SPCE, with the same procedure for comparison of the operation of PdAu/PPI–MWCNTs/SPCE.

2.4 *Analytical procedure*

2.4.1 *Cyclic voltammetry at PdAu/PPI–MWCNTs/SPCE*

The PdAu/PPI–MWCNTs/SPCE was first activated in the blank solution (NaOH, 0.1M) by cyclic voltammetric sweeps between -0.8 and +1.0V until stable cyclic voltammograms were obtained. Then the electrode was used for the oxidation of glucose in the same supporting electrolyte solution. For comparison, bare SPCE, MWCNTs/SPCE, and PPI–MWCNTs/SPCE were used with the same procedure.

2.4.2 *Chronoamperometric studies*

The PdAu/PPI–MWCNTs/SPCE was inserted into the solution and left at open circuit under quiescent conditions for an incubation period of 100 s. A potential step (0.1 V vs. Ag/AgCl) was then applied, and the resulting current was monitored. For calibration studies, chronoamperograms were recorded in supporting electrolyte (NaOH, 0.1M) containing glucose concentrations within the range of 0.03 – 3.0 mM, using a 100-s incubation time, and the current from the resulting chronoamperograms was sampled at 100 s.

2.4.3 *Detection of glucose in real samples*

The plasma samples were 20-fold diluted with 0.1M NaOH prior to the measurements, and each sample was analyzed using the chronoamperometric method. Also, the recovery tests for glucose determination were performed by adding known amounts of glucose to the diluted serum samples.

3. Results and Discussion

3.1 *Direct electrochemistry of modified electrodes*

Various modified electrodes were fabricated and used, as detailed in the experimental section. CVs of SPCE, MWCNTs/SPCE, PPI-MWCNTs/SPCE, Au/PPI-MWCNTs/SPCE, Pd/PPI-MWCNTs/SPCE, and PdAu/PPI-MWCNTs/SPCE in 0.1 M NaOH over the potential range from -0.8 to 1.0 V are shown in Figure 1. As can be seen, no redox peak was observed at the SPCE (A), MWCNTs/SPCE (B), and PPI-MWCNTs/SPCE (C), although using the MWCNTs (B and C), the background current was increased, which was attributed to the large surface area and high electrocatalytic activity of CNTs. For Au/PPI-MWCNTs/SPCE (D) and Pd/PPI-MWCNTs/SPCE (E), some significant oxidative and reductive peaks were observed. A broad oxidation peak observed above +0.40 V corresponds to the oxidation of metal nanoparticles and metal oxide formation. Also, the cathodic peaks that appear in the return cycles are related to the reduction of gold and palladium oxides and the regeneration of metals. PdAu/PPI-MWCNTs/SPCE showed the same behavior (F), but the cathodic peaks of Au and Pd were seen as two overlapped peaks at about -0.3 V. These results confirm that the Pd and Au surfaces of the PdAu/PPI-MWCNTs are electrochemically active.

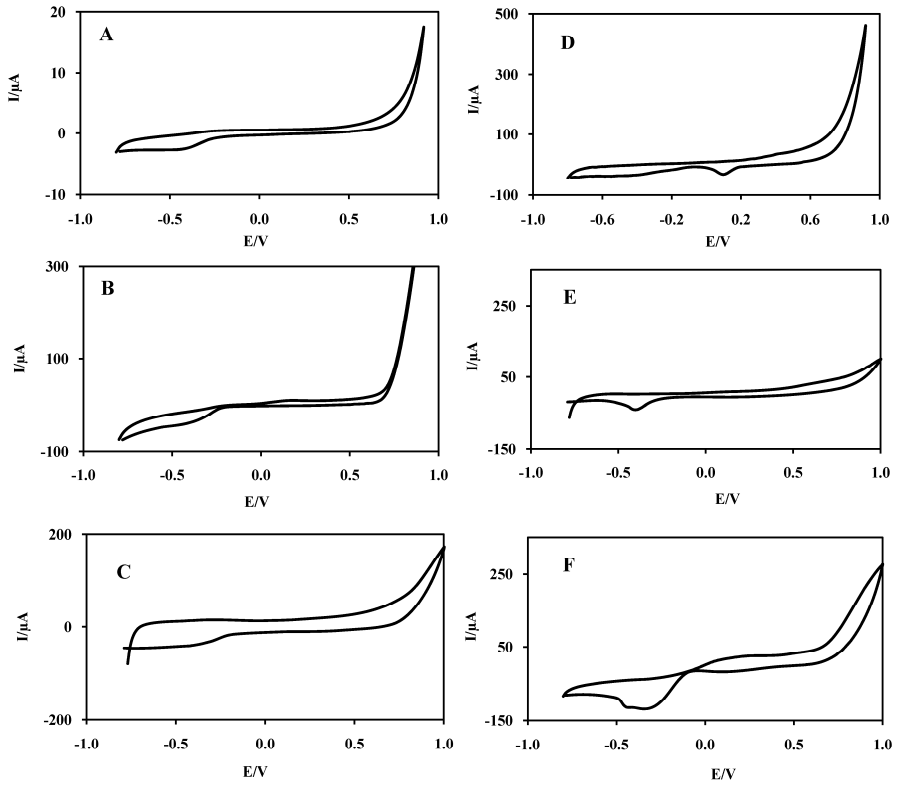

Figure 1. Cyclic voltammograms of (A) SPCE, (B) MWCNTs/SPCE, (C) PPI-MWCNTs/SPCE, (D) Au/PPI-MWCNTs/SPCE, (E) Pd/PPI-MWCNTs/SPCE, and (F) PdAu/PPI-MWCNTs/SPCE in 0.1M NaOH solution; scan rate 50 mVs^{-1}

3.2 *Electrocatalytic oxidation of glucose at the PdAu/PPI-MWCNTs/SPCE*

In the alkaline solution, glucose can be oxidized through a two-electron mechanism according to the following reaction:

$$C_6H_{12}O_6 + 2OH^- \rightarrow C_6H_{12}O_7 + H_2O + 2e^-$$

The simultaneous presence of Au and Pd in the bimetallic nanocomposite can provide more catalytic active sites and the synergistic effect, which more promote the above electrochemical oxidation.

To demonstrate the performance of the PdAu/PPI-MWCNTs/SPCE, we compared the CVs of glucose on the surface of different modified electrodes. Figure 2 shows the voltammograms of the background and 2 mM glucose using various modified electrodes.

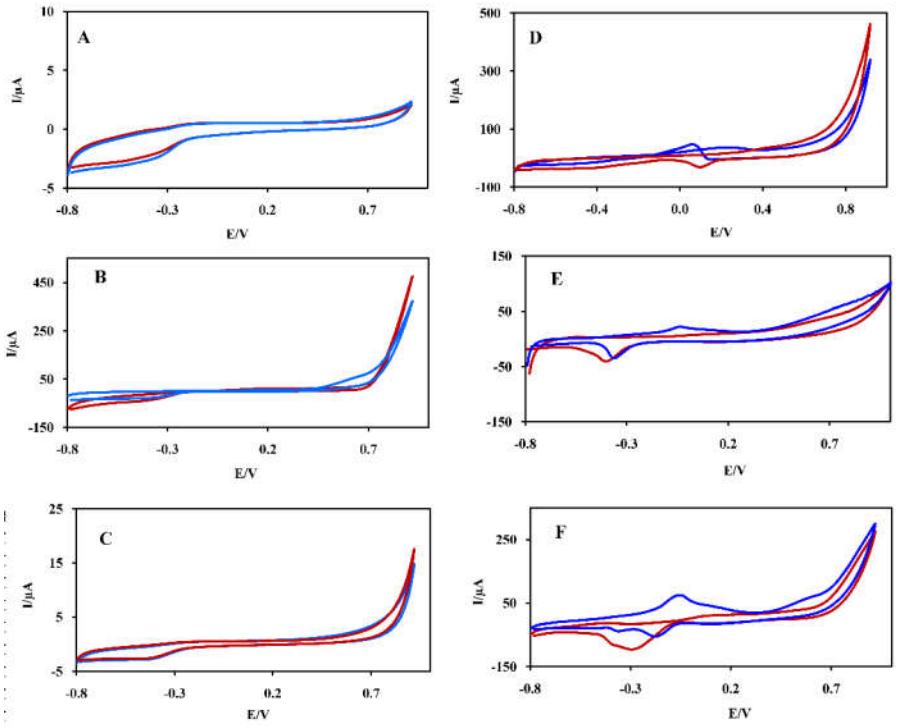

Figure 2. Cyclic voltammograms of (A) SPCE, (B) MWCNTs/SPCE, (C) PPI-MWCNTs/SPCE, (D) Au/PPI-MWCNTs/SPCE, (E) Pd/PPI-MWCNTs/SPCE, and (F) PdAu/PPI-MWCNTs/SPCE in the presence of 2 mM glucose in 0.1M NaOH solution; scan rate 50 mVs^{-1}

As seen, no redox peak was observed on the surface of the bare SPCE (A), while a very weak oxidation peak was obtained on the surface of MWCNTs/SPCE (B) and PPI-MWCNTs/SPCE (C) at about 0.65 V. For Au/PPI-MWCNTs/SPCE (D) and Pd/PPI-MWCNTs/SPCE (E), clear and distinguished anodic peaks were observed at about 0.1 V. Also, a decrease in the cathodic peaks of these electrodes is related to the overlap of this peak with the anodic peak that appears in the negative potential scan. In the case of the PdAu/PPI-MWCNTs/SPCE (F), the glucose oxidation potential decreased, while the peak current increased in comparison with the other mentioned electrodes. This behavior indicates that the PdAu/PPI-MWCNTs/SPCE possesses an excellent electrocatalytic activity for glucose oxidation.

3.3 *Selectivity, repeatability, and stability of the modified electrode*

The selectivity of the sensor was evaluated by studying the interference of the poisoning effect of chloride ions and the oxidation of biological species, such as ascorbic acid (AA ~ 0.1 mM), uric acid (UA ~ 0.02 mM), and *p*-acetamidophenol (AP ~ 0.1 mM).

The poisoning effect of chloride ions on the response of the modified electrodes has been investigated using cyclic voltammetry. Figure 3 shows the voltammograms of the modified electrode in 0.1 M NaOH containing 2 mM glucose in the presence (curve a) and in the absence (curve b) of 0.1 M NaCl. As it is seen, the glucose response remained unchanged under the presence of chloride ions. Therefore chloride ions had no observable poisoning effects on the electrocatalytic activity of PdAu/PPI-MWCNTs/SPCE.

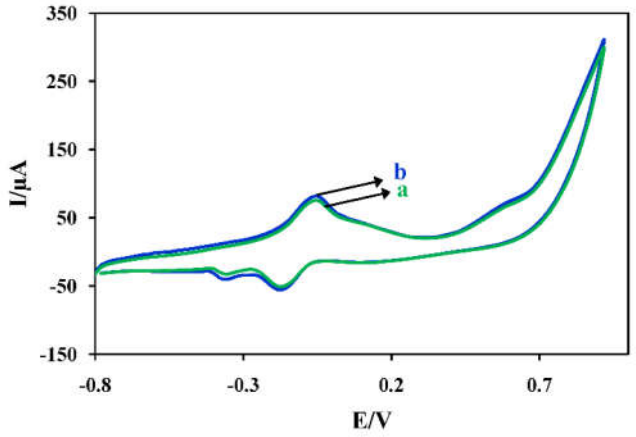

Figure 3. Voltammograms of the PdAu/PPI-MWCNTs/SPCE in 0.1 M NaOH containing 2 mM glucose in the presence (curve a) and in the absence (curve b) of 0.1 M NaCl

The interferences of oxidizable species have been investigated using chronoamperometry. The chronoamperometric responses of the modified electrode at the applied potential of 0.1 V in 0.1M NaOH containing 2 mM glucose in the absence and also in the presence of 0.1 mM AA, 0.02 mM UA, and 0.1 mM AP were evaluated, respectively. The results demonstrated that in the potential of 0.1 V, the interferences caused by AA, UA, and AP were negligible, and the sensor showed excellent selectivity for glucose oxidation.

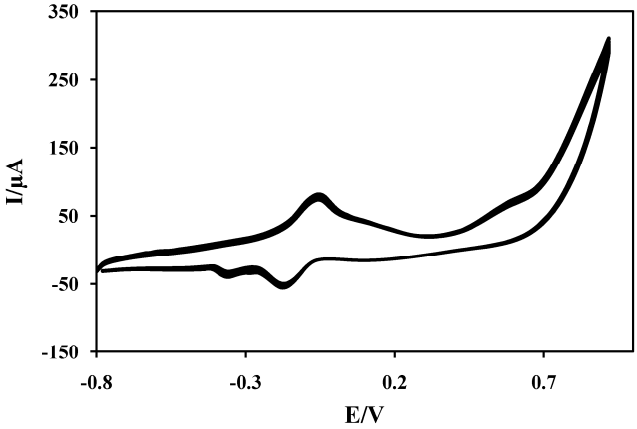

Figure 4. Ten different cyclic voltammograms of the PdAu/PPI-MWCNTs/SPCE at a single electrode in the presence of 2 mM glucose in 0.1M NaOH; scan rate 50 mVs^{-1}

Furthermore, the stability and repeatability of the electrocatalytic signals on PdAu/PPI-MWCNTs/SPCE were investigated using cyclic voltammetry. Figure 4 shows ten different cyclic voltammetry measurements for 2 mM glucose with 30 min interval times at a single electrode. As can be seen in this figure, the electrocatalytic characteristics of the PdAu/PPI-MWCNTs/SPCE toward the oxidation of glucose are fairly stable and repeatable (R.S.D. of 4.3%) since the cyclic voltammograms remain almost invariable after ten measurements. In addition, no significant change was observed after storing the electrode in the supporting electrolyte solution for ten days. Therefore, the PdAu/PPI-MWCNTs nanocomposite exhibited acceptable stability and repeatability in the potential range where it was used as an electrocatalyst for the oxidation of glucose.

3.4 *Chronoamperometric studies*

The chronoamperometric method at a working potential of 0.1 V *vs.* Ag/AgCl was used to examine the performance of the PdAu/PPI-MWCNTs/SPCE towards the detection of glucose.

Figure 5 shows the chronoamperometric responses of the modified electrode, which were recorded in the supporting electrolyte (0.1M NaOH) containing glucose with concentrations within the range of 0.03 – 3.0 mM.

As shown in Figure 5 (Inset), the plot of current versus glucose concentration is linear in the range of 0.03 – 3.0 mM with 0.01 mM as the limit of detection at a signal-to- noise ratio of 3.

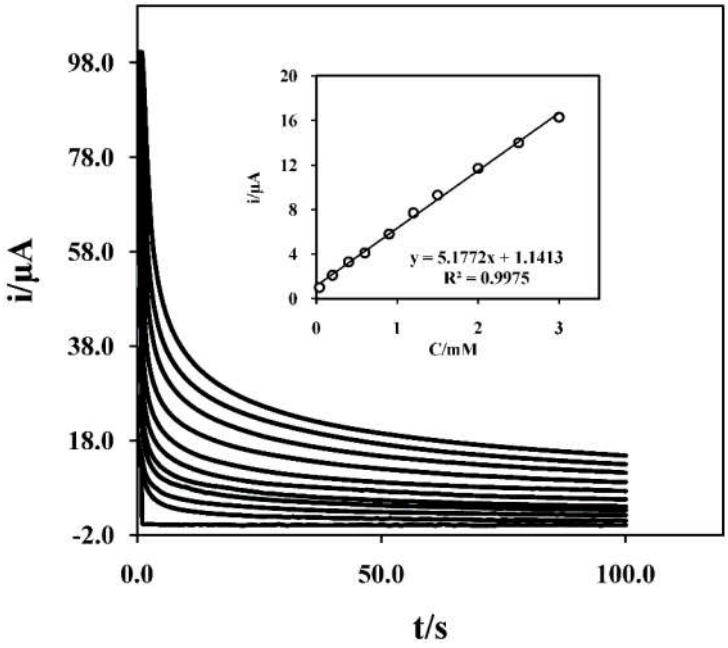

Figure 5. A) The effect of glucose concentration on the chronoamperometric response of the PdAu/PPI-MWCNTs/SPCE at 0.1 V in 0.1 M NaOH and inset, calibration plot of the current at 100th s versus glucose concentration

3.5 *Real sample analysis*

The applicability of the modified electrode was investigated for glucose determination in three human plasma samples. The recoveries (Table 1) were in the range of 94.12 to106.06%, indicating that the PdAu/PPI-MWCNTs/SPCE provides sensitive, accurate and precise results for monitoring the glucose concentrations in human blood.

Table 1. Determination of glucose in diluted human plasma samples

Sample	Found in solution (mM)	Glucose in blood (mM)	Added (mM)	Found (mM)	Recovery (%)
1	0.23	4.6	0.10	0.35	106.06
			0.30	0.51	96.23
			1.00	1.20	97.56
2	0.26	5.2	0.10	0.38	105.56
			0.30	0.58	98.21
			1.00	1.28	101.59
3	0.19	6.8	0.10	0.28	96.55
			0.30	0.51	104.08
			1.00	1.12	94.12

In addition, a comparison between the analytical performance of the proposed sensor with some gold or palladium-based modified electrodes is shown in Table 2. According to this table, the analytical performances of the proposed sensor are comparable with previous sensors. These results indicated that the PdAu/PPI-MWCNTs composite could greatly improve the electrocatalytic response of glucose oxidation.

Table 2. Comparison of nonenzymatic electrochemical determination of glucose at some gold or palladium electrodes

Electrode	Technique	Linear range (mM)	LOD (mM)	Ref.
Pd-Gr/GCE[1]	Amperometry	0.01- 5	0.001	[33]
Pd-SWNT/GCE[2]	Amperometry	0.5-17	0.0002	[11]
Porous Au film	Amperometry	2 – 10	0.005	[12]
Conventional Au disk	Amperometry	0.5 – 20	0.01	[34]
Pd/EPAgE[3]	3-Step coulometry	1–20	-	[35]
Au nanoparticles/GCE	Amperometry	2 – 38	0.01	[36]
Pd/TiO_2[4]	Chronoamperometry	7–35	-	[37]
PtPd/MCV/GCE[5]	Amperometry	1.5 to 12	0.12	[38]
PdAu/PPI–MWCNT/SPCE [6]	Chronoamperometry	0.03 to 3.0	0.01	This work

[1)] Palladium nanoparticles–graphene nanohybrids modified glassy carbon electrode; [2)] Palladium nanoparticles-single walled carbon nanotubes modified glassy carbon electrode; [3)] Palladium nanoparticles modified epoxy-silver electrode; [4)] Nanoporous Pd-modified TiO_2 electrode; [5)] PtPd nanoparticles/onion-like mesoporous carbon vesicle modified glassy carbon electrode; [6)] Screen-printed carbon electrode modified with bimetallic palladium–gold nanoparticles encapsulated on dendrimer grafted multi-wall carbon nanotubes.

4. Conclusions

In this work, the PdAu/PPI–MWCNTs/SPCE was successfully fabricated and examined in the electrocatalytic oxidation of glucose. The results showed that the PdAu/PPI–MWCNTs/SPCE exhibited a distinctly enhanced electrocatalytic activity towards the oxidation of glucose, even in the presence of high concentrations of chloride ions. The fabricated glucose sensor displayed excellent selectivity and reproducibility, long-term stability, short response time, and acceptable detection limit. Therefore, the novel PdAu/PPI–MWCNTs composite film has an application potential to develop nonenzymatic glucose sensors.

Conflicts of Interest

The author declares that he has no conflict of interest.

Acknowledgements

Financial support from the Research Affairs of the University of Zabol is gratefully appreciated (grant number UOZ-GR-9718-18).

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How to cite this article: Ahmar H. Nonenzymatic Electrochemical Detection of Glucose Using Screen-Printed Electrode Modified with Pd–Au Nanoparticles Encapsulated on Dendrimer Grafted Multi-Wall Carbon Nanotubes. *Curr. Appl. Sci.*, 2024, 2(2):67-78. https://doi.org/10.22034/cas.2022.343533.1019